

CHITOSAN AS A MULTIPURPOSE EXCIPIENT IN DIRECTLY COMPRESSED MINITABLETS

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Dedicated to my family:
The late Jean Roi my dearest father,
Cornelia my mother,
Alet and Jean Roi my siblings
and to
“Ouma Plaas”
who is a great example of how one should live
life to the full

“A man reaps what he sows. The one who sows to please his sinful nature, from that nature will reap destruction; the one who sows to please the Spirit, from the Spirit will reap eternal life.”

Gal 6:7,8

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ABSTRACT

CHITOSAN AS A MULTIPURPOSE EXCIPIENT IN DIRECTLY COMPRESSED MINITABLETING

The development of direct compression as the choice method of tablet manufacture can be promoted from two major advantages posed by the process. The method is a phenomenal time saving process due to its relative simplicity (blending of components followed by tableting directly afterwards). The consequence of these phenomena is acceleration of the production rate of tablets, ultimately resulting in vast economical benefits (Otto, 2002:X).

The growing interest for multiple-unit dosage forms, especially minitables (2-3 mm in diameter), and their increasing share of the pharmaceutical product market are claimed by their proponents to stem from advantages they offer over single-unit dosage forms, such as more uniform transit, less variability and a smaller risk of dose dumping. Other advantages like their large surface area and uniformity adds to their wide field of applications. The use of minitables would therefore constitute a potential economic saving through the use of less excipients, few and inexpensive production steps, and if to be coated, reduced amounts of coating material required.

Chitosan is a natural polysaccharide that is obtained by the partial deacetylation of chitin, the second most abundant natural polymer. Since chitosan combines unique physicochemical characteristics, *in vivo* biodegradability, biocompatibility, non-toxicity, and antimicrobial action, it has been widely investigated over the last few years for potential applications in the medical and pharmaceutical fields. As a pharmaceutical excipient, its main contribution seems to be as an absorption enhancer of large molecular drugs from the gastrointestinal tract (through "tight junctions") and as a fat absorber in dietary products. Although it is present in various tablet preparations, little if any is known about the tabletability or inclusion of this versatile polymer into minitables.

Characterisation of chitosan powder revealed a large particle size and a narrow particle size distribution which had a detrimental effect on the flowability of the powder, however, did not preclude the employment of chitosan. Due to its density results it could be predicted that chitosan would produce significantly compacted tablets of minimum weight, thus questioning its compactibility propensity. During preformulation studies it was found that the production

of pure chitosan tablets were impossible without the inclusion of other excipients, however, it was possible to produce minitables which did not necessitate the presence of a lubricant. The chitosan minitables did however, not disintegrate and were of poor mechanical strength. It was these properties that provided the challenges that were circumvented in this study. The addition of the sparingly water-soluble, hydrophobic drug, furosemide, perplexed the formulations of chitosan minitables, however, provided a mode of evaluation of the effects of formulation variables on tracer substance dissolution behaviour.

The physical stability of chitosan minitables indicated sensitivity towards varying ambient conditions regardless the inclusion of Kollidon® VA64 as chitosan easily dehydrate. It was furthermore evident that the presence of moisture (sorbed water) had a considerable influence on the tableability and disintegration of chitosan powder. Furthermore, the stability of chitosan powder deteriorated to a greater extent than the chitosan minitables which were subjected to the same conditions. It is therefore recommended that chitosan minitables should be stored at a temperature not exceeding 25 °C at a relatively low humidity (60%). It was also evident that the presence of Kollidon® VA64 as well as the method of inclusion of this binder influenced the stability of chitosan tablets. Thus, direct compression of chitosan granules which comprised of 5% w/w Kollidon® VA64 produced the optimal system in terms of product stability. Additionally, evaluation of chitosan tablets should be performed at consistent intervals as these tablets showed a time-dependent behavioural pattern regarding their physical properties.

Systematic evaluations of various commercially available excipients were investigated to determine their effects on the physical tablet properties as well as on tracer (furosemide) dissolution.

The tablet weight and dimensions of the different formulations did not vary discernibly, regardless the method used for production of the filler-system. Thus, exceptional good flowability was illustrated despite the extensive percentage chitosan, and the inclusion of furosemide into the formulations. The inclusion of furosemide did, however, portray a slightly detrimental effect on the crushing strength of the minitables compressed at the same compression setting. Furthermore, the crushing strengths of all the minitab formulations were very low (> 80 N) and the addition of other excipients did play a significant role in the crushing strengths of the different formulations. It might be suggested that the inclusion of a binder could not be seen as the only contributor to tablet strength. Friability results for all of the formulations were in congruence with the results obtained for tablet strength. Friability

and tablet hardness do not, however, play an important role in minitablets as these tablets are encapsulated and are thus, not subjected to intensive handling. The efficiency of the minitabulet formulations in this study were, however, determined and set apart through the disintegration, and more importantly, the dissolution properties of the different formulations.

Most of the formulations resulted in tablets that disintegrated well within the set limit of 15 minutes. The production of a 5% w/w Kollidon[®] VA64 filler-system through granulation proved detrimental to the disintegration process for all the different formulations. Interestingly, formulations containing a buffer disintegrated faster than formulations where a disintegrant was incorporated. It was furthermore demonstrated that formulations containing ascorbic acid disintegrated slower than formulations containing sodium bicarbonate, whereas formulations containing Kollidon[®] CL disintegrated significantly slower than formulations containing Ac-Di-Sol[®]. These results indicated that Ac-Di-Sol[®] is the preferred choice of disintegrant when used in these concentrations and in combinations with various buffers. Furthermore, the level of disintegrant or buffer determined the efficiency of disintegration to a large extent. The higher level imparted a more significant enhancement of disintegration effectiveness than did the type of excipient incorporated. When considering formulations where sodium bicarbonate and a disintegrant were combined, formulations which contained 2% w/w sodium bicarbonate disintegrated the fastest. It would seem that the inclusion of sodium bicarbonate imparted a more significant enhancement of disintegration effectiveness than did any other variable and that this buffer should be included in formulations containing Ac-Di-Sol[®] so as to enhance the disintegration process.

Comparison of the dissolution parameters of all of the formulations clearly indicated the dependency of the area under the dissolution curve (AUC) on the initial dissolution rate (DR_i). The decreased DR_i-values of both furosemide and chitosan, however, were contradictory to the prodigious disintegration of all the chitosan formulations which validated that tablet disintegration is no absolute guarantee for drug dissolution. It was further evident that formulation variables played a much more pronounced role during dissolution testing than with the physical properties.

The incorporation of 5% w/w Kollidon[®] VA64 into the different formulations through physically mixing provided tablets with enhanced dissolution properties for both furosemide and chitosan, whereas Methocel[®] K100M inhibited the onset of dissolution. The high AUC_n values of all of the formulations containing a buffer, regardless the concentration used, suggested dissolution conditions comparable and even superior to that of a furosemide

suspension. This could be attributed to thorough dispersion of the primary drug particles throughout the medium before settling at the bottom of the dissolution vessel. Incorporation of 0.5% w/w ascorbic acid led to an increased dispensation of furosemide due to increased chitosan solubility. However, the ascorbic acid created an unfavourable microenvironment for the furosemide to be able to dissolve promptly. A more significant increase in dissolution of furosemide was produced through the incorporation of sodium bicarbonate into the minitablen formulations. The observed increase in the DR_i of furosemide from these tablets was attributed to a pH-increase in the microenvironment and an increase in solvent penetration into the tablets due to the creation of capillaries. However, an increase in sodium bicarbonate concentration had a negative influence on the microenvironment of the chitosan which only dissolves in an acidic environment, thus inhibiting prompt dissolution.

It could clearly be seen that the incorporation of a disintegrant resulted in the most significant change in the dissolution profiles and the efficiency of these super-disintegrants were indeed concentration-dependent. Furthermore, it could be concluded that formulations containing Ac-Di-Sol[®] depicted faster dissolution rates and more pronounced extent of dissolution than formulations containing Kollidon[®] CL. The formulations containing 0.625% w/w Ac-Di-Sol[®] illustrated a significant positive linear dependence of $(AUC)_n$ on $(DR_i)_n$, whilst Kollidon[®] CL formulations illustrated a slightly less apparent dependence. However, an increase in concentration of Ac-Di-Sol[®] resulted in an inadequate correlation coefficient, thus, indicating that the $(AUC)_n$ were less dependant on the $(DR_i)_n$ with increasing concentrations of this disintegrant.

There was no discernable vindication for the inclusion of a combination of a disintegrant and ascorbic acid into the formulations. It was rather the inclusion of a disintegrant which played a more pronounced role in the dissolution parameters. It was however clear that the inclusion of 2.0% w/w sodium bicarbonate into formulations containing Ac-Di-Sol[®] rendered tablets with exceptional dissolution properties. Ac-Di-Sol[®] and sodium bicarbonate had a synergistic effect on the dissolution of both furosemide and chitosan in an acidic medium. The formulation (CF/36) which contained a physically mixed filler-system (consisting of chitosan and 5% w/w Kollidon[®] VA64) with 1.25% w/w Ac-Di-Sol[®] and 2% w/w sodium bicarbonate derived the most suitable dissolution properties (84.18% furosemide dissolved). It is thus evident that the undesirable properties of chitosan could be modified to comply with the set standards of directly compressed minitables.

The incorporation of the CF/36 formulation into capsules proved successful as the capsules disintegrated within ± 60 seconds releasing and dispersing their contents into the dissolution medium. It was only due to the lag time that the disintegration of the capsules and the Avicel[®] PH-101 matrix provided that the end furosemide concentration dissolved, had not yet been reached. However, these dissolution profiles still followed the same curve as the profile of the CF/36 minitabets. Capsules containing CF/36 tablets and Avicel[®] PH-101 did not separate as promptly as capsules which did not contain a protective filler and contrary to previous findings these capsules depicted slightly less favourable dissolution profiles than capsules containing only the minitabets. This was probably due to the formation of a rather viscous microcrystalline cellulose matrix around the minitabets which first had to disintegrate preceding disintegration of the minitabets.

Chitosan does not rank highly regarding its employment as first choice filler. Undesirable properties that limit its utilisation include its hydration propensity influencing stability, problematic tablet strength characteristics and the availability of fillers that circumvent some of the undesirable properties of chitosan. This study, however, proved that chitosan could serve as a relatively cheap alternative to other direct compression fillers especially in the formulation of minitabets, if the formulations were carefully optimised. Furthermore, its mucoadhesive and absorption enhancing properties could also benefit and improve bioavailability of various poorly absorbed compounds or active ingredients. Based on these results, the development of an optimised chitosan multifunctional excipient seems an attainable and relevant objective which is comparable and even superior to commercial products.

UITTREKSEL

KITOSAAN AS 'N MEERDOELIGE HULPSTOF IN DIREK SAAMGEPERSDE MINITABLETTE

Die ontwikkeling van direkte samepersing as metode van keuse tydens tabletvervaardiging hou twee groot voordele in, naamlik tydbesparing as gevolg van die relatiewe eenvoud daarvan (die meng van komponente gevolg deur die tabletering van die mengsel direk daarna), en versnelling van die vervaardigingstempo, wat uiteindelik groot ekonomiese voordele inhou.

Die toenemende belangstelling in veelvuldige eenheid doseersvorme, veral minitabette (2-3 mm in deursnee), asook die stygende aanspraak op die farmaseutiese produksiemark, spruit voort uit die voordele wat dit inhou bo enkeleenheds-doseervorme, byvoorbeeld 'n meer eenvormige vrystelling, kleiner variansie en 'n kleiner risiko vir "dose dumping". Ander voordele, soos hul groot oppervlakarea en eenvormigheid, dra by tot 'n wye toepassingsveld. Die gebruik van minitabette het dus die potensiaal om groot ekonomiese besparings teweeg te bring deur die gebruik van minder hulpstowwe, minder en goedkoper vervaardigingstappe en, indien bedek, 'n verminderde hoeveelheid bedekkingsmateriaal wat benodig word.

Kitosaan is 'n natuurlike polisaggarië wat verkry word deur die gedeeltelike deasetilering van chitien, die tweede mees bekombare natuurlike polimeer. Aangesien kitosaan unieke fisies-chemiese eienskappe, soos bio-degradeerbaarheid, bio-verenigbaarheid, non-toksisiteit en antimikrobiese aksie kombineer, is dit gedurende die afgelope paar jaar wyd ondersoek vir potensiële toepassings in die mediese en farmaseutiese velde. As 'n farmaseutiese hulpstof is die grootste waarde daarvan skynbaar dat dit die absorpsie van groot molekulêre geneesmiddels deur die gastroïntestinale kanaal (deur "tight junctions") verbeter en dat dit dien as 'n vetabsorbeerder in dieëtoprodukte. Hoewel dit voorkom in verskeie tabletformules is min, indien enigiets, bekend oor die tableteerbaarheid of insluiting van hierdie veelsydige polimeer in minitabette.

Die spesifieke kitosaangrondstof wat in hierdie betrokke studie gebruik is, beskik oor 'n relatief groot gegranuleerde deeltjiegrootte met 'n klein deeltjiegrootte verspreiding. Dit het egter nie die gebruik daarvan as tablethulpstof uitgeskakel nie. Met behulp van die digtheidsresultate kon voorspel word dat die gebruik van kitosaan tablette van minimum

gewig tot gevolg sal hê, wat die per eenheidsvolume saampersbaarheid daarvan bevraagteken. Gedurende preformuleringsstudies is gevind dat die vervaardig van suiwer kitosaantablette onmoontlik is sonder die insluiting van ander hulpstowwe, hoewel dit moontlik was om minitablette te vervaardig wat nie die teenwoordigheid van 'n smeermiddel genoodsaak het nie. Geen disintegrasië het egter plaasgevind nie en die meganiese sterkte van die kitosaan-minitablette was swak. Inkorporering van 'n swak wateroplosbare, hidrofobiese geneesmiddel, furosemied, het die formulerings van kitosaan-minitablette gekomplekseer, maar dit het 'n evalueringsmetode daargestel om die effekte van formuleringsveranderlikes op die dissolusiegedrag te vergelyk.

Ongeag die insluiting van 'n bindmiddel (Kollidon[®] VA64) het die fisiese stabiliteit van kitosaan-minitablette sensitiviteit ten opsigte van variërende omgewingstoestande getoon, aangesien kitosaan maklik dehidreër. Dit was verder duidelik dat die teenwoordigheid van vog ("sorbed water") 'n beduidende invloed het op die tabletteerbaarheid en disintegrasië van kitosaanpoeier. Die stabiliteit van die poeier was beduidend swakker as die kitosaan-minitablette wat aan dieselfde omstandighede blootgestel is. Dit word dus aanbeveel dat kitosaan-minitablette teen 'n temperatuur van nie meer as 25 °C en by 'n relatiewe lae humiditeit (60%) gestoor moet word. Verder was ook duidelik dat die teenwoordigheid van Kollidon[®] VA64 asook die metode van insluiting van hierdie bindmiddel die stabiliteit van kitosaantablette beïnvloed. Direkte samepersing van kitosaangranules bestaande uit 5% m/m Kollidon[®] VA64 het dus die optimale sisteem in terme van produkstabiliteit tot gevolg gehad. Wat ook in gedagte gehou moet word, is dat die evaluering van kitosaan-minitablette met reëlmatige intervalle gedoen behoort te word, aangesien die tablette 'n tydseafhanklike gedragpatroon ten opsigte van hul fisiese eienskappe toon.

Sistematiese evaluering van verskeie kommersieel beskikbare hulpstowwe is ondersoek om hul invloed op die fisiese tableteienskappe asook dissolusiegedrag te bepaal.

Tabletgewig en afmetings van die verskillende formules het nie beduidend verskil nie, ongeag die metode wat gebruik is tydens die vervaardig van die vulstofsisteem. Uitsonderlike goeie vloeibaarheid is verkry ten spyte van die groot persentasie kitosaan en die insluiting van furosemied in die formulerings. Die insluiting van furosemied het egter wel 'n geringe nadelige uitwerking gehad op die breeksterkte van die minitablette. Verder was die breeksterkte van al die minitabletformulerings beduidend laag (> 80 N) en die byvoeging van ander hulpstowwe het ook 'n betekenisvolle rol in die breeksterkte van die verskillende formulerings gespeel. Dit kan dus genoem word dat die insluiting van 'n bindmiddel nie

gesien kan word as die enigste bydrae tot tabletsterkte nie. Afsplytingsresultate vir al die formulerings was in ooreenstemming met die resultate wat vir breeksterkte verkry is. Afsplyting en tablethardheid speel egter nie 'n belangrike rol in minitabette nie, aangesien hierdie tablette geënkapsuleer word en dus nie onderhewig is aan intensiewe hantering nie. Die effektiwiteit van die minitabetteformules in hierdie studie is egter bepaal en vergelyk t.o.v. disintegrasië en, belangriker, dissolusie-eienskappe.

Meeste van die formules het tablette gelewêr wat binne die gestelde tydspan van 15 minute disintegreer. Die vervaardiging van 'n 5% m/m Kollidon® VA64- vulstofsisteem deur granulering het geblyk nadelig te wees vir die disintegrasiëproses van al die verskillende formules. Interessant genoeg het formules wat 'n gasvormende buffer bevat het, vinniger gedisintegreer as formules waar 'n disintegreermiddel ingesluit is. Formules wat askorbiensuur bevat het, het stadiger gedisintegreer as formules wat natriumbikarbonaat bevat, terwyl formules wat Kollidon® CL bevat, beduidend stadiger gedisintegreer het as formules waarby Ac-Di-Sol® gevoeg is. Hierdie resultate dui dus daarop dat Ac-Di-Sol® die voorkeur-disintegreermiddel is wanneer dit gebruik word in hierdie konsentrasies asook in hierdie kombinasie met verskillende buffers. Daar is ook gevind dat die konsentrasie van die disintegreermiddel of buffer die effektiwiteit van disintegrasië tot 'n groot mate bepaal het. Dit was eerder die hoër konsentrasie as die tipe hulpstof geïnkorporeer wat 'n betekenisvolle verbetering op die disintegrasië-effektiwiteit getoon het. In die geval waar natriumbikarbonaat en 'n disintegreermiddel gekombineer is, het formules wat 2% m/m natriumbikarbonaat bevat het, die vinnigste gedisintegreer. Uit die resultate kon afgelei word dat die insluiting van natriumbikarbonaat 'n groter invloed gehad het op die disintegrasië-effektiwiteit as enige ander veranderlike, en daarom dan die afleiding dat hierdie buffer ingesluit behoort te word in formules wat Ac-Di-Sol® bevat, ten einde die disintegrasiëproses te verbeter.

'n Vergelyking van die dissolusiegedrag van die formules het die afhanklikheid van die mate van dissolusie (AUC) op die aanvanklike dissolusiesnelheid (DR_i) getoon. Die verminderde DR_i -waardes van beide furosemied en kitosaan was egter teenstrydig met die vinnige disintegrasië van al die kitosaanformules, wat 'n aanduiding daarvan is dat tablettdisintegrasië nie 'n absolute voorvereiste vir geneesmiddeldissolusie is nie. Dit was ook duidelik dat formulerings 'n groter rol gespeel het gedurende dissolusietoetse as tydens evaluering van die fisiese eienskappe.

Die insluiting van 5% m/m Kollidon[®] VA64 in verskillende formules deur fisiese vermenging, het tablette met verhoogde dissolusie-eienskappe vir beide furosemied en kitosaan tot gevolg gehad, terwyl Methocel[®] K100M die aanvanklike dissolusiesnelheid vertraag het. Die hoë AUC_n-waardes van alle formules wat 'n buffer bevat het, ongeag die konsentrasie gebruik, het gedui op dissolusietoestande wat vergelykbaar was met, en selfs beter was as dié van 'n furosemiedsuspensie. Dit kan toegeskryf word aan die deeglike verspreiding van die primêre geneesmiddeldeeltjies deur die medium voor dit onderin die dissolusiebak tot rus kom. Die insluiting van 0.5% m/m askorbiensuur het gelei tot 'n toename in die dispersie van furosemied as gevolg van verhoogde kitosaanoplosbaarheid. Die askorbiensuur het egter 'n ongunstige mikro-omgewing geskep wat vinnige dissolusie van furosemied voorkom het. 'n Meer betekenisvolle toename in die dissolusie van furosemied is verkry deur die insluiting van natriumbikarbonaat in die minitab-let-formules. Die waargenome toename in die DR_i van furosemied vanuit hierdie tablette is toegeskryf aan 'n pH-verhoging van die mikro-omgewing asook 'n toename in oplosmiddelpenetrasie deur lugspasies in die tablette. Verhoging van die natriumbikarbonaat-konsentrasie het egter 'n negatiewe invloed gehad op die mikro-omgewing van die kitosaan, wat slegs in 'n suur omgewing oplos, en het dus vinnige dissolusie onderdruk.

Dit was duidelik dat die insluiting van 'n disintegreermiddel die mees betekenisvolle verandering in die dissolusieprofiel getoon het, en die effektiwiteit van hierdie superdisintegreermiddels was inderdaad konsentrasie-afhanklik. Verder kon die afleiding gemaak word dat formules wat Ac-Di-Sol[®] bevat, vinniger dissolusietempo's en 'n meer prominente mate van dissolusie getoon het as formules wat Kollidon[®] CL bevat. Die formules wat 0.625% m/m Ac-Di-Sol[®] bevat het, het 'n betekenisvolle positiewe lineêre afhanklikheid van (AUC)_n op (DR_i)_n getoon, terwyl Kollidon[®] CL-formules 'n effens minder ooglopende afhanklikheid getoon het. 'n Toename in die konsentrasie van Ac-Di-Sol[®] het egter 'n onvoldoende korrelasie-koëffisiënt tot gevolg gehad, wat 'n aanduiding was dat die (AUC)_n minder afhanklik van die (DR_i)_n is met toenemende konsentrasies van hierdie disintegreermiddel.

Daar was geen betekenisvolle regverdiging vir die insluiting van 'n kombinasie van 'n disintegreermiddel en askorbiensuur in die formules nie. Dit was eerder die insluiting van 'n disintegreermiddel wat 'n groter rol in die dissolusiegedrag gespeel het. Die insluiting van 2.0% m/m natriumbikarbonaat in formules wat Ac-Di-Sol[®] bevat, het minitabelle met uitsonderlike dissolusie-eienskappe tot gevolg gehad. Ac-Di-Sol[®] en natriumbikarbonaat het 'n sinergistiese werking op die dissolusie van beide furosemied en kitosaan in 'n suur medium

tot gevolg gehad. Die formule (CF/36) wat 'n fisiese gemengde vulstofsisteem (bestaande uit kitosaan en 5% m/m Kollidon[®] VA64) met 1.25% m/m Ac-Di-Sol[®] en 2% m/m natriumbikarbonaat bevat het, het die mees gepaste dissolusie-eienskappe getoon (84.18% furosemied opgelos). Dit is dus duidelik dat die ongewenste eienskappe van kitosaan gewysig kan word om te voldoen aan die gestelde standarde vir direk saamgepersde minitablette.

Insluiting van die CF/36-formule in kapsules blyk suksesvol te wees, aangesien die kapsules binne ± 60 sekondes gedisintegreer het en die inhoud daarvan vinnig deur die dissolusiedmedium versprei het. Die rede waarom die volledige dissolusie nie plaasgevind het nie, kan toegeskryf word aan die disintegrasielag "lag time" van die kapsules en die Avicel[®] PH-101 tabletmatriks. Hierdie dissolusieprofiel het egter steeds dieselfde kurwe as die profiel van die CF/36-minitablette gevolg. Kapsules wat CF/36-tablette en Avicel[®] PH-101 bevat het, het nie so vinnig gedisintegreer soos kapsules wat nie 'n beskermende vulstof bevat het nie, en in teenstelling met vorige bevindings het hierdie kapsules effens minder gunstige dissolusieprofiel getoon as die kapsules wat slegs die minitablette bevat het. Dit was waarskynlik as gevolg van die vorming van 'n redelike viskeuse mikrokristallynsellulosematriks om die minitablette wat eers moes disintegreer voor die minitablette kon disintegreer.

Kitosaan word nie baie hoog geag in terme van die gebruik daarvan as 'n eerste keuse vulstof nie. Ongewenste eienskappe wat die benutting daarvan beperk, sluit in kitosaan se geneigdheid tot hidrasie wat die stabiliteit beïnvloed, problematiese tabletsterkte-eienskappe en die beskikbaarheid van vulstowwe wat sommige van die ongewenste eienskappe van kitosaan kan teenwerk. Hierdie studie het egter bewys dat kitosaan as 'n relatiewe goedkoop alternatief tot ander direk saampersbare vulstowwe gebruik kan word, veral in die formulering van minitablette indien die formules deeglik geoptimaliseer word. Boonop kan die mukoklewende en absorpsieverbeterende eienskappe daarvan ook die biobeskikbaarheid van verskeie verbindings of aktiewe bestanddele met swak orale absorpsie bevoordeel en verbeter. Gebaseer op hierdie resultate blyk die ontwikkeling van 'n geoptimaliseerde kitosaan-multifunksionele hulpstof na 'n bereikbare en relevante doelwit wat vergelykbaar is met en selfs beter is as kommersiële produkte.

INTRODUCTION AND AIM OF STUDY

Aim

In recent years chitosan has attracted a great deal of attention and has become well known as a multifunctional biopolymer and a potential absorption enhancer across mucosal epithelia, with a wide range of applications both in the medical and pharmaceutical field. The main driving force in the development of new applications for chitosan lies in the fact that the polysaccharide is not only naturally abundant, but it is also non-toxic and biodegradable. Unlike oil and coal, chitosan is a naturally regenerating resource (e.g., crab and shrimp shells) that can be further enhanced by artificial culturing. The myriad of challenges that is presented in the production of directly compressed minitables that disintegrate instantaneously, and that contain chitosan as filler so as to produce mucoadhesive properties, is the subject of this study. The evaluation of these challenges, the characterisation of chitosan powder and the determination of the effect that chitosan has on drug release will be presented systematically in the following chapters of this dissertation.

Background

Chitosan is no longer just a waste by-product from the seafood-processing industry. Since chitosan combines unique physicochemical characteristics, in vivo biodegradability, biocompatibility, non-toxicity, and antimicrobial action, it has been widely investigated over the last few years for potential applications in the medical and pharmaceutical fields. Because of these unique properties chitosan may be administered orally, nasally or through any other delivery route, enabling, for example, prolonged drug release or improved bioavailability of poorly absorbed compounds such as peptides. With the wide range of potential applications and the rate of scientific progress being made, chitosan is truly a multifunctional designer polymer in future biotechnology.

Although chitosan is present in various tablet preparations, little is known about its tabletability and function in tablet formulations. Furthermore, commercially available chitosan is not always well characterised, especially its physicochemical properties. It is therefore expected that knowledge about these factors that influence the behaviour of chitosan

will play an increasingly important role in the potential use of this polymer as a filler in direct compression formulations of minitables.

Accounting for 70% of all ethical pharmaceutical preparations produced, tablets are still the most popular dosage form because of its numerous advantages. Since chitosan is a naturally abundant and relatively inexpensive polymer the application thereof in tablet formulations will prove to be advantageous from an economical point of view (Aucamp, 2004:VI).

The production of tablets, and especially minitables, is a unique process and some prerequisites are to be met by both materials and machinery that are employed in the manufacturing process. Moreover, direct compression has become an interesting alternative for the tablet manufacturer due to the intensive research in tableting machinery and the development of potent, new excipients over the last thirty years. Direct compression excipients should, however, meet extensive requirements to render them suitable for this manufacturing process.

The growing interest for multiple-unit dosage forms and their increasing share of the pharmaceutical product market are claimed by their proponents to stem from advantages they offer over single-unit dosage forms. The chief advantage being that once ingested, the particles will be distributed over an ever increasing area as they pass down the gastrointestinal tract, thereby compensating for the local variations in milieu conditions and individual unit imperfections. As a result, toxic side effects will be minimised.

Minitables in particular, have received increasing interest over the last 10 years. It was found that certain active ingredients could not be tableted into normal sized tablets, but the production of minitables was indeed possible with the same active ingredients. These tablets rendered a mechanical stability equal to, and at high pressures higher than, that of normal-sized tablets. This multi-unit dosage form may be produced on rather conventional tableting machines, utilising common methods to control the process. Another advantage of minitables is that they are quite suitable for coating in order to sustain drug release. Furthermore, the drug release rate from minitables is more consistent than from granules. The use of minitables would therefore constitute a potential economic saving through the use of less excipients, few and inexpensive production steps, and if to be coated, reduced amounts of coating material per tablet required.

Objectives

To achieve the aim of the study the following investigations were undertaken:

- Characterisation of chitosan powder i.e. molecular weight, nuclear magnetic resonance spectroscopy analysis, infrared analysis, relative viscosity, and thermoanalytical characterisation of chitosan
- Determination of the micromeritic properties of chitosan i.e. flow and compactability of the powder through preformulation studies.
- Amend the compactability of chitosan through the production of minitables and through the addition of binders in formulations containing a tracer drug. Determine the ideal type and optimum concentration of the binder.
- Improve the flowability and compressibility of chitosan through the implementation of wet granulation of the filler and determine its applicability as processing variable. Evaluate the physical and dissolution properties of these minitables.
- Establish disintegration and improving dissolution properties of chitosan minitables by incorporation of buffering agents, i.e. ascorbic acid and sodium bicarbonate. Determine their efficiency in combination with previously optimised auxiliaries.
- Improvement of disintegration and dissolution properties of chitosan through the incorporation of super-disintegrants and evaluate their efficiency in combination with previously optimised auxiliaries.
- Evaluate the influence of the incorporation of 10 minitables into hard gelatin capsules on the dissolution properties of the chitosan minitable formulation with the most desirable tablet properties.
- Comparison of the dissolution properties of the chitosan minitable formulation mentioned above, with reference tablets containing a familiar filler as well as with commercially available furosemide tablets.
- Investigate the effect of ambient conditions on the short- and long-term stability of chitosan powder and chitosan minitables prepared through various techniques.

CHAPTER 1

IMPORTANT PRINCIPLES OF TABLET MANUFACTURE AND MINITABLETING

1.1 Introduction

Various complex drugs (i.e. peptides) are commonly administered by parenteral routes, which are often complex, difficult, painful and in some cases also dangerous. Hence, the need for efficacious non-invasive delivery systems for such drugs is becoming more and more important, especially as the number of potential candidates increases. Among non-invasive galenics, those choosing the oral route of administration are generally the most favoured by the patients, mainly because of the great flexibility in its design (Guggi *et al.*, 2003:125-135; Santos *et al.*, 2002:154).

The Arabic medical literature contains the earliest reference to a dosage form resembling the tablet which was described as a presentable dosage form of drug particles being compressed between the ends of engraved ebony rods and a force being applied to it by means of a hammer. In December 1843, however, a patent was granted to the Englishman, William Brockedon, for a machine to compress powders to form compacts. This invention caught the imagination of various pharmaceutical companies, and Wellcome, in Britain was the first company to describe this compressed dosage form as a tablet. The use of the tablets rapidly increased, especially in the USA, where the demand for large quantities of medical supplies during the civil war spurred its development. Power-driven presses replaced Brockedon's hammer, and by 1874, there existed both rotary and eccentric presses that in their mode of operation were fundamentally similar to those in use at present (Armstrong, 2000:648; Rubinstein, 2000:304).

Tablets are defined in the *British Pharmacopoeia* (BP) as being circular in shape with either flat or convex faces and prepared by compressing the medicament or mixture of medicaments, usually with added substances. Accounting for 70% of all ethical pharmaceutical preparations produced, tablets are now the most popular dosage form. This arises from the numerous advantages associated with its use. Accurate dosing, easy transport, acceptable stability and

an elegant appearance of the final product are considered to be major perks of this dosage form. Furthermore, tablets offer advantages to the manufacturing industry due to its relative ease of manufacture and lower manufacturing cost compared to other dosage forms (Andres *et al.*, 1995:1875-1885; Armstrong, 2000:648; Rubinstein, 2000:304).

1.2 Fundamental principles of tablet manufacture

1.2.1 Required properties of powders intended for tableting

Marshall (1986:66) described compaction as: "The general term used to describe the situation in which materials are subjected to some level of mechanical force". In the pharmaceutical industry the effects of such forces are particularly important in the manufacture of tablets and granules, in the filling of hard-shell gelatine capsules, and in powder handling in general. The physics of compaction may be simply stated as: "The compression and consolidation of a two-phase (particulate solid-gas) system due to the applied force". Compression is regarded as a reduction in the bulk volume of the material as a result of displacement of the gaseous phase, whereas consolidation is an increase in the mechanical strength of the material resulting from particle/particle interactions (Karehill *et al.*, 1990:35; Leuenberger, 1982:41). In the field of tableting technology, it is still not possible to predict with certainty how a given material will behave during compression (Rubinstein, 1988:306).

In practice, two or more excipients are usually mixed together with the active ingredient to facilitate tablet manufacture and to ensure tablets with satisfactory characteristics. Among these excipients are fillers/diluents, binders, lubricants and disintegrants. Added benefits arising from blending is the surmounting of capping and lamination propensities as well as augmentation of tablet strength to prevent damage caused during handling and to produce predictable, reproducible drug release properties (Otto, 2002:1; Mattsson, 2000:8; Andr s *et al.*, 1995:1876; Vromans & Lerk, 1988:183).

Excipients account for the major proportion of the final tablet constitution. In cases of highly potent drugs, excipients can contribute up to 95% (w/w) of the final tablet formulation. The selection of appropriate excipients or processing thereof prior to tableting, is therefore paramount to optimise tablet performance, since excipients will influence the physicochemical properties of the end product to a great extent. Fluidity and compressibility are considered the major physiochemical properties of powders intended for compression. Unfortunately, relatively few substances possess these essential properties without some

preliminary treatment (Steckel & Mindermann-Nogly, 2004:108; Armstrong, 2000:649; Rubinstein, 2000:306; Bryan & McCallister, 1992:2030).

The drug substance may govern the method of production, but where a choice is available, economic factors will play an important role. Therefore, all factors should be considered carefully (Armstrong, 2000:649; Mattsson, 2000:8; Rubinstein, 2000:306).

1.2.1.1 Flow properties (fluidity)

The importance of fluidity can be explained as follows: uniform powder flow allows for consistent filling and particle arrangement in the die cavity; as a consequence, tablets of uniform weight and active ingredient content are produced. The physiochemical properties of tablets will also be comparable for tablets produced from these mixtures and will result in production of tablets of predictable quality. Adequate mixing of components and maintained fluidity are, therefore, critical considerations (Steckel & Mindermann-nogly, 2004:107-114; Staniforth, 2000:600). Powder flow can, however, be improved through the use of vibrators, the incorporation of a glidant into the formulation, spray-drying, and the use of spheronisation machines or by means of granulation (Rubinstein, 2000:306).

According to Martin *et al.* (1983:517) a bulk powder is somewhat analogous to a non-Newtonian liquid, which exhibits plastic flow and sometimes dilatancy. Pharmaceutical powders may be broadly classified as free-flowing or cohesive. Changes in particle size, density, shape, electrostatic charge, surface texture, and adsorbed moisture, which may arise from processing of formulation, significantly affect most flow properties. Poorly flowing powders or granulations present many difficulties to the pharmaceutical industry.

Particles with a high density and a low internal porosity tend to possess free-flowing properties, while elongated or flat particles tend to pack, albeit loosely, to give powders with a high porosity. Surface roughness leads to poor flow characteristics due to friction and cohesiveness. Occasionally, poor flow may result from the presence of moisture, in which case drying of the particles will reduce the cohesiveness. Particle flow through an orifice, however, is restricted when relatively small particles (less than 10 μm) occur in the powder mass. This is because of the cohesive forces between particles that are of the same magnitude as gravitational forces. These latter forces are a function of the diameter raised to the third power and thus become more significant as the particle size increases and flow is facilitated. A maximum flow rate is reached, after which the flow decreases as the size of the particles approach that of the orifice. The powder flow properties may be improved if a powder

contains a reasonable amount of small particles, by removing the “fines” or adsorbing them onto the larger particles (Martin *et al.*, 1983:517).

Researchers found that as granule size is reduced, the variation in tablet weights decreases (Martin *et al.*, 1983:518). If the granule size is further reduced, the granules will flow less freely and the tablet weight variation will increase. Thus, the particle size distribution affects the internal flow and segregation of the granulate. It was observed that the flow rate of a tablet granulation increased with an increase in the quantity of fines added. An increase in the amount of lubricant also raised the flow rate, and the combination of lubricant and fines appeared to have a synergistic action.

1.2.1.2 The importance of the presence of moisture

One of the most significant parameters contributing to the behaviour of many tablet formulations is the level of moisture during manufacture as well as the residual of moisture in the product. For example, there is increasing evidence to suggest that moisture levels may be very critical in minimising certain faults occurring during compression (such as lamination) (Nokhodchi *et al.*, 1995:13; Marshall & Rudnic, 1991:358). The presence of moisture in pharmaceutical powders can play a significant role in the consolidation properties and often produces changes in flow properties particularly after storage. Occasionally moisture is added deliberately in order to produce a cohesive mass suitable for further processing. Moisture can be present in powders in different physical forms:

- As absorbed monolayers or multi-layers on the particle surfaces
- As condensed water on the surface
- As physically absorbed water within the particles, or
- As strongly bound chemisorbed water.

The presence and distribution of moisture in the above forms will depend considerably on the chemical nature of the particulate material, its physical properties such as particle size and porosity, and on the ambient relative humidity which determines the equilibrium moisture content (Garr & Rubinstein, 1992:188).

Garr and Rubinstein (1992:187-192) found that the mean yield pressure decreased with increasing moisture content due to the overall plasticising effect of moisture, whilst relative powder density increased due to the lubrication effects of moisture smoothing surface micro-irregularities and so reducing frictional forces. Increasing moisture contents by up to 6% (w/w) in their study, progressively increased compact strength due to the hydrodynamic

lubrication effects of moisture promoting optimum transmission and utilisation of compaction force and the formation of strong hydrogen bonds. A higher moisture content decreased tablet strength due to the hydrostatic resistance of the excess moisture in the void spaces which reduces particle-particle contact areas, surface energy and adhesive forces. The capping pressure similarly increased with moisture content at up to 6% (w/w) as the surface molecular role of moisture promotes strong hydrogen bonding.

Moisture can thus have marked effects on surface tension, compression, tablet tensile strength and tablet toughness, density of the materials, binding capacities and lubrication properties in addition to having hydrodynamic properties. Therefore, moisture content must be optimised in a formulation to achieve good powder compressibility (Nokhodchi *et al.*, 1995:13; Garr & Rubinstein, 1992:188; Marshall & Rudnic, 1991:358).

1.2.2 Granulation

Excipients and/or active ingredient(s) are processed through two major methods of precompression treatment known as granulation or direct compression (Otto, 2002:2). Granulation prior to compression is the process of particle size enlargement and is done to confer fluidity and compressibility of mixtures. In addition, the granulate is capable of being subjected to high compression pressures without forming defects. It is able to have a uniform distribution of all the ingredients in the formulation, while during direct compression it is necessary to ensure effective powder mixing. Granulation proves very useful in cases of formulation of small quantities of highly potent drugs and is manufactured through wet granulation or slugging. Wet granulation employs a liquid phase to form cohesion between particles whereas slugging is a so-called dry method whereby granules are formed through compression and milling of the formed ("slugged") compacts (Milosovich, 1963:557).

Despite its advantages, granulation poses considerable drawbacks as a processing procedure of powder components. A number of time-consuming steps are for example involved. These numerous operations are labour intensive and make extensive use of space and equipment (Henderson & Bruno, 1970:1336). Furthermore, granulation can affect the chemical stability of ingredients especially the active ingredients such as antibiotics that are susceptible to hydrolysis in aqueous granulation media or ingredients which are thermolabile. An additional disintegration step is also required to release drug particles from granules, after tablet disintegration has been completed (Milosovich, 1963:558). Thus, during the development of a tablet formulation, all the physical variables, which can affect the resultant granules, have to

be considered so as to minimise the effect of process variation on the quality of the final product (Armstrong, 2000; Rubinstein, 2000:309).

1.2.3 Tablet manufacture through direct compression

Direct compression is a tablet technology that was developed in the 1960's. It is however only in recent years that it has received increased attention, as it has become an ever expanding field of research and application in tablet manufacture. Many direct compressible vehicles (DCVs) have been introduced and still many others are under development for better performance and/or lower costs. (Nada & Graf, 1998:347; Patel *et al.*, 1994:203; Schmidt & Rubensdörfer, 1994:2900; Lerk, 1993:2359). Apart from an economic viewpoint and the simplicity of the process, it offers various advantages in terms of improved process reliability and product stability (Schmidt & Rubensdörfer, 1994:2900).

Sakr *et al.* (1979:1187) and Bolhuis and Lerk (1973:469) described the direct compression technique of tablet manufacture as the compression of tablets directly from powdered materials without modifying their physical nature, thus saving space, machinery, personnel and time. In addition to economical advantages, this method of tablet manufacture can offer the advantage of improved chemical and physical stability compared to tablets produced by wet granulation. Formerly, direct compression as a method of tablet manufacture was reserved for a small group of crystalline chemicals having all the physical characteristics required for the formulation of a good tablet. These materials possess cohesive and flow properties which make direct compression possible. However, the majority of active ingredients cannot be tableted without the addition of a direct compression vehicle to aid compression. It is for these reasons that much effort has been put into the modification of existing dry binders to make them suitable for direct compression and even into the development of special directly compressible vehicles. (Armstrong, 2000:647; Rubinstein, 2000:307).

The ideal excipient for direct compression should possess the following properties: it should be physiologically inert, possess high fluidity or flowability, be highly compressible and compatible with all types of active ingredients, not show any physical or chemical change during aging and should be stable to air, moisture and heat. It should furthermore, be colourless and tasteless; accept colourants uniformly; be relatively inexpensive; possess proper mouth feel; it should not interfere with the biological availability of the active ingredients; be capable of being re-worked without loss of flow or compressibility; have a good pressure hardness profile; possess an optimum bulk density and lastly it should have a

high capacity which is generally expressed in terms of percentage of non-compressible material or as optimum drug to excipient ratio. Despite these difficulties, the majority of drugs can be tableted via direct compression, by choosing the correct equipment and machinery (Armstrong, 2000:665; Schmidt & Rubensdörfer, 1994:2899; Sakr *et al.*, 1978:1188). The quantity of a drug that can be mixed with the carrier so that direct compression properties are retained is governed by the capacity of the carrier. The more compressible the active ingredient, the greater the proportion that can be carried successfully by the vehicle. Normally, one considers the active ingredient to be non-compressible when considering the capacity potential of the filler. Generally, unless the drug itself is easily compressible, the amount of drug present is limited to a maximum of about 25% of the tablet weight.

In practice, the requirements discussed are quite extensive and no single material is known to comply with all these prerequisites (Armstrong, 2000:647-649; Henderson & Bruno, 1970:1336). In 1993 Shangraw and Demarest (1993:40-44) did a survey in which it was concluded that direct compression was the overwhelming choice of tablet manufacture followed by wet massing-fluid bed drying and wet massing-tray drying. However, when the responses were segmented into innovator companies vs. generic companies, this preference for direct compression was not found to be industry-wide. Due to long lead time from discovery to market and the uncertainty of drug properties, the innovator industry may be more likely to prefer granulation of one type or another. This however, was not true of the generic industry. Furthermore, both the vitamin and non-prescription drug industries are heavily committed to direct compression. It is thus clear that direct compression is becoming more and more the preferred choice of tablet production whereas wet granulation is slowly being phased-out.

1.2.4 Compaction and compressibility behaviour of powders

Compressibility of powders is an essential property, since a stable, intact mass (tablet) needs to be produced on application of pressure to the powder mixture (Otto, 2002:2). The behaviour of a material to resist an external force or pressure is expressed as the yield pressure (or yield stress) which is defined as the reciprocal of the slope values obtained from the Heckel plots, where the logarithm of the reciprocal of porosity is plotted versus the maximum compaction pressure. Fragmentation is the main consolidation mechanism when the value for the yield pressure is considered high (e.g., a yield pressure of approximately 300

MPa for an applied pressure of approximately 150 MPa), and this, therefore, means that there is a high resistance towards the deformation of the powder bed (Schlack *et al.*, 2001:794).

Powders almost invariably differ in their compressibility behaviour and this may present an array of problems regarding tableting properties of powder mixtures (Steckel & Mindermann-nogly, 2004:107-114; Rubinstein, 2000:306) including mechanical strength and excipient compactibility (Armstrong, 2000:649). Much research has, however, been of an empirical nature in which the role of the fundamental mechanical properties and the deformation characteristics have not been fully clarified (Wong *et al.*, 1991a:240; Wong *et al.*, 1991b:659-661; Carstensen *et al.*, 1985:1239-1241;).

Compaction can be described as a process during which the barriers that hinder close contact between spontaneously attracting entities are destroyed or removed. The compact consists of packed particles that have several contacts with near “neighbours”. The innate interpartical attraction, primarily dispersion forces, at these contacts results in a perimeter in tension and a mid-region in compression – Born repulsion. The at-rest compact has attraction forces equal the repulsion forces. These contacts are solid “bridges” (connections). Other mechanisms may act to enlarge the contact area. The relative strength of the bonding is measured as the magnitude of a tensile stress that produces fracture. Since all materials are Mohr bodies, the observed strength depends on the method of measurement used. There are a myriad of observations that indicate the prominence of plastic deformation enabling bonding. For example, all die wall vs. punch pressure plots, attest to this. Also, all organic materials studied have viscoelastic characteristics both in compression and tension conditions. This is a clear indication that a (thermodynamically) irreversible process, plastic deformation, is involved. Since not all contact sites are equal, their strengths are not equal. Furthermore, some contacts may fracture in a brittle manner while their immediate neighbours may undergo ductile extension. With so many contact conditions existing simultaneously at different contact sites, the complexity of calculating the tensile strength becomes formidable. Mechanical properties are the controlling properties; they determine success or failure of tableting (Hiestand, 1997:241).

Normally there is a reduction in bulk volume through rearrangement (consolidation) of particles in the die when external mechanical forces are applied to the powder mass. This is a result of one or more effects. Closer repacking of the powder particles usually accompanies the onset of loading, and in most cases, this is the main mechanism of initial volume reduction. Rearrangement becomes more difficult as the load increases, and further compression involves some type of particle deformation (fig1.1)

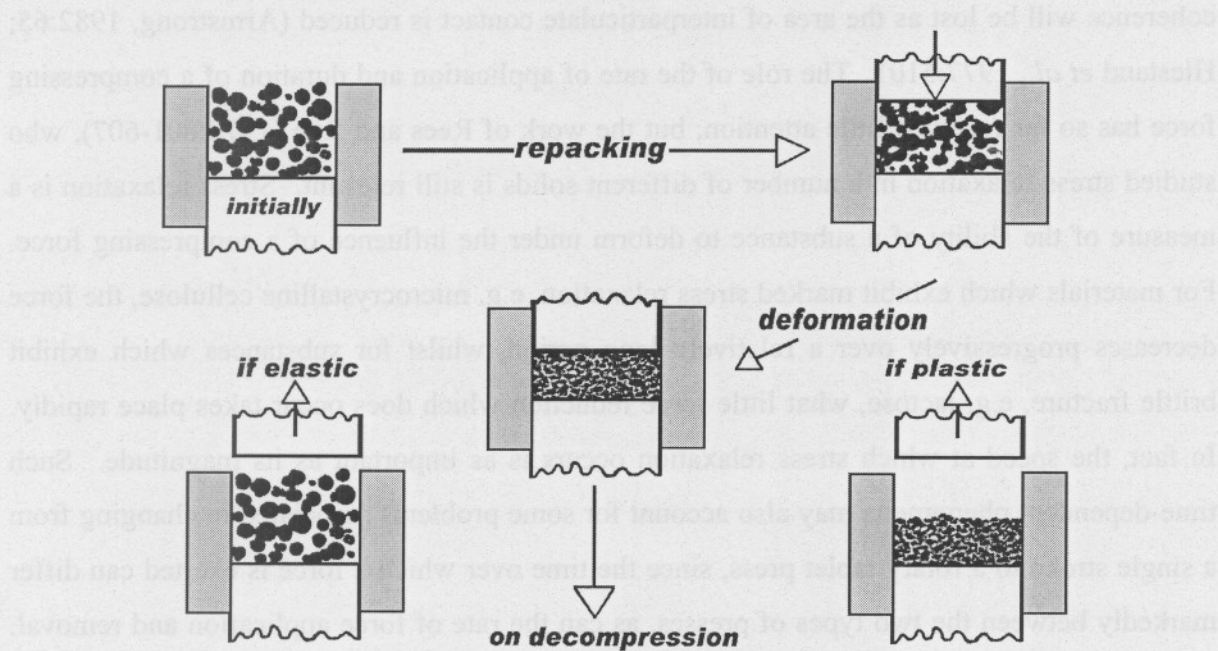


Figure 1.1: *Diagram of the effect of compressional force on a bed of powder (Marshall, 1986:72).*

As early as 1950 the importance of plastic flow in the production of compacts by powder compression was recognised and it was suggested that the more plastic a material, the more likely it is to form compacts (Rees & Rue, 1978:601). The effect of removing the compressing force varies from the type of deformation. When the shear strength is greater than the tensile or breaking strength, particles may be preferentially fractured. Obviously fragmentation is permanent and there is no way in which the fragmented particles will recombine into the original particles when the force is removed. The smaller fragments help to fill up any adjacent air space. This is most likely to occur with hard, brittle particles and is known as brittle fracture (Marshall, 1986:72). Fragmentation has been defined as the formation of smaller, discrete particles from an initial grain (Hiestand, 1997:241; De Boer, 1986:148; Armstrong, 1982:64).

However, when the deformation is to a large extent spontaneously reversible, i.e., if it behaves like rubber, then it is said to be elastic. All solids undergo some elastic deformation when subjected to external forces. If, on the other hand, plastic deformation has occurred (where particles resemble the behaviour of modelling clay), then by definition this is permanent and the particles will remain deformed even after the force has been removed. In this case the shear strength is less than the tensile strength. The area of interparticulate contact and hence the magnitude of interparticulate forces of attraction will remain constant, and hence tablet strength will be maintained. If, however, the particles tend to revert to their former shape,

coherence will be lost as the area of interparticulate contact is reduced (Armstrong, 1982:65; Hiestand *et al.*, 1977:510). The role of the rate of application and duration of a compressing force has so far received little attention, but the work of Rees and Rue (1978:601-607), who studied stress relaxation in a number of different solids is still relevant. Stress relaxation is a measure of the ability of a substance to deform under the influence of a compressing force. For materials which exhibit marked stress relaxation, e.g. microcrystalline cellulose, the force decreases progressively over a relatively long period, whilst for substances which exhibit brittle fracture, e.g. lactose, what little force reduction which does occur takes place rapidly. In fact, the speed at which stress relaxation occurs is as important as its magnitude. Such time-dependent phenomena may also account for some problems associated in changing from a single stroke to a rotary tablet press, since the time over which a force is exerted can differ markedly between the two types of presses, as can the rate of force application and removal. Thus, a powder which responds quickly to a compressing force is less likely to pose problems when changing from one type of press to the other (Armstrong & Palfrey, 1989:151; Armstrong, 1982:65; Rees & Rue, 1978:601). When the shear strength is, however, greater than the tensile or breaking strength, particles may be preferentially fractured. The smaller fragments then help to fill up any adjacent air space. This is most likely to occur with hard, brittle particles and is known as brittle fracture (Ilkka & Paronen, 1993:183; Marshall, 1986:72). Fragmentation thus results in exposure of a high contact surface-area between particles and offers a multitude of advantages to the compaction process. Fragmenting materials are less sensitive to variations in particle size and shape of the starting material (Wong & Pilpel, 1990:152; Duberg & Nyström, 1982:434). Traditionally, deformation mechanisms were accepted as the major compaction mechanism, but fragmentation is now widely acknowledged for its contribution to compaction (Eriksson & Alderborn, 1995:1035).

As previously discussed, all materials possess both an elastic and a plastic component. The volume reduction mechanism which will dominate for a specific material is dependent on factors such as temperature and compaction rate. For example: lower temperatures and faster loading during compression will generally facilitate consolidation by fragmentation, therefore, the compaction characteristics of materials are pivotal in determining the physical properties of tablets. Compaction of powders leads to the establishment of interparticulate contact and therefore attraction and it is the characterisation of these forces that provides information regarding the bonding mechanisms of powders (Nyström *et al.*, 1993:2146; Hiestand *et al.*, 1977:519).

1.2.5 Bonding mechanisms of solid particles

According to Nyström *et al.* (1993:2195) co-adherence mechanisms of particles can be distinguished into five classes namely: solid bridges, bonding attributed to movable liquids, non-freely movable binder bridges, interparticulate attractions (distance forces), and shape-related bonding (mechanical interlocking).

The presence of moisture and the type or chemical structure of the material can influence any of these mechanisms (Mattsson, 2000:11). Particles may consist of simple ions that form an ionic crystal e.g. sodium chloride. An ionic material may consist of more complex ions bonded by covalent interactions e.g. hydrogen phosphate and this ion may interact with other ions, e.g. calcium. Calcium hydrogen phosphate is an example of a compound formed in this system. Additional hydrogen bonding and Van der Waals mechanisms may also contribute to bonding in these complex systems (Adolfsson *et al.*, 1997:244). Figure 1.2 summarises the primary mechanisms of bonding (as revised by Otto, 2002:7).

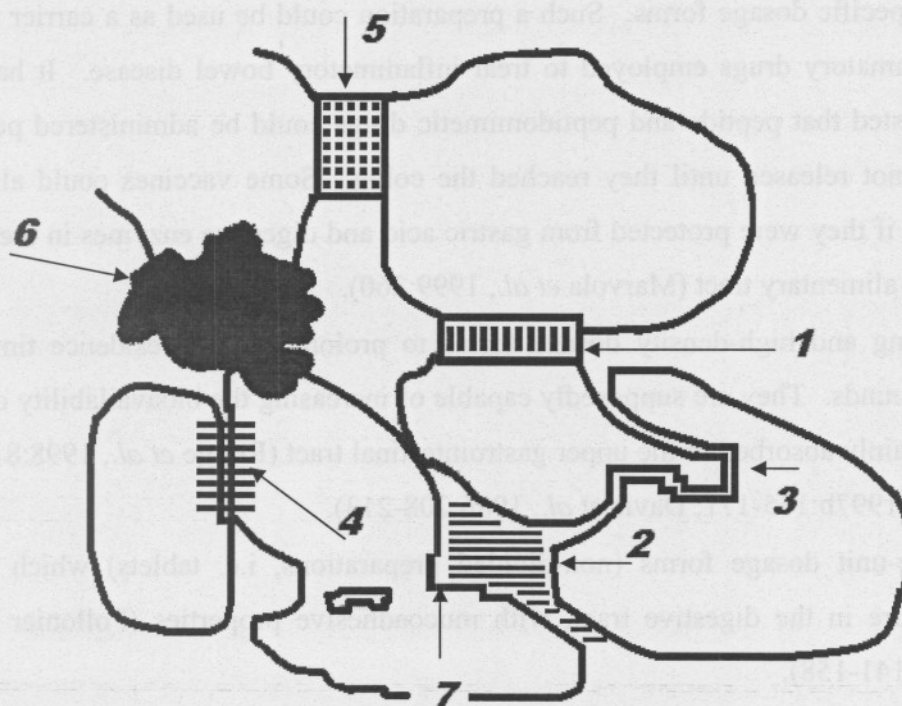


Figure 1.2: *Simplified description of binding forces. 1 = surface tension of liquid bridges. 2 = capillary force in liquid filled cavities. 3 = mechanical interlocking. 4 = dispersion forces between particles. 5 = dispersion forces of binder. 6 = lattice forces of incrustation. 7 = lattice forces after sintering processes (List & Muazzam, 1979:162).*

1.3 Minitablets: An interesting multi-unit dosage form

1.3.1 Introduction

It has been reported that about 40% of the compounds being developed by the pharmaceutical industry are poorly water soluble. A limiting factor to the in vivo performance of poorly water soluble drugs after oral administration is their inadequate ability to be wetted by and dissolved into the fluid in the gastrointestinal (GI) tract. Furthermore, the gastrointestinal transit time of ingested objects depends on their physicochemical properties and the physiological conditions of the gastrointestinal tract. Most drugs are mainly absorbed in the small intestine. Therefore, the time during which an orally administered drug delivery system resides in the stomach has an important effect on the absorption of drugs (Hu *et al.*, 2004:233; Ichikawa *et al.*, 1991:1153).

Due to these problematic challenges, new developments in traditional pharmaceutical dosage forms had to be made. Some of these developments, to name but a few, are:

- Site-specific dosage forms. Such a preparation could be used as a carrier for the anti-inflammatory drugs employed to treat inflammatory bowel disease. It has also been suggested that peptide and peptidomimetic drugs could be administered per os if they were not released until they reached the colon. Some vaccines could also be given orally if they were protected from gastric acid and digestive enzymes in the upper parts of the alimentary tract (Marvola *et al.*, 1999:260).
- Floating and high-density dosage forms to prolong gastric residence time of active compounds. They are supposedly capable of increasing the bioavailability of drugs that are mainly absorbed in the upper gastrointestinal tract (Rouge *et al.*, 1998:81-87; Rouge *et al.*, 1997b:165-171; Davis *et al.*, 1986:208-213).
- Single-unit dosage forms (non-divided preparations, i.e. tablets) which retain their structure in the digestive tract, with mucoadhesive properties (Follonier & Doelker, 1992:141-158).
- Multiple-unit dosage forms (i.e. pellets, microspheres, granules, minitables etc.; figure 1.3) which consist of numerous sub-units, filled into hard gelatin capsules or sachets, or made into tablets which can disintegrate instantaneously or which can provide sustained release of the drug (Follonier & Doelker, 1992:141-158).

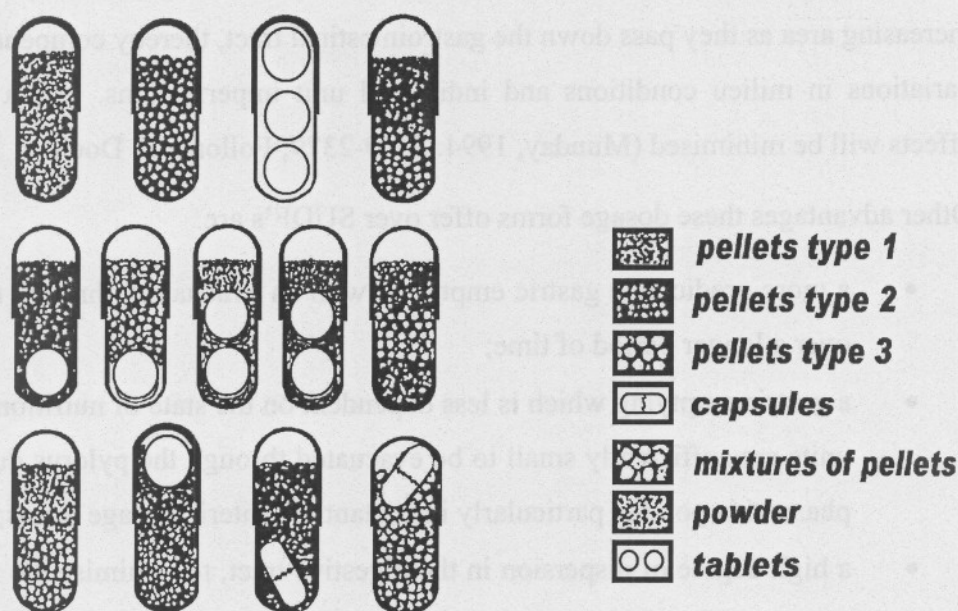


Figure 1.3: *Various types of multiple-unit sustained release capsules (Follonier & Doelker, 1992:142).*

The growing interest for multiple-unit dosage forms such as minitables, and their increasing share of the pharmaceutical product market are claimed by their proponents to stem from advantages they offer over non-divided (single-unit) dosage forms. These advantages will be discussed in the following section.

Minitables, however, are tablets with a diameter equal to or smaller than 2-3 mm. They constitute an alternative to pellets as they can be easily produced with defined form, size and strength, through ordinary reciprocating or rotary tableting machines, using a multiple tooling. Process steps like moisturising, extruding, spheronising, and drying may thus be avoided. Minitables can be filled into hard gelatin capsules, directly or after coating, for the production of multiple unit dosage forms. Several papers have already been published describing matrix minitables based on hydrophilic as well as hydrophobic materials (Van der Merwe *et al.*, 2004:86; De Brabander *et al.*, 2000a:195-203; Lennartz & Mielck, 1998:75-78; Flemming & Mielck, 1995:2239-2251; Munday, 1994:2369-2379).

1.3.2 Advantages and technological difficulties

Much work has been carried out to develop multiple-unit sustained release dosage forms for various drug compounds. From a technological standpoint, multiple-unit dosage forms (MUDF) offer a number of advantages over single-unit dosage forms (SUDF) such as tablets.

The main advantage being that once ingested, the particles will be distributed over an ever increasing area as they pass down the gastrointestinal tract, thereby compensating for the local variations in milieu conditions and individual unit imperfections. As a result, toxic side effects will be minimised (Munday, 1994: 2369-2379; Folloner & Doelker, 1992:142).

Other advantages these dosage forms offer over SUDF's are:

- a more predictable gastric emptying, with an evacuation through the pylorus spread over a longer period of time;
- a gastric emptying which is less dependent on the state of nutrition, because the sub-units are sufficiently small to be evacuated through the pylorus during the digestive phase; this point is particularly important for enteric dosage forms;
- a high degree of dispersion in the digestive tract, to minimise the risks of high local concentrations which could damage the mucosa;
- a lesser risk of a sudden dose dumping;
- a lesser inter- and intra-subject variability, owing to the improvement in both transit reproducibility and dispersion along the digestive tract;
- the possibility of controlling the release profile by combining spheroids of different natures in the same preparation (for example an immediate-release and a slow-release component, or spheroids of differing sensitivities to pH), of separating active ingredients which are incompatible, or of combining several substances with their own kinetics of release characteristics (Steckel & Mindermann-Nogly, 2004:107-114; De Brabander *et al.*, 2000a:195-203; De Brabander *et al.*, 2000b:81; Lennartz & Mielck, 1998:75-78; Rouge *et al.*, 1997a:388; Follonier & Doelker, 1992:141-158).

These advantages of multiple-unit dosage forms can be anticipated on the grounds of a behaviour which is different from that of non-divided dosage forms, i.e. statistical instead of stochastic (all or nothing) (Follonier & Doelker, 1992:141-158).

Conversely, MUDF's suffer several drawbacks: their manufacture is technically more complicated, generally requires more time, and is more expensive than that of non-divided forms. The quality control requirements, both in the process and on the finished product, are more stringent, because of the more complicated nature of the manufacturing processes. Moreover, manufacturing is hampered by problems of reproducibility between batches (conformity), and even within the same batch (homogeneity). A uniform filling of the capsules can prove difficult to achieve, in particular where mixtures with a significant size

variation are concerned. As to the tableting of micro-capsules or of pellets, this technique is quite tricky when release is controlled by a coating membrane, because of risks of breakage in the process (Folloner & Doelker, 1992:142).

However, Munday (1994: 2369-2379) concluded that encapsulated multi-unit dosage forms containing regular units, such as minitables within the capsule, produced more reliable sustained release dosage forms than those containing irregular units such as pellets, granules or particles. Moreover, since the minitables contained uniform weights of the active ingredient, the exact number of minitables could be more easily adjusted to facilitate individual dose titration. Minitables may be produced on rather conventional tableting machines, utilising common methods to control the process. However, production with rotary machines necessitates very fine mechanical adjustments if a uniform filling of the matrices is to be achieved and damage to the punches avoided (Folloner & Doelker, 1992:142). During direct compression the raw materials, especially excipients, also have to meet special requirements (Flemming & Mielck, 1995:2241)

A further advantage of minitables used as a MUDF, is that they are quite suitable for coating in order to sustain drug release. For example, it is necessary to coat granules with about 3 times more ethylcellulose or Eudragit® RL to achieve the same drug release rate as that from coated minitables. Furthermore, the drug release rate from minitables is more consistent than from granules. The use of minitables would therefore constitute a potential economic saving by a reduction in the amount of coating polymer required (De Brabander *et al.*, 2000a:195-203; Flemming & Mielck, 1995:2239-2251; Munday, 1994: 2369-2379).

1.3.3 Production and production requirements of minitables

Minitables are competing with pellets to produce the ideal multi-unit dosage form. Even though the process of production of minitables is exactly the same as for traditional tablets, the tooling used, as mentioned before, must meet special requirements regarding precision and mechanical stability. This is due to the higher die-wall friction compared with normal tooling. If the fitting is not exact, a high amount of abrasion could occur. Hershberg (1965) was the first to describe the problems connected with the durability of small-sized punches in a patent. The particle size of the tableting mixtures must have a definite upper limit. Therefore, direct compression is the most appropriate method for minitableting, if a mixture with sufficient flow properties can be obtained (Flemming and Mielck, 1998:76).

Furthermore, the narrow diameter of the tablet die requires excellent flowability as well as a strict limit for the maximum particle size, in order to avoid blocking of the die opening by coarser particles. Flowability is thus, again, an important powder-technological property, which also affects the performance in sieving, mixing, granulating, and content uniformity of the final product (Flemming & Mielck, 1995:2240). The tableting behaviour of tablet mixtures using small diameters seems to provide advantages. A patent of the Nordmark company (Pich and Mowest, 1989) points out that they were able to produce minitables, but not 10 mm tablets, with a content of 99.5% pancreatin.

The size dependence of tensile strength has also been described as early as 1977 in the literature by Stanley and Newton (1977:13-19). They found a clear indication that the strength of the material of tablets of different size varies significantly. Several reasons were suggested for this observation, such as structural differences caused by friction effects and different stress distribution due to different tablet-forming conditions or tablet dimensions. Varying flaw distributions were also discussed. Kennerley *et al.* (1977:39p) prepared cylindrical tablets of two different volumes, but with identical ratio of diameter to thickness. They found a decrease in mean fracture stress with decrease in size. Jacob and Hüttenrauch (1982:44-52), on the other hand, explained that the mechanical activation decreases with increasing tablet diameter, leading to a lower strength following the activation theory of tablet formation.

Another possible reason for an increased strength of smaller tablets is a better force transmission due to less material between the punches with decreasing tablet height, resulting in a greater overall pressure. However, it was shown that the force transmission became smaller when the diameter of the tablets was decreased to values fewer than 3 mm, due to a higher amount of friction. Therefore force transmission should not be the reason for a higher mechanical strength of minitables (Lennartz & Mielck, 1998:75-85).

Lennartz and Mielck (1998:75-85) conducted a study where they tried to improve the compactability of paracetamol. These authors found that the mechanical stability of minitables was equal to, and at high pressures higher than, that of normal-sized tablets and that the weakening influence of paracetamol was lower. The often proposed reason for a higher strength of smaller tablets i.e. the flaw distribution inside the tablet, could not completely explain these results. They conclude that the reason for the higher mechanical stability of minitables, as demonstrated by the compression profiles and increase in capacity of a direct compressible excipient derived therefrom, as well as reduced capping tendency, may lie in the main difference between minitables and normal-sized tablets, namely the ratio

between the outer surface and volume of the tablet. This ratio increased with decreasing tablet size, leading to a higher relative amount of material exposed to the surfaces of the punches and die. Consequently, the region of volume exposed to shear stress, which is connected with die wall friction, is larger in relation to the total volume of the mass in the die forming the tablet. This will result in a higher relative amount of shear stress which leads to a wider distribution of relative density over the volume of the tablet and thus locally to more binding sites without necessarily increasing the elastic deformation of the particles. This possibly forms a protecting shell around the tablet, which enhances strength and prevents capping. Such a shell could be expected for all tablet sizes, but with decreasing tablet size it may become more predominant, especially in ranges with diameters smaller than 3 mm.

1.4 Conclusion

Accounting for 70% of all ethical pharmaceutical preparations produced, tablets are now the most popular dosage form because of its numerous advantages. The production of tablets is a unique process and some prerequisites are to be met by both materials and machinery that are employed in the manufacturing of tablets. Moreover, direct compression has become an interesting alternative for the tablet manufacturer due to the intensive research in tableting machinery and the development of potent, new excipients over the last thirty years.

Direct compression excipients should meet extensive requirements to render them suitable for this manufacturing process. The most essential properties of direct compression excipients are good flowability, sufficient powder compressibility and optimum moisture content, thereby rendering tablets of high tensile strength and toughness, low friability and uniform mass. In addition, these excipients should be compatible with a variety of drug substances and it should promote rapid and complete disintegration. In practice, unfortunately, relatively few substances possess these essential properties, and thus, oblige the optimisation of these materials through appropriate processing measures. For example, granulation prior to compression is the process of particle size enlargement and is done to confer fluidity and compressibility of mixtures.

The growing interest for multiple-unit dosage forms and their increasing share of the pharmaceutical product market are claimed by their proponents to stem from advantages they offer over single-unit dosage forms. The chief advantage being that once ingested, the particles will be distributed over an ever increasing area as they pass down the gastrointestinal

tract, thereby compensating for the local variations in milieu conditions and individual unit imperfections. As a result, toxic side effects will be minimised.

Minitablets in particular, have received increasing interest over the last 10 years. They are tablets with a diameter equal to or smaller than 2-3 mm and constitute an alternative to other multi-unit dosage forms. Minitablets proved to be more reliable sustained release dosage forms than those containing irregular units such as pellets, granules or particles and can be easily produced with defined form, size and strength. It was found that certain active ingredients could not be tableted into normal sized tablets (i.e. 10 mm diameter), but the production of minitables was indeed possible with the same active ingredients. These tablets rendered a mechanical stability equal to, and at high pressures higher than, that of normal-sized tablets. The reason for the higher mechanical stability being the main difference between minitables and normal-sized tablets, i.e. the ratio between the outer surface and volume of the tablet.

This Multi-unit dosage form may be produced on rather conventional tableting machines, utilising common methods to control the process. However, production with rotary machines necessitates very fine mechanical adjustments if a uniform filling of the matrices is to be achieved and damage to the punches avoided. Another advantage of minitables is that they are quite suitable for coating in order to sustain drug release. Furthermore, the drug release rate from minitables is more consistent than from granules. The use of minitables would therefore constitute a potential economic saving through the use of less excipients, few and inexpensive production steps, and if to be coated, reduced amounts of coating material required.

The myriad of challenges that is presented in the production of minitables that disintegrate instantaneously, and that contain chitosan as filler (described in chapter 2), so as to produce mucoadhesive properties, is the subject of this study and the evaluation of these challenges will be presented systematically in the following chapters of this thesis.

CHAPTER 2

CHITOSAN IN THE PHARMACEUTICAL INDUSTRY

2.1 Introduction

A variety of synthetic or natural polymers have been employed in various drug delivery systems (Kawaguchi, 2000:1174; Miyazaki *et al.*, 1981:3067). Natural produced polymers such as cellulose, starch, pectin and alginate represent biodegradable and toxicological harmless raw-materials of low costs. They have therefore been used as abundant excipients in various pharmaceutical formulations for many decades. Because of progress in pharmaceutical sciences leading to more and more sophisticated drug delivery systems, however, the features of these polymers became in many cases insufficient, which has intensified the search for new, more specific and suitable polymers. A promising strategy in this direction is the chemical modification of natural produced polymers e.g. the development of cellulose derivatives such as methylcellulose or sodium carboxymethylcellulose (Mi *et al.*, 2002a:181; Bernkop-Schnürch, 2000:1-2; Hugerth *et al.*, 1997:150).

In the past thirty years, substantial progress has been made on fundamental and applied research in chitosan technology, a monograph of which was included in the fourth edition of the European Pharmacopoeia in 2002 (chitosan hydrochloride) (Säkkinen *et al.*, 2004:145). One of the driving forces behind this rapid development has been the continuing decrease in the supply of natural resources. At the same time there has been an increased realisation that there are abundant alternative bioresources. Two factors – economics and versatility – have also stimulated interest in chitosan's utilisation in various fields (Li *et al.*, 1992:3).

Chitosan has already been approved as a food additive in Japan and is believed to be non-toxic. Except for the food industry, chitosan has also been applied in the agricultural, wound healing, biotechnological, textile and cosmetic industry. Derivatives of chitosan such as glucosamine (endogenous aminosaccharide) have found markets as dietary supplements for osteoarthritis, and itself is recommended for weight loss and hypercholesterolemia (Muzzarelli, 2000:3). Although

it has been widely used by these industries it was only in recent years that its potential application in the pharmaceutical field has been identified by several scientists. The controlled release properties of drugs exhibited by chitosan dosage forms as well as the favourable characteristics shown during transfection studies with genetic material enforces the applicability of similar novel dosage forms in the future (Mura *et al.*, 2003:67-68; van der Merwe, 2003:19; Win *et al.*, 2003:305; Paul & Sharma, 2000:5; Kotzé *et al.*, 1999b:344; Felt *et al.*, 1998:981; Onishi *et al.*, 1997:205).

It has been predicted that the world market for chitin and chitosan will flourish by 2005 of which the major share will be used for health care products including drug delivery systems, wound healing dressings and ointments, sutures and artificial ligaments etc. (Paul & Sharma, 2000:6). The main driving force in the development of new applications for chitosan lies in the fact that this cationic polysaccharide is not only naturally abundant, economically processed and chemically modified, but it is also non-toxic, biodegradable and multifunctional. Moreover, it is potentially more useful than cellulose for developing advanced functions because of the presence of amino groups (Mao *et al.*, 2004:45; Sandri *et al.*, 2004:351; Säkkinen *et al.*, 2002:33; Felt *et al.*, 1998:980; Calvo *et al.*, 1997:125; Imai *et al.*, 1991:12).

2.2 Chemical identity of chitin and chitosan

Chitosan was reportedly first discovered by Rouget in 1859 (Onishi *et al.*, 1997:206) when he boiled chitin in a concentrated potassium hydroxide solution. This resulted in the *N*-deacetylation of chitin [$\alpha(1-4)$ -2-acetamido-2-deoxy- β -D-glucan] (Miyazaki *et al.*, 1981:3067). Fundamental research on chitosan, however, did not start in earnest until about a century later. In 1934, two patents, one for producing chitosan from chitin and the other for making films and fibers from chitosan, were obtained by Rigby (Rigby, 1934a:1; Rigby, 1934b:2). In the same year, the first X-ray pattern of a well-oriented fiber made from chitosan was published by Clark and Smith (Li *et al.*, 1992:3). Since then, knowledge about chitosan has been greatly advanced by the work of pioneers such as Muzzarelli (1973:144-176).

The term 'chitinosans' (figure 2.1) embraces the spectrum of acetylated poly(*N*-glucosamines), ranging from chitin or poly(*N*-acetyl-glucosamine), where the degree of deacetylation is 0 %; to chitosan or poly(glucosamine), with a 100 % degree of deacetylation. In general then, commercially available 'chitosans,' $(C_6H_{11}O_4N)_n$ are heterogeneous, poorly characterised for

which the degree of deacetylation ranges from 60 to 90 %. The heterogeneity of these chitinosans is the result of the relatively uncontrolled commercial processing of native chitin involving both *N*-deacetylation and depolymerisation as previously mentioned (Mao *et al.*, 2004:45; Romøren *et al.*, 2003:116; Sawayanagi *et al.*, 1982:2935; Onishi *et al.*, 1997:206; Rege *et al.*, 1999:50).

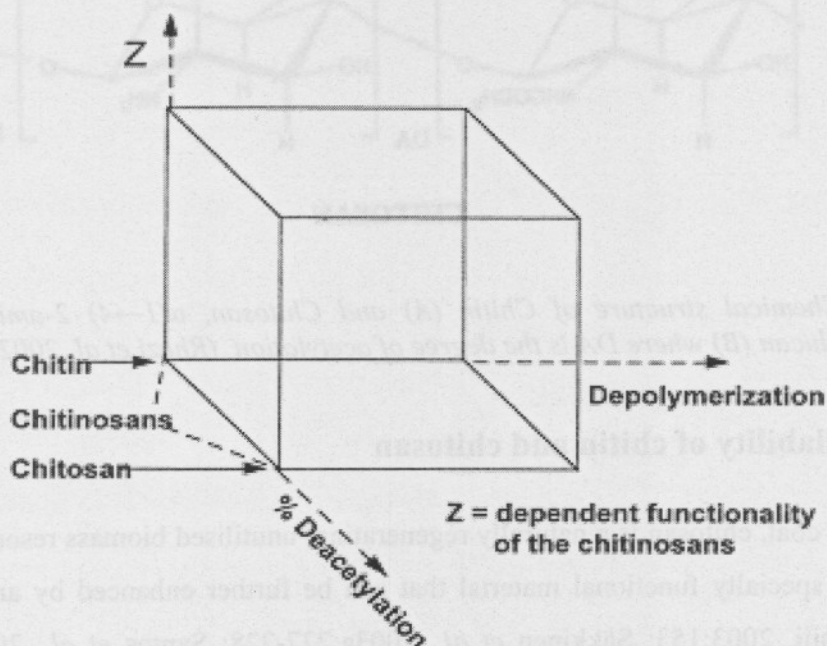


Figure 2.1: *The chitosan spectrum (Rege et al. 1999:50).*

Chitosan, $(C_6H_{11}O_4N)_n$, depicted in figure 2.2 has a sugar backbone consisting of linear 1-4 linked 2-acetamido-2-deoxy- β -D-glucopyranose (GlcNAc) and 2-amino- β -D-glucopyranose (GlcN) units, a structure very similar to that of cellulose, the only difference being the replacement of the hydroxyl moieties by amino groups through alkaline *N*-deacetylation on the C-2 position. Thus, chitosan is poly(*N*-acetyl-2-amino-2-deoxy-D-glucopyranose), where the *N*-acetyl-2-amino-2-deoxy-D-glucopyranose (or Glu-NH₂) units are linked by (1 $\rightarrow$ 4)- β -glycosidic bonds (Hejazi & Amiji, 2003:151; Mura *et al.*, 2003:67-68; Paul & Sharma, 2000:6; He *et al.*, 1999:55; Felt *et al.*, 1998:980; Carreño-Gómez & Duncan, 1997:232; Kas, 1997:689; Borchard *et al.*, 1996:132; Kawashima *et al.*, 1985b:2107).

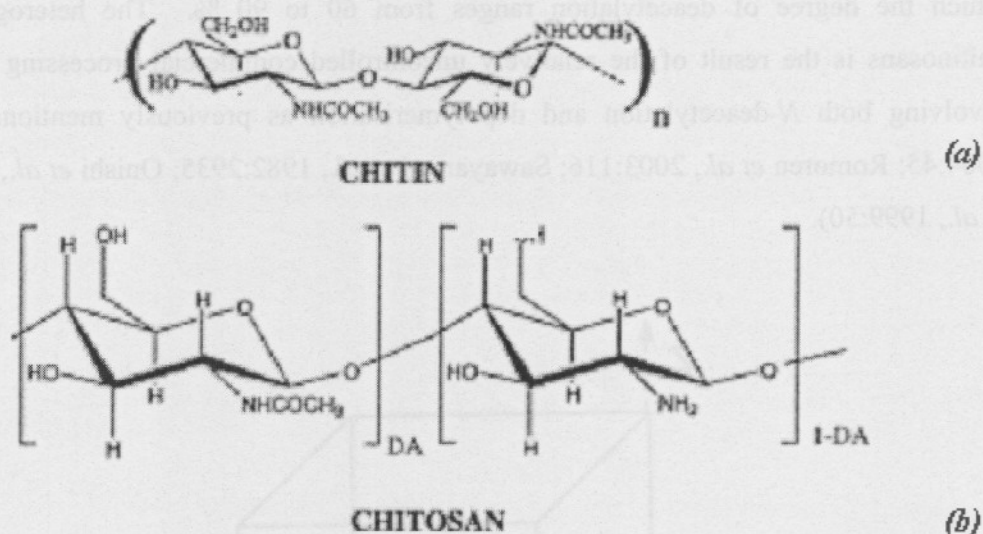


Figure 2.2: Chemical structure of Chitin (A) and Chitosan, $\alpha(1 \rightarrow 4)$ 2-amino-2-deoxy- β -D-glucan (B) where DA is the degree of acetylation (Rhazi *et al.*, 2002:1524).

2.2.1 Availability of chitin and chitosan

Unlike oil and coal, chitosan is a naturally regenerating, unutilised biomass resource, as well as a novel type of specialty functional material that can be further enhanced by artificial culturing (Hejazi & Amiji, 2003:153; Säkkinen *et al.*, 2003a:227-228; Santos *et al.*, 2002:153; Paul & Sharma, 2000:5-8; Felt *et al.*, 1998:980; Akbu, 1994:217-218; Li *et al.*, 1992:3; Miyazaki *et al.*, 1981:3067-3069). It was also reported that chitosan and chitin are contained in vivo in cell walls of fungi such as *Aspergillus niger* (Felt *et al.*, 1998:980) or the order *Mucorales*. The advantage of using fungi is the easy handling, harvesting and controlling to produce high quality chitosan. Furthermore, the manipulation of characteristics of chitosan, such as molecular weight (MW) and the grade of deacetylation (DD), through fungi culturing are possible. The MW of the chitosan produced so far from fungi is high, indicating that fungi produce these high polymerised carbohydrates naturally. In this point, the chitosan from fungi is comparable with chitosan from crab shells, which is also normally produced with a high MW (Niederhofer & Müller, 2004:102).

Chitin (figure 2.2a), however, is more widely distributed in nature than chitosan and can be found in mushrooms, yeasts, and the hard outer shells of insects and crustaceans. It was reported, for example, that about 50-80 % of the organic compounds in the shells of crustacean and the cuticles of insects consists of chitin. At present, most chitosan for practical and commercial use comes from the production of deacetylated chitin, with the shells of crab, shrimp, and krill (the

major waste by-product of the shellfish-processing industry) being the most available sources of chitosan (Smith *et al.*, 2004:44; Hejazi & Amiji, 2003:151; Win *et al.*, 2003:305; He *et al.*, 1999:54; Carreño-Gómez & Duncan, 1997:232; Li *et al.*, 1992:4).

Depending upon its crystalline structure, chitin exists in several forms with each having their specific properties. Among these forms, α -chitin is the most abundant crystalline, tightly compacted and stable form. It is extracted from shrimp or crab shells and has an antiparallel structure with strong intermolecular hydrogen bridges. β -Chitin is extracted from squid or loligo pens and exhibits weaker intermolecular hydrogen bonding due to the parallel arrangement of the polymer chains, whereas in the γ -chitin there are two up chains for every down chain. α -Chitin is found where extra hardness proves essential, whereas β - and γ -chitin provide toughness, flexibility and mobility together with control over electrolytes and polyanionic characteristics (Van de Velde & Kiekens, 2004:1; Paul & Sharma, 2000:6). An analysis of the diffraction spectra of chitin and chitosan revealed a structural resemblance between the two polymers. It has been suggested that the conformation of chitosan was similar to that of α -chitin (Li *et al.*, 1992:5).

2.2.2 Chemical methodology for preparation of chitosan

Shrimp or crab shell proteins are removed by treatment with 3-5% w/v sodium hydroxide (NaOH) aqueous solution at 80-90 °C for a few hours, or at room temperature overnight (decalcification). The inorganic constituents of the product are then removed by treatment with 3-5% w/v hydrochloric acid (HCl) aqueous solution at room temperature (deproteination) giving a white to beige coloured sample of chitin. This chitin is then deacetylated in a boiling aqueous sodium hydroxide solution (40-45% w/v) at 90-120 °C for 4-5 hours. The insoluble precipitate is washed with water to give a crude sample of chitosan (Hejazi & Amiji, 2003:152; Snyman, 2000:5). The conditions used for deacetylation will determine the polymer molecular weight and the degree of deacetylation. The crude sample is dissolved in aqueous 2% w/v acetic acid, and then the insoluble material is removed giving a clear supernatant solution which is neutralised with NaOH solution resulting in a purified sample of chitosan as a white precipitate (Hirano, 1996:238). Further purification may be necessary to prepare different medical pharmaceutical grades of chitosan, some of which may be ultra pure and even well suited for implantation. The quality and properties of these grades depend on the manufacturing process, which is briefly outlined in figure 2.3, and are assessed by determining the levels of heavy metals and proteins

present in the chitosan, as well as the pyrogenicity and cytotoxicity of the chitosan (Kas, 1997: 690).

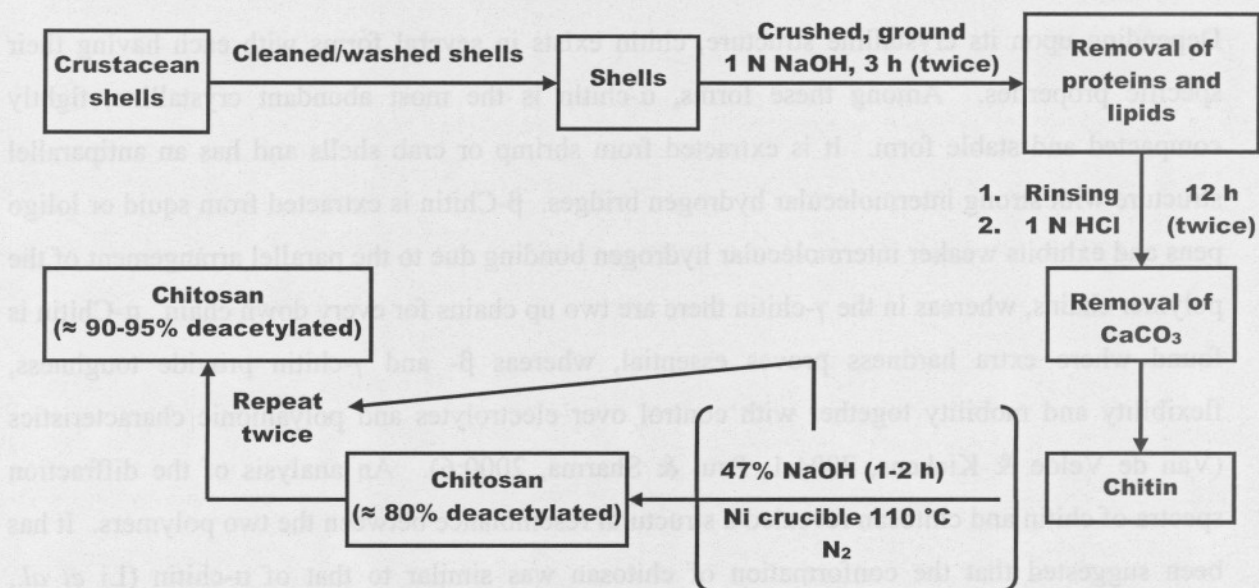


Figure 2.3: Production of chitosan (Singla & Chawla, 2001:1048).

Different methods of production on small scale may be used, of which the methods of Horowitz (1957:5046), Wolfrom (1959:1764), Broussignac (1968:1241) and Fujita (1970:599) have been described. Horowitz's (1957:5046) technique for example, where chitin is treated with solid potassium hydroxide for 30 minutes at 180 °C, resulted in the highest removal (95%) of acetyl groups. Production of chitosan from chitin on a large scale is also based on these methods and has been thoroughly reviewed by Muzzarelli (2002:233).

2.2.3 Physico-chemical and biological properties

As previously mentioned the word chitosan refers to a large number of polymers, which differ in their (1) degree of *N*-deacetylation (40-98%), which is the share of nitrogen sites occupied by acetyl groups (each nitrogen atom can react with one acetyl group), and (2) molecular weight (50,000-2,000,000 Da). These two characteristics are very important to the physico-chemical properties of the chitosans and hence, they have a major effect on the biological properties (Van de Velde & Kiekens, 2004:1; Carreño-Gómez & Duncan, 1997:232; Aspen *et al.*, 1995:69; Sannan *et al.*, 1976:3589-3600). As the properties of the specific chitin/chitosan polymer depend

on the degree of acetylation (DA) it is important to accurately determine this variable with the use of infrared analysis (figure 2.4) (Van de Velde & Kiekens, 2004:1; Knapczyk *et al.*, 1989:659; Felt *et al.*, 1998:990; Paul & Sharma, 2000:6). When the DA is lower than 50%, the chitin becomes soluble in aqueous acidic solutions and is named chitosan (Le Dung *et al.*, 1994:209). Furthermore, due to the polymer characteristics of chitosan, a wide range of molecular weights is available, from high molecular weight polymers to oligomers of only a small number of subunits. This is important during characterisation as the polydispersity of the polymer (distribution of molecular weight) influences various other properties such as viscosity and reactivity (Aspden *et al.*, 1995:69). Rege *et al.* (1999:51) found that the moisture content of chitosans ranges from between 7-11% and does not seem to be dependant on the degree of deacetylation or molecular weight of the polymer.

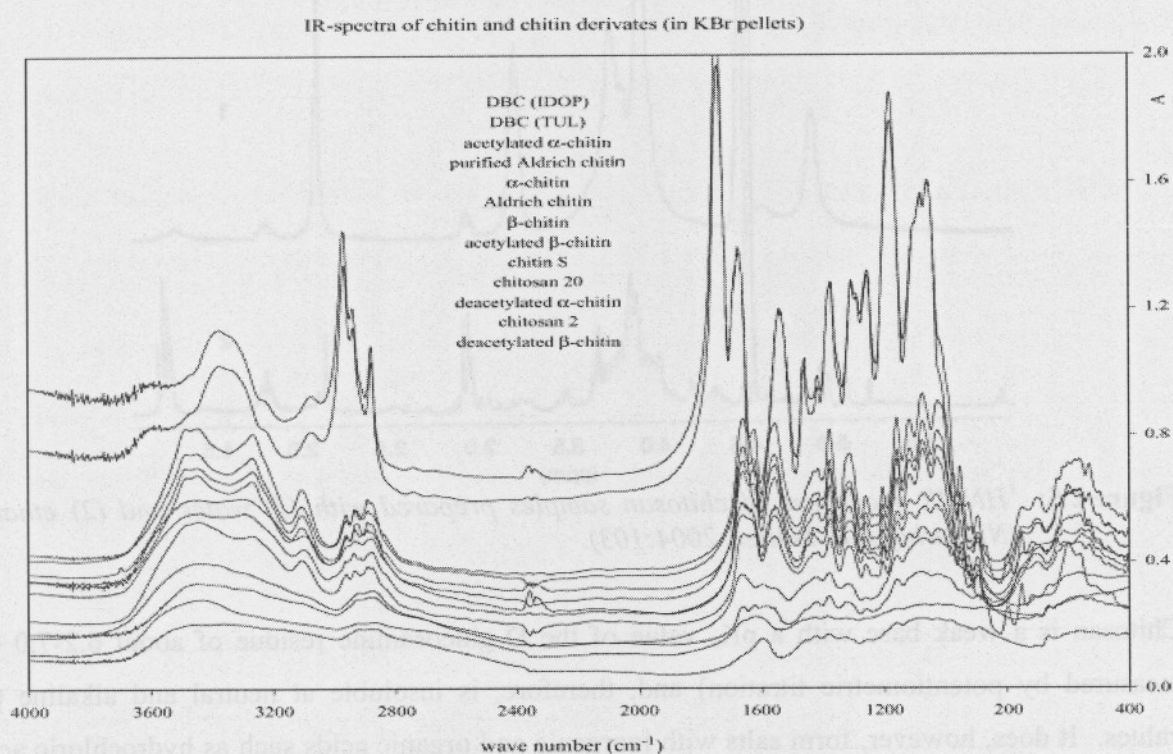


Figure 2.4: FT-IR spectra of chitin and chitin derivatives (Van de Velde & Kiekens, 2004:4).

Methods for checking the removal of acetyl groups in chitosan include infrared spectroscopy, titration, gas chromatography, and dye absorption (Li *et al.*, 1992:6). Among these, Muzzarelli (1977:46-50) suggested that first derivative ultraviolet spectrophotometry at 199 nm was probably the best method for non-destructively and accurately determining the degree of acetylation in chitosan samples. With this technique the *N*-acetylglucosamine absorbance

readings were linearly dependent on concentration and were not influenced by the presence of acetic acid. Other experimental procedures that may be helpful during the characterisation of these polymers especially the degree of deacetylation and molecular weight include: ^1H -NMR (figure 2.4), X-ray diffractometry, size exclusion chromatography as well as light scattering and viscosity measurements (Aspden *et al.*, 1995:69; Li *et al.*, 1992:7). Among these, viscometry is the most simple and rapid method for determination of molecular weight. Although it was indicated that the molecular weight of chitosan was not always directly related to its viscosity because of the presence of colloidal particles, the viscometry method is still widely employed for determining the relative molecular weight of chitosan (Li *et al.*, 1992:8).

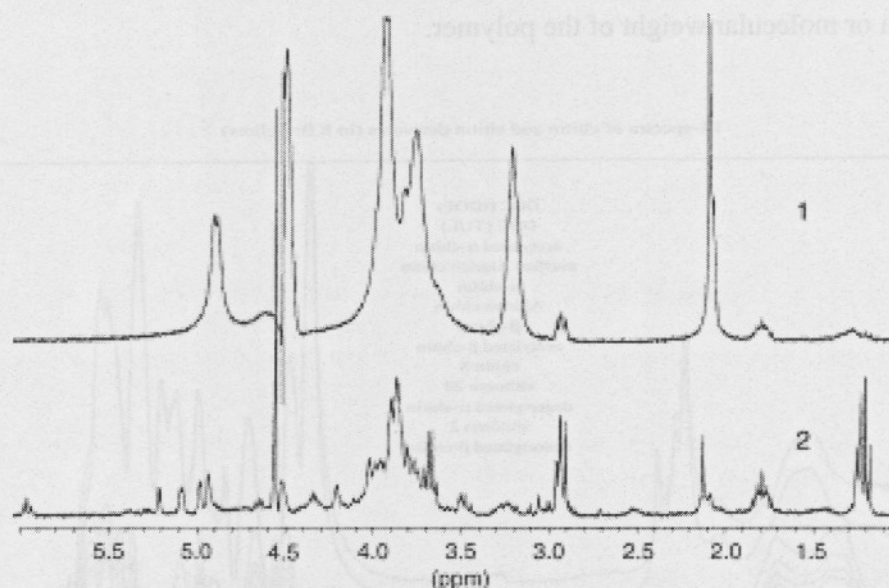


Figure 2.5: ^1H NMR spectra of chitosan samples prepared with (1) water and (2) ethanol (Niederhofer & Müller, 2004:103).

Chitosan is a weak base with a pK_a value of the D-glucosamine residue of about 6.2-7.0 (as measured by potentiometric titration) and, therefore, is insoluble at neutral and alkaline pH values. It does, however, form salts with inorganic and organic acids such as hydrochloric acid, acetic acid, glutamic acid, and lactic acid. In an acidic medium, protonation of the amino group of the D-glucosamine monomeric units results not only in a soluble, positively charged polysaccharide that has a high charge density (one charge for each D-glucosamine unit), but it also causes the chitosan chain to uncoil and become soluble. Hence, chitosan-based drug delivery devices release most of the active ingredients in the stomach (He *et al.*, 1998:76; Agnihotri & Aminabhavi, 2004:246). As pH values increase above its pK_a of approximately 6.5,

chitosan loses this charge, coils up and is likely to precipitate from the solution. It can also form gels in an acidic medium by interacting with different types of divalent and polyvalent anions. This is because chitosan is hydrophilic and can retain water in its structure (Leane *et al.*, 2004:242; Hejazi & Amiji, 2003:153; Säkkinen *et al.*, 2003a:227; Säkkinen *et al.*, 2002:33; Santos *et al.*, 2002:154; Felt *et al.*, 1998:981; Junginger & Verhoef, 1998:374; Schipper *et al.*, 1996: 1686-1692; Knapczyk *et al.*, 1989:659; Domard *et al.*, 1986:105).

Salts of chitosan are soluble in water, the solubility depending on the degree of deacetylation and the pH of the specific solution. Chitosans with a low degree of deacetylation ($\leq 40\%$) are soluble up to a pH of 9, whereas highly deacetylated chitosans ($\geq 85\%$) are soluble only up to a pH of 6.5. Addition of salts to the solution interferes with the solubility of chitosans. The higher the ionic strength, the lower the solubility as charge neutralisation occurs by increasing concentrations of counterion. By increasing the degree of deacetylation the viscosity is increased. This can be explained by the fact that high and low deacetylated chitosans have different conformations in aqueous solution. Chitosan has an extended conformation with a more flexible chain when it is highly deacetylated, because of the charge repulsion in the molecule. However, the chitosan molecule has a rod-like shape or coiled shape at a low degree of deacetylation due to the low charge density in the polymer chain (Snyman, 2000:5; Illum, 1998:1326-1331; Errington *et al.*, 1993:113-117). The viscosity of chitosan solution is also affected by factors such as concentration, degree of deacetylation, molecular weight, ionic strength and temperature. As the chitosan concentration increases and the temperature decreases, the viscosity increases. The nearly linear negative dependence of viscosity on temperature is due to the enhanced chain flexibility and reduced root-mean-square unperturbed end-to-end distance of the polymer chains with increasing temperature (Muzzarelli, 1977:53).

Chitosan in solution exist in the form of quasi-globular conformation stabilised by extensive intra- and inter-molecular hydrogen bonding. The hydrogen bonding in chitosan chains due to the presence of amine and hydroxyl groups causes the high viscosity of chitosan solutions. Chitosans with lower degrees of deacetylation have lower hydrogen bonding densities because of the lower number of amino groups in the polymer chains. Since urea is capable of breaking the hydrogen bonding, the chain conformation of chitosan changes from a spherical structure in acetic acid-NaCl to a random coil conformation in acetic acid-NaCl-urea system. Intermolecular hydrogen bonding in chitosan is responsible for the film and fiber-forming properties of the polymer. According to Chen *et al.* (1994:41-46), the degree of deacetylation of chitosan, which

will determine the number of intermolecular hydrogen bonds, was found to affect the rigidity of this polymer film. For chitosan hydrogels, the extent of dissociation of the hydrogen bonding may affect the swelling kinetics of the gels. At low pH, the hydrogen bonding dissociated due to the protonation of the amine groups leading to faster swelling. When the salt form of chitosan is neutralised, it can form interpenetrating polymer networks with other neutral polymers, e.g. poly(ethylene oxide), poly(*N*-vinylpyrrolidone), and poly(vinyl alcohol), by intermolecular hydrogen bonding.

Thermogravimetric analysis and differential scanning calorimetry were performed on chitosan by Agnihotri and Aminabhavi (2004:5-246). They concluded that chitosan started decomposing after 100 °C and weight loss remained constant up to 310 °C. A sudden weight loss, however, was observed after 310 °C, and the weight loss around 400 °C was 47.4%. Pure chitosan has shown an endothermic peak at 53 °C, which corresponded to its transition temperatures.

The pharmaceutical requirements of chitosan are: particle size < 300 µm, density between 1.35 and 1.4 g/cm³, pH 6.5 – 7.5, insoluble in water, and partially soluble in acids (Hejazi & Amiji, 2003:154; Sandford, 1989:657). The chemical and biological properties of chitosan are summarised in table 2.1.

Table 2.1: *The chemical and biological properties of chitosan (Hejazi & Amiji, 2003:154).*

Chemical properties	Biological properties
Cationic polyamine	Biocompatibility
High charge density at pH < 6.5	Natural polymer
Adheres to negatively charged surfaces	Biodegradable to normal body constituents
Forms gels with polyanions	Safe and non-toxic
High molecular weight linear polyelectrolyte	Haemostatic, bacteriostatic, and fungistatic
Viscosities, high to low	Spermicidal
Chelates certain transitional metals	Anticancerogen
Amiable to chemical modification	Anticholesteremic
Reactive amino/hydroxyl groups	Reasonable cost
	Versatile

2.2.3.1 Toxicity of chitosan

To date, most studies assume chitosan's biocompatibility and non-toxicity, and proceed to describe the performance of chitosan formulations designed for controlled release. Smith *et al.* (2004:44), Hejazi and Amiji (2003:155) and Schipper *et al.* (1996:1686), however, mentioned

that chitosan depicted low oral toxicity with an LD₅₀ in rats of 16 g/kg. They further indicated that the toxicity of chitosan might depend on different factors such as degree of deacetylation, molecular weight, purity, the specific counter ion, and route of administration.

Carreño-Gomez and Duncan's (1996:232), preliminary studies led them to believe that soluble chitosan (like other cationic polymers) displayed concentration-dependant toxicity towards cells in vitro and also caused red blood cell lysis (with the exception of L 110 in the B16F10 cytotoxicity experiments). They found that cytotoxicity was clearly related to the salt used, and this was not surprising as the nature of the counterion will govern interaction of the protonated amine function of the chitosan polymer with negatively charged cellular components. Chitosan hydrochloride (CL 210) was the most cytotoxic in the B16F10 assay, having an IC₅₀ value of 0.21 ± 0.004 mg/ml and was only fourfold less toxic than poly-L-lysine (Sgouras & Duncan, 1990:61-68). Chitosan cytotoxicity could be ranked in the order chitosan hydrochloride > hydroglutamate > glycol chitosan > hydrolactate.

Other studies have shown chitosan to be biologically active. Berscht *et al.*, (1995:201-205) examined several different chitosan salts fabricated as fleeces destined for use as wound dressings. After a 72 hour incubation with fibroblast cells, chitosan hydrolactate, hydrochloride and hydroglutamate inhibited cell growth by 70-80%. In contrast, a chitosan methylpyrrolidinone fleece caused only 35% incubation. Exposure of the same materials to fibroblasts during in their logarithmic growth phase resulted generally in less toxicity; chitosan methylpyrrolidinone fleeces reduced growth by 10%, hydrolactate by 21%, hydrochloride by 48%, but the chitosan hydroglutamate fleeces still caused a 76% inhibition. Although Kotzé *et al.*, (1996:425-426) found that chitosan hydrochloride and hydroglutamate had no damaging effect on Caco-2 cells (MTT assay), the incubation duration was only 4 hours.

Another factor was that all the chitosans caused red blood cell lysis in a time- and molecular weight-dependent manner (all chitosans that had molecular weight of > 100 K were more toxic towards B16F10 cells). At 1 hour, relatively little lysis was seen except for the hydroglutamate and hydrochloride salts. However, over 24 hours, lysis was so extensive that the haemoglobin released into the solution was actually precipitated, even at chitosan concentrations as low as 1 µg/ml. The data reported here suggest that soluble chitosans of high molecular weight would not make ideal candidates for intravenous administration (Huang *et al.*, 2004:344; Mao *et al.*, 2004:45-54; Carreño-Gomez and Duncan, 1996:236-237).

Hirano *et al.*, (1988:897-901) on the other hand, showed that intravenous injection of low molecular weight chitosan to rabbits at a dose of 4.5 mg/kg (approximately 10 mg/animal) daily for 11 days produced no abnormal changes, whereas a 50 mg/kg/day dose caused death after 3 days (one of two animals). This was probably due to blood cell aggregation. In this study it was also shown that oral dosing of chitosan for 239 days (0.7-0.8 g/kg) to rabbits or hens, produced little evidence of toxicity (loss of appetite occurred in the hens which was regained after chitosan feeding stopped). Despite the association of high molecular weight and degree of deacetylation to the cytotoxic and haemolytic properties of chitosan, a systematic study of the effects of these two parameters on the cytotoxicity of chitosan has not been reported (Huang *et al.*, 2004:344).

Säkkinen *et al.* (2004:145-147) found during adhesion studies in isolated porcine oesophagus that tablets containing conventional chitosan or various grades of microcrystalline chitosan had marked tendencies to adhere to oesophageal mucosa. It was thus, suggested that chitosans might be among the excipients that should not be used in formulations containing drugs associated with oesophageal injury. In addition to its use in pharmaceutical preparations, chitosan is used throughout the world in parapharmaceutical products, as a fat binder in cholesterol-lowering formulations and in slimming formulations. Failure of capsules and tablets to move rapidly through the oesophagus is a problem associated not only with pharmaceutical formulations but also with non-medical natural products. It has, for example, been reported that guar gum in a slimming product hydrated and formed a large viscous mass in the oesophagus, resulting in oesophageal obstruction. Caution might be needed in relation to parapharmaceutical products, because the amounts of chitosan in it are high, the daily intake usually amounting to several grams. The substantial amounts of gel-forming agent and the large size of the product could make them likely to stagnate in the oesophagus.

Thus, careful selection of the chitosan salt used (hydrolactate has been found the least toxic) and polymer molecular weight will improve chances of good biocompatibility.

2.2.4 Derivatives of chitosan and the need thereof

A promising strategy in the direction of obtaining more specific and suitable polymers is the chemical modification of these naturally produced materials. Again, chitosan has received much attention on this subject (Van der Merwe *et al.*, 2004:85; Bernkop-Schnürch *et al.*, 2000:2). Due to the primary amino group at the C-2 position of each polymer-subunit further chemical

modifications are easy feasible. By these modifications, the features of chitosan can be optimised according to a given task in delivery systems focusing on specific pharmaceutic-technological challenges (Bernkop-Schnürch *et al.*, 2000:2).

One such challenge is where most macromolecular pharmaceuticals such as peptide and protein drugs are indicated for chronic administration. Here, the drugs are mostly absorbed in the more basic environments of the large intestine and colon, and therefore, possibilities for the potential use of chitosans are limited as it is insoluble and precipitates from solution in these environments. Kotzé *et al.* (1997a:1197), however, stated that chitosan derivatives with different physicochemical properties, especially water solubility at neutral and basic pH values, will be of particular interest as they might prove to be useful as absorption enhancers in these environments. The nasal cavity also has a pH in the order of 7.40, and thus, possibilities exist for the use of such chitosan derivatives as nasal absorption enhancers (Kotzé *et al.*, 1999b:351).

It was the hypothesis of Kotzé *et al.* (1999b:351) that unmodified chitosan with a primary amino group may not be optimal, but that derivatives with different substituents, different basicities, or different charged densities will have the same or even increased efficacy in opening tight junctions. In the past several chitosan derivatives have been synthesised and the production of chitosan salts such as chitosan hydrochloride and chitosan glutamate, for example, proved extremely beneficial in improving the solubility of chitosan (Kotzé, 1999a:145), while retaining both the mucoadhesive and absorption enhancing capabilities of chitosan (Kotzé, 1999c:253).

In several reports it has been shown that the next generation of chitosan derivatives such as *N*-trimethyl chitosan chloride (TMC) is a partially quaternised derivative with superior water solubility, compared with other chitosan salts (Junginger & Verhoef, 1998:375). TMC has intensely been studied and described by Kotzé *et al.* (1999b:351) who came to the conclusion that this polymer was able to decrease the TEER of Caco-2 cell monolayers and that it increased the transport of several hydrophilic model compounds such as [¹⁴C]-mannitol (MW 182) and [¹⁴C]-polyethylene glycol (MW 4000) and the peptide drugs buserelin (MW 1300), 9-deglycinamide, 8-arginine vasopressin (MW 1412) and insulin (MW 5778) across Caco-2 cell monolayers (Kotzé *et al.*, 1997b:243-253; Kotzé *et al.*, 1997a: 1197-1202; Kotzé *et al.*, 1998a:1-20). The charge density of this polymer, as determined by the degree of quaternization, could be varied by chemical manipulation and this might be beneficial in absorption enhancement in neutral and alkaline environments. It was therefore, concluded that TMC could contribute significantly to the

effective delivery of hydrophilic compounds such as protein and peptide drugs (Hamman, 2001:51).

The ability of chitosan to crosslink with other polymers lead to favourable properties such as decreased release rate of drugs from different formulations. The degree of cross-linking may be changed to obtain the specific properties as needed. Glyceraldehyde has been successfully evaluated as a cross-linking agent for chitosan (Conti *et al.*, 1998:87) since it is non-toxic and biocompatible. Other derivatives such as mono-*N*-carboxymethyl chitosan and chitosan cyclodextrin analogues were also developed to display certain characteristics such as drug incorporation and DNA transfection etc. (Thanou *et al.*, 2001:828).

The development of these (and other) derivatives, however, took place in a relatively short time, leaving shortcomings in basic research on the behaviour and physicochemical character of these polymers. Investigating these shortcomings will, therefore, lead to a better understanding of previous results from drug release, absorption enhancement, toxicity and similar experiments.

2.3 Applications of chitosan

The industrial production and use of chitosan has been steadily increasing since the 1970s. In Japan, for example, the production of chitosan increased 37% each year from 1978 to 1983, the total annual amount reaching 311 tons by 1983 and 1,270 tons by 1986. At that time, the major applications of chitosan were centred on sludge dewatering, food processing, and metal ion chelation. The present trend, in industrial applications, however, is toward producing high value products, such as cosmetics, drug carriers, feed additives, semi-permeable membranes, and pharmaceuticals (table 2.2) (Win *et al.*, 2003:306; Aspen, 1995:70; Li *et al.*, 1992:11; Hirano *et al.*, 1988:897). Chitosan has been used, for example, in the pharmaceutical industry as a tablet binder (Upadrashta *et al.*, 1992:1707) as well as a disintegrant (Ritthidej *et al.*, 1994:2130). The fungicidal and antimicrobial activities of chitosan have also been well investigated (Meyer *et al.*, 2002:157). The uses and applications in pharmaceutical biotechnology are endless.

The difference in value between the products and the low-cost polymer is one of the main driving forces pushing studies on new applications of chitosan. Biotechnology is currently attempting large-scale production of high-value bioproducts like monoclonal antibodies, which were projected at about 1.2 billion dollars (U.S.) on the world market in 1991. Immobilisation techniques have been proven to be an effective way to increase cell density, product

concentration, and hence, productivity in a culturing system. Chitosan membranes and gels have great potential for use in immobilised cell culture systems (Win *et al.*, 2003:306; Knapczyk, 1993a:233; Li *et al.*, 1992:11).

Table 2.2: *Applications of chitosan (Li et al., 1992:11).*

Applications	Examples
Agriculture	Seed coating; Leaf coating; Hydroponics/Fertiliser; Controlled agrochemical release.
Biotechnology	Enzyme immobilisation; Protein separation; Chromatography; Cell recovery; Cell immobilisation; Glucose electrode.
Cosmetics	Make-up powder; Nail Polish; Moisturisers; Fixtures; Bath lotion; Face, hand and body creams; Toothpaste; Foam enhancing.
Food	Removal of dyes, solids, acids; Preservatives; Colour stabilisation; Animal feed additive.
Medical	Bandages; Sponges; Artificial blood vessels; Blood cholesterol control; Tumor inhibition; Membranes; Dental/plaque inhibition; Skin burns/artificial skin; Eye humor fluid; Contact lens; Controlled release of drugs; Bone disease treatment.
Membranes	Reverse osmosis; Permeability control; Solvent separation.
Pulp and paper	Surface treatment; Photographic paper; Carbonless copy paper.
Water treatment	Removal of metal ions; Flocculant/coagulant of proteins, dyes or amino acids; Filtration.

2.3.1 Biotechnological applications

2.3.1.1 Mucoadhesive properties of chitosan

Bioadhesion can be defined as the state in which two materials, at least one biological in nature, are held together for an extended period of time by interfacial forces (Ahuja *et al.*, 1997:489; Hendriksen *et al.*, 1996:231; Peppas & Buri, 1985:257) when applied to a mucosal epithelium. A bioadhesive polymer adheres primarily to the mucus layer, and this phenomenon is referred to as mucoadhesion (Junginger, 1990:111). Adhesion is an interfacial phenomenon influenced by surface energies. When an adhesive bond is established between two materials the total surface energy of the system is diminished, destroying two free surfaces and creating a new interface. Bioadhesive bonds may be physical or mechanical bonds, secondary chemical bonds or primary

chemical bonds. Of these, secondary chemical bonds (polar forces, Van der Waals forces, and hydrogen bonding) have been shown to be desirable for the development of bioadhesive strength (Jadhav *et al.*, 2004:195-203; Junginger, 1990:111). The new generation of mucoadhesive polymers, however, is capable of forming covalent bonds to the mucus layer (Bernkop-Schnürch *et al.*, 2004:177).

2.3.1.1.1 Importance of muco/bioadhesion

The adhesive properties of certain compounds have led to increasing interest in the development of slow-release dosage forms with prolonged residence times and decreased clearance rates of drugs from the chosen absorption route (e.g. gastrointestinal and urogenital tract, airways, ears, nose and eyes) and thereby allow a longer contact time with the absorptive epithelium. This might be beneficial, especially for hydrophilic drugs or for drugs with a high molecular weight such as peptides and proteins (Snyman *et al.*, 2003:61-69). Delivery of these drugs with sites of action in the stomach has been the main objective, but other suitable routes include: the buccal, nasal, ocular, rectal, sublingual and vaginal delivery routes (Ahuja *et al.*, 1997:490).

Possible benefits of slow-release dosage forms are, however, still under investigation. Dosage forms that release drugs slowly in the upper regions of the gastrointestinal tract could be of value if bioavailability of the drugs concerned were low or if toxic metabolites were formed distally in the intestine. If the bioavailability of a drug is low because it is specifically absorbed from sites in the upper regions of the gastrointestinal tract (i.e. furosemide), use of chitosan as an excipient could result in amounts of drug absorbed being increased as a result of prolongation of gastric residence time (Sandri *et al.*, 2004:351; Säkkinen *et al.*, 2003a:228; Takeuchi *et al.*, 2003:235; Smart *et al.*, 1984:295).

Incorporation of a bioadhesive agent into a formulation has thus been considered useful because of:

- Longer residence time of the dosage form on mucosal tissues. This will improve absorption of the drug and increase the drug's bioavailability.
- Higher drug concentration at the site of adhesion-absorption, which will create a driving force for the paracellular passive uptake.
- Immediate absorption from the bioadhesive drug delivery system without previous dilution and possible degradation in the luminal fluids.

- Enhancement of topical action of certain drugs such as antibiotic against certain bacteria that colonise the stomach such as *H. pylori*. Better stability and longer residence time allow more of the antibiotic to penetrate through the gastric mucus layer to act on *H. pylori*. Chitosan as well as some of its derivatives possess bio- and mucoadhesive properties and are therefore useful candidates for these dosage forms (Hejazi & Amiji, 2003:158; Lehr *et al.*, 1992a:70; Borchard *et al.*, 1996:131; Snyman *et al.*, 2003:65).

2.3.1.1.2 Factors influencing mucoadhesion

It was found that a wide range of factors, not standardised in previous mucoadhesive testing systems, could crucially influence the strength of the observed mucoadhesive bond (Tobyn *et al.*, 1995:235). These variables should be kept at constant values during the measurement of mucoadhesive properties of polymers, in order to improve the validity of the results. A few of these factors include:

- Initial contact and prehydration time (Jadhav *et al.*, 2004:195-203; Ponchel *et al.*, 1987:129-132).
- Selection of the model substrate surface (Ahuja *et al.*, 1997:494; Rossi *et al.*, 1995:1074-1078; Ponchel *et al.*, 1987:140).
- Applied strength (Ahuja *et al.*, 1997:494)
- pH of the medium (Ahuja *et al.*, 1997:494).
- Concentration (Ahuja *et al.*, 1997:493; Ponchel *et al.*, 1987:129; Breimecker, 1983:417).
- Molecular weight (Ahuja *et al.*, 1997:493; Lehr *et al.*, 1992b:46; Gurny *et al.*, 1984:336).
- Flexibility of polymer chains (Ahuja *et al.*, 1997:493).
- Spatial conformation (Ahuja *et al.*, 1997:493).

2.3.1.1.3 Chitosan as a muco/bioadhesive

According to Säkkinen *et al.* (2003b:346) adsorption of hydrophilic chitosan onto mucosal surfaces, and dehydration of mucosa are involved in the adhesion process, but so far, very little information on the *in vivo* adhesive behaviour of chitosan formulations is available. In the 1980s Peppas and Buri (1985:258) believed that (I) strong hydrogen-bonding groups (-OH, -COOH), (II) strong anionic charge, (III) high molecular weight (IV) sufficient chain flexibility and (V) surface energy properties favouring spreading onto mucus, were responsible for mucoadhesion.

This theory, however, had to be changed at the beginning 1990s, when Hassan and Gallo (1990:491-495) demonstrated that the positively charged polymer chitosan also displayed strong mucoadhesive properties. This phenomenon could be explained by electrostatic interactions of the polymer with negatively charged groups such as sialic acid moieties of the mucous layer (Säkkinen *et al.*, 2003b:346; Bernkop-Schnürch, 2000:8; Kotzé *et al.*, 1999b:347; He *et al.*, 1998:75-88; Lehr *et al.*, 1992b:43; Hassan & Gallo, 1990:491-495; Smart *et al.*, 1984:295). Due to the use of mucoadhesive chitosan-coated liposomes, for instance, the bioavailability of perorally administered insulin could be strongly improved (Takeuchi *et al.*, 1996:899-901).

On the other hand, for anionic polymers such as chitosan-EDTA mucoadhesion could be explained by the hydrogen bond formation of their carboxylic acid groups with the mucus gel layer. The combination of cationic as well as anionic moieties on the same polymer might compensate both effects. These results were in good accordance with earlier investigations demonstrating that the adhesive properties of chitosan-EDTA conjugates were strongly reduced if there were remaining primary amino groups on the polymer conjugate (Bernkop-Schnürch *et al.*, 1998:207-214). Similar observations were made by Lueßen *et al.*, (1996:1666-1672) after the peroral administration of buserelin in rats. Whereas the co-administration of chitosan hydrochloride lead to a strongly increased intestinal buserelin bioavailability, it was much lower using a mixture of the cationic polymer chitosan with the anionic polymer carbomer. Reasons for these results can be seen in reduced mucoadhesive and or absorption enhancing properties based on a reduced charge density which has to be taken into consideration for the chitosan-DTPA conjugate as well.

Miyazaki *et al.* (1995:257-258) examined adhesion of tablets to human cheek mucosa, during preliminary experiments. They showed that it adhered within a few seconds and remained in place for at least one hour. The results of this study showed a significant improvement of bioavailability of diltiazem administered sublingually compared to that achieved by oral administration.

Generally ocular formulations are preferred as solutions or suspensions, for reasons of patient compliance. A major problem is that extensive drug loss results from the instillation of solutions into the eye, due to an efficient drainage of solutions via the tear ducts to the nasal mucosa (Hendriksen *et al.*, 1996:232). Hui and Robinson (1985:203-213) investigated the use of bioadhesive acrylic acid polymers to deliver progesterone to the eye, and obtained a significantly improved drug bioavailability. Recently, it has been shown that the co-administration of

bioadhesive polymers with nanoparticles to the eyes of rabbits gave an improved pharmacological response when compared with other viscous polymers (Zimmer *et al.*, 1995:31-46). Another preliminary trial in rabbits by Skaugrud (1995:104) showed that the clearance rate for chitosan compared to saline solutions was significantly lower. It was evident that the chitosan in this trial was not easily washed off, but stuck to the negatively charged surface of the cornea and also influenced the drainage of the lacrimal fluid, resulting in a lowered clearance rate. In view of this, the bioadhesive properties of chitosan in solution make this polymer an interesting candidate for ocular drug delivery (Hendriksen *et al.*, 1996:232).

The bioadhesive properties of chitosan have also been studied in the form of microspheres (Gallo & Hassan, 1988:300-304) and solution (Hassan & Galo, 1990:495) with complexation and viscometric methods. Takayama *et al.* (1990:1997) studied the adhesion force of hydrated chitosan sodium hyaluronate tablets in different buffers, whereas Miyazaki *et al.* (1995:257-263) measured the adhesion force of partly hydrated chitosan sodium alginate tablets. Other formulations of chitosan that have been tested include films (Lehr *et al.*, 1992b:43-48), gels (Needleman & Smales, 1995:617-624) and liposomes (Takeuchi *et al.*, 1994:1954-1956). These formulations represent differing degrees of hydration, from wetted tablets to chitosan solutions, and have been assessed by widely different methods. The most promising results seem to have been achieved where the polymer was solvated (Needleman & Smales, 1995:623; Takeuchi *et al.*, 1994:1955; Hassan & Gallo, 1990:494) or in a film that includes an acid to give a locally low pH environment allowing hydration (Lehr *et al.*, 1992b:48).

2.3.1.2 Chitosan as an absorption enhancer for hydrophilic drugs

Permeation across any epithelial membrane is a fundamental step for absorption and systemic availability. The permeation process can be passive, active, or carrier-mediated and transcellular or paracellular. Transcellular absorption of protein and peptide drugs is hindered by their high molecular weight ($>30 \text{ \AA}$) and hydrophilicity, whereas their entry through the paracellular route is nearly completely restricted by the tight junctions. Low oral bioavailability leads to sub-therapeutic plasma levels and high inter- and intra-subject variability. For that reason, many of these therapeutic drugs are administered invasively by injection, a route that leads to poor patient compliance. Thus, it is important to enhance the oral bioavailability of macromolecular drugs to obtain therapeutic plasma levels. To achieve higher peroral peptide delivery, a system is required to improve the residence time of the drug, maintain the drug stability until it reaches its

absorption site, and enhance the permeability of the drug into the systemic circulation through the regulation of the integrity of the tight junctions (Leane *et al.*, 2004:241; Hejazi & Amiji, 2003:162; Bernkop-Schnürch, 2000:2; Kotzé *et al.*, 1999a:145; Kotzé *et al.*, 1999b:341; Junginger & Verhoef, 1998:370; Lee, 1991:56).

An ideal penetration enhancer should have the following desirable attributes:

- The absorption-enhancing action should be immediate and unidirectional, and the duration of the effect should be specific, predictable and suitable.
- After removal of the material from the applied membrane, the tissue should immediately fully recover its normal barrier property.
- It should show no systemic and toxic effects.
- It should not irritate or damage the applied membrane surface.
- It should be physically compatible with a wide range of drugs and pharmaceutical excipients.
- Effectivity in the low pH of the stomach, the neutral pH of the small intestine, as well as the basic pH of the colon may be an advantage, although not a prerequisite.

As evident from the above points, we are currently far from having an absorption enhancer available that fulfils all the requirements (Sandri *et al.*, 2004:351-359; Kotzé *et al.*, 1999a:146; Kotzé *et al.*, 1999b:341; Junginger & Verhoef, 1998:370; Kotzé *et al.*, 1998:35).

Generally, permeation enhancers can be divided into surfactants, fatty acids, salicylates, chelating agents and swellable polymers (Aungst *et al.*, 1996:19-31). The latter ones – especially if they are mucoadhesive – offer the advantage of remaining concentrated at the absorption site of the drug. Chitosan is reported to display such permeation enhancing capabilities (Sandri *et al.*, 2004:352; Smith *et al.*, 2004:43; Kotzé *et al.*, 1997a:1197; Borchard *et al.*, 1996:131; Artursson *et al.*, 1994:1358; Illum *et al.*, 1994:1188), but it needs to be in solution or gelled to exert its absorption-enhancing action (Leane *et al.*, 2004:242).

Chitosan induces redistribution or translocation of cytoskeletal F-actin and the tight junction protein ZO-1, thereby altering the integrity of the tight junctions that is associated directly or indirectly with the F-actin filaments. It increases the paracellular transport of several peptide drugs through an interaction of a positively charged amino group of the C-2 position of chitosan with negatively charged sites on the cell membranes and tight junctions of the mucosal epithelial cells, thereby activating the protein kinase C pathway. This results in transient and reversible

opening of the intercellular tight junctions followed by enhanced paracellular transport of compounds. The "bioadhesive nature" of chitosan may also play a role by prolonging the contact between the drug compound and the membrane (Leane *et al.*, 2004:242; Smith *et al.*, 2004:43; Huang *et al.*, 2002:1488; Steenkamp, 2002:34; Soane *et al.*, 1999:56; Junginger & Verhoef, 1998:370; Schipper *et al.*, 1997:923-929; Schipper *et al.*, 1996: 1686-1692). However, the mechanism of penetration enhancement via the buccal and vaginal mucosae, which lack tight junctions, has still to be clarified. (Sandri *et al.*, 2004:351-359).

In previous studies it has been shown that chitosan is able to reduce the transepithelial electrical resistance *in vitro* of a cultured intestinal epithelial cell line (Caco-2) (Borchard *et al.*, 1996:137). Chitosan was also able to increase the transport of hydrophilic molecules such as [¹⁴C]-mannitol and fluorescein-dextran significantly in Caco-2 cell monolayers (Artursson *et al.*, 1994:1358-1361; Borchard *et al.*, 1996:137). Similarly, the transport of the peptide drug 9-desglycinamide, 8-arginine vasopressin (DGAVP, MW 1299.5) was increased markedly after co-administration with chitosan glutamate in a Caco-2 cell culture (Lueßen *et al.*, 1997:15-23). Chitosan salts, i.e. chitosan glutamate and chitosan hydrochloride have been used *in vivo* as absorption enhancers for peptide drugs. Examples are for instance the nasal application of insulin with chitosan glutamate at a pH of 4.4 which led to a significant reduction in the blood glucose levels of rats and sheep (Illum *et al.*, 1994:1188), while the intraduodenal application of buserelin and chitosan hydrochloride in a gel formulation at pH 6.7, increased the absolute bioavailability of buserelin from 0.1 ± 0.1 % to 5.1 ± 1.5 % (Kotzé *et al.*, 1999a:146; Lueßen *et al.*, 1996:1168).

However, the increased transport of all these compounds was obtained in acidic conditions, where the pH was less or in the order of the pK_a (6.0 to 6.5) of chitosan (Yalpani & Hall, 1984:272-281). This suggests that the charge density of chitosan is an important factor for the enhancement of mucosal transport (Kotzé *et al.*, 1996:425; Schipper *et al.*, 1997:928). As previously mentioned, chitosan is a weak base and in neutral and basic environments the chitosan molecules will lose its charge, coil up, and precipitate from solution. In these conditions chitosan will be ineffective as an absorption enhancer, limiting its use in the more basic environment of the large intestine and colon (Artursson *et al.*, 1994:1359).

Schipper *et al.* (1996:1686-1691) could further demonstrate that the structural properties of chitosan, i.e. molecular mass and degree of deacetylation, dictate absorption enhancing properties and toxicity to a large extent. Chitosans exhibiting a high degree of deacetylation (> 80%) and high molecular mass display the comparatively highest increase in epithelial permeability as

illustrated in figure 2.6 (Smith *et al.*, 2004:43; Bernkop-Schnürch, 2000:3). They concluded that as the features of chitosan determining absorption enhancement are not correlated with those determining toxicity, a selection of chitosans with maximal effect on absorption and minimal toxicity seems to be feasible.

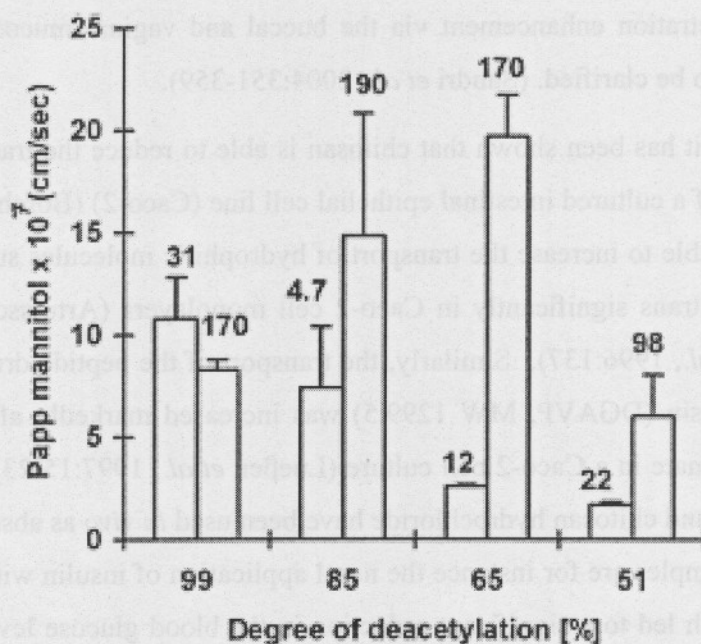


Figure 2.6: The mean apparent permeability coefficient (P_{app}) of [^{14}C]-mannitol across Caco-2 cell monolayers during exposure to 50 $\mu\text{g/ml}$ chitosan. Indicated numbers associated with the bars in the graph show the molecular mass of studied chitosan in kDa (Bernkop-Schnürch, 2000:3).

2.3.1.3 Gene transfection

Genetic immunisation techniques have gained increasing interest following the documentation a decade ago that DNA could elicit immune response. There are several reasons why plasmid vectors are attractive for the development of vaccines and, among others, the high stability of plasmid DNA, the relatively easy manufacturing process, the ability of the vaccine to elicit both humoral and cellular immune responses, the lack of infection risk (which is associated with attenuated viral vaccines) and low manufacturing costs are often mentioned (Romøren *et al.*, 2003:115). Chitosan is considered as a safe candidate for non-viral gene delivery (Thanou *et al.*, 2002:153; Huang *et al.*, 2000:337; Carreño-Gómez & Duncan, 1997: 239) due to its low toxicity and, in addition, the polymer can be obtained in, or modified to, different molecular weights and different degrees of acetylation (Huang *et al.*, 2000:337).

However, delivery into a target cell is but one step in a whole series of essential steps in DNA delivery. In order for DNA to have its desired effect, it needs to be delivered into the cell, escape from the endosomal compartments and be translocated through the cytoplasm and across the nuclear membrane to a position in the nuclear compartment. The first problem to overcome is to reduce the size of DNA, which is normally available in the form of a circular plasmid. Chitosan is able to compact and condense the plasmid DNA into small particles in defined conditions, as well as to form complexes with the DNA and protect it against degradation (Snyman 2003:11). Also, chitosan could be useful as an oral gene carrier, as well as in oral vaccinations because of its adhesive and transport properties in the GI tract (Hejazi & Amiji, 2003:158). Many formulation parameters, like salt concentration, pH, polymer charge density, polymer molecular weight and the charge ratio, may influence the polymer-DNA complexation and the characteristics of the complexes (Davis & Illum, 2000:153; Richardson *et al.*, 1999:242). Chitosan derivatives have also been investigated as possible transfection agents for gene therapy in diseases such as cystic fibrosis (Florea *et al.*, 2000:7315).

2.3.1.4 Controlled/sustained release

Sustained release dosage forms employ polymers as shells for microencapsulated drugs, as erodible and nonerodible matrices, as barrier membranes to regulate the release of drugs by diffusion, as ion-exchange resins and as backbones for pendent drug molecules attached by labile bonds. Combining a drug with a polymer for controlled release characteristics improves the patient compliance as well as the therapeutic success of the drug (Conti *et al.*, 1998:87). As early as the 1980's, chitosan has been assessed for its potentiality in the development of controlled-release systems and for its propensity for targeting drugs to specific sites (Paul & Sharma, 2000:5-22; Felt *et al.*, 1998:992; He *et al.*, 1998:75-88; Carreño-Gómez & Duncan, 1997:231).

Chitosan has already been evaluated as a matrix for sustained release granules (Hou *et al.*, 1985:3986; Miyazaki *et al.*, 1981:3067-3069). Miyazaki *et al.* (1981:3067-3069) and Sawayanagi *et al.* (1982:2935) noticed the usefulness of chitosan as a sustained release vehicle when tablets including this polymer were submitted to dissolution tests in acid media. Later, Kawashima *et al.* (1985a:264-268) and Akbuga *et al.* (1993:19-24) confirmed this. Goskonda and Upadrashta, (1993:915-927) showed that spherical beads could be produced by a combination of Avicel RC-591 and different viscosity grades of chitosan raising the possibility of designing beads with release modifying properties. Other sustained release dosage forms

include: pellets (Santos *et al.*, 2002:154), hydrogel-based controlled-release systems (Säkkinen *et al.*, 2002:33; Knapczyk, 1993a: 233-237; Kristl *et al.*, 1993:13-19; Miyazaki *et al.*, 1981:3067), membranes (Thacharodi and Rao, 1993:177-181) and film matrixes (Chandy & Sharma, 1991:745-760; Kawashima *et al.*, 1985a:264-268).

The potentiality of chitosan in sustained release systems has been assigned to its polymeric character including its gel and film forming properties. It has to be mentioned that chitosan demonstrates slow release properties due to its swelling nature in acid media, on the other hand, it has been found to act as a disintegrant when in medium pH 6.8 or higher. Attempts have, however, been made to diminish the medium dependent disintegrant activity of chitosan (Santos *et al.*, 2002:154).

2.3.1.5 Gastro retention

Although many gastric retentive devices have been developed, few were successful in improving gastric residence time. Consequently, attention has turned to retaining dosage forms within the stomach. This area of research has been termed gastro retention (Davis & Illum, 2000:141). The various gastric retentive systems could be divided into: high-density systems, expandable systems, super-porous hydrogel systems, intragastric floating systems, and mucoadhesive systems. Floating systems have a density lower than the density of the gastric juice. Thus, gastric residence time and hence, the bioavailability of drugs that are absorbed in the upper part of the gastrointestinal tract will be improved. (Davis & Illum, 2000:144).

When chitosan granules were added to acidic and neutral (deionised distilled water) media, they were immediately buoyant and provided controlled release systems for various drugs. The diffusion of the drug molecules out of the chitosan granules was enhanced because of the swelling and gel-formation at low pH. Laminated preparations prepared by coating the chitosan granule's layer with chitosan membranes were also immediately buoyant. These dosage forms take the form of small air filled and bioadhesive particles that remain in the stomach for extended periods of time, even in the fasted state (Heazi & Amiji, 2003:160). Intragastric floating dosage forms are therefore, useful for the administration of drugs that have a specific absorption site, are insoluble in the intestinal fluid, or are used for the treatment of gastric diseases such as *H. pylori* infections (Hejazi & Amiji, 2004:99-108; Davis & Illum, 2000:144). By increasing the local concentration and contact time, stomach-specific delivery systems may also minimise the

resistance problems associated with systemic administration of antibiotics (Hehazi & Amiji, 2002:87-94; Akiyama *et al.*, 1995:397-405).

2.3.1.6 Chelating capacity

One of the earliest applications of chitosan was for chelating cholesterol, fats, proteins, tumour cells and harmful metal ions, such as cadmium, copper, lead, mercury, nickel, zinc and uranium, from waste-water (Li *et al.*, 1992:11). This is because of its much higher selectivity than usual commercial chelating resins and high loading capacity (Bernkop-Schnürch, 2000:4). It has been shown that the hydroxyl and carbonyl groups are involved with the metal ion chelating mechanism (Anthonsen *et al.*, 1993:193) and its flexible structure enables chitosan to take suitable configuration for the complexation with metal ions. Chelation with chitosan has successfully been applied to areas of food preparation, health care, water purification processes, dialysis, and pharmaceuticals. However, the development of chemically modified chitosans ensured much higher complexing properties (Bernkop-Schnürch, 2000:5).

2.3.2 Biological applications

As mentioned before, chitin and chitosan are considered as excellent resource compounds because of their favourable properties including availability, biocompatibility, biodegradability, non-toxicity, adsorption properties and so forth. The fungicidal and antimicrobial activities of chitosan have also been well investigated (Meyer *et al.*, 2002:157). As chitosan is soluble in acidic aqueous solutions and various derivatives may be readily prepared, the uses and applications in pharmaceutical biotechnology are endless (Snyman, 2003:4).

2.3.2.1 Routes of application

2.3.2.1.1 Oral route

The oral route is the most popular and the most practical way to administer a therapeutic agent, particularly from the point of view of the patient. However, it is not always the most suitable route for some active compounds for various known reasons. Furthermore, the possibility to control drug delivery after oral administration is very limited since it depends on the residence time of the dosage form in the gastrointestinal tract. Indeed, the drug will follow the gastric

emptying rate, a physiological parameter that is subject to significant interindividual variability (Felt *et al.*, 1998:979-993).

For these reasons, *recherche* have tried new excipients for manufacturing tablets or to develop drug carrier systems capable of controlling drug delivery after oral administration. As chitosan is digestible, it has for several years been evaluated as a potential vehicle for drugs administered orally (Senel *et al.*, 2002:77; Carreño-Gómez & Duncan, 1997: 239; Kristl *et al.*, 1993:13-19; Imai *et al.*, 1991:11-20). The mucoadhesive properties of chitosan also enable potential oral preparations for site specific targeting, and prolonged residence time in the gastro-intestinal tract may increase the absorption of various drugs such as furosemide (Felt *et al.*, 1998:979-993; Leußen, 1996:12). As described before, chitosan tend to adhere to the mucosa, decreasing the distance of diffusion for absorption of a drug and increasing the probability of absorption (Lehr *et al.*, 1992a:70). Chitosan was also shown to open tight junctions and increase the absorption of large hydrophilic drugs including peptides and proteins (Kotzé *et al.*, 1999c:253).

Oral vaccination poses (I) no pain for the patient, (II) no local side effects, and (III) ease of application on a large scale. Microencapsulation of antigens into liposomes and polymer microspheres of chitosan were shown to provide an effective immune response (Jani *et al.*, 1989:809). Other examples concerning the oral route and containing chitosan were researched by Hejazi and Amiji (2003:151-165). They described the local delivery of drugs and other bioactive agents directly into the periodontal pocket in treating moderate to severe periodontal diseases. They found that being a muco/bioadhesive polymer, chitosan was considered a good candidate for oral cavity drug delivery as well for buccal and sublingual delivery systems. In addition to its non-toxicity, biocompatibility, and biodegradability, chitosan itself possesses antibacterial activity. The antibacterial activity of chitosan could be due to the electrostatic interactions between the amine groups of chitosan and the anionic sites on bacterial cell walls because of the presence of carboxylic acid residues and phospholipids.

Specific drug delivery to the colon is important for the treatment of local diseases such as Crohn's disease, ulcerative colitis, carcinomas and infections as well as for other systemic indications. The degradability and adhesiveness of chitosan to the gastrointestinal mucosa improves the specific and persistent delivery of the drug (Snyman, 2003:7).

2.3.2.1.2 Transdermal route

It has been increasingly recognised that intact skin represents an interesting way to provide controlled delivery of drugs to the systemic circulation when the first pass metabolism through the liver, enzymatic degradation and effects on the gastrointestinal tract are considered. Transdermal delivery devices are designed to support the passage of drug substances from the surface of the skin into the systemic circulation. The benefits of intravenous drug administration can be duplicated by transdermal drug delivery, avoiding the risk and disadvantages, of intravenous infusion. In spite of its well-known film-forming property, only a few studies have been performed on the usefulness of chitosan membranes as transdermal devices (Felt *et al.*, 1998:989). Both chitosan and its derivatives, however, have already been incorporated in ointments, creams, lotions, patches and gels.

Thacharodi and Panduranga Rao (1993: 177-181; 1996: 1307-1311) evaluated the efficacy of chitosan membranes as rate-controlling membranes by testing a series of hydrophilic and hydrophobic drugs. They concluded that water-soluble drugs such as propranolol could be transported through chitosan membranes principally via a pore mechanism, whereas hydrophobic solutes such as nifedipine would be influenced by both partition and pore mechanisms operating concurrently. Regarding the kinetic characteristics of several films described in the literature, it can be seen that permeability coefficients are affected by different parameters, including the degree of cross-linking, the acid or base nature of the drug, the molecular volume of the drug (Sawayanagi *et al.*, 1982:2935-2940), the pH of the medium, and the thickness of the membrane (Kim *et al.*, 1992:1823-1828).

2.3.2.1.3 Nasal route

Nasal drug delivery represents an interesting alternative to the parenteral route for administration of drugs that show poor oral bioavailability, such as peptides and proteins. In addition, the nose has further advantages for the absorption of drugs in that it has a large epithelial surface area available due to the presence of numerous microvilli and a low level of enzymatic degradation (Snyman, 2003:1-28; Paul & Sharma, 2000:9; Carreño-Gómez & Duncan, 1997: 231). On the other hand, one of the major drawbacks of the nasal cavity is its rapid mucociliary clearance mechanism, which can reduce the bioavailability of drugs given intranasally. A possible strategy to circumvent this problem, without using absorption enhancers, is to prevent the clearance of the

delivery system from the nasal cavity and thereby prolong contact between the drug and the mucosa. This aim can be reached by employing bioadhesive systems that form a gel-like structure at the contact of the mucus.

The good mucoadhesive and absorption enhancing properties of chitosan make it a promising candidate for development of nasal delivery systems that exert drug controlled release. In fact, chitosan is able to swell and form a gel-like layer in an aqueous environment (here, for example by absorbing water from the mucous layer in the nasal cavity), which is favourable for the interpenetration of polymer and glycoprotein chains of the mucus (Felt *et al.*, 1998:987).

Aspden and colleagues (1995b:550) studied the effect of five types of chitosan (with varying molecular weights and degrees of deacetylation) on the nasal epithelium and the beat frequency of the cilia using a frog palate model. They concluded that chitosan with a low degree of acetylation (15 %) demonstrated good absorption enhancement at both low and high molecular weights. Although chitosan was non-toxic when applied to nasal mucosa, dose dependant toxicity was reported. Chitosan with a 35 % degree of acetylation and high molecular weight exhibited low toxicity and absorption enhancement. The first experiment based on a chitosan nasal delivery system containing a model drug has, however, been described by Illum *et al.* (1994:1186). The main goal was the enhancement of the systemic bioavailability of insulin after intranasal instillation in animals' models (rats and sheep). The authors have shown that a concentration of 0.5 % polymer is sufficient to improve plasma insulin levels greatly. The low molecular weight of chitosan oligomers also possess favourable characteristics such as low viscosity and may be suitable for drug inclusion matrices that necessitates an increased rate of drug release compared to the high molecular weight chitosan polymers (Felt *et al.*, 1998:987).

2.3.2.1.4 Ophthalmic route

Conventional aqueous solutions topically applied to the eye have the disadvantage that most of the instilled drug is lost within the first 15-30 seconds after instillation due to reflex tearing and to drainage via the nasolacrimal duct. One of the goals in ophthalmic research has been directed towards an increase of drug absorption and duration of contact time. The most frequent approach to achieve improved drug efficacy is exemplified by the use of viscosified solutions (Felt *et al.*, 1998:987). Again, chitosan proved to be a very efficient cationic polymer for sustained release ophthalmic preparations and as previously explained has superior mucoadhesiveness owing to its ability to develop molecular attraction forces by electrostatic interactions with the negative

charges of the mucus (Genta *et al.*, 1997:737). The molecular weight and degree of acetylation also plays an important role (Paul & Sharma, 2000:10). Shah and Mowbray (1992:1) estimated that a satisfactory *N*-acetylation range was between 30 % and 50 %. In addition, Lehr *et al.* (1992b:43) demonstrated that the mucoadhesive performance of chitosan was significantly higher in a neutral or slightly alkaline medium; the pH values of tear fluid reported in the literature range from 7.0 to 7.4.

Since chitosan is endowed with an appreciable film-forming capacity, it has been suggested as a biopolymer of choice for the development of contact lenses. Principally, it has been evaluated for the manufacture of ocular bandage lenses used as protective devices for acutely or chronically traumatised eyes. The lenses obtained have been claimed to possess desirable physical properties such as tensile strength, water content, protein deposition, and oxygen permeability (Felt *et al.*, 1998:988; Shah & Mowbray, 1992:1).

2.3.2.1.5 Chitosan implants

A possible approach to provide sustained release of a medication is to place implants in body tissues surgically. The constant contact between the systems and living tissues implies specific requirements. Some requirements that the polymer should meet is non-toxicity, sterilisability, good mechanical properties, harmless degradation of products, ease of film formation, controlled release properties, adequate drug storage capacity and it should ensure drug stability. Since chitosan possess these characteristics, attempts have been made to develop implantable dosage forms of anti-tumoral drugs based on chitosan or its derivatives (Felt *et al.*, 1998:989).

Chitosan can also be crosslinked to present various properties. Research is underway to develop a combined polymer and mineral to produce a material with the toughness and flexibility of a polymer but with the strength and hardness of a mineral deposit (Muzzarelli & Muzzarelli, 2002:233). This may be potentially used as a hard tissue substitute for bone composites and implantation that improves bone growth and recovery time.

2.3.2.1.6 Other delivery routes

Among alternative routes, the injectable and the vaginal routes have been investigated (Sandri *et al.*, 2004:351). Principally, the injectable route is a viable alternative for drugs that are strong irritants when taken orally and for substances not absorbed or inactivated in the gastrointestinal

tract. Although pharmaceuticals administered parenterally, generally exert a rapid effect, some injectable systems, such as microspheres and macromolecular conjugates, are intended to provide sustained drug release. Macromolecular drug carrier systems have been largely studied, particularly in the field of cancer chemotherapy, in an attempt to improve therapeutic effects and to reduce nonselective toxic effects associated with antitumor agents. The rationale for the utilisation of chitosan lies, in part, in the presence of free amino groups, which allow its conjugation with some drugs (Felt *et al.*, 1998:985). Other studies concentrated on using chitosan derivatives in subcutaneous and intramuscular parenteral routes (Jameela *et al.*, 1998:17). These formulations have the ability to prolong the delivery of drugs and hormones such as progesterone for an extended period of time.

To fulfil the therapeutic requirements, formulations designed for vaginal administration should possess the following features: good mucoadhesive properties and high resistance to physiological removal mechanisms, in order to permit intimate and prolonged contact of the formulation with the diseased mucosa; prolonged drug release properties, in order to reduce dosing and to improve patient compliance; good penetration properties, in order to enable the drug to reach the deeper layers of the mucosa (Sandri *et al.*, 2004:352). Other routes also under investigation include the pulmonary route.

2.3.2.2 Pharmaceutical delivery systems

Chitosan may be used as an excipient in various formulations, for a multitude of purposes. Chitosan beads, microspheres and microcapsules, for example, were developed in the early 1990's and have typically been used for the prolonged release of drugs (He *et al.*, 1998:76; Wan *et al.*, 1994:195-200). On the other hand, small particle size ($< 5 \mu\text{m}$) chitosan microspheres, containing anticancer agents such as 5-fluorouracil (5-FU) (Ohya *et al.*, 1993:1-9), and magnetic microspheres (Hassan *et al.*, 1992:390-397) have been described for site specific delivery. Chitosan polymers also offer many advantages as an excipient used in implants, granules, pellets, microparticles, gels, films, as a stabilising agent in emulsions and as a binder and lubricant in wet granulated tablets (Muzzarelli, 2000:23; Paul & Sharma, 2000:7; He *et al.*, 1998:76; Illum 1998:1326). A summary of some of the existing formulations incorporating chitosan that were developed to date is given in table 2.3. Chitosan is used for its properties such as a release modifying polymer, absorption enhancer, mucoadhesive polymer, film characteristics etc. in the following dosage forms (Snyman, 2003:7).

Table 2.3: Summery of recent chitosan formulation studies (Paul & Sharma, 2000:8).

Drug	Formulation	Advantage	Reference
Ampicillin	Microparticles	Prolonged release	Giunchedi <i>et al.</i> , 1998:157
Aspirin	Tablets	Prolonged release	Kawashima <i>et al.</i> , 1985b:2107
	Microparticles	Controlled release	Vasudev <i>et al.</i> , 1997:375
Captopril	Transdermal	Prolonged release	John <i>et al.</i> , 1995:133
Diclofenac sodium	Tablets	Prolonged release	Acartörk <i>et al.</i> , 1989:547; Sabinis <i>et al.</i> , 1997:243
	Microparticles	Controlled release	Remunan-Lopez <i>et al.</i> , 1998a:49; Lorenzo-lamosa <i>et al.</i> , 1998:109
Diltiazem	Tablets	Mucoadhesive	Miyazaki <i>et al.</i> , 1995:257
	Microparticles	Controlled release	Bayomi <i>et al.</i> , 1998:187
DNA	Chitosan-DNA	Increased transfection	Thanou <i>et al.</i> , 2002:153; Sato <i>et al.</i> , 2001:2075
	Microparticles	Increased vaccination	Van der Lubben <i>et al.</i> , 2001:687
	Microspheres	Increased transfection	Aral <i>et al.</i> , 2000:83
Furosemide	Microparticles	Prolonged release	Akbu <i>et al.</i> , 1994:217
Indomethacin	Granules	Sustained release	Miyazaki <i>et al.</i> , 1988:642; Shiraishi <i>et al.</i> , 1993:217
Insulin	Tablets	Patient compliance	Bernkop-Schnürch <i>et al.</i> , 1998:207
	Microparticles	Improved absorption	Tozaki <i>et al.</i> , 1997:1016
	Membranes	Controlled release	Kim <i>et al.</i> , 1992:1823
Ketoprofen	Tablets	Mucoadhesive	Miyazaki <i>et al.</i> , 1995:257
	Microparticles	Improved absorption	Genta <i>et al.</i> , 1998:779
Metotrexate	Microparticles	Prolonged release	Lee <i>et al.</i> , 1998:775; Singh <i>et al.</i> , 1998:581
Nifedipine	Tablets	Mucoadhesive	Remunan-Lopez <i>et al.</i> , 1998b:143
	Beads	Sustained delivery	Chandy <i>et al.</i> , 1992:949
	Microparticles	Improved solubility	Filipovic-Gricic <i>et al.</i> , 1996:183
	Transdermal	Controlled release	Thacharodi <i>et al.</i> , 1996:1307
Prednisolone	Tablets	Sustained release	Inouye <i>et al.</i> , 1989:55
	Microspheres	Sustained release	Mooren <i>et al.</i> , 1998:58
Progesterone	Microparticles	Prolonged release	Jameela <i>et al.</i> , 1998:17
Propanolol	Tablets	Sustained release	Remunan-Lopez <i>et al.</i> , 1998b:143
	Transdermal	Controlled release	Thacharodi <i>et al.</i> , 1995:145
Theophylline	Tablets	Mucoadhesive	Miyazaki <i>et al.</i> , 1994:745
	Beads	Controlled release	Gosconda <i>et al.</i> , 1993:915
	Microparticles	Prolonged release	Lin <i>et al.</i> , 1992:500

2.3.2.2.1 *Tablets*

Since tablets are still considered as the dosage form of choice for reasons such as low cost of manufacturing or good stability, early studies have led to the use of chitosan as a tableting excipient. It has mainly been used as a diluent or filler in direct tablet manufacturing. However, virtually all formulations developed to date necessitate the addition of other ingredients to facilitate compression (Rege *et al.*, 1999:49-60). Directly compressed tablets may result in either immediate release of the drug from the formulation or sustained release, that are influenced by the excipients used (Bernkop-Schnürch, 2000:9; Paul & Sharma, 2000:10). The mucoadhesive properties of these formulations also affected the residence time in the intestines and, therefore, these tablets may be used for oral, intraoral or sublingual drug delivery (He *et al.*, 1998:75-88)

Henriksen *et al.* (1993:181) suggested preparing chitosan formulations by standard wet granulation. They found that a chitosan salt (chitosan malate) was retarding the drug release rate, whereas chitosan base had the opposite effect by enhancing the dissolution of the active compound. This observation was not in agreement with the results of Sawayanagi *et al.* (1982:2940), who noticed that water-soluble drug release from tablets based on chitosan followed a zero-order or a pseudo-zero-order pattern and provided controlled release. This difference could be explained either by the manufacturing process or by chitosan origin.

Although chitosan has been mostly used as a diluent in tablet manufacturing, it has also been proposed as a binder (Upadrashta *et al.*, 1992:1701), a lubricant (Paul & Sharma, 2000:10; Sawayanagi *et al.*, 1982:2940) and as a potential disintegrant owing to its water uptake property (Leane *et al.*, 2004:241-249; Knapczyk, 1993b:1-7; Knapczyk, 1992:9-14). Upadrashta *et al.* (1992:1701) found that the higher the compressional pressure and the concentration of chitosan, the harder the tablets produced and the more sustained the release of a drug.

2.3.2.2.2 *Microspheres*

Over the last few decades, research workers have tried to develop systems capable of delivering active compounds at a specific target site. For example, in cancer chemotherapy, for which it is imperative to have efficient delivery to the desired site of action due to the high toxicity, poor stability, and short biological half-life of antitumoral drugs. Chitosan microspheres loaded with anti-neoplastic agents appear to be promising drug delivery systems for the treatment of cancer (Felt *et al.*, 1998:982).

Microspheres have been modified for oral, nasal and parental delivery of drugs. However, most of the studies conducted with chitosan microspheres involved oral delivery systems which provide a constant therapeutic level of the drug and, in this way, avoid frequent administration of the solid oral dosage form (Felt *et al.*, 1998:982; Mooren *et al.*, 1998:58). Microspheres effectively increase the active surface area of the formulation as well as providing the possibility of controlled release of drugs (Paul & Sharma, 2000:10). Chitosan has been used for the preparation of drug loaded microspheres (Arica *et al.*, 2002:71). Novel formulations have also been developed (Calis *et al.*, 2002:65) based on alginate microspheres incorporating the drug coated with a chitosan layer to avoid the initial burst effect. This retains the mucoadhesive properties of chitosan as well as the absorption enhancing properties while providing a useful platform with a large surface area for the delivery of the drug. However, chitosan microspheres formed by electrostatic interaction between a polyion and counterion become unstable in gastric fluid. A potential way to overcome this problem is irreversible chemical cross-linkage (Felt *et al.*, 1998:982). Non crosslinked chitosan microspheres cannot be kept suspended in water because of swelling and dissolution. Particle size and drug release is also reduced with an increase in the amount of cross-linking agent. The effect of chitosan molecular weight on the release rate of the drug is minimal (He *et al.*, 1999:55).

2.3.2.2.3 Granules and beads

Matrices based on polysaccharides have been widely used for therapeutic drug targeting. Owing to its gel-forming ability at low pH, chitosan has been thought to be suitable to provide oral sustained release. Thus, the use of chitosan to develop a bioerodible matrix, granules and/or beads, has been reported by some authors (Felt *et al.*, 1998:983).

Various in vitro and in vivo results confirmed that chitosan granules are systems well suited to provide sustained release for oral administration. In spite of the different animal models selected and the different drugs tested, the conclusions were quite similar, revealing delayed, prolonged, and higher plasma levels of drugs after administration of chitosan granules compared with conventional solid oral dosage forms (Felt *et al.*, 1998:979-994).

Beads with varying size and porosity are produced through various drying techniques and preparation procedures. Different characteristics may also be prepared with various methods to produce a useful formulation for drug delivery. Micro-beads may enable the researcher to

increase or decrease the dissolution rate of a drug from a polymer matrix to suit the specific release profile (Wan *et al.*, 1994:195; Lubbe, 2002:60).

In vitro data of Chandy and Sharma (1992: 949-952.) have demonstrated a near zero-order release of nifedipine and ampicillin from chitosan beads and a first-order release of the same compounds from granules. The mechanism of drug release was probably diffusion from beads, whereas it was disintegration of the matrix in the granules.

2.3.2.2.4 Gels

Chitosan hydrogels may be prepared by various methods (Chen *et al.*, 2002:101; Li *et al.*, 1992:11). Ofori-Kwakye and Fell (2003:252) for example used chitosan in film-coating formulations. The film coating systems were found to have potential as a carrier for the biphasic delivery of drugs. The leaching of the large molecular weight created aqueous channels or water-filled pores which allowed the diffusion of drug molecules through the film coatings of the dosage forms. Chitosan can also be used in the preparation of coatings of other formulations such as beads, microspheres, films and sheets with specific properties.

2.3.2.2.5 Membranes

Chitosan is a good cationic polymer for membrane formation. In early research it was shown that membranes formed from the polymer could be exploited for water clarification, filtration, fruit coating, surgical dressing, and controlled release (Li *et al.*, 1992:8). In 1978, for example, Hirano (1978:166-168) showed that *N*-acetyl chitosan membranes were ideal for controlled agrochemical release. The development of systems containing chitosan to obtain biphasic release patterns has also been described in the literature (Ofori-Kwakye & Fell, 2003:252). Sawayanagi *et al.* (1982: 2935-2940) found greater permeability through chitosan membranes for acidic drugs than basic drugs.

The use of chitosan as a wound dressing has gained popularity due to the advantages over other membranes due to characteristics including sustained release of the drug to the wound. Incorporation of silver sulphadiazine into a asymmetric dressing with two layers (one of which is a sponge-like sub-layer containing silver sulphadiazine) provides the ability to control the release rate with factors such as the thickness and porosity of the sub-layer (Mi *et al.*, 2002b:139).

Chitosan membranes may also be used for dialysis membranes due to its hydrophilicity, high chemical reactivity and biocompatibility (Chandrkrachang *et al.*, 2002:145).

2.3.2.2.6 Other delivery systems

Chitosan solutions may be used to prepare films and for the coating of particles for various applications as well as for the preparation of emulsions (Thacharodi *et al.*, 1996:1307).

As orally administered peptide drugs are very poorly absorbed, partly because of enzymatic degradation in the gastrointestinal tract, the use of carrier systems is preferable. Liposomes are a suitable vehicle for the peptide drugs, and many studies have been carried out to investigate aspects such as drug encapsulation, absorption in vivo etc. (Takeuchi *et al.*, 2003:236; Carreño-Gómez & Duncan, 1997:232; Hendriksen *et al.*, 1994: 227-236). The ability of chitosan to interact with liposomes has two positive consequences: the stabilisation of the liposomes and the possibility of targeting the vesicle to a specific site due to its mucoadhesion property (Felt *et al.*, 1998:979-993; He *et al.*, 1998:75-88; Takeuchi *et al.*, 1996:236). These properties have been exploited by Takeuchi *et al.* (1996:896-901), who prepared mucoadhesive chitosan-coated liposomes to improve oral absorption of insulin. After in vivo administration of the liposomes to male Wister rats, the hypoglycaemic response was prolonged over a period of up to 12 hours. This sustained effect has been explained by the mucoadhesiveness of the system, which increased the duration of contact of insulin with the intestinal mucosa, thus leading to an increased probability of insulin absorption. Hence, chitosan-coated liposomes could represent an interesting concept for the administration of poorly absorbed drugs as is the case for peptides (Bernkop-Schnürch, 2000:11).

2.3.3 Therapeutic indications of chitosan

Chitosan was traditionally used in the Orient for the treatment of abrasions and in America for the healing of machete gashes (Allen *et al.*, 1984:119). A recent analysis of the varnish on one of Antonio Stradivarius's violins even showed the presence of a chitinous material (Li *et al.*, 1992:3). It has also been used in the cosmetic industry and it is said to have anti-acidic, (Steckel & Mindermann-Nogly, 2003:5) and intrinsic anti-ulcer properties; chitosans' effect being through the adhesive action on gastric epithelial cells and/or by neutralising the hydronium ions in the gastric fluid (Hejazi & Amiji, 2004:100; Ito *et al.*, 2000:218-225).

It could further be a source of glucosamine after oral absorption. Glucosamine is an anti-inflammatory compound naturally present in the body and is synthesised from glucose incorporated into glycosaminoglycans and glycoproteins. While the therapeutic effect of glucosamine is not as high as for other anti-inflammatory drugs, the side effects are non-existing, and therefore, chitosan is also indicated for the treatment of osteoarthritis (Haupt *et al.*, 1999:2423).

Unregulated accumulation of cholesterol is cytotoxic and will result in gallstone disease and atherosclerosis, among others. Maezaki *et al.* (1993:1439) and Furda (2000:41) found that when chitosan was given to volunteers in a study over 4 weeks, the serum total cholesterol level significantly decreased, whereas the serum HDL-cholesterol increased. Steckel and Mindermann-Nogly (2003:5) as well as Ormrod *et al.* (1998:329) came to the same conclusions in their individual studies.

Chitosan salts are currently available for dietary use (fat binders, fat beaters) to reduce body weight, especially in obese patients (Junginger & Verhoef, 1998:375). As it is non-toxic and safe for oral administration, there are no observed side effects. Some criticism were made regarding the possibility that chitosan may deprive the diet of liposoluble vitamins and trace metal ions, but recent clinical trials observed that no alteration in the serum levels of liposoluble vitamins A and E, and iron took place (Wadstein *et al.*, 2000:65; Wuolijoki *et al.*, 1999:357).

2.4 Conclusion

In closing, we can safely state that chitosan is no longer just a waste by-product from the seafood-processing industry. Since chitosan combines unique physicochemical characteristics, *in vivo* biodegradability, biocompatibility, nontoxicity, and antimicrobial action, it has been widely investigated over the last few years for potential applications in the medical and pharmaceutical fields. Because of these unique properties chitosan may be administered orally, nasally or through any other delivery route, enabling, for example, prolonged drug release or improved bioavailability of poorly absorbed compounds such as peptides. With the wide range of potential applications and the rate of scientific progress being made, chitosan is truly a multifunctional designer polymer in future biotechnology.

However, commercially available chitosan is not always well characterised, particularly regarding molecular weight. It is therefore expected that knowledge about the factors that

influence the behaviour of chitosan and its derivatives, such as the chemistry and physicochemical properties of the polymers, will play an increasingly important role in the potential use of these polymers. Understanding the differences between these polymers and the effect of these differences on polymer properties will therefore improve the possibilities and development of similar custom-made polymers.

CHAPTER 3

EXPERIMENTAL METHODS

3.1 Introduction

The formulation of a solid dosage form often requires precise processing control of the powder mixture to ensure a volumetric delivery of a homogeneous aliquot. Thus, various excipients are gravimetrically added to form the bulk powder with which uniformity is achieved through optimum mixing and good flow of the mixture into the tablet die (Shah & Mlodozeniec, 1977:1377).

This chapter deals with the choice of and motivation for the drug and excipients used in various formulations of this study. It also describes the experimental procedures employed to determine the effect of these excipients on the physical properties of tablets as well as on the dissolution profile of a sparingly water-soluble and poorly water-wettable drug.

3.2 Choice of furosemide as model drug

Furosemide with a pK_a of 3.6 was chosen as a model drug representing a group of drugs, which exhibits poor dissolution due to low water-solubility and poor water-wettability (Boles Ponto & Schoenwald, 1990:305). This is probably due to the fact that drug particles are cohesive and agglomerate spontaneously (De Villiers, 1988:39; De Villiers *et al.*, 1993:160).

The dissolution process limits the absorption of sparingly water-soluble drugs, and the surface-area of the drug in contact with the surrounding medium is of primary importance in terms of optimum dissolution. The liberation of the maximum surface-area of the drug exposed to the surrounding medium determines both the rate and extent of drug dissolution, and is to a large extent dependent on the disintegration process (Marais, 2000:60).

Various authors found that the appropriate employment of excipients should, therefore, impart properties to the drug formulations that avert or minimise the challenges that these physicochemical properties pose towards dissolution (Rubinstein & Price, 1977:5P;

Rubinstein & Rughani, 1987:543-550; Boles Ponto & Schoenwald, 1990:385; Akbuga, 1991:858; Marais & Van der Watt, 1991:1718; Steyn, 1994:36; Shin *et al.*, 1998:18). The relative contributions of each excipient to dissolution could, therefore, be distinguished by evaluation of various formulation and processing variables.

3.3 Materials

The materials used in the study are presented in table 3.1.

Table 3.1: *Materials, lot numbers and manufacturers used in the study.*

Compound	Lot number	Manufacturer
Ac-Di-Sol®	T017C	FMC Corporation, Philadelphia, Pennsylvania, USA
Ascorbic acid	440619/1 53502	Fluka Chemika, Sigma-Aldrich Chemie GmbH, Steinheim, Switzerland
Chitosan	021010	Warren Chemicals, WCI 7109, Durban, South Africa
Cibacron Brilliant Red 3B-A dye	04006PQ	Aldrich Chem. Co., Milw., WI, 53201
Furosemide	3017 HJ11	Adcock Ingram Ltd, Wadeville, South Africa
Glacial acetic acid	UN27898/32B2	Labchem (Pty) Ltd., Edenvale, South Africa
Glycine	441845/1 54902100	Fluka BioChemika, Sigma-Aldrich Chemie GmbH, Steinheim, Switzerland
Hydrochloric acid	UN1789/5b	Labchem (Pty) Ltd., Edenvale, South Africa
Kollidon® VA 64	62-8826	BASF Aktiengesellschaft, Ludwigshafen, Germany
Kollidon® CL	30-1411	BASF Aktiengesellschaft, Ludwigshafen, Germany
Methogel K100M Premium (HPMC)	QI25012N11	Colorcon limited, Kent, England
Sodium bicarbonate	444227/1 50903126	Fluka Chemika, Sigma-Aldrich Chemie GmbH, Steinheim, Switzerland
Sodium Chloride	431517/1 44102367	Fluka BioChemika, Sigma-Aldrich Chemie GmbH, Steinheim, Switzerland

3.4 Physical characterisation of chitosan

The physical properties of powders could influence the tableability of the various formulations. The primary physical excipient properties of importance are flowability, compactibility and compatibility. The properties that were evaluated are described in the following sections.

3.4.1 Determination of the molecular weight

Since the mucoadhesive properties of chitosan is a function of the molecular weight up to a value of 100 000 dalton (Gurny *et al.*, 1984:336) it is important to determine the molecular weight of the chitosan used. Size exclusion chromatography (SEC) coupled to a laser light scattering device is an accurate way to determine the absolute molecular weight of large polymer macromolecules.

Objects can be conformationally characterised by making deductions from the differences and quantity of light scattered from them. The scattered light is measured as a function of the angle between the detector and the incident beam direction. Multi-angle laser light scattering (MALS) instruments are able to determine the absolute molar weights of polymers and biopolymers from below 100 g.mole⁻¹ (dalton) to millions of datons.

The method of working with MALS-instruments may be summarised as follows. A vertically polarised laser beam passes through the sample, either in a flow-through cell or in a static (batch) container. The sample of polymer macromolecules scatters light into all angles. Each detector, placed at different angular positions around the sample, provides a response directly proportional to the intensity of the scattered light it receives. The analogue light scattering signals are digitised and transmitted to a computer for processing. The computer software performs the analysis necessary to calculate the absolute molar mass from the data.

3.4.1.1 Method for the determination of molecular weight

The molecular weight of the chitosan samples was measured using a size exclusion chromatograph (Hewlett Packard 1100, USA) connected to a multi-angle laser light scattering detector (MALS) that consisted of a laser photometer (Dawn DSP, Wyatt Technology Corporation, USA) connected to a refracting index detector (ERC 7515A, Japan).

The chitosan samples were dried at 40 °C in a vacuum oven for at least 24 hours and prepared in solutions of 5 mg.cm⁻³ from which 0.8 cm³ samples were collected in chromatographic sample vials. The mobile phase consisted of 0.2 M ammonium acetate and the pH was adjusted to 4.50 by means of acetic acid. The experimental set-up consisted of a HP 1100 vacuum degasser, isocratic pump and auto sampler connected to a TSK-guard PWH (Toso Haas, Japan) inline column. The size exclusion columns included a TSK G6000 PW (Toso Haas, Japan, inside diameter = 7.5 mm, length = 30 cm, particle size > 17 µm, pore size >

1000 Å) column connected in series with a TSK G5000 PW (Toso Haas, Japan, inside diameter = 7.5 mm, length = 30 cm, particle size > 17 µm, pore size > 1000 Å) column. Samples of 100 µm³ were injected at a flow rate of 0.8 cm³.min⁻¹ and was analysed with the laser photometer (He/Ne, λ = 633 nm) and the refracting index detector. The data from the detector was interpreted with a computer using Astra[®] for Windows (Wyatt Technology Corporation, USA). The computer software performs the analysis necessary to calculate the absolute molecular weight from the data with the following equation:

$$\frac{K^*c}{R(\theta)} = \frac{1}{M_w P(\theta)} + 2A_2c \quad [3.1]$$

Where:

R(θ) is the excess intensity of scattered light at Dawn angle θ, **c** is the sample concentration, **M_w** is the weight-average molecular weight, **A₂** is the second virial coefficient, **K*** is the optical parameter equal to $4\pi^2 n^2 (dn/dc)^2 / (\lambda_0^4 N_A)$, **n** is the solvent refractive index, **dn/dc** is the refractive index increment, **N_A** is Avogadro's number and **λ₀** is the wavelength of the scattered light in vacuum.

The function **P(Θ)** describes the angular dependence of scattered light. The expansion of **1/P(Θ)** to first order gives:

$$\frac{1}{P(\Theta)} = 1 + \left(\frac{16\pi^2}{3\lambda^2} \right) \langle r_g^2 \rangle \cdot \sin^2\left(\frac{\Theta}{2}\right) + f_4 \sin^4\left(\frac{\Theta}{2}\right) = \dots \quad [3.2]$$

At low angles of angular dependence of light scattering depends only on the mean square radius $\langle r_g^2 \rangle$ (alternatively called radius of gyration) and is independent of molecular conformation or branching. A plot of $K^*c/R(\Theta)$ vs. $\sin^2(\Theta/2)$ (Zimm plot) yields a curve which intercept gives **M_w** and which slope at low angles gives $\langle r_g^2 \rangle$ (Wyatt Technology, 1999:4).

3.4.2 Nuclear magnetic resonance (¹H-NMR) spectroscopy analysis

The proton behaviour was investigated with ¹H-NMR spectrometry and is based on the theory that under appropriate conditions in a magnetic field, a sample can absorb electromagnetic radiation in the radio frequency region at frequencies governed by the characteristics of the sample (Silverstein *et al.*, 1991:165). This absorption can be plotted in a spectrum that is

specific to the sample, and may be used as identification for, and characterisation of, that sample.

^1H -NMR-spectra were measured in D_2O at $80\text{ }^\circ\text{C}$, using a 300 or 600 BRUKER DMX-600 MHz spectrometer (Bruker, Switzerland). ^{13}C -NMR-spectra were measured in D_2O at $80\text{ }^\circ\text{C}$ at 150 MHz. No attempts were made to remove the residual water from the NMR sample, because at $80\text{ }^\circ\text{C}$ this peak does not interfere with the spectrum of the polymer.

3.4.3 Infrared (IR) analysis

The stretching and bending vibrations of different groups on a molecule can be analysed and identified with the use of infrared spectrometry. These spectra may also be used to identify compounds, as they are unique to those compounds. IR spectra were recorded on a Nicolet Nexus 470 FT IR ESP spectrometer (Madison, USA) over a range of $4000 - 400\text{ cm}^{-1}$ using the KBr reference technique. Samples weighing approximately 2 mg were collected after drying for 1 hour at $40\text{ }^\circ\text{C}$ and mixed with 200 mg of KBr (Merck, Germany) before analysis.

3.4.4 Measurement of relative viscosity

Two methods have been developed to determine the viscosity of liquids. They are the capillary viscometer and the Couette viscometer (not used in this study). The capillary viscometer is a simple and inexpensive glass construction, which is easy to use. A fluid of which the viscosity needs to be determined, is elevated above a certain level in the viscometer and the time taken for the fluid to descend through the capillary is taken as the sample flow-rate and is compared against the flow-rate of a reference solvent (Schott, 1993:601).

The relative viscosity of chitosan was measured according to the British Pharmacopoeia (2002:A115-A118) for dextran with a size D glass U-tube viscometer at $25\text{ }^\circ\text{C}$. Solutions of the chitosan were prepared in concentrations of 0.5, 0.2, 0.1, 0.05 and 0.025% in 0.2% v/v acetic acid (Merck, South Africa). The temperature of the solutions ($25\text{ }^\circ\text{C}$) was kept constant by means of a waterbath throughout the experiment. The flow-through time of the solutions and pure solvent were measured in the U-tube. The relative viscosities (η_{rel} , equation 3.3) as well as the reduced (η_{red} , equation 3.4) and inherent (η_{inh} , equation 3.5) viscosities of the polymer was determined (Hamman & Kotzé, 2001:370).

$$\eta_{rel} = \frac{\eta}{\eta_0} \quad [3.3]$$

$$\eta_{red} = \frac{(\eta_{rel} - 1)}{c} \quad [3.4]$$

$$\eta_{inh} = \frac{(\ln \eta_{rel})}{c} \quad [3.5]$$

Where:

η_{rel} is the relative viscosity of the polymer solution, η is the viscosity of the chitosan polymer solution, η_0 is the viscosity of the pure solvent and c is the concentration of the solution.

The extrapolated value (where $c = 0$) of the straight-lines when η_{red} and η_{inh} is plotted as a function of c , is the intrinsic viscosity ($[\eta]$) of the polymer, which gives an indication of the molecular weight according to equation 3.6 (Mark-Houwink equation).

$$[\eta] = KM^\alpha \quad [3.6]$$

Where:

K and α are constants for a given polymer-solvent system indicative of polymer conformation and M is the molecular weight of the polymer.

3.4.5 Differential scanning calorimetry (DSC) analysis

A Shimadzu DSC (Shimadzu, Japan) was used to obtain DSC spectra of chitosan. A mass, not exceeding 2 mg was weighed into aluminium pans. Lids with a small pinhole were placed into position and crimped onto the pans with the aid of a Du Pont crimper. A similar sealed, empty pan was used as reference. DSC-curves were obtained at a heating rate of 10 °C per minute under a nitrogen purge of 30 cm³ per minute.

3.4.6 Thermogravimetric analysis (TGA)

Thermogravimetric (TGA) analysis was done on chitosan at different temperatures (0-200 °C) and time intervals. TGA thermograms were recorded with a Shimadzu TGA-50 instrument (Shimadzu, Kyoto, Japan). The sample weight was approximately 5-8 mg and heating rates of 10 °C.minute⁻¹ under nitrogen gas flow of 35 cm³.minute⁻¹ were used.

The theoretical weight loss during different conditions for chitosan was calculated (in percentage) and compared.

3.4.7 Karl-Fisher

The moisture content of chitosan was determined with a Metler DL18 Karl Fischer titrator. The Karl Fischer solution was calibrated against a predetermined mass of water. 250 mg ($\pm$ 0.4 mg) of the powder was accurately weighed and added to methanol, previously neutralised with the Karl Fischer solution, in the titration beaker. The mixture was stirred magnetically and titrated with the Karl Fischer solution. The experiments were done in duplicate and the percentage water (w/w) calculated.

3.4.8 Morphology of powder particles

3.4.8.1 Introduction

The analysis of the physical powder and tablet properties is often the observation of various macroscopic phenomena. An additional means of characterisation of the physicochemical properties of powders and tablets is by SEM observations. Information is gathered on a micro – level that might direct a better understanding of the macroscopic phenomenon e.g. the environment in which excipients exert their actions. The electronmicrographs were included in the result chapters of this study where applicable.

3.4.8.2 Experimental conditions, sample preparation and apparatus

Powder and tablet samples were affixed on double-sided conductive carbon tape to a sampling tray and dusted with an inert gas. Samples were consequently sputter-coated with a mixture of gold/palladium (80:20) to form a layer of approximately 28 nm on the surface of the samples. An Eiko[®] ion coater (model IB-2, Eiko Engineering, Japan) was used in all coating procedures and operated under a vacuum better than 0.06 Torr. Samples were studied using a Philips[®] XL 30 DX 4i SEM microscope (Eindhoven, The Netherlands).

3.4.9 Particle size analysis and size distribution

Particle size analysis of chitosan powder was conducted by method of laser diffraction using a Malvern® Mastersizer X (Malvern Instruments Ltd., Worcestershire, UK) fitted to a MSX14 sample suspension unit and a 300 mm lens. A constant velocity setting of 5 was selected and governed the rate of the sample pump, cell stirring, ultrasonic stirring and suspension stirring. This setting was maintained for all evaluations.

Samples were prepared by suspending 2 g of Chitosan in 10 cm³ distilled water. The suspended sample was transferred to the sample unit and cycled through the apparatus by addition of 300 cm³ of dispersion liquid. The analysis was performed in fourfold and the result reflects the mean value of these determinations.

A sieve analysis was also performed in fourfold on chitosan powder. Mesh sieves of 500, 355, 250, 212, 180, 150, 125, 106, 90 and 63 µm (Labotec test sieve, Industria, Johannesburg, South Africa) were used in a vibrator sieve (Fritsh® analysette, Germany, TYPE 03.502) for 10 minutes at an amplitude of 6 ampere.

3.4.10 Flow properties

Flow behaviour can often be described best by quantification of the process of flow. Numerous methods have been described, either directly, using dynamic or kinetic methods, or indirectly, generally by measurements carried out on static powder beds (Staniforth, 2000:601). These include the angle of repose, hopper flow rate, Carr's index, and Hausner ratio.

3.4.10.1 Angle of repose

The angle of repose has been used as an indirect method of quantifying powder fluidity (Staniforth, 2000:601). Particles will start to slide when the angle of inclination is large enough to overcome the friction forces between particles. An amount of powder (100 g) was poured into a cylindrical Perspex container fitted with a shutter containing a 18.8 mm orifice (the fixed-height funnel method) (Lavoie *et al.*, 2002:887-893). The shutter was opened and the powder discharged from a height of 15 cm onto a horizontal glass surface. The height (h) and diameter (d) of the resulting cone was measured and, using equation 3.7, the angle of repose was calculated.

$$\tan \theta = \frac{h}{r} \quad [3.7]$$

Where:

h represents the height of the powder cone and **r** is ½ x diameter of the cone base.

The flow rate of a powder is proportional to the angle of repose. A large angle is indicative of poor flowability and a small angle of superior flow. The time of the powder discharged from the container was also noted to determine a flow rate through the 18.8 mm orifice (Staniforth, 2000:603).

3.4.10.2 Hopper flow rate

The simplest direct method of measuring powder flow properties is the hopper flow rate. This method determines the rate at which a mass of powder discharges from a hopper.

A hopper, fitted with a closed shutter at the bottom, was filled with a predetermined amount of powder (approximately 100 g). Consequently, the shutter was opened and the time required to complete the discharge of the powder mass was recorded (Lavoie *et al.*, 2002:887-893). Division of the mass of the powder by the discharge time was performed to calculate the flow rate. The procedure was performed in triplicate using different samples (each of 100 g) of chitosan.

3.4.10.3 Carr's index and Hausner ratio

Both the Carr's index and Hausner ratio are calculated from the bulk and tapped densities. These properties provide a better understanding of the flowability of powders.

The Carr's index (%) was calculated using equation 3.8.

$$\rho_t = \left(\frac{\rho_b}{\rho_t} \right) \times 100 \quad [3.8]$$

Whereas the Hausner ratio is represented by equation 3.9.

$$\text{Hausner} = \frac{\rho_t}{\rho_b} \quad [3.9]$$

Where:

P_t is the tapped density (g.cm^{-3}) and P_b is the bulk density (g.cm^{-3}).

3.4.11 Density

Density is defined as the weight-volume ratio of a substance or tablet, expressed in g.cm^{-3} . Several parameters are used to gain knowledge of powder density and commonly used descriptors of density include true density, bulk density, tapped density and porosity. The determinations of these descriptors are consequently discussed.

3.4.11.1 True density

Only the solid portion of the powder particles is taken into consideration when the true density is measured, and therefore, the effect of void spaces in the bed is negated. Samples were prepared by degassing (vacuum) an accurately weighed sample of powder (20 cm^3) in a container of known volume, followed by analysis of the sample using a Quantachrome® stereopycnometer (model no SPY-4, Quantachrome Corp, Boyton Beach).

3.4.11.2 Bulk (poured) density (ρ_b)

The bulk (or poured) density of a powder is defined as the ratio of the weight of a powder to the volume it occupies. This density term accounts not only for the volume of the solid portion of the particles, but also for the voids within a particle and the voids between particles. Poured density is essentially the same as bulk density. The bulk density of the filler was determined by pouring a predetermined weight of powder (100 g) into a graduated cylinder and measuring the volume it occupied. The density was calculated using equation 3.10.

$$\rho_b = \frac{w}{V_b} \quad [3.10]$$

Where:

P_b is the bulk density or tapped density (g.cm^{-3}); w is the weight (g) and V_b is the volume (cm^3) of the powder.

3.4.11.3 Tapped density (ρ_t)

The tapped density (accounting for the settling of powder particles due to handling or vibration) of chitosan was determined by pouring a predetermined weight (100 g) into a graduated cylinder. The cylinder was placed on a vibrating surface (Fritsch® analysette) set at an amplitude of 5 ampere and vibrated for 5 minute intervals (up to 20 minutes). Repetitions of the procedure were performed until the powder volume reached a constant level (after 10 minutes). The average tapped density was calculated using equation 3.10.

3.4.11.4 Porosity (ϵ)

Porosity (ϵ) is defined as the volume occupied by spaces (voids) between particles in a powder bed (Lantz, 1988:120). The porosity or voids is determined as the ratio of the void volume to the bulk volume according to equation 3.11 and is expressed in percentage, ϵ (100).

$$\frac{V_b - V_t}{V_b} = 1 - \frac{V_t}{V_b} \quad [3.11]$$

Where:

V_b is the bulk volume (cm^3) and V_t the true volume (cm^3), obtained individually from the bulk density and the true density.

3.5 Preparation and evaluation

3.5.1 Mixture composition

The composition of the various mixtures prepared during this study is presented in table 3.3.

Table 3.3: *Composition of furosemide formulations.*

Compound	Function	%w/w
Furosemide	Model drug	14.29*
Chitosan	Filler (multipurpose excipient)	Qs to 100
Ac-Di-Sol® or Kollidon® CL	Disintegrants	0; 0.625 or 1.25
Ascorbic acid	Acidifier	0 or 0.5
Kollidon® VA 64 or Methogel K100M Premium (HPMC)	Binders	0; 3.0 or 5.0
Sodium bicarbonate	Gas-evolving buffering agent	0; 2.0 or 4.0

* Comprising 2 mg furosemide per tablet.

Fifty gram (50 g) mixtures of each formulation were prepared in 250 cm³ glass containers fitted with a screw cap. Parafilm[®] was used to seal the openings of these containers prior to mixing. All mixing procedures employed a Turbula[®]-mixer (model T2C W.A. Bachofen, Bâle, Switzerland) at 69 rpm as the standard method. The total mixing time, however, depended on the filler system used. The standard mixing method was divided into two phases of 5 minutes each. Furosemide was always added during the last step of mixture preparation after it was sieved, ensuring that the particles were of the same size and did not agglomerate. In order to assure proper mixing of the active ingredient and prevention of it sticking to the container walls or lid, a small indent was made in the surface of the mixture. The active ingredient was placed in this hole, which was carefully covered before mixing commenced. The final mixtures were transferred to glass bottles, which was covered with Parafilm[®] before the screw caps were fitted, and stored in a dark cabinet at room temperature for at least 24 hours prior to tableting.

3.5.1.1 Physically mixed chitosan filler-system

The chitosan and other excipients used in the different formulations were pre-mixed for 5 minutes (first phase). This filler-system was then mixed with the active ingredient for another 5 minutes as described earlier (second phase).

3.5.1.2 Granulated chitosan filler-systems

Four hundred millilitres (400 cm³) granulation solution was prepared for each granulation process. This solution contained either 3 or 5% w/v of the binder, which was dissolved in either 90% v/v ethanol for Kollidon[®] VA64, or distilled water (which was heated to assist the solubility of the binder) for HPMC.

Two hundred grams (200 g) chitosan were weighed and transferred to a variable speed planetary Kenwood mixer (Kenlyte, Kenwood limited, Hampshire, Britain). The granulation solution was slowly added to the chitosan at setting 3. The speed was increased to a setting of 6 after the total granulation solution was added and maintained until the binder was thoroughly distributed through the mixture.

The wetted mass was forced through a 10 mesh stainless steel sieve using a spatula. The granules containing Kollidon[®] VA64 were dried in an oven fitted with a fan at 80 °C ± 2 °C for at least an hour, whereas the granules containing HPMC were dried for at least 3 hours.

After drying, the granulate was forced through a 5 mesh stainless steel sieve to produce small granules. The filler-system was prepared by mixing the granulate and the extra-granular excipients (if any) for 5 minutes. Furosemide was then added in the second phase as described in section 3.5.1.

3.5.2 Compression of tablets

Mixtures were tableted directly after mixing to a weight of approximately 14 mg at a specific upper punch (applied load) setting (50). Reference to compression settings was designated as the terms, setting or settings. The fill volume of the die was maintained at a constant level by adjustment of the lower punch setting. Slightly biconcave compression tooling was utilised to manufacture tablets that presented a slightly biconvex curvature. The tooling was chosen to produce tablets of approximately 3 mm in diameter. An eccentric single press (Cadmach[®]) was employed during all tableting procedures.

The first twenty tablets compressed of each mixture were disposed of. After manufacturing was completed, tablets were transferred to glass bottles, which were sealed with Parafilm[®] before closure with screw caps, and stored in a dark cabinet at room temperature (25 ± 2 °C) for at least 24 hours preceding the analysis.

3.5.3 Analysis of physical tablet properties

The following physical properties of the tablets from each formulation were determined:

- weight variation
- radial crushing strength
- diameter
- thickness
- friability
- disintegration

3.5.3.1 Weight variation

Twenty tablets from each formulation were randomly selected, lightly dusted with an art brush and weighed on a Precisa[®] analytical balance (model 240A, PAG OERLIKON AG,

Zurich, Switzerland). The average weight, standard deviation and the percentage relative standard deviation were calculated and evaluated.

3.5.3.2 Radial crushing strength, diameter and thickness determinations

Evaluation of crushing strength, diameter and thickness was determined for 10 tablets of each formulation. A Pharma Test[®] (model PTB-311) tablet test unit (Pharma Test, Switzerland) was employed to determine all the values of the tested parameters.

3.5.3.3 Determination of friability

Ten tablets were randomly selected from the batch and carefully dusted with an art brush and accurately weighed on an analytical balance. The tablets were transferred to a Roche[®] friabilator. The friabilator was operated for a duration of 4 minutes at 25 rpm. After completion of the test, the tablets were carefully removed from the friabilator and lightly dusted. They were weighed again and the weight loss was expressed as a percentage of the original total weight of the tablets. Equation 3.12 was used to calculate the percentage friability.

$$\%F = 100 \frac{W_b - W_a}{W_b} \quad [3.12]$$

Where:

%F is the calculated percentage friability, W_b is the total weight of dusted tablets before rotation is initiated and W_a is the total weight of dusted tablets after completion of rotation.

3.5.3.4 Measurement of disintegration time

The disintegration times of six tablets from each formulation were determined using a Manesty[®] tablet disintegration test unit (Manesty Machines Ltd., Liverpool, England). The tests were performed without fitting discs to the disintegration tubes after the tablets were transferred to the tubes. The official limit of 15 minutes was employed and all formulations that did not meet the standard were considered non-disintegrating. The disintegration medium was distilled water and was maintained at a temperature of 37 ± 0.5 °C by a thermostat.

3.5.4 Dissolution studies

3.5.4.1 Apparatus and dissolution conditions

Dissolution studies were performed in a six-station Erweka[®] (model DT6R) dissolution apparatus that was fitted with a thermostat and a synchronous motor (Erweka[®], Heustenstamm, Germany) with adjustable speed settings. Dissolution tests were performed in 900 cm³ of 0.1 M HCl (pH ~ 1.00) as the dissolution medium. The thermostat regulated the temperature of the medium at 37 ± 0.5 °C and the synchronous motor maintained the rotational speed at 50 rpm.

Changes made to the dissolution apparatus:

For the analysis of mini-tablets, the standard USP-paddles were removed from the rods and replaced with stainless steel rotating baskets (United States Pharmacopoeia, 2002:194-195). Standard USP-paddles were, however, used when the capsules and reference tablets were analysed.

3.5.4.2 Rational for the choice of the dissolution medium

Furosemide is a weak acidic drug with a pKa of 3.6. The solubility of this sparingly water-soluble and poorly wettable drug is pH-dependant. Rubinstein and Price (1977:5P) concluded that the pH of the medium played an important role in detecting differences between good and poor formulations. Prasad *et al.* (1982:85) and Akbuga and Gürsoy (1987a:2204) showed that as the pH of the dissolution medium increased away from the pKa of the drug (i.e. 3.6-3.9), the differences in dissolution decreased and at pH 7.4 it virtually disappeared. Therefore, the dissolution studies were done in a discriminating medium to be able to determine the effect of various formulation variables on dissolution.

3.5.4.3 Method of tablet dissolution

3.5.4.3.1 Dissolution of compressed tablets

The dissolution vessels were filled with the dissolution medium and left to equilibrate at the specified temperature. Prior to each dissolution run, the rods were pulled up and the baskets,

containing ten mini-tablets in each, were clipped in place. The motor was started and as soon as the rotational speed reached 50 rpm, the rods were pushed down, resulting in the rotating baskets being submerged into the medium, about 25 ± 2 mm from the bottom of the glass beaker. The timing sequence was initiated at a starting point of $t = 0$. The sampling of the analytes (furosemide and chitosan) from the vessels followed the time schedule of $t = 1, 2, 4, 5, 6, 10, 20, 30, 45, 60, 75, 90, 105, 120, 135$ and 150 minutes and these experiments were performed in fourfold. Samples of approximately 5 cm^3 were withdrawn at equal heights from each vessel and this volume was replaced immediately after sampling with fresh, preheated dissolution medium. The sampling was performed through a filter unit fitted with a $0.3 \text{ }\mu\text{m}$ Millipore[®] prefilter and the samples were transferred to clean 10 cm^3 glass polytops. From these polytops $300 \text{ }\mu\text{m}^3$ of each sample was withdrawn and transferred to disposable cuvettes to be able to analyse the amount of chitosan in solution. The furosemide concentrations in solution were analysed, using the samples in the 10 cm^3 glass polytops. The samples were subsequently analysed.

After withdrawal of the last sample (after 150 minutes) the motor was stopped, the rods removed and the remaining parts of tablets (if any) carefully loosened from the baskets and transferred to the dissolution medium. Each beaker was placed in an ultrasonic bath for 30 minutes to ensure maximum drug solution after which a 5 cm^3 sample (and again a $300 \text{ }\mu\text{m}^3$ sample) was withdrawn.

3.5.4.3.2 *Dissolution of capsules and references*

Dissolution tests were performed to evaluate the effect that chitosan mini-tablets, incorporated into capsules, would have on the dissolution rate and extent of furosemide. The capsules were filled with ten mini-tablets each, prior to the dissolution test. Some of these capsules contained Avicel[®] PH101 as filler, whereas the other capsules did not contain any filler. Dissolution studies were performed in four fold as described in the previous sections (3.5.4.1 to 3.5.4.3.1). The standard USP-paddles were used in these analyses.

Dissolution studies were also performed on tablets containing 20 mg and 40 mg furosemide and these tablets were used as references. The studies were again performed as described in sections 3.5.4.1 to 3.5.4.3.1. The standard USP-paddles were used.

Table 3.4: *Reference tablet formulations.*

Tablet content	Furosemide amount (mg)	Tablet weight (mg)
Furosemide, Avicel [®] PH101, 1.25% w/w Ac-Di-Sol	20	245 mg $\pm$ 1 mg
Furosemide, Avicel [®] PH101, 1.25% w/w Ac-Di-Sol, 2% w/w NaHCO ₃	20	245 mg $\pm$ 1 mg
Lasix [®] (Aventis, batch nr. 22C)	20	80 mg $\pm$ 1 mg
Puresis [®] (Aspen Pharmacare, batch nr. E15302T)	40	170 mg $\pm$ 1 mg

3.5.4.3.3 *Analysis of furosemide*

Furosemide absorbs ultraviolet light between 275 and 345 nm and fluoresces at 405 to 417 nm. The fluorescence is pH-dependent and quantum yield is maximised in the pH-range of 2 to 3 (United States Pharmacopoeia, 2002:778; Chungi *et al.*, 1979:36; Boles Ponto & Schoenwald, 1990:381-383). Therefore, the analysis was performed by cycling the samples through a 1 cm³ flow-through quartz cell by a super sipper, connected to a Helios α Unicam[®]-spectrophotometer (Unicam Ltd, Cambridge, UK) and measuring the UV-absorbance of each sample in duplicate at 277 nm. 0.1 M HCl was used as a blank. During dissolution calculations, a correction was made for the amount of drug lost through sampling (section 3.5.4.7).

3.5.4.3.4 *Colorimetric analysis of chitosan*

For chitosan to be active as an absorption enhancer, the polymer should be in solution. Therefore, the amount of the polymer released from the dosage form should be determined parallel to the amount of furosemide released. A colorimetric method for determining chitosan in an aqueous solution was previously described by Muzzarelli (1998:255). This method was used as the basis for the present chitosan colorimetric assay.

A solution of Cibacron brilliant red 3B-A (Aldrich Chem Co., Milwaukee, WI, USA) was prepared in deionised water, at a concentration of 1.5 mg. cm⁻³. Aliquots (5 cm³) of the stock solution were made up to a 100 cm³ with 0.1 M hydrochloric buffer solution (pH 3.2). The final concentration of the dye solution was 75 μ g.cm⁻³.

The glycine hydrochloride buffer solution was prepared by dissolving glycine (1.87 g) and sodium chloride (1.46 g) in 250 cm³ deionised water. Aliquots (81 cm³) of this solution were made up to 100 cm³ with 0.1 M HCl, with a final pH of 3.2.

The amount of chitosan that dissolved was spectrophotometrically analysed at 575 nm on a Unicam[®]-spectrophotometer (Unicam Ltd, Cambridge, UK) by adding exactly 3 cm³ of the dye solution to each cuvette. 0.1 M HCl together with 3 cm³ dye solution in a cuvette was used as a blanco. During dissolution calculations, a correction was made for the amount of chitosan lost through sampling (section 3.5.4.7).

3.5.4.4 Dissolution of a furosemide suspension

A suspension was thought to produce the optimum dissolution of furosemide since it contains finely dispersed and well-wetted drug particles with the optimum surface-area available for contact with the surrounding dissolution medium. The dissolution rates of furosemide from the various tablet formulations were compared to that from the suspension, in order to evaluate the effect of the various formulation and processing variables on the dissolution of the drug.

3.5.4.4.1 *Preparation of the furosemide suspension*

An excess of furosemide was added to 50 cm³ of the dissolution medium (0.1 M HCl) containing 0.184% sodium lauryl sulphate (surfactant). The suspension was placed in a rotating bottle apparatus (rotated at 25 rpm at 37 °C) for 3 hours to ensure proper dispersion and wetting of the drug particles.

3.5.4.4.2 *Dissolution test on furosemide from a suspension*

Prior to the dissolution test, 50 cm³ of dissolution medium was removed from each dissolution vessel. At time = t_0 , 50 cm³ of the suspension was introduced into the dissolution medium and samples were withdrawn as described in section 3.5.4.3.

3.5.4.5 Standard curve of furosemide

Standard curves were constructed prior to each set of dissolution tests. Standard solutions were prepared to range from 2 to 20 µg.cm⁻³ and were diluted from a mother solution that was prepared by dissolving 50 mg furosemide in ± 20 cm³ absolute ethanol and 0.1 M HCl to 250 cm³. The UV-absorbance of each standard solution was measured at 277 nm against 0.1 M HCl. These absorbance values were plotted against the appropriate concentration, and linear

regression analysis of this data produced the best-fitted straight line through these coordinates. All standard curves complied with the principles of Beer's law in the concentration range that was utilised and excellent correlation coefficients were obtained ($r^2 \geq 0.9999$). Additional parameters that were derived by regression, the slope (m) and y-intercept (c), were utilised to calculate the furosemide concentration of the samples.

3.5.4.6 Standard curve of chitosan

Stock solutions (300 μ l) of chitosan were prepared prior to each set of dissolution tests by dissolving oven dried chitosan powder (30 mg) in 100 cm^3 deionised water containing 2% (v/v) acetic acid. For the chitosan standard curve, different volumes of stock solution (25, 50, 100, 150, 200, 250 and 300 μ l) were pipetted directly into disposable cuvettes, after which the volume in each cuvette was filled up to 300 μm^3 with 0.1 M HCl (pH $\sim$ 1.00). Aliquots of the dye solution (exactly 3 cm^3) were added to each cuvette and the absorbance was recorded within 5 minutes, after adding the dye, at a wavelength of 575 nm with a Helios α Unicam[®] - spectrophotometer (Unicam Ltd, Cambridge, UK). The chitosan standard curves were constructed with the results obtained from the absorbance data.

3.5.4.7 Computation of dissolution data

The concentration of furosemide and chitosan dissolved ($\mu\text{g}.\text{cm}^{-3}$) at each sampling time was calculated using equation 3.13 and the amount of drug lost during sampling was corrected by using equation 3.14.

$$x = \frac{y^* - c}{1000m} \quad [3.13]$$

Where:

y^* is the corrected absorbance value (as from equation 3.14); x is the drug concentration ($\mu\text{g}.\text{cm}^{-3}$) and m and c are the slope and y-intercept respectively obtained from the standard curve.

$$y_n^* = y_n + \frac{V_s}{V_m} \cdot \sum^{n-1} y^* \quad [3.14]$$

Where:

y^* is the corrected absorbency of n^{th} sample; y_n is the measured absorbency of n^{th} sample; V_s is the sampling volume; V_m is the dissolution medium volume and $\Sigma^{n-1}y^*$ is the sum of all the corrected absorbencies prior to the n^{th} sample.

Dissolution profiles in this study are presented as the concentration of furosemide that dissolved ($\mu\text{g}.\text{cm}^{-3}$) as a function of time (minutes). These concentrations are the means of four determinations for each formulation.

3.5.4.8 Dissolution parameters, DR_i and AUC_n

The initial slope of the dissolution curve between t_0 and t_6 was proposed to be a fair approximation of the initial dissolution rate (DR_i) ($\mu\text{g}.\text{cm}^{-3}.\text{min}^{-1}$) of furosemide and chitosan from the various tablet formulations. The significance of this parameter is discussed later. The area under the dissolution curve (AUC) was calculated from t_0 up to completion of the dissolution test at 150 minutes (t_{150}) and would give an indication of the extent of dissolution of the active ingredient during this period. The calculation of the AUC ($\mu\text{g}.\text{min}^{-1}.\text{cm}^{-3}$) was made by application of the trapezoidal rule and the value was determined for the time interval $t_0 - t_{150}$. The trapezoidal rule is described by equation 3.15.

$$AUC = 0.5 \sum_{t=n}^{t=0} (t_n - t_{n-1})(c_n + c_{n-1}) \quad [3.15]$$

Where:

$t_n - t_{n-1}$ is the time difference between two consecutive sampling intervals and c_n and c_{n-1} is the concentration ($\mu\text{g}.\text{cm}^{-3}$) in samples at sampling times corresponding to t_n and t_{n-1} .

The use of the area under the dissolution profile as a method to compare the effects of formulation or processing variables on drug release profiles from tablets is based in the following assumption: If two formulations do not differ much in the **rate** and **extent** to which they make the drug available in vitro, they will not differ much in their area under the concentration/time curves obtained from dissolution tests (Banakar, 1991:437; Rescigno, 1992:925).

In order to compare the different formulations in terms of their respective AUC's, each individual AUC was related to the AUC of the furosemide suspension. The calculated value, termed the normalised AUC, AUC_n , is a dimensionless parameter (Vadas *et al.*, 1984:782; Marais, 2000:48-52) and was calculated as follows:

$$AUC_n = \frac{AUC_{suspension}}{AUC_{formulation}} \quad [3.16]$$

3.6 Short- and long-term stability testing of chitosan powder, granules and tablets

An independent experiment was conducted to evaluate the effect of varying ambient conditions on the physical properties of chitosan powder, chitosan tablets, mixtures of chitosan with a binder and chitosan granules. The results of this study could provide insight into the factors that control the stability of and determine the optimum storage conditions for chitosan powder and tablets.

3.6.1 The influence of moisture content, temperature and time of exposure on the physical stability of chitosan powder (short-term stability)

An experiment was conducted to evaluate the influence of moisture content, temperature and time of exposure on the physical properties of chitosan powder. These results could provide insight into the effect that the environmental conditions could have on the formulation of chitosan tablets.

3.6.1.1 Preparation of chitosan samples for physical stability testing

Various 2 kg batches of chitosan powder were subjected to elevated temperatures of 30, 40, 50 and 60 °C (± 2 °C) in an oven fitted with a fan. Samples consisting of 50 g chitosan powder were taken at different time intervals over an 8 hour time period (at 1, 2, 4 and 8 hours). From each of these samples 5 g of chitosan powder was taken and Karl Fischer titration (section 3.4.7), DSC analysis (section 3.4.5) and TGA analysis (section 3.4.6) were conducted on the samples taken at each temperature and time interval prior to tableting.

The 45 g chitosan samples were transferred into 250 cm³ glass containers. Furosemide (14.29% w/w) and Kollidon® VA64 (5% w/w) were added to the samples and the containers were fitted with a screw cap. In order to assure proper mixing of the active ingredient and prevention of it sticking to the container walls or lid, a small indent was made in the surface of the chitosan powder. The active ingredient was placed in this hole, which was carefully

covered before mixing commenced. Parafilm[®] was used to seal the openings of these containers prior to mixing. All mixing procedures employed a Turbula[®]-mixer (model T2C W.A. Bachofen, Bâle, Switzerland) at 69 rpm as the standard method for a total of 5 minutes.

Mixtures were tableted directly after mixing into tablets weighing approximately 80 mg at a specific upper punch setting (50). The fill volume of the die was maintained at a constant level by adjustment of the lower punch setting. Slightly biconcave compression tooling was utilised to manufacture tablets that presented a slightly biconvex curvature. The tooling was chosen to produce tablets of approximately 5 mm in diameter. An eccentric single press (Cadmach[®]) was employed during all tableting procedures.

The first twenty tablets compressed of each mixture were disposed of. After manufacturing was completed, tablets were transferred to glass bottles, which were sealed with Parafilm[®] before closure with screw caps, and stored in a dark cabinet at room temperature (25 ± 2 °C) for at least 24 hours preceding the analysis. All of the physical properties were analysed according to section 3.5.3.

3.6.2 Evaluation of long-term stability testing on the physical properties of chitosan powder, chitosan tablets, as well as mixtures and granules of chitosan

3.6.2.1 Preparation prior to storage

Glass containers (500 cm³) were filled with approximately 200 g chitosan powder and were sealed with Parafilm[®] prior to storage.

Tablets containing only chitosan powder; chitosan powder mixed with 5% w/w Kollidon[®] VA64 and chitosan granules (prepared through granulation with 5% w/w Kollidon[®] VA64) were prepared. These tablets weighed approximately 80 mg and were tableted at a specific upper punch setting (50). Slightly biconcave compression tooling was utilised to manufacture tablets that presented a slightly biconvex curvature. The tooling was chosen to produce tablets of approximately 5 mm in diameter. An eccentric single press (Cadmach[®]) was employed during all tableting procedures and approximately 1500 tablets were produced. All

of the different tablets were packaged in glass containers that were sealed with Parafilm[®] prior to examination or storage.

3.6.2.2 Storage conditions

The different chitosan tablets were preserved under controlled conditions of humidity and temperature. Two sets of conditions were evaluated namely; 25 °C/60% relative humidity and 40 °C/75% relative humidity. Tablets for each set of conditions were sampled every month for a period of 6 months (1, 2, 3, 4, 5 and 6) and analysed, following the procedures described in section 3.5.3 to monitor the aging process of the tablets. A specific quantity of chitosan powder (50 g) was also sampled from the sealed containers for each set of conditions. The chitosan powders were then tableted and transferred to glass bottles, which were sealed with Parafilm[®] before closure with screw caps, and stored in a dark cabinet at room temperature (25 ± 2 °C) for at least 24 hours preceding the analysis.

3.6.2.3 Tablet sampling and evaluation

Continued, monthly analysis was performed for 6 months to examine the potential long-term effects of ambient conditions on the physical stability of chitosan. Since placebo tablets were produced, dissolution studies were negated in the stability assessment study.

3.7 Calculations

All calculations were made using Microsoft[®] Excel[™] XP for Windows[™] (Microsoft[®] Corporation, Seattle, Washington, USA).

CHAPTER 4

CHARACTERISATION AND STABILITY OF CHITOSAN FROM THE PERSPECTIVE OF PHYSICAL ANALYSIS OF THE POWDER AND TABLETS

4.1 Introduction

For successful application of direct compression in pharmaceutical processes three vital properties of the excipients are demanded: (I) the particles must be sufficiently free-flowing so that they will uniformly flow into a relatively small volume in the die in a very short time and no segregation should take place, (II) the particles, when subjected to a force from the punches, should cohere to form a compact of adequate strength and (III) whilst the particles must cohere, adhesion by the tablet to the punches and dies must be avoided, otherwise damage to both tablet and press will ensue when attempts are made to remove the tablet from the die. Unfortunately, relatively few substances possess these essential properties without some preliminary treatment (Marshall & Rudnic, 1991:357-358; Armstrong, 1988:648).

Optimal preparation of these excipients for tableting is not possible without definite knowledge of the influence of pharmaceutical unit operations upon particle properties, and of particle parameters upon tablet properties. The interrelationship between pre-treatment processes, parameter spectrum of starting material and tablet characteristics is very complex (Figure 4.1).

Each of the operations involved have an impact on a whole range of particle parameters, and each particle factor affects several tablet parameters, so that the different steps of the overall process have a cascade-like effect in quite a number of ways. Optimal properties of the excipients are tantamount to optimal conditions of the process (Andrès *et al.*, 1995:1875; Flemming & Mielck, 1995:2240; Hüttenrauch, 1983:435-436).

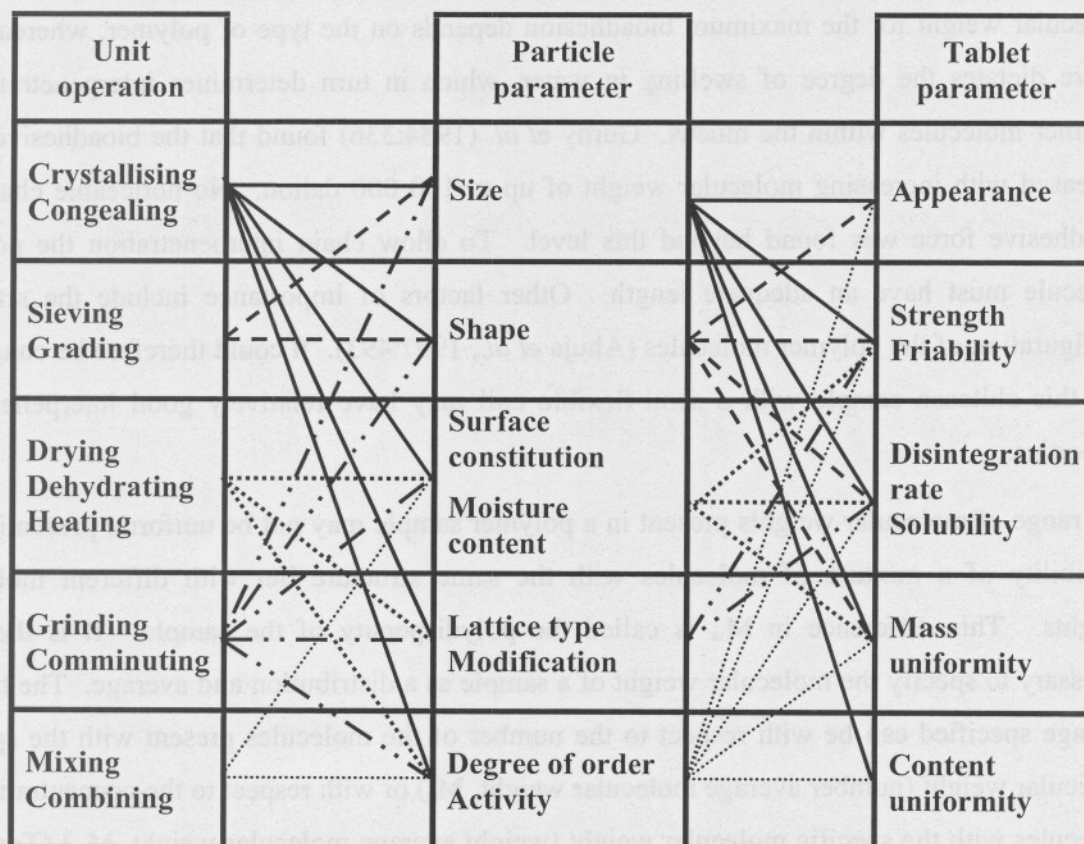


Figure 4.1: Relations between modifying operations, influenced particle parameters and consequences to tablet properties (adopted from: Hüttenrauch, 1983:435).

This chapter deals with the powder properties of chitosan including both microscopic and macroscopic aspects, in order to determine the inherent physical properties and its contribution to the physical properties of the tablets. The interpretation of the results obtained by these evaluations provided some insight into the choice of experimental procedures and excipients that were employed in the remainder of the study.

4.2 Molecular weight

Results for molecular weight (M_w) are summarised in annexure A.1. The M_w for this particular sample of chitosan powder was performed according to section 3.4.1 and was found to be approximately 125 600 dalton which is a relatively low to medium molecular weight sample. Numerous studies have indicated a certain molecular weight at which bioadhesion reaches a maximum. The interpenetration of polymers is favourable for the low molecular weight polymer, whereas entanglements are favoured for high molecular weight polymers. The optimum

molecular weight for the maximum bioadhesion depends on the type of polymer, whereas their nature dictates the degree of swelling in water, which in turn determines interpenetration of polymer molecules within the mucus. Gurny *et al.* (1984:336) found that the bioadhesive force increased with increasing molecular weight of up to 100 000 dalton. No noticeable change in bioadhesive force was found beyond this level. To allow chain interpenetration the polymer molecule must have an adequate length. Other factors of importance include the size and configuration of the polymer molecules (Ahuja *et al.*, 1997:493). It could therefore be concluded that this chitosan sample with a semi-flexible coil may have relatively good interpenetration properties.

The range of molecular weights present in a polymer sample may not be uniform, presenting the possibility of a mixture of molecules with the same structure but with different molecular weights. This difference in M_w is called the polydispersity of the sample. It is therefore necessary to specify the molecular weight of a sample as a distribution and average. The type of average specified can be with respect to the number of the molecules present with the specific molecular weight (number average molecular weight, M_n) or with respect to the concentrations of molecules with the specific molecular weight (weight average molecular weight, M_w) (Tombs & Harding, 1998). The ratio of polydispersity is used as an indication of molecular weight distribution. A polydispersity of 1.0 indicates a monodisperse sample where the molecular weights of the molecules are identical. The greater the polydispersity of the sample, the greater the distribution of molecular weight in that sample. This particular sample had a polydispersity of 1.920 ± 0.095 .

The dn/dc value is the specific refractive index increment of the dissolved molecules and was taken as 0.170, whereas the mean square radius of the molecule (41.2) measured in nanometres, is an indication of the physical molecular size at the specific ionic strength of the aqueous media. The relatively low standard deviation (5.0%) observed for the mean square radius of the polymers indicated the small range of polymer sizes for that sample.

4.3 Nuclear magnetic resonance spectroscopy analysis (¹H NMR)

Most of the physical and chemical properties of chitosan depend greatly on the degree of deacetylation and it has been repeatedly claimed in the literature that a technique to measure this parameter accurately is highly desirable. All techniques, except for NMR based measurements, require an accurate weighing of chitosan. Therefore, moisture needs to be eliminated carefully and the purity of the samples must be determined separately. Also, many of these non-NMR techniques are inaccurate, long or complicated to perform. In the ¹H NMR method, the amount of chitosan used does not need to be known accurately and the purity of the sample does not need to be determined as long as the impurity peaks do not overlap with the relevant peaks of chitosan. Sample preparation is furthermore very simple and ¹H NMR has been found to be precise and accurate for the quantification of high degree of deacetylation, which is usually difficult to measure with conventional techniques like infrared analysis or titration. Additionally, the degree of deacetylation can be calculated using different combinations of peaks in order to verify that the method is consistent (Lavertu *et al.*, 2003:1149-1158).

The analysis of ¹H-NMR-spectra of chitosan powder (section 3.4.2) is illustrated in figure 4.2. The assignments of chitosan resonances were according to Lavertu *et al.* (2003:1149-1158). They assigned the peak at 4.92 ppm to the proton H-1 (D), whereas a water peak could clearly be observed between 4.0 and 4.4 ppm. The resonances assigned to the acetyl group at 2.26 ppm representing *N*-acetylglucosamine units could hardly be observed. This was due to a high degree of deacetylation.

Next, the degree of deacetylation was calculated using integrals of the peak of proton H1 of the deacetylated monomer (H1-D) and of the peak of the three protons of the acetyl group (H-Ac):

$$DDA (\%) = \left(\frac{H1D}{H1D + \frac{HAc}{3}} \right) \times 100 \quad [4.1]$$

Equation 4.1 is normally used for powders with a high degree of deacetylation (DDA > 90%). According to this calculation, the degree of deacetylation was 91.416% and this result confirmed the high degree of deacetylation (± 91%) depicted in the certificate of analysis for this specific chitosan batch (Annexure A.2).

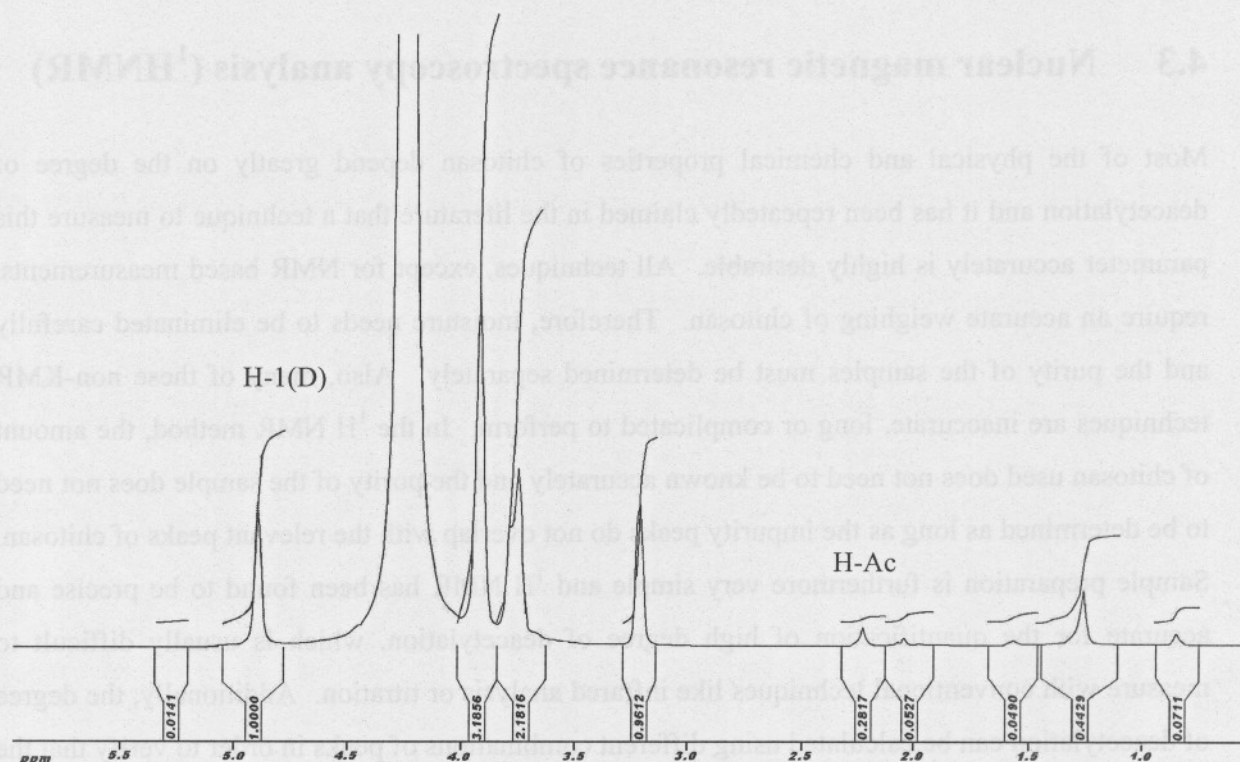


Figure 4.2: ^1H -MNR spectrum of chitosan in $\text{D}_2\text{O}/\text{DCL}$ (at a temperature of $80\text{ }^\circ\text{C}$).

4.4 Infrared (IR) analysis

Infrared analysis was done according to section 3.4.3. Figure 4.3 depicts the IR spectra of chitosan. The strong band found at $3450\text{--}3400\text{ cm}^{-1}$ and the weak bands found around 2300 cm^{-1} may be assigned to --OH stretching, indicating intermolecular hydrogen bonding, and atmospheric carbon dioxide respectively, whereas the bands between $1220\text{--}1020\text{ cm}^{-1}$ are usually assigned to C--N stretching vibrations. The C=O stretching (amide I) peak near 1655 cm^{-1} representing the structure of *N*-acetylglucosamine as well as the NH_2 stretching peak at 1600 cm^{-1} representing the glucosamine functional group was exhibited in the spectrum of chitosan powder. A further variation was found at around 900 cm^{-1} , but because of the fact that the band occurs inside the fingerprint area, the identification of peaks would not be accurate. Further studies of this area are needed to characterise the smaller bands accurately (Nunthanid *et al.*, 2004:20; Silverstein *et al.*, 1991:124).

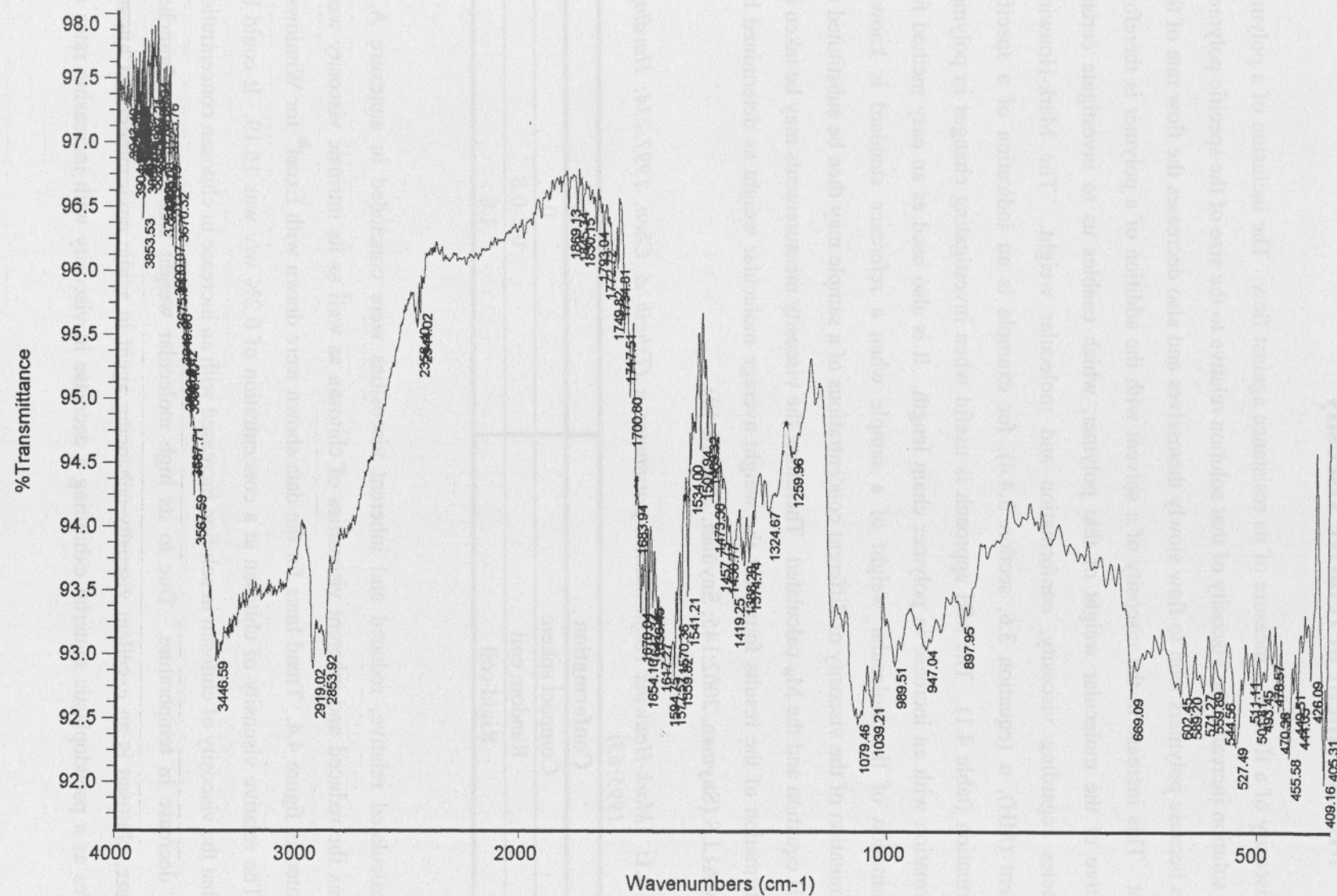


Figure 4.3: Infrared spectra of chitosan between 4000 and 500 cm^{-1} .

4.5 Measurement of relative viscosity

The viscosity of a fluid is a measure of its resistance against flow. The inclusion of a polymer into a solution increases the viscosity of that solution relative to the size of the specific polymer. This is because polymers tend to flow slowly themselves and also decreases the flow rate of the solvent. This increase in the viscosity of a solvent with the addition of a polymer is therefore indicative of the molecular weight of that polymer, which enables us to investigate certain tendencies regarding viscosity, conformation and molecular weight. The Mark-Houwink exponent (MH), α (equation 3.6, section 3.4.4), for example is an indication of a specific conformation (table 4.1). The MH approach is useful when investigating changes in polymer conformation with an increase in polymer chain length. It is also used as an easy method for determination of the molecular weight of a sample when a reference standard is known. Determination of the viscosity of different concentrations of a sample may then be substituted in the MH equation and the M_w calculated. Therefore, the viscosity measurements may be taken as a confirmation of the results found for the weight average molecular weight as determined by SEC/MALLS (Snyman, 2002:145; Snyman, 2003:18-19).

Table 4.1: *Mark-Houwink conformation parameter α (Tshaih & Chen, 1997:234; Harding, 1995:63).*

Conformation	α
Compact sphere	0
Random coil	0.5-0.8
Rigid coil	1.8

The calculated relative, reduced and inherent viscosities were concluded in annexure A.3, whereas the reduced and inherent viscosities of chitosan as well as its intrinsic viscosity were illustrated in figure 4.4. Trend lines for the data shown were drawn with Excel[®] for Windows[®] XP. The relative viscosity of chitosan at a concentration of 0.2% w/v was 16.19. It could be seen that the viscosity of chitosan in solution increased with an increase in chitosan concentration and a decrease in temperature. Due to its high molecular weight and a linear unbranched structure, chitosan is an excellent viscosity-enhancing agent in acidic environments. Chitosan behaves as a pseudoplastic material exhibiting a decrease in viscosity with increasing rates of shear.

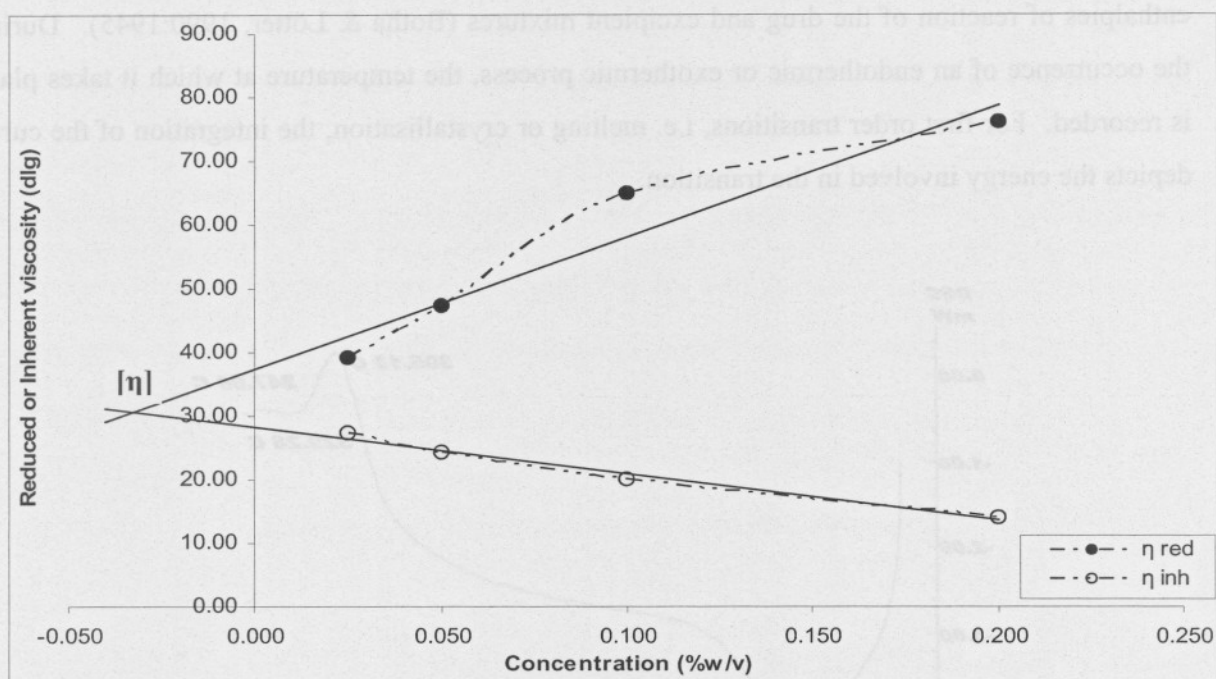


Figure 4.4: Plots of reduced (η_{red}) and inherent (η_{inh}) viscosities for chitosan, whereas $[\eta]$ is an indication of the intrinsic viscosity.

It is furthermore important to note the difference between chitosan with a high degree of deacetylation and those with a low degree of deacetylation. With a decrease in the number of acetyl groups on the polymer backbone, an increase in the deacetylated groups will cause an increase in the number of fixed positive charges on the chitosan polymer when in solution. This increase will cause electrostatic repulsion between groups on the polymer, resulting in a decrease in flexibility (Şenel & McClure, 2004:1469). Therefore, the conformation of this chitosan sample with a high degree of deacetylation would be less flexible than that of chitosan with a low degree of deacetylation. Results proved this as the chitosan sample presented a Mark-Houwink exponent (α) of 0.569. This exponent increases as the flexibility of the polymer decreases and was thus indicative of random coil conformation ($\alpha = 0.5 - 0.8$). This was similar to results found previously for chitosan (Tshaih *et al.*, 1997:234; Harding *et al.*, 1991:63).

4.6 Thermoanalytical characterisation of chitosan

Differential scanning calorimetry analysis (DSC) (section 3.4.5) usually allows the fast evaluation of possible incompatibilities for it shows changes in the appearance, shift or disappearance of melting endotherms and exotherms, and/or variations in the corresponding

enthalpies of reaction of the drug and excipient mixtures (Botha & Lötter, 1990:1945). During the occurrence of an endothermic or exothermic process, the temperature at which it takes place is recorded. For first order transitions, i.e. melting or crystallisation, the integration of the curve depicts the energy involved in the transition.

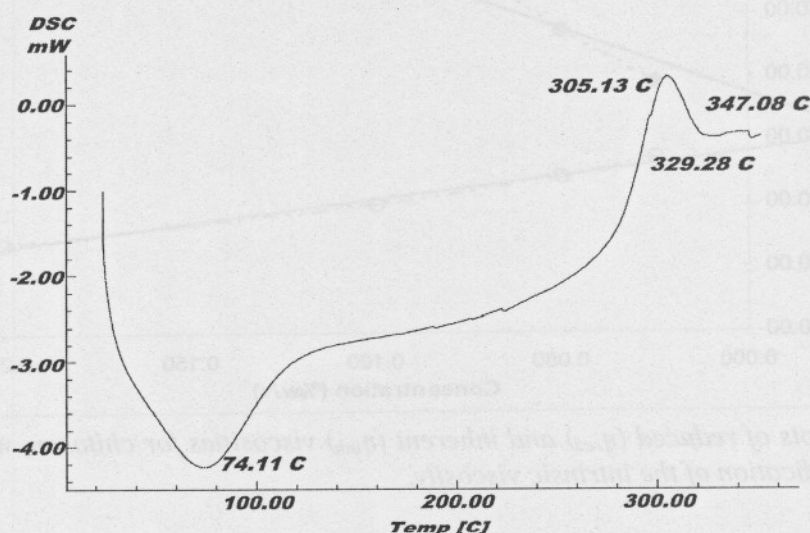


Figure 4.5: DSC thermogram of chitosan.

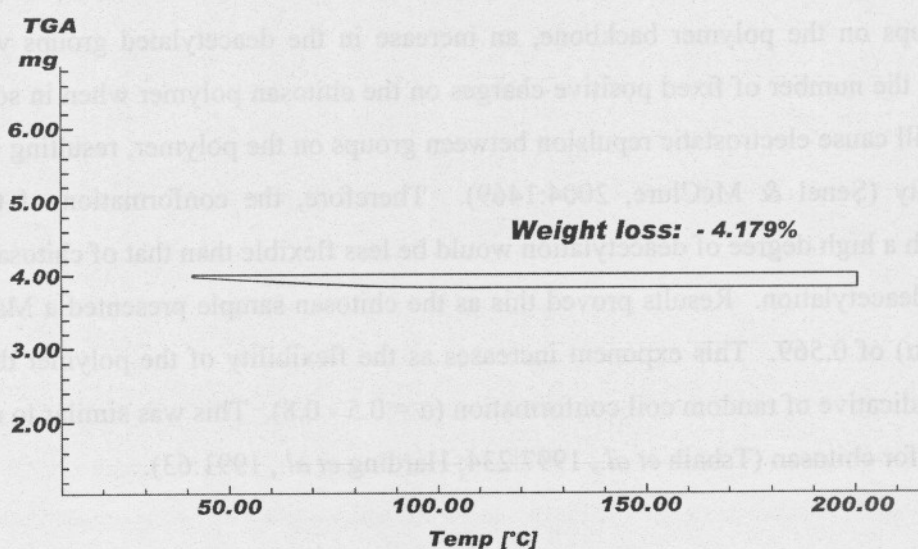


Figure 4.6: Thermogravimetric analysis of chitosan.

The DSC thermogram (conducted at 0-350 °C) of chitosan is depicted in figure 5.5. It exhibited a characteristic endothermic peak of 74.11 °C and an exothermic peak at 305.13 °C was observed, corresponding to the decomposition temperature of this polymer. Although the

endothermic peak around 74.11 °C seems to be attributable to the elimination of water, it was observed that water was absent from the chitosan after drying. Therefore, the endothermic peak may be related to the melting of the chitosan sample.

The thermal properties of chitosan were further characterised by thermogravimetric analysis (TGA) (figure 4.6) as well as the Karl-Fisher method (annexure A.4). Both TGA and Karl-Fisher (sections 3.4.6 and 3.4.7) depicted an insignificant resultant weight loss, due to loss of water, of 4.18% and 3.99% respectively. This was in agreement with the DSC results showing that the endothermic peak found was indeed due to melting and not water loss.

4.7 Micromeritic properties of chitosan

The flow properties, particle size and size distribution, density and porosity of chitosan powder were assessed according to the protocols described in section 3.4. Table 4.2 summarises the results obtained for these experiments and annexure A.5 provides the data obtained for each run. As was seen from these results, the flow properties of chitosan were not commendable.

Table 4.2: *Micromeritic properties of chitosan.*

Property	Chitosan
Mean particle size (Malvern analysis) (µm)	221.66
Mean particle size (Sieve analysis) (µm)	233.81
Angle of repose (°)	36.342
Hausner ratio	1.4127
Carr's index (%)	29.214
Flow rate (g.s ⁻¹)	7.9188
Uniformity of flow (%RSD) *	6.6917
Bulk density (g.cm ⁻³)	0.1835
Tapped density (g.cm ⁻³)	0.2593
True density (g.cm ⁻³)	1.4420
Void volume (cm ⁻³)	159.33
Porosity (%)	87.275

* Percentage relative standard deviation (= $STD/Average \times 100\%$)

The angle of repose indicated sufficient to relatively good flow properties, however, angle of repose tend to be variable and are not always representative of flow under specific conditions.

Hausner found that the ratio $\frac{\rho_{Tapped}}{\rho_{Bulk}}$ was related to interparticle friction and, as such, could be used to predict powder flow properties. Carr, on the other hand, found that the percentage compressibility of a powder is a direct measure of the potential powder arch or bridge strength and stability (Staniforth, 2002:207). Therefore, Carr's index and the Hausner ratio were also taken into consideration and from these results it could be concluded that chitosan powder is indeed not free flowing, but rather relatively cohesive flakes with poor flowability. The flow rate was comparatively low and the uniformity of flow (%RSD of 6.6917) indicated that different samples of the powder possessed relatively poor reproducible flow rates. These properties suggested that the die cavity would not be filled consistently during each compression cycle, resulting in tablets which will probably vary in weight.

Staniforth (2000:604) suggested that the larger the particle size and the more uniform the particle shape, the better the fluidity of an excipient, however, this is only possible to some extent where large size results in poor flow. Both the scanning electron photomicrograph (figure 4.7) and the analysis of the mean particle size revealed that chitosan consisted of large amorphous particles ($\pm 228 \mu\text{m}$) of irregular size and shape with rough surfaces. Chitosan powder furthermore, depicted a narrow particle size distribution (annexure A.5). These results had a detrimental effect on the flowability of the powder, however, did not preclude the employment of chitosan.

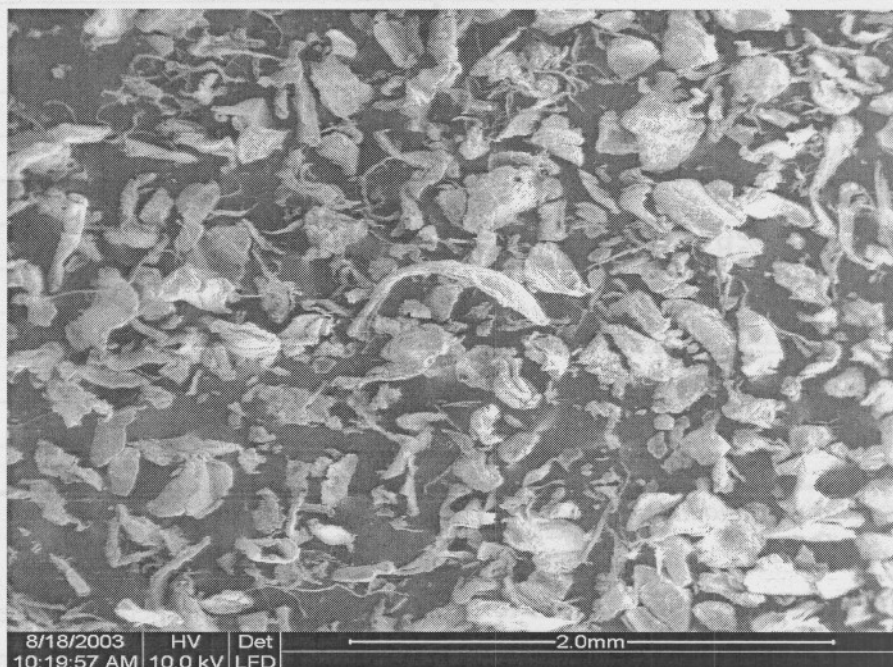


Figure 4.7: *Representative scanning electron photomicrographs of chitosan powder.*

When considering the densities of chitosan powder it could be concluded that this excipient will produce tablets of minimum weight. The ratio of the bulk and tapped density and the tapped and true density was approximately 1.4 and 5.6 respectively, which, together with the void volume and porosity, suggests that chitosan powder may be compressed into significantly compacted tablets. This may, therefore, influence disintegrant efficiency and subsequent drug dissolution due to restricted contact between disintegrant and drug particles, and solvent molecules (a process necessary for optimum drug release and eventual drug dissolution). Another factor of importance during disintegration and dissolution is the particle shape and as mentioned before, chitosan particles are irregular in shape and therefore, this may also restrict contact between the drug particles and the dissolution medium.

4.8 Preformulation studies on compactibility of chitosan

4.8.1 Assessment of tablets of chitosan-mixtures

4.8.1.1 Compactability

Preformulation studies were conducted in order to determine whether chitosan could be tableted into tablets with acceptable properties. During these studies it was concluded that tablets with a diameter of 10 mm which contained only chitosan could not be produced, as these tablets were too fragile and pulverised easily. It was therefore, decided that studies would further be conducted on tablets with smaller diameters (i.e. 5.5 mm).

Mixtures of chitosan with other conventional fillers, i.e. Avicel[®] PH 200 (microcrystalline cellulose), Prosolv[®] 50 or Prosolv[®] 90 (silicified microcrystalline cellulose) were mixed in 30:70 and 70:30 ratios to establish the compactability of chitosan (sections 3.5.1 and 3.5.2). After concluding that tablets could be produced from these mixtures, tablets containing only chitosan were also evaluated. In the previous chapters it was noted that chitosan possesses some lubrication properties and this was confirmed in the preformulation studies, for the chitosan tablets could be tableted without the inclusion of any lubricant. Sticking and lamination were effectively averted and the surfaces of the compacts exhibited a satisfactory appearance with no indication of frictional damage on the tablet edges. Continued experimentation, therefore, excluded a lubricant in any of the formulations.

Tableting was preceded by the determination of appropriate compression settings at a constant die fill volume (obtained through changing the depth of the upper punch into the die) to accommodate the different mixtures. It was found that the different mixtures of chitosan produced tablets of varying weight. The 30:70 ratio mixtures produced tablets of approximately 100 mg, whereas the 70:30 ratio mixtures produced tablets of approximately 80 mg. Tablets containing only chitosan, however, weighed approximately 25 mg. The interpretation of the results obtained by physical analysis of the tablets, could in some cases, be correlated to the properties of the powder. The decline in tablet weight with the augmentation of chitosan confirmed suspicions in section 4.7, where it was suggested that when considering powder densities and porosity, chitosan powder would produce tablets of minimum weight.

The physical properties of the tablets were evaluated according to previously explained procedures (section 3.5.3). Tablets were evaluated in terms of weight variation, crushing strength, thickness (tablet height), diameter, friability, and disintegration time. Tables A.6.1 and A.6.2 in annexure A summarises the results that were obtained for the chitosan mixtures.

The flowability of the mixtures could be evaluated indirectly by analysis of the weight variation. As was described earlier (section 1.2.1.1), uniform flowability of powders leads to the production of tablets of uniform weight. It was indicated that tablet weight did not vary discernibly for the chitosan mixtures which contained 30 and 70% chitosan respectively, prepared with different mixing times and at different compression settings. Furthermore, the percentage relative standard deviation (%RSD) indicated insignificant intrabatch weight variation that illustrated relatively good flow properties of these mixtures. However, tablets containing only chitosan exhibited poor flowability as the weight variation of these tablets were considerable (%RSD = 24.22). This was due to the relatively cohesiveness of the chitosan flakes and their irregular size and shape.

The crushing strength of tablets containing chitosan mixtures indicated insignificant intrabatch variation with changes in the duration of mixing time; however, tablets obtained from the 20 minute mixing time were slightly harder (tables A.6.1 and A.6.2). Figure 4.8 illustrates an example of the distinct changes in crushing strength as the compression setting increased for tablets containing Prosolv[®] 50 and mixed for 10 minutes. An increase in compression pressure clearly coincided with an increase in crushing strength. Furthermore, mixtures containing 30% chitosan were significantly harder. The sharp positive curvature in the profile (up to setting 40) suggested that this particular system reached a limiting crushing strength value, and approached a

setting where the crushing strength of these tablets seemed unresponsive to a further increase in compression force (above setting 40). On the other hand, mixtures containing 70% chitosan depicted a linear increase ($R^2 = 0.9776$) in crushing strength when the compression force increased. Therefore, the ideal machine setting to provide tablets of maximum crushing strength had not yet been reached for these particular systems.

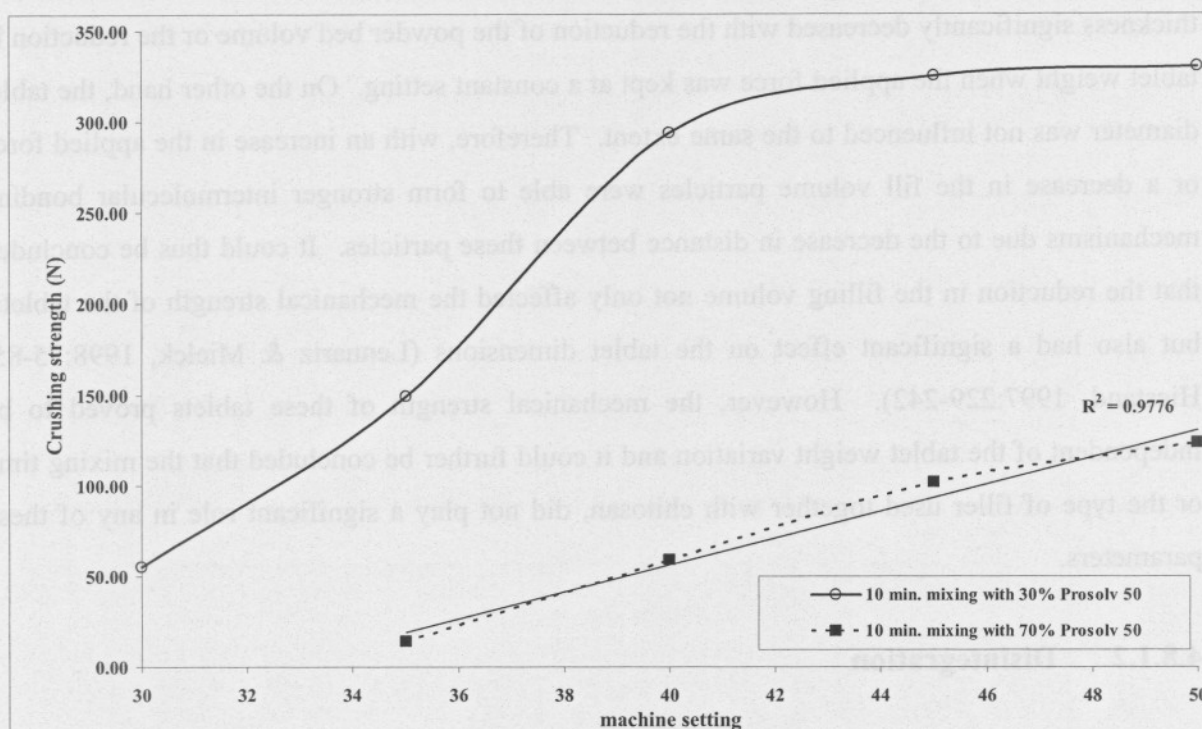


Figure 4.8: Example of the effect of machine upper punch setting (related to compression force) on the crushing strength (N) of tablets containing mixtures of 30 and 70% chitosan respectively, and Prosolv® 50.

As with crushing strength, tablet friability is also an important factor that should be considered when examining tablet characteristics because it is also indicative of the degree of mechanical strength of tablets. The friability of these tablets was markedly affected by compaction pressure, whereas other factors, such as mixing time, did not play an important role. This decrease in friability could be attributed to an increase in the crushing strength of the tablets, resulting in tablets that were less fragile due to single crystals that were “glued” together during compression. All of the tablets of chitosan mixtures did however, portrayed acceptable friability values ($< 1.00\%$) despite the low crushing strengths. The pure chitosan powder could only be tableted at the maximum machine setting (50) and yet, still depicted poor crushing strengths (± 34.79 N).

However, their friability also proved acceptable (0.237%). These results might be due to the binding mechanism of chitosan powder that could be the combination of interlocking of the particles, as well as weak hydrogen bonding.

Other tablet parameters that were also affected by the manufacturing process were the thickness and diameter measurements. Alteration of these parameters is usually depicted with the increase or decrease in the machine setting when applying a compression force to the powder bed. Tablet thickness significantly decreased with the reduction of the powder bed volume or the reduction in tablet weight when the applied force was kept at a constant setting. On the other hand, the tablet diameter was not influenced to the same extent. Therefore, with an increase in the applied force or a decrease in the fill volume particles were able to form stronger intermolecular bonding mechanisms due to the decrease in distance between these particles. It could thus be concluded that the reduction in the filling volume not only affected the mechanical strength of the tablets, but also had a significant effect on the tablet dimensions (Lennartz & Mielck, 1998:75-85; Hiestand, 1997:229-242). However, the mechanical strength of these tablets proved to be independent of the tablet weight variation and it could further be concluded that the mixing time or the type of filler used together with chitosan, did not play a significant role in any of these parameters.

4.8.1.2 Disintegration

A generalised prerequisite for the bioavailability of drugs after oral administration is that the drug must be in solution in the gastrointestinal fluids. Pharmaceutical availability relates to the fraction or percentage of an orally administered drug dose in solution in the gastrointestinal fluids and at the absorption-site. Therefore, the rate and extent of drug release from the specific dosage form (disintegration) can have a significant effect on the bioavailability of the drug. This includes the time of onset of the therapeutic action as well as the duration and intensity of the effect. The pharmaceutical availability of an orally administered drug from tablets gives an indication of both the rate and extent of drug release from the dosage form. Most conventional tablet formulations are designed and manufactured to produce rapid and complete drug release from the tablets followed by the dissolution of the drug (Bühler, 1993:157; Abdou, 1989:554).

Disintegration is defined as the break-up of a tablet into its primary components when the tablet is exposed to an aqueous environment. The disintegration of the tablet can be regarded as the

first step on the path to bioavailability and to the pharmacological action of the drug taking effect (Rudnic & Kottke, 1996:341-342; Bühler, 1993:157; Gordon & Chowhan, 1990:438).

From tables A.6.1 and A.6.2 it could be concluded that most of the formulations resulted in tablets that only disintegrated at the lowest machine settings, or did not disintegrate at all. It should be noted that these tablets swelled considerably, but did not break up into smaller particles. This could pose a major problem regarding pharmaceutical availability of active ingredients from tablets intended as immediate release dosage forms. These formulations would, therefore, probably necessitate the incorporation of a disintegrant with a higher capacity for liquid-uptake and/or stronger swelling properties in order to instigate tablet disintegration. Interestingly, formulations containing Avicel® provided tablets with the poorest results (independent of the concentration incorporated), although it is generally renowned for its good disintegration properties (Doelker *et al.*, 1995:643-661). It could therefore be concluded that the chitosan powder properties played the most predominant role during disintegration. Chitosan is a very adhesive powder, especially when it comes into contact with water and this adhesiveness probably prevented the breaking up of the tablets. Furthermore, it seemed that chitosan tablets in the disintegration medium presented an elastic structure which could "stretch" to eliminate and/or accommodate the swelling properties of the microcrystalline cellulose particles. It was also observed that prolongation of the mixing time had a detrimental effect on the disintegration time of the chitosan tablets.

4.9 Conclusion

Chitosan powder consists of large, irregular size and shape particles with rough surfaces. The powder depicted a narrow particle size distribution which had a detrimental effect on the flowability of the powder, however, did not preclude the employment of chitosan. When considering the densities of chitosan powder it could be concluded that this excipient will produce significantly compacted tablets of minimum weight.

Preformulation studies had to be conducted in order to determine whether chitosan could be tableted into tablets with acceptable properties. During these studies it was concluded that tablets with a diameter of 10 mm which contained only chitosan could not be produced, as these tablets were too fragile and pulverised easily. However, during production of smaller tablets this material did not necessitate the presence of a lubricant as sticking and lamination were effectively

averted. Despite its poor reproducible flow rates, it was indicated that tablet weight did not vary discernibly for the chitosan mixtures which contained 30 and 70% chitosan respectively, prepared with different mixing times and at different compression settings. Furthermore, %RSD indicated insignificant intrabatch weight variation that illustrated the relatively good flow properties of these mixtures. Tablets containing only chitosan, however, proved to have poor flowability as the weight variation of these tablets were considerable.

An increase in compression pressure clearly coincided with an increase in crushing strength. Furthermore, mixtures containing less chitosan were significantly harder and all of these tablets portrayed acceptable friability values despite the low crushing strengths. The pure chitosan tablets could only be tableted at the maximum machine setting, producing tablets of insufficient tablet strength although friability proved acceptable. The addition of a dry binder is suggested to circumvent this problem. The mechanical strength of chitosan tablets was independent of the tablet weight variation and it could further be concluded that the type of filler used together with chitosan, did not play a significant role in any of the tablet properties. However, prolongation of the mixing time had a detrimental effect on the disintegration time, but did not significantly influence any of the other tablet properties.

Another drawback was illustrated by the fact that the tablets possessed little disintegration propensity, but rather swelled considerably. It seemed that chitosan tablets in the disintegration medium presented an elastic structure which could “stretch” to eliminate the swelling properties of the microcrystalline cellulose particles. This could seriously affect the pharmaceutical availability of active ingredients from immediate release tablets and the use of the filler is, therefore, questioned without addition of an appropriate disintegrant in these cases.

The characterisation of the powder material and tablets provide the stepping-stone from which modifications of the undesirable properties of the filler can now commence (Otto, 2002:70). A systematic evaluation of excipients will be conducted in chapter 5 to improve the tablet(ing) properties of chitosan through the production of minitables (3 mm diameter) as well as through the incorporation of various dry binders and disintegrants, whilst the effect of formulation preparation via physical mixing or wet granulation will also be evaluated.

CHAPTER 5

INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE PHYSICAL STABILITY OF CHITOSAN

5.1 Introduction

The physical evaluation of tablets is an integral part or component of the stability of the finished product. One of the most significant parameters contributing to the behaviour of many tablet formulations is the level of moisture during manufacture as well as the residual of moisture in the product. The presence of moisture in pharmaceutical powders can play a significant role in the consolidation properties and often produces changes in flow properties particularly after storage (Garr & Rubinstein, 1992:188). It is therefore imperative to investigate the accelerated deterioration of chitosan powder during elevated temperatures over a certain period of time. The properties of chitosan have been discussed in chapter 2 and the results in chapter 4 revealed some characteristics of the physical behaviour of the filler. Stability testing was performed in order to determine the effect of storage conditions (specifically temperature and moisture) on the raw material and finished product, and was conducted on both a short- and long term basis as described in section 3.6.

5.2 Short-term stability: Effect of temperature and moisture

Chitosan raw material was exposed to 30, 40, 50 and 60 °C (section 3.6.1) and sampled after 1, 2, 4 and 8 hours and tableted. Karl Fischer titration, DSC analysis and TGA analysis of the samples at each temperature and time interval were done as described in sections 3.4.5, 3.4.6 and 3.4.7 to establish the moisture content. Formulations consisting of the different chitosan samples, 14.29% w/w furosemide and 5% w/w Kollidon[®] VA64 were mixed for 5 minutes and tableted into 5 mm tablets. Crushing strength, friability and disintegration were used to determine and compare changes in tablet characteristics. A detailed representation of the stability data is presented in annexure D.1.

5.2.1 Physical analysis

As indicated by Nokhodchi *et al.* (1995:13) and Marshall and Rudnic (1991:358) moisture content plays an important role in the tableability of a material. Ahlneck *et al.* (1989:131) observed the variation in tablet strength after tableting, associated with the presence of water in the powder mass. They concluded that the presence of moisture in pharmaceutical powders can play a significant role in the consolidation properties due to the indirect volume reduction of the powder mass and often produces changes in flow properties particularly after storage. Moisture or the lack thereof may also have marked effects on surface tension, compression, tablet tensile strength and tablet toughness, density of the materials, binding capacities and lubrication properties in addition to having hydrodynamic properties. Malamataris and Karidas (1994:115-123) found for example that the tensile strength of tablets compressed at a certain pressure level increased with moisture content, reached a maximum at about 10% w/w moisture and then decreased while the tensile strength of tablets at fixed packing fraction exhibited an initial plateau up to 6% w/w moisture content and then decreased. They furthermore found that the profiles of particle packing versus moisture content were sigmoidal, possibly due to the lubrication effect of tightly bound moisture up to 6% w/w, and the tensile strength maxima were attributed to the combined effect of closer packing of particles and softening of interparticle bonds. They also confirmed that tensile strength begins to decrease when the moisture content is about double that corresponding to tightly bound or "monomolecular" water.

Therefore, moisture content must be optimised in a formulation to achieve good powder compressibility as the presence of moisture may aid in the binding of particles during compression by means of the formation of liquid bridges between the particles; or it may have the opposite effect, resulting in mechanically weak tablets (Nokhodchi *et al.*, 1995:13; Garr & Rubinstein, 1992:188; Marshall & Rudnic, 1991:358). A distinct correlation exists therefore between the moisture content and physical properties of the produced tablets.

From table D.1 (annexure D) and figure 5.1 it was evident that temperature and time were two variables which had a significant influence on the moisture content of chitosan powder as the exposure to elevated temperatures resulted in dehydration of the powder ($p < 0.05$). The dehydration of the chitosan powder was more prominent at the lower temperatures, whereas variation in time did not play such a significant role ($p > 0.05$). At 30 °C for example, no variation in moisture content was found over the time period of 8 hours ($p > 0.05$).

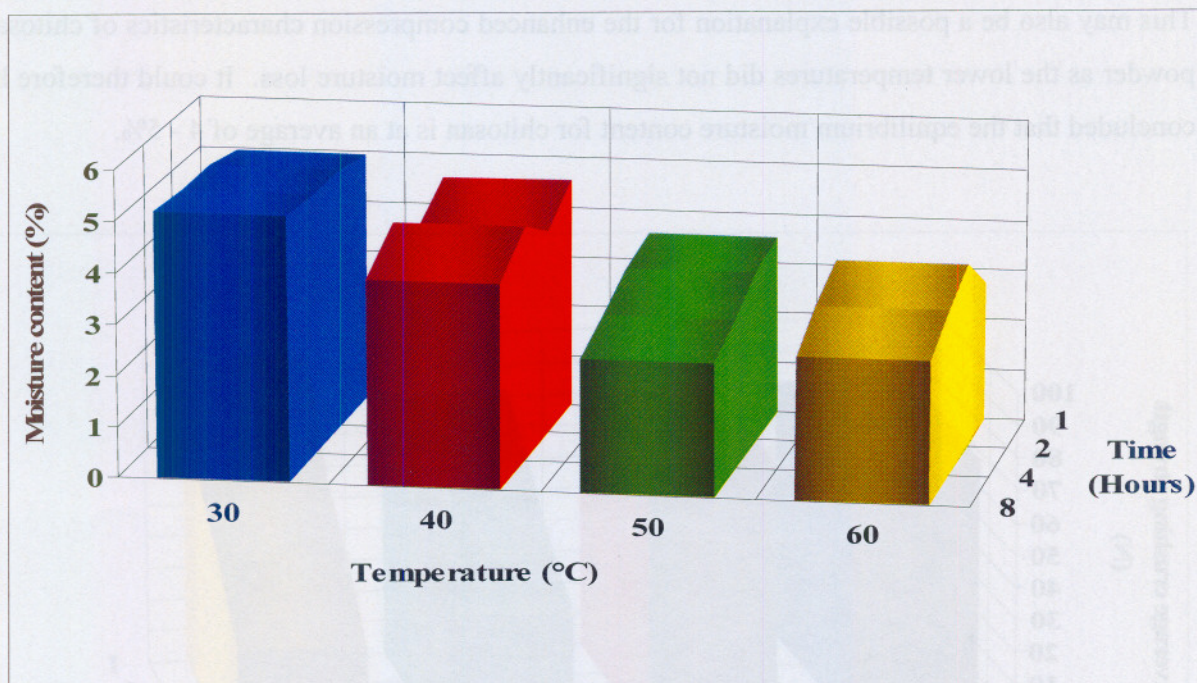


Figure 5.1: *The effect of elevated temperatures and time on the moisture content (%) of chitosan powder.*

Considering the mechanical tablet strength of the tablets produced from these chitosan samples, it was clear that temperature had an effect on the crushing strength with the elongation of time (figure 5.2). Interestingly, figure 5.2 followed the same trend as figure 5.1 emphasising the correlation between moisture content and the mechanical strength of the tablets.

Chitosan powder exposed to 30 and 40 °C produced tablets with relatively high crushing strengths when compared to samples exposed to 50 and 60 °C. Furthermore, the average crushing strength of these tablets showed the lowest degree of variation ($p > 0.05$) with time. Overall, exposure to higher temperatures (50 and 60 °C) negatively affected the crushing strength of the tablets. It could therefore be concluded that lower temperatures were advantageous in terms of storage of chitosan powder. As mentioned in chapter 1, Garr and Rubinstein (1992:187-192) found that the mean yield pressure decreased with increasing moisture content due to the overall plasticising effect of moisture, whilst relative powder density increased due to the lubrication effects of moisture smoothing surface micro-irregularities and so reducing frictional forces. Increasing moisture contents by up to 6% (w/w) in their study, progressively increased compact strength due to the hydrodynamic lubrication effects of moisture promoting optimum transmission and utilisation of compaction force and the formation of strong hydrogen bonds.

This may also be a possible explanation for the enhanced compression characteristics of chitosan powder as the lower temperatures did not significantly affect moisture loss. It could therefore be concluded that the equilibrium moisture content for chitosan is at an average of 4 - 5%.

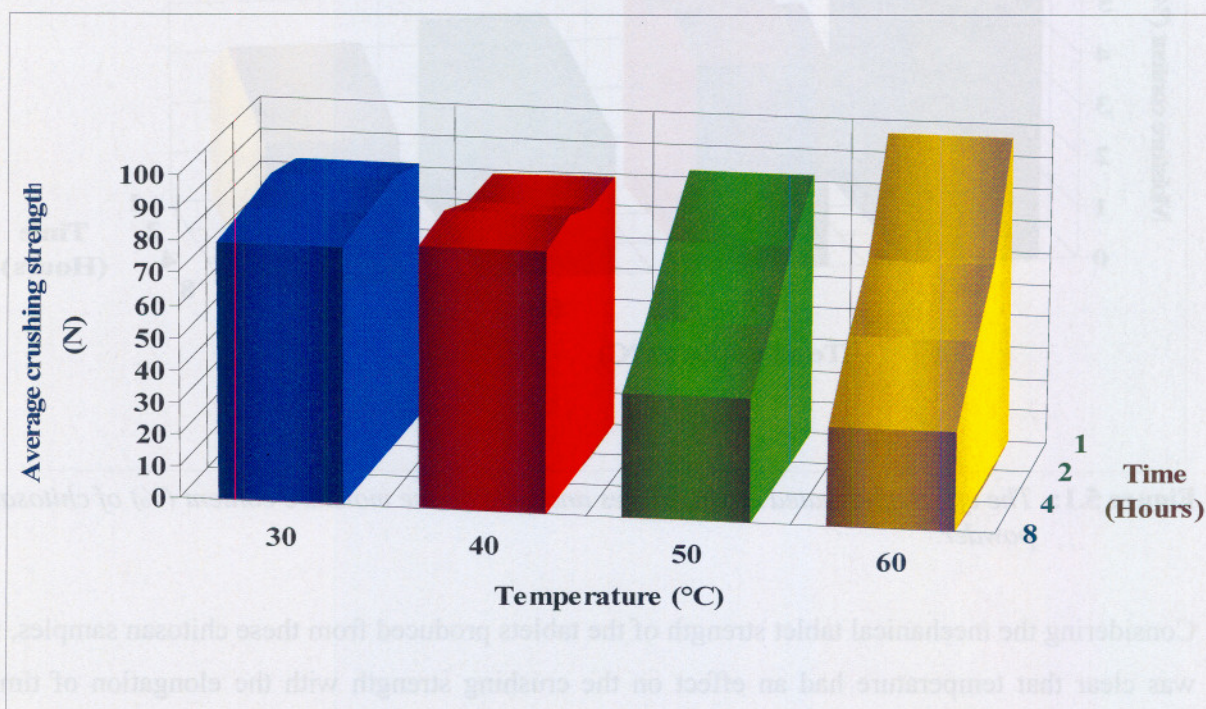


Figure 5.2: The effect of elevated temperatures and time on the average crushing strength of tablets containing chitosan.

Varying time at lower temperatures did not significantly affect the crushing strength of tablets produced from these different chitosan samples. However, an explicit detrimental effect on the average crushing strength of tablets produced from the various chitosan samples was found at higher temperatures. Thus, the time chitosan powder was exposed to higher temperatures played an important role in the tablet strength. This was probably due to the extended time in which dehydration of the material could commence.

Nokhodchi *et al.* (1995:13-20) explained the increase in tensile strength with increasing moisture content through two possible mechanisms. Firstly, adsorbed water could function as a surface restructuring medium leading to an increased amount of solid bridges. An increase in mechanical strength with increasing moisture content for sodium chloride has been suggested to be a result of a restructuring of surfaces in the tablet. Another possible explanation for an increase in tensile strength is that immobile water layers sorbed at particle surfaces can enhance the particle-particle

interaction. According to this theory, an adsorbed water vapour layer can contribute in two ways to the strength of the interaction: (a) tightly bound water vapour layers can be regarded as a part of the particles which reduces the interparticular surface distances and thus results in increased intermolecular attraction forces; (b) adsorbed layers can touch or penetrate each other which will increase the attraction forces between neighbouring particles. A decrease in tensile strength has been explained as a result of the formation of multilayers of water at the particle surfaces. Such layers may then disturb or reduce intermolecular attraction forces and thereby reduce the tablet strength.

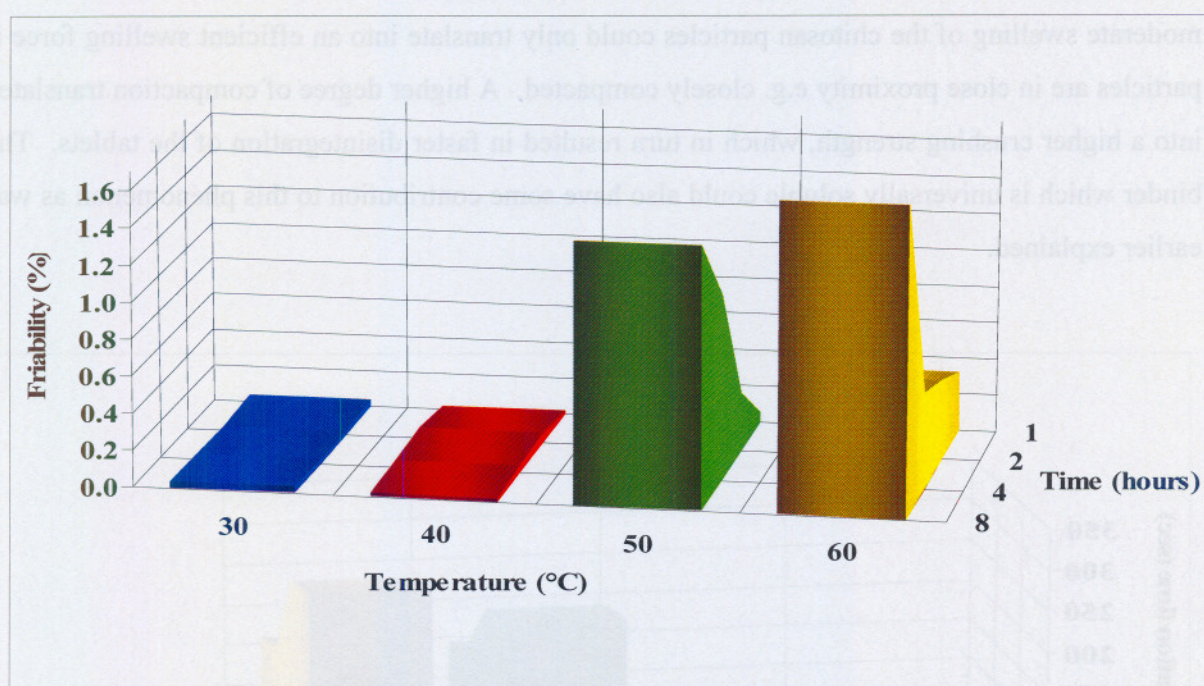


Figure 5.3: *The effect of elevated temperatures and time on the percentage friability of tablets containing chitosan.*

Hardness and friability are both parameters that can be used in the measurement of the mechanical strength of tablets. A ratio or index which incorporates both these two parameters, namely the hardness-friability index (HFI), can therefore be determined and used to equate the effect of exposure to temperature on the mechanical strength of tablets (de Kock, 2002:78, 88). Generally a higher crushing strength would be accompanied by a decrease in friability, thus resulting in an increase in the HFI. It was evident that tablets of the different chitosan samples signified ultimate HFI at a temperature of 30 °C after 2 hours (6000.00 N.%⁻¹), whereas the lowest HFI-value was found at 60 °C after 8 hours (18.36 N.%⁻¹). A conclusion could be drawn,

namely, that tablets produced from chitosan samples and exposed to 30 and 40 °C, produced optimal results in terms of the HFI.

Despite the exclusion of a disintegrant, all of the tablets produced from the chitosan samples illustrated prodigious disintegration (figure 5.4). However, exposure to elevated temperatures with the elongation of time seemed to have an effect on the disintegration of the different tablets. The higher temperatures (50 and 60 °C) proved slightly detrimental to the disintegration process compared to the lower temperatures. Explanations for this phenomenon could relate to the enhancement in tablet strength afforded by the concentration moisture of the chitosan samples. A relationship between these parameters could thus be suggested under these conditions. The moderate swelling of the chitosan particles could only translate into an efficient swelling force if particles are in close proximity e.g. closely compacted. A higher degree of compaction translated into a higher crushing strength, which in turn resulted in faster disintegration of the tablets. The binder which is universally soluble could also have some contribution to this phenomenon as was earlier explained.

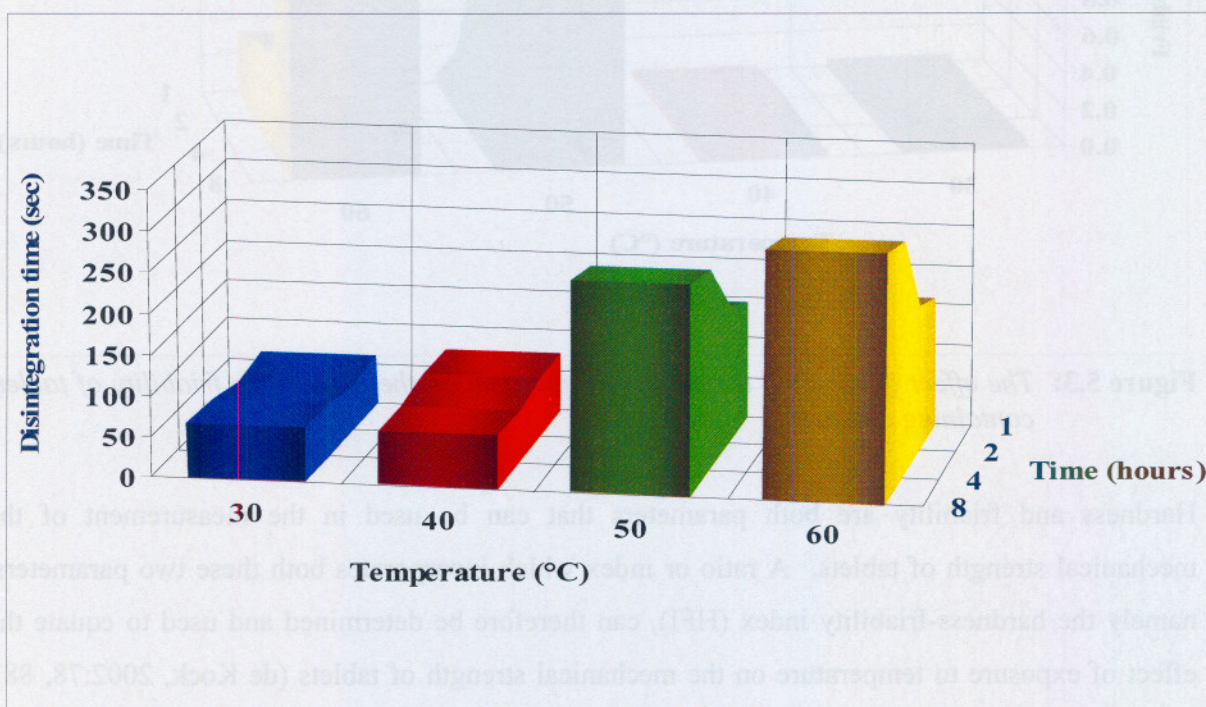


Figure 5.4: The effect of elevated temperatures and time on the disintegration time of tablets containing chitosan.

Varying times at lower temperatures did not significantly affect the disintegration process ($p > 0.05$), which was in concurrence with results found for the variation in the average crushing strength of the tablets (figure 5.2) at these temperatures. However, an explicit detrimental effect ($p < 0.05$) on the average disintegration time of tablets produced from the various chitosan samples was found at higher temperatures. Thus, the time chitosan powder was exposed to higher temperatures also played an important role in the disintegration process of the chitosan tablets. This was again, probably due to the extended time in which dehydration of the material could commence. The dehydration of the chitosan samples led to decreased crushing strengths due to decreased particle-particle interaction, therefore, inhibiting efficient swelling and thus, extending the disintegration process.

5.2.2 Concluding remarks

It was clear that the exposure of chitosan powder to elevated temperatures for a period of 8 hours resulted in marked differences in the physical properties of the tablets. In addition, it was evident that the presence of moisture (sorbed water) had a considerable influence on the tabletability and disintegration of chitosan powder. Furthermore, it could be deduced that chitosan is a polymer, which exhibits time-dependent tableting and disintegration performance sensitivity to temperature fluctuation. These results were in accordance with results found by Aucamp (2004:106).

5.3 Long-term stability

Accelerated stability testing with temperature and humidity as the stress conditions enhances product ageing (simulating long term storage conditions but a shorter time span). Evaluation of tablets under extreme conditions can simulate the aging of the product under the conditions for which tablets were manufactured in the first place (e.g. air-conditioned pharmacy) or for extremely inappropriate conditions (storage in extreme humidity).

The stability of chitosan raw material (powder), pure chitosan tablets, chitosan- Kollidon® VA64 tablets (prepared from both physical mixtures and granulations) was investigated for 6 months at 20 °C/60% RH and 40 °C/75% RH according to the method described in section 3.6.2. The formulations were tableted into tablets with a diameter of 5 mm prior to or after subjection to varying conditions. Samples were collected monthly. Sampled chitosan tablets and compressed

raw material of both sets of stress conditions were analysed for assessment in the influence of elevated temperature and relative humidity on the physical properties of chitosan tablets and chitosan raw material. The weight variation, crushing strength, percentage friability and disintegration results of the tablets and raw material were evaluated and are presented in annexure D.2.

5.3.1 Evaluation of long-term stability testing on the physical properties of pure chitosan powder and chitosan tablets

The explanation of the differences seen in weight variation for the evaluated period is better explained from the perspective of a graphical display (figure 5.5). Considering the total tablet weight of the 5 mm tablets, it was noted that a significant difference in weight variation was detected for the two sets of conditions. The 40 °C/75% RH condition (40/75) seemed to be affected slightly more than the 25 °C/60% RH (25/60) condition. The chitosan powder which was tableted after being subjected to the different conditions also presented noteworthy differences from the chitosan tablets subjected to the same conditions. Relatively small standard deviations (tables D.2.1 to D.2.4 in annexure D.2) indicated that all tablets were equally affected by ambient conditions.

The most pronounced effect was observed for chitosan powder samples at 40 °C/75% RH (40/75). A considerable decrease in tablet weight was observed from month 0 to 6. The decrease in tablet weight for this period represented a weight loss of almost 26%. The limited presence and distribution of moisture in various forms was clearly illustrated in figure 5.5. Tablets subjected to the 25/60 condition depicted the smallest decrease in tablet weight (7.17% w/w). As mentioned before, moisture can be present in powders in different physical forms i.e. as absorbed monolayers or multi-layers on the particle surfaces, condensed water on the surface, physically absorbed water within the particles, or as strongly bound chemisorbed water. The presence and distribution of moisture in these forms will depend considerably on the chemical nature of the particulate material, its physical properties such as particle size and porosity, and on the ambient relative humidity which determines the equilibrium moisture content (Garr & Rubinstein, 1992:188). Dehydration of residual moisture from chitosan tablets was less prominent due to limitation of water vapour escaping the tablets. This was probably a result of an increase in particle-particle contact areas, surface energy and adhesive forces.

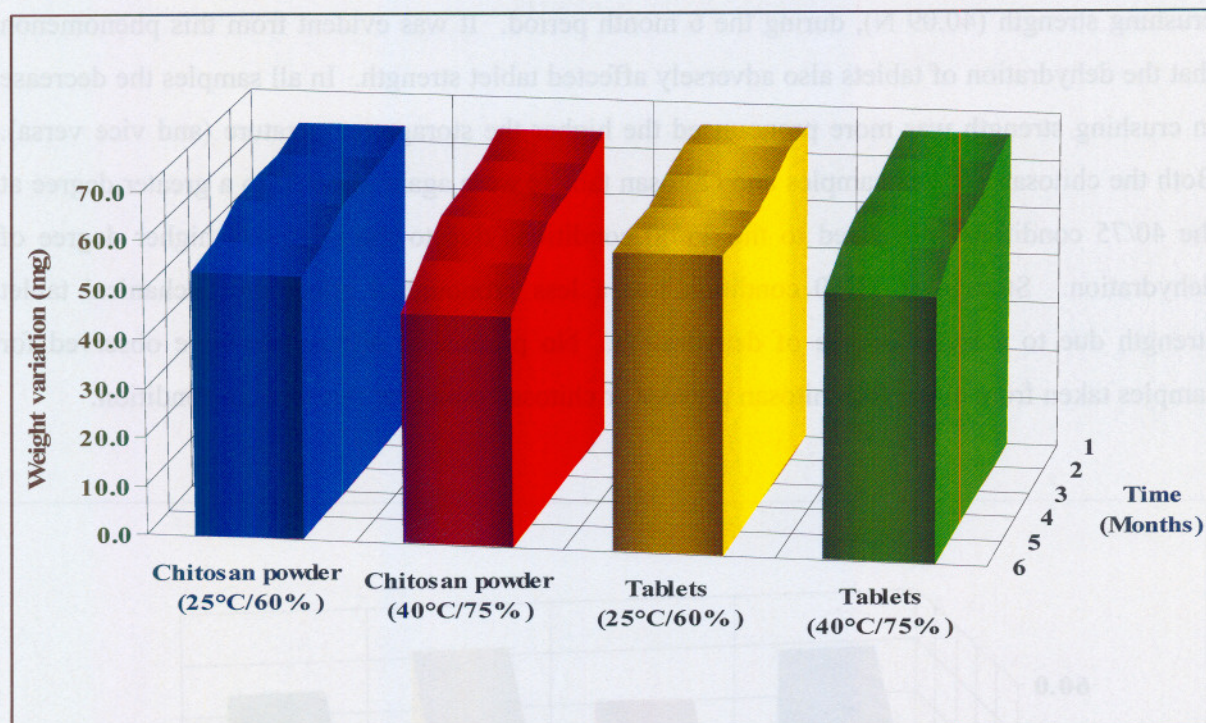


Figure 5.5: *Weight variation of tablets compressed from chitosan powder after subjection to ambient conditions for 6 months and chitosan tablets subjected to the same conditions and time period.*

From comparison with the 25/60 storage condition, it was evident that a relatively higher ambient temperature (40/75) had a detrimental effect on tablet weight (11.4% w/w). Tablets stored at the 25/60 condition seemed to be the most stable, indicating that storing chitosan tablets at room temperature and 60% RH will provide the most stable conditions for storage and therefore limit the progressive deterioration of the tablets. Furthermore, tablet weight did not vary significantly during the evaluation of the long term stability testing. The chitosan powder and the chitosan tablets produced samples of comparable weight. Due to the relatively low ambient temperature the dehydration of lattice water (Otto, 2002:66) cannot be catalysed to the same extent as at higher temperatures and therefore, the lesser degree of weight loss at the 25 °C condition compared to the 40 °C set up was observed.

A comparable deteriorating effect on the crushing strength for all the samples could be observed over a period of 6 months (figure 5.6). This deterioration of tablet strength could be attributed to changes of bound particles due to the dehydration and aging of particles. The most marked decrease in terms of crushing strength was illustrated during the first monthly interval (month 1 – 2). Chitosan powder subjected to the 40/75 condition depicted the most significant decrease in

crushing strength (40.09 N), during the 6 month period. It was evident from this phenomenon that the dehydration of tablets also adversely affected tablet strength. In all samples the decrease in crushing strength was more pronounced the higher the storage temperature (and vice versa). Both the chitosan powder samples and chitosan tablets were again affected to a greater degree at the 40/75 conditions compared to the 25/60 conditions due to the relatively higher degree of dehydration. Storage at 25/60 condition had a less pronounced effect on mechanical tablet strength due to a lesser degree of dehydration. No prominent differences were observed for samples taken from either the chitosan powder or chitosan tablets at this storage condition.

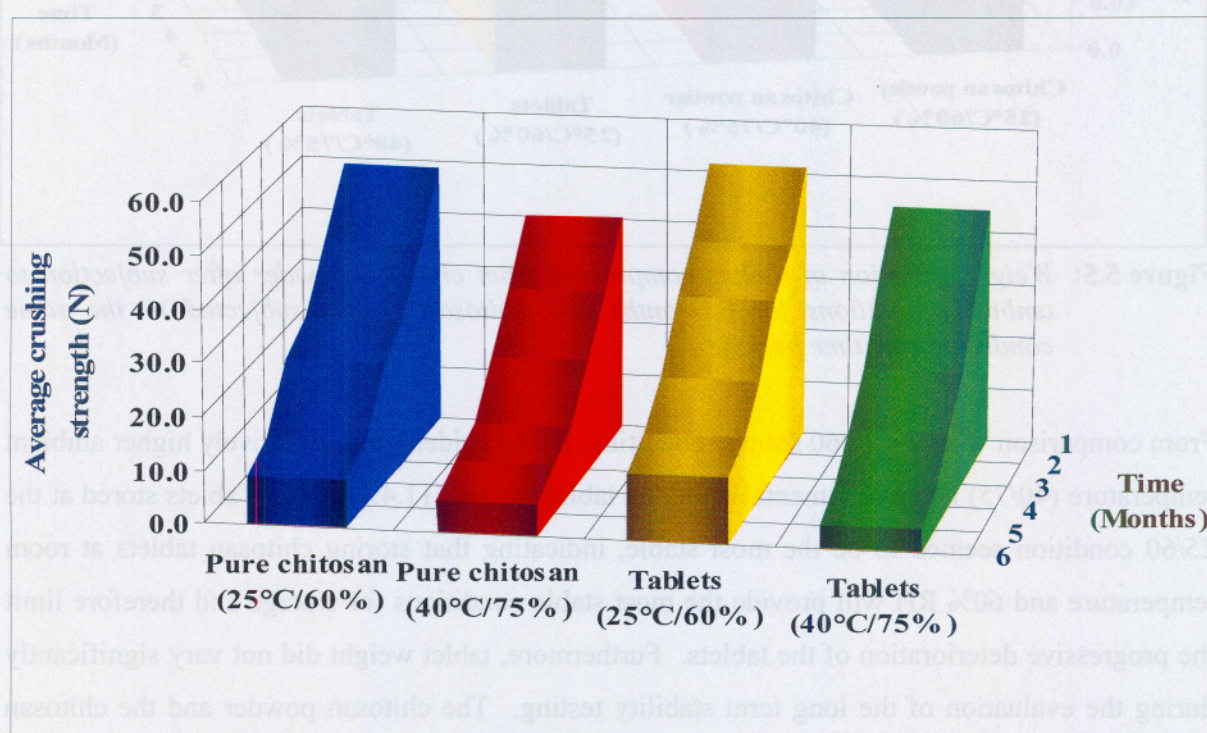


Figure 5.6: Average crushing strength of tablets compressed from chitosan powder after subjection to ambient conditions for 6 months and chitosan tablets subjected to the same conditions and time period.

As described in chapter 4, tablet friability is also an important factor to be considered when examining tablet characteristics because it is also an indication of the degree of mechanical strength of tablets. A significant increase in friability was observed, with the 40/75 samples once again producing the most significant changes in friability (figure 5.7). Friability for the tablets changed relatively slow up to the third month after which the friability increased more rapidly

during the last 3 months. The chitosan powder, however, showed a continuous increase in friability from the start.

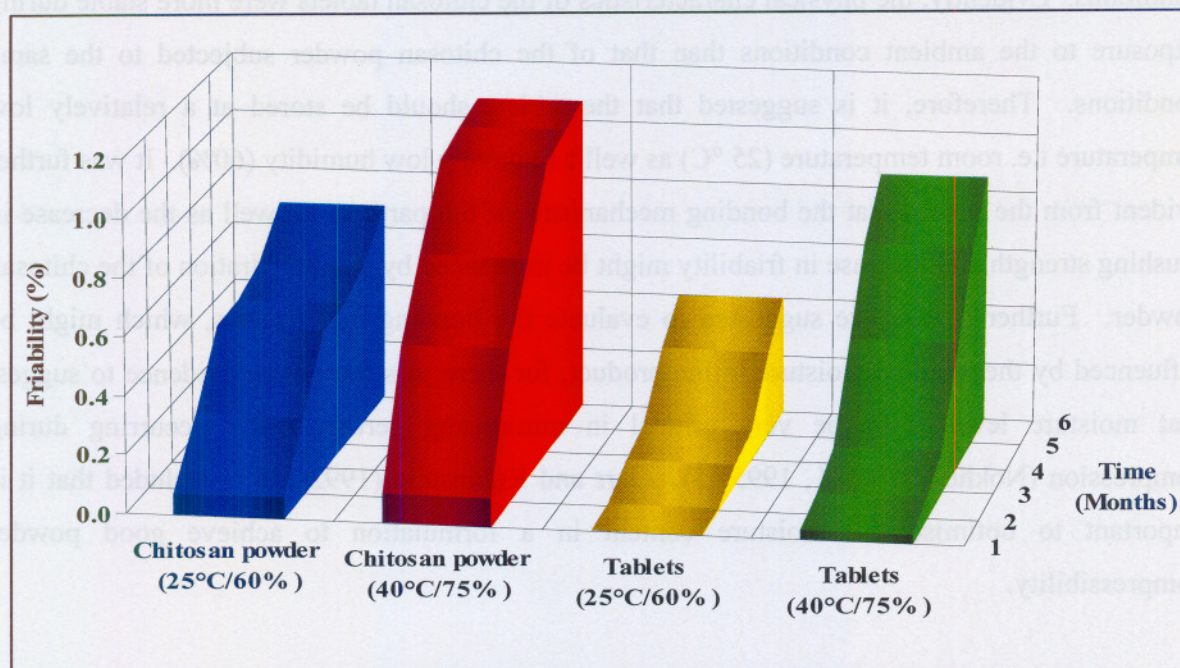


Figure 5.7: *Friability of tablets compressed from chitosan powder after subsection to ambient conditions for 6 months and chitosan tablets subjected to the same conditions and time period.*

When considering tablet quality, the tableting of chitosan seemed to be advantageous to the chitosan powder stored at the same ambient conditions for the same time period. Unacceptable tablets were produced from chitosan powder subjected to the 40/75 condition only after 5 months. None of the other samples exposed to stress conditions produced unacceptable tablets after 6 months. This would suggest that care should be taken when storing chitosan powder. Chitosan tablets, on the other hand, were less prone to friability when exposed to these conditions. Once again, a correlation was observed between mechanical strength and moisture loss as a function of time (section 5.2.1).

None of the samples exposed to the different conditions disintegrated within the set time limit, but the tablets did break into various pieces which were too large to fall through the disintegration baskets. This was probably due to the lack of the inclusion of a disintegrant as well as insufficient swelling of the chitosan particles due to dehydration.

5.3.2 Concluding remarks

The long-term stability of the raw material was significantly affected by varying ambient conditions. Evidently, the physical characteristics of the chitosan tablets were more stable during exposure to the ambient conditions than that of the chitosan powder subjected to the same conditions. Therefore, it is suggested that the tablets should be stored at a relatively low temperature i.e. room temperature (25 °C) as well a relatively low humidity (60%). It was further evident from the results that the bonding mechanisms of the particles as well as the decrease in crushing strength and increase in friability might be influenced by the dehydration of the chitosan powder. Further studies are suggested to evaluate the bonding mechanisms, which might be influenced by the residual moisture in the product, for there was increasing evidence to suggest that moisture levels may be very critical in minimising certain faults occurring during compression (Nokhodchi *et al.*, 1995:13). Garr and Rubinstein (1992:188) concluded that it is important to optimise the moisture content in a formulation to achieve good powder compressibility.

5.3.3 Evaluation of long-term stability testing on the physical properties of chitosan mixtures and chitosan granules

Since it was shown that chitosan tablet formulations necessitate the inclusion of a binder, it was imperative to investigate the stability of mixtures and granulations of chitosan with a binder. Therefore, chitosan was mixed with 5% w/w Kollidon® VA64 (chitosan mixtures) as described in section 3.6.2 and tableted into tablets with a diameter of 5 mm. Chitosan granules comprising of 5% w/w Kollidon® VA64 were also produced as described in section 3.5.1.2 and tableted. These tablets were stored at 25 °C/60% RH and 40 °C/75% RH for 6 months. Monthly samples were collected with a subsequent physical analysis of the tablets.

5.3.3.1 Physical analysis of tablets

During the 6 month period a significant gradual deterioration in tablet weight was observed for the two sets of conditions. The (40/75) condition seemed to be affected slightly more than the (25/60) condition and there were distinct differences noted between tablets comprising of chitosan mixtures and chitosan granules (figure 5.8). Relative small standard deviations (tables

D.2.5 to D.2.8 in annexure D) indicated that all tablets were equally affected by ambient conditions.

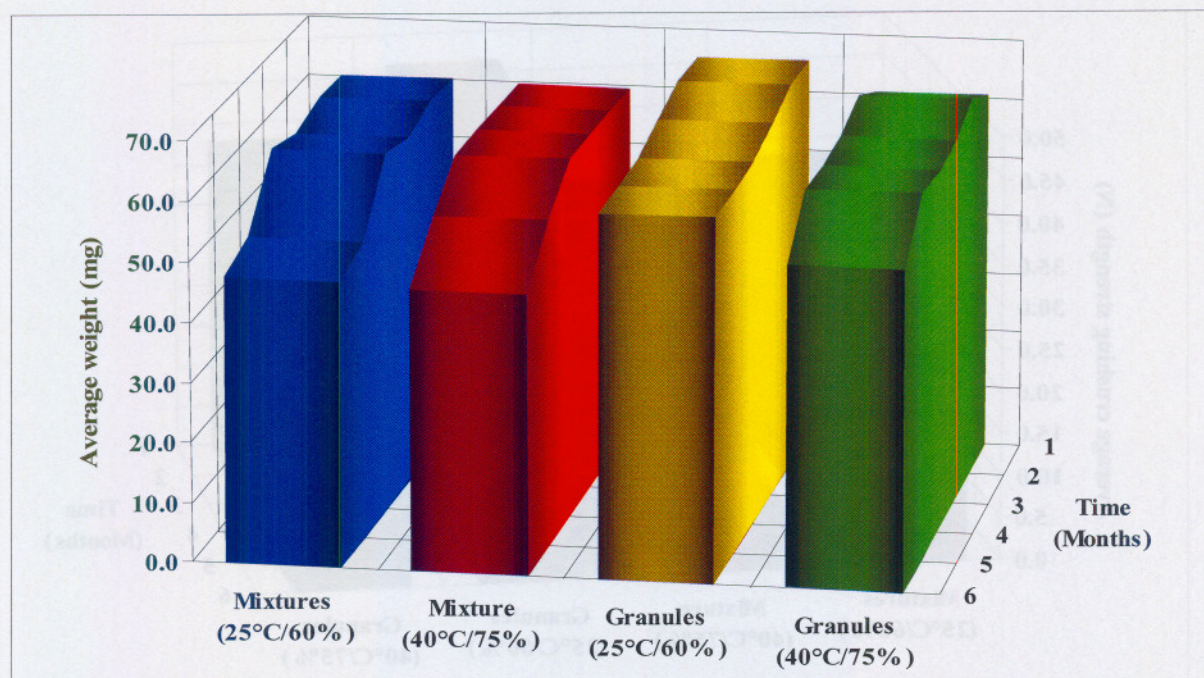


Figure 5.8: Weight variation of tablets compressed from chitosan mixtures and chitosan granules after subjection to ambient conditions.

The most pronounced weight loss (12.6 mg) over 6 months was seen for the tablets comprising of the chitosan mixtures that were stored under the 40/75 condition. However, chitosan mixtures stored under the 25/60 condition depicted approximately the same weight loss (12.5 mg) over the same time period. In comparison, tablets comprising of chitosan granules subjected to the (25/60) condition depicted a significantly smaller decrease in tablet weight (7.07% w/w) over the same time period. It was therefore clear that chitosan granules were less affected by ambient conditions than chitosan mixtures as less dehydration of these tablets were observed over a 6 month period.

Marked differences in crushing strength were also illustrated for the tablets evaluated in the long-term stability tests (annexure D.2 and figure 5.9). It was evident that the crushing strength was negatively affected by ambient conditions in a time dependent manner. This decrease in crushing strength was in concurrence with the decrease in tablet weight (figure 5.8), and this deterioration

of tablet strength could again be attributable to changes of bound particles due to the dehydration and aging of particles.

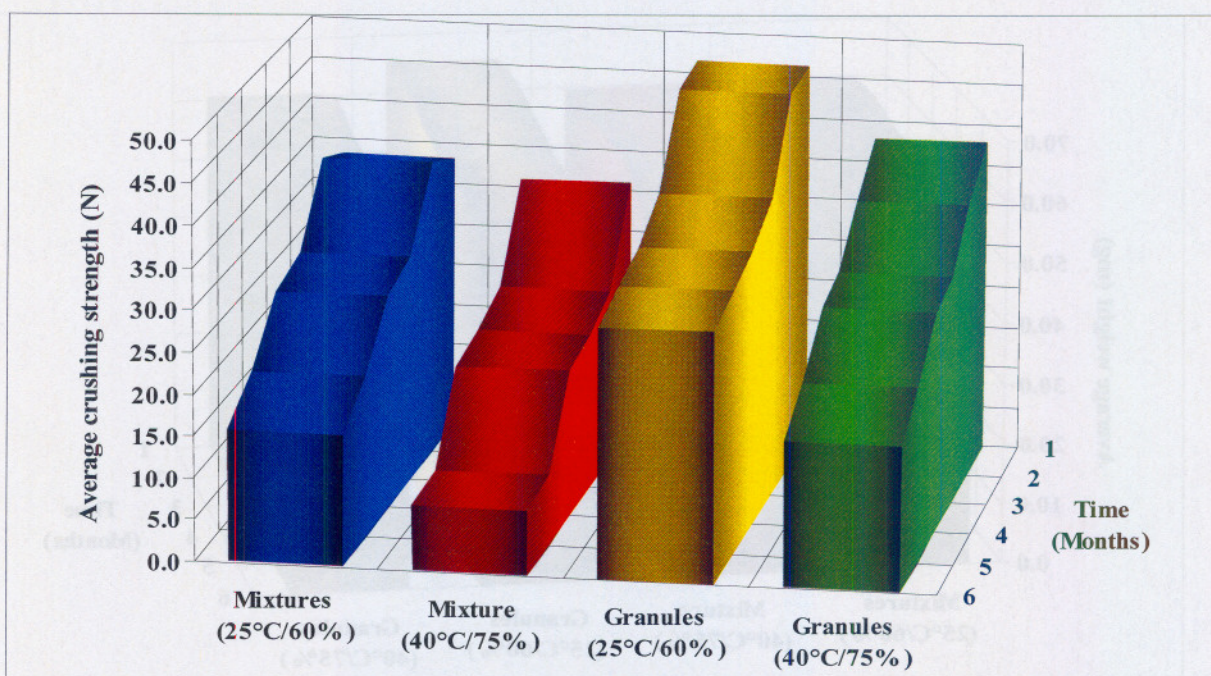


Figure 5.9: Average crushing strength of tablets compressed from chitosan mixtures and chitosan granules after subjection to ambient conditions.

The first two monthly intervals produced the most marked decreases in terms of crushing strength for tablets produced from either chitosan mixtures or chitosan granules. Tablets produced from chitosan mixtures subjected to the 40/75 condition depicted the most significant decrease in crushing strength, with a total decrease of 23.14 N, whereas tablets produced from chitosan granules stored at the 25/60 condition depicted the most stable results (decrease of only 16.3 N). The effect of ambient temperature was illustrated by the relatively larger decrease in crushing strength for the higher temperature analysis than for the lower temperature. Therefore, again a correlation existed between the dehydration of tablets and tablet strength. Furthermore, mixtures or granules of chitosan proved to be less affected by ambient conditions than chitosan powder or tablets. However, when crushing strength is taken into consideration, it was clear that chitosan granules proved superior when stored under these conditions.

Considering the friability, a more objective conclusion could be drawn in terms of the tablet strength as seen in figure 5.10. As expected, the 40/75 samples produced tablets of the highest

friability tendency, whereas the 25/60 condition did not affect the tablets to the same extent. This effect was more pronounced for tablets produced from the chitosan mixture.

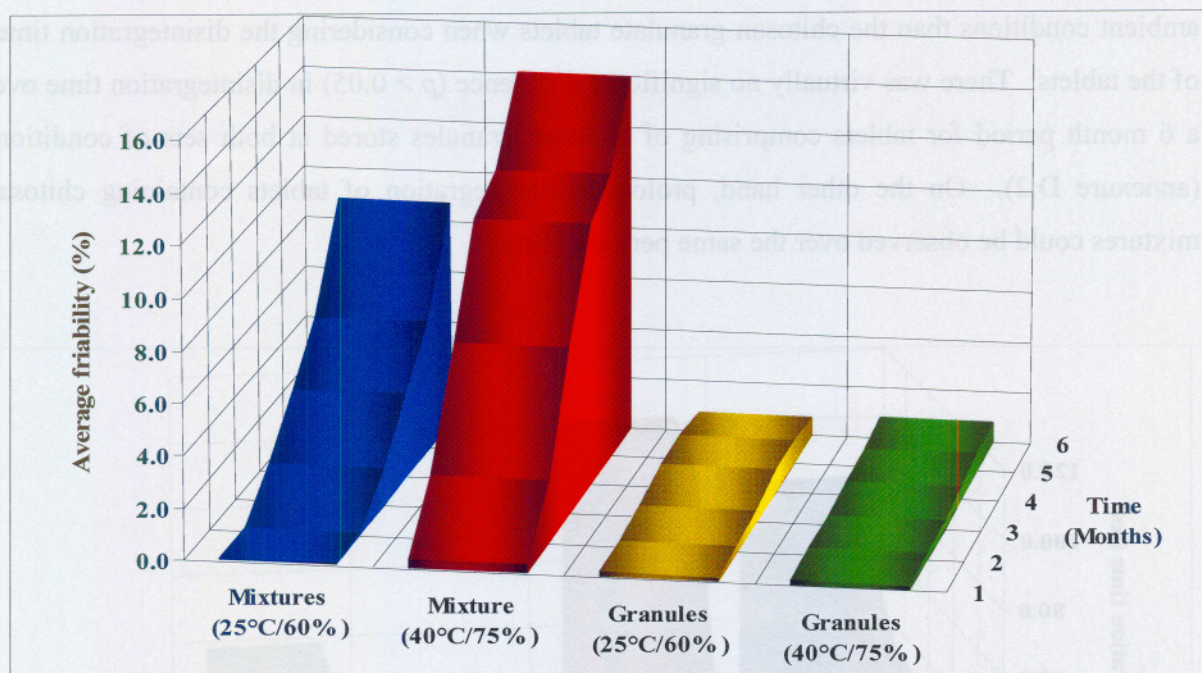


Figure 5.10: Friability of tablets compressed from chitosan mixtures and chitosan granules after subjection to ambient conditions.

Tablets comprising of the chitosan mixture were more significantly affected by ambient conditions in terms of friability and produced tablets that was unacceptable when sampled after 3 months. This phenomenon was probably due to the lower crushing strengths attained by tablets from the chitosan mixture. The tablets produced from chitosan granules on the other hand, depicted friability values after 6 months that were still acceptable (annexure D.2, i.e. < 1%). Furthermore, the analysis of tablets containing granulate in terms of friability revealed that these tablets compared favourably or even superior to tablets which contained only chitosan.

Unlike the chitosan powder or chitosan tablets, both sets of tablets containing either chitosan mixture or chitosan granules disintegrated within the set time limit of 15 minutes (900 seconds). This was probably due to inclusion of Kollidon[®] VA64 which is universally soluble as well as to the enhancement in tablet strength afforded by the concentration moisture of the chitosan samples. A relationship between these parameters could thus again be suggested under these conditions. The moderate swelling of the chitosan particles could only translate into an efficient

swelling force if particles are in close proximity e.g. closely compacted. Thus, deterioration in crushing strength resulted in slower disintegration of the tablets.

It was evident from figure 5.11 that tablets from chitosan mixtures were more sensitive to ambient conditions than the chitosan granulate tablets when considering the disintegration times of the tablets. There was virtually no significant difference ($p > 0.05$) in disintegration time over a 6 month period for tablets comprising of chitosan granules stored at both sets of conditions (annexure D.2). On the other hand, prolonged disintegration of tablets containing chitosan mixtures could be observed over the same period of time.

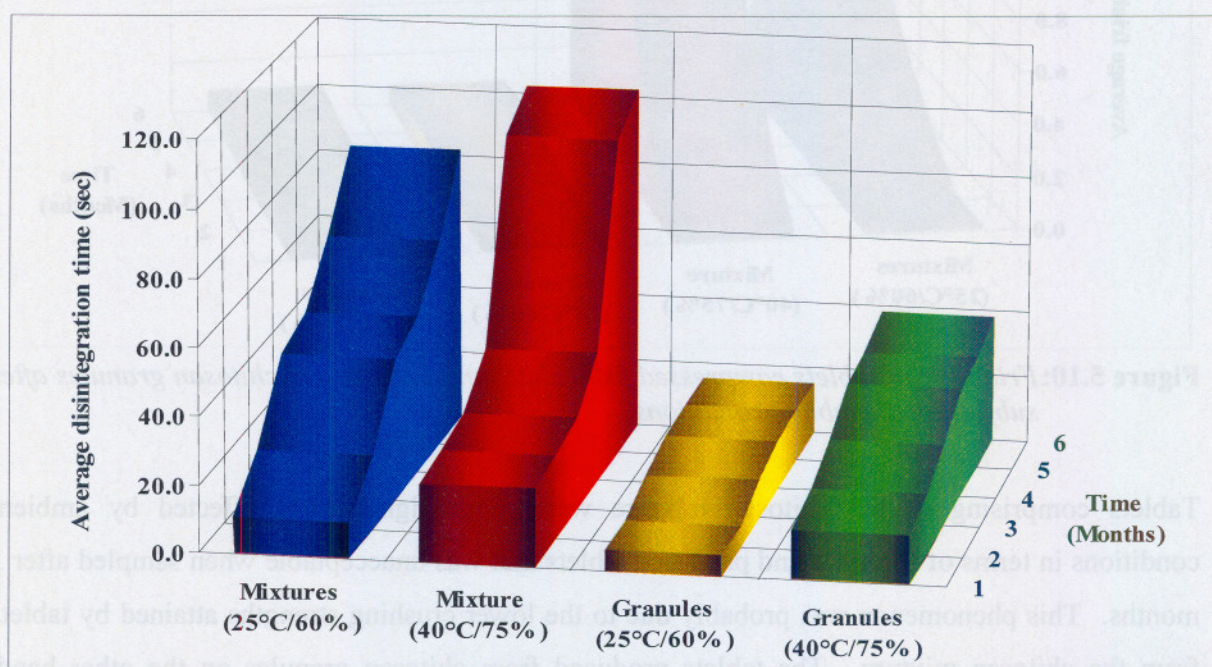


Figure 5.11: Average disintegration time of tablets compressed from chitosan mixtures and chitosan granules after subjection to ambient conditions.

The 40/75 condition seemed to have a slightly more pronounced time-dependent effect on both sets of the tablets. Thus, the time chitosan mixtures are exposed to ambient conditions also play an important role in the disintegration process. This was again, probably due to the extended time in which dehydration of the material could commence. The dehydration of the chitosan mixtures was more pronounced (evident from the tablet weight results) than the chitosan granules due to decreased particle-particle interaction, therefore, inhibiting efficient swelling and thus, extending the disintegration process.

5.3.4 Concluding remarks

The long-term stability of chitosan mixtures seemed to be affected to a larger extent by ambient conditions than chitosan granules was. However, tablets produced from either mixtures or granules of chitosan proved more stable during the analysis than pure chitosan powder or tablets. The optimum conditions for storage of these tablets also required a low temperature (25 °C) and a low humidity (60%). It was further evident from the tests conducted that the bonding mechanisms of the particles as well as the decrease in crushing strength and increase in friability and disintegration might be influenced by the dehydration of the chitosan mixture or granules. Additionally, it was evident that the presence of Kollidon® VA64 as well as the method of inclusion of this binder influenced the stability of chitosan tablets.

5.4 Conclusion

Moisture may have marked effects on surface tension, compression, tablet tensile strength and tablet toughness, density of the materials, binding capacities and lubrication properties in addition to having hydrodynamic properties.

The stability of chitosan tablets was significantly affected by varying ambient conditions regardless of the inclusion of Kollidon® VA64, as chitosan easily dehydrate. It was furthermore evident that the presence of moisture (sorbed water) had a considerable influence on the tabletability and disintegration of chitosan powder. Therefore, it is suggested that the chitosan tablets should be stored at relatively low temperatures (± 25 °C), which also coincides with a relatively low humidity (60%). Another interesting observation was that the presence of Kollidon® VA64 as well as the method of inclusion of this binder influenced the stability of chitosan tablets. Direct compression of chitosan granules, which comprised of 5% w/w Kollidon® VA64, produced the optimal system in terms of product stability. Moisture content must thus be optimised in chitosan formulations to achieve good powder compressibility and evaluation of chitosan tablets should also be performed at consistent time intervals due to the fact that there is a time-dependent influence on the physical properties.

CHAPTER 6

EVALUATION OF THE EFFECT OF EXCIPIENTS ON THE TABLET PROPERTIES OF CHITOSAN MINITABLETS

6.1 Introduction

Successful application of direct compression in pharmaceutical processes depends on the appropriate selection of suitable excipients that are free-flowing, highly compressible, soluble, physiologically inert and chemically compatible with the active ingredient(s). The physicochemical properties of directly compressible filler/binders mainly determine the physical properties of tablets due to their high concentration in the final tablet mass.

From chapter 4 it was concluded that chitosan depicted poor flowability and produced tablets of insufficient tablet strength. Another drawback was that the tablets possessed little disintegration propensity and this could seriously affect the pharmaceutical availability of active ingredients from immediate release tablets. Furthermore, chitosan is a cationic polyelectrolyte, which forms a gel structure in acidic pH mediums and has also been found to interact with anionic compounds (chapter 2). Therefore, the possibility exists that chitosan may interact with either the drug or excipients, consequently influencing the dissolution of the drug or even that of chitosan. Modifications of the undesirable properties of the filler are thus imperative.

A method of improving the mechanical strength is to reduce the tablet size through the production of minitables. Chapter 1 described the reason for these tablets rendering mechanical stability equal to, and at high pressures higher than, that of normal-sized tablets, as being the ratio between the outer surface and volume of the tablet. Another method is to include a binder through either physical mixing, or granulation of the filler and binder. Granulation might also improve the flowability of the chitosan filler-system and the inclusion of buffering agents or disintegrants, should enable the establishment or improvement of disintegration properties of chitosan. Therefore, the production of chitosan minitables and a systematic evaluation of the

inclusion of various excipients via different methods were conducted in this chapter. Dissolution studies were furthermore imperative in order to determine the effect that the inclusion of excipients and the inherent properties of chitosan would have on drug release.

6.2 Improvement of the compactability of chitosan

Results from the previous chapters illustrated some contradictory properties regarding the mechanical strength of chitosan tablets. It was concluded that although the mechanical strength of the tablets was relatively low, the tablets exhibited acceptable to good friability. Both these strength parameters are to some extent dependent on the compactibility of the filler and modification of this attribute might prove beneficial to performance and stability of the finished product. A method of modification of the compactibility properties of the filler is through the inclusion of excipients that are recognised for their bonding capability. In the case of direct compression, these excipients are commonly known as binders since their effect are to be exerted by blending and compression procedures alone (Otto, 2002:71).

6.2.1 Background to binders in tablet formulations

Binding agents are frequently incorporated in formulations that produce tablets of inadequate mechanical strength and it is generally considered that binders should be highly compactible. Alternatively, it has been suggested that binders which are amorphous in structure and highly deformable, provide a large surface-area for bonding. This deformation should preferably occur by plastic deformation (Kolter & Flick, 2000:1160). The addition of binders, therefore, results in an increase in particle cohesiveness (Joneja *et al.*, 1999:1130; Otto, 2002:37), however, the exact mechanism of the improvement of mechanical strength of tablets remains uncertain (Mattsson, 2000:18). Ideal binders are highly deformable and show limited elastic recovery. The intrinsic tensile strength of the binders is an important contributor to tablet tensile strength, especially for binders that are not highly deformable. These considerations could prove paramount in the prevention of capping and lamination of tablets during compression (Mattsson & Nyström, 2001:184; Sugimori *et al.*, 1989:1067).

As with other excipients, the choice of an appropriate binder is a rational one. Extensive knowledge of the properties of the binding material is prerequisite for evaluation of its effect on

mechanical strength. The binder should be evaluated in combinations with other compression materials to determine its interaction with these substances. One property that is impeded by the addition of binders, even water-soluble ones, is the disintegration efficiency and therefore the use in tablet formulations should be limited, not because binders are unnecessary, but because of the two-way action of the binders themselves (Hwang & Parrott, 1993:507; Joneja *et al.*, 1999:1130). The functionality of the binder should thus be revealed by these interactions and should guide the formulator to choose the most suitable binder (Mattsson, 2000:18). Dry binders are in general less effective compared to their binding capacity in wet granulation compositions (Nyström *et al.*, 1982:209).

Commercially available binders include: hydroxypropylcellulose, hydroxyethylcellulose, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, povidone, and starch (Joneja *et al.*, 1999:1130). Various polyethylene glycol grades are also employed as binders (Mattsson & Nyström, 2001:182; Larhrib & Wells, 1998:200), however, in this study only two binders which may be used in wet granulation or direct compression, namely Kollidon[®] VA64 and Methogel[®] K100M were evaluated and compared in chitosan formulations due to their different contributions to the tablet properties.

6.2.1.1 Vinylpyrrolidone-vinyl acetate copolymer (Kollidon[®] VA64)

Water-soluble vinylpyrrolidone-vinyl acetate copolymer contains the two components, vinylpyrrolidone and vinyl acetate, in a ratio of 6:4. It is produced in the same way as soluble polyvinylpyrrolidone, by free-radical polymerisation reaction where a chain structure is obtained. In contrast to the soluble grades of Kollidon[®], the number, 64 in the trade name, is not a K-value but the mass ratio of the two monomers. The K-value, in actual fact, approximately corresponds to that of Kollidon[®] 30 (Bührer, 1993:191).

A pronounced difference is seen in the hygroscopicity between the copolymer and the other Kollidon[®] grades. The copolymer absorbs three times less moisture under equivalent humidity conditions. This property can lead to an improvement in tablet stability compared to the other grades (Bührer, 1993:199). The lack of moisture sorption renders this material superior to the other Kollidon[®] grades concerning its film coating application. This is due to the vinyl acetate subunit that is comparatively more hydrophobic than the vinylpyrrolidone, thus, leading to the formation of less brittle films than those produced by native polymer. Because of the ratio of

vinylpyrrolidone to vinyl acetate in Kollidon[®] VA64, it is almost as universally soluble as povidone. It dissolves in extremely hydrophilic liquids such as water as well as in more hydrophobic solvents such as butanol. This gives the product its favourable properties as a soluble binder and film-forming agent, particularly for solid dosage forms (Bührer, 1993:193).

The particle size distribution provides Kollidon[®] VA64 with good flow properties and the bulk and tap density is relatively low and does not increase much with powder bed movement. The concentration range of effective binding capacity ranges between 2-5% w/w. Another important property of Kollidon[®] VA64, is its degree of plasticity, a property that povidone does not possess. Plasticity provides granules and mixtures that are less susceptible to capping during compression and tablets that are less brittle. A smaller incidence of sticking of tablets that contain the polymer, to compression tooling is also seen under humid conditions (Bührer, 1993:215). As is the case for povidone, copovidone is extremely suitable for direct compression and ensures superior final product stability due to its low moisture sorption (Bührer, 1993:219).

6.2.1.2 Hydroxypropylmethyl cellulose (Methocel[®] K100M Premium EP)

Hydroxypropylmethylcellulose (HPMC) polymers are widely used as binders (more regularly as wet binders) or in the production of hydrophilic tablet matrices in order to control drug release. Various grades of HPMC are available which differ in molecular size, chemical substitution and particle size, allowing selection of the most suitable HPMC for the desired tablet properties. Methocel[®] K100M Premium EP contains a low methoxy/hydroxypropyl ratio of approximately 2.65 (represented by the K in the trade name) and has an apparent viscosity of 80000-120000 cP (nominal value 100 000 cP i.e. the 100 in the trade name). This powder grade depicts a higher apparent density and tends to comprise of irregularly shaped particles with fewer fibres, undergoing a somewhat more pronounced fragmentation during compaction. However, the fragmentation propensity may be considered as low compared to many other pharmaceutical excipients. The resulting compacts have a larger surface area, and thereby a larger number of potential interparticulate bonding points, i.e. hydrogen bonds. Relatively strong tablets with a high porosity are formed. In addition, mechanical interlocking may also contribute to the tensile strength, which together with porosity seems to reach a plateau when compacted at 100 MPa. Further increase in compaction load only results in elastic energy which does not assist the development of bonds (Colorcon, 2000; Gustafsson *et al.*, 1999:183; Nyström *et al.*, 1993:2143-2196; Pesonen & Paronen, 1990).

Dahl *et al.* (1990:1-10) suggested that the high tensile strength of Methocel® is the result of the relatively low methoxy group content, the high hydroxypropyl group content and the relatively high moisture content of the powder (6.2%), all of which may contribute to the development of relatively strong hydrogen bonds within the tablets. As water may act as a plasticizer and a binder as well as contributing to the hydration rate of the polymer, the water content may affect the tablet strength and also drug release from the matrices. In addition, Dahl *et al.* (1990:1-10) suggested that differences in the synthetic and recrystallisation procedures for the polymers might affect the dissolution profiles and that the high content of the hydroxypropyl substituent would favour the hydration rate making the domains of the HPMC particles more amorphous and thereby enhancing the dissolution process through increased swelling of the tablets and increased matrix gel strength (Gustafsson *et al.*, 1999:183; Mitchell *et al.*, 1993:154; Alderman, 1984:9).

6.2.2 Evaluation and comparison of the incorporation of two binders into chitosan minitabiet formulations

The initial phase of this stage of the study comprised of the comparison of the properties of minitabiet prepared from chitosan, physically mixed chitosan-filler systems (physical mixtures) and granulated chitosan-filler systems (granulated mixtures) which is described in section 3.5. The compositions of the mixtures prepared are summarised in table 6.1. The binders were included into the filler-systems either through physical mixing for 5 minutes or through wet granulation in two concentrations (3 and 5% w/w). Furosemide was then added and mixed for an additional 5 minutes. The mixtures were tableted at a compression setting of 50 (the highest and optimum setting) producing tablets of approximately 14 mg. Furosemide was included to monitor drug release properties (rate and extent of dissolution) of the different formulations and the influence that each binder had on the dissolution properties of a sparingly water-soluble and poorly water-wettable active ingredient. The inclusion of a lubricant was again disregarded as the mixtures did not necessitate any lubricant action.

Table 6.1: *Composition of chitosan mixtures.*

Component	Excipient	Filler mixture composition (% w/w)
Active ingredient	Furosemide	14.286
Filler	Chitosan	Qs to 100%
Binder	Kollidon [®] VA64 or Methocel [®] K100M	0, 3 or 5

6.2.2.1 Physical characterisation

The physical properties of the tablets were again evaluated according to previously explained procedures (section 3.5.3). Evaluation in terms of weight variation (an indirect evaluation of the flowability), crushing strength, thickness (tablet height), diameter, friability, and disintegration time was conducted. The results that were obtained for the different chitosan filler-systems are summarised in table 6.2.

All of the tablets exhibited surfaces of satisfactory appearance with no indication of frictional damage on the tablet edges. No significant differences ($p > 0.05$) were found in the tablet thickness or diameter of the different formulations. Furthermore, it is indicated that the tablet weight did not vary discernibly for the different mixtures despite the extensive percentage chitosan, and the inclusion of furosemide. The percentage relative standard deviation obtained for weight variation (%RSD < 3.15%) indicated insignificant intrabatch weight variation which illustrated exceptional flowability. The physically mixed formulations containing Kollidon[®] VA64 as binder portrayed slightly more desirable flow; however, this difference was omissible.

The inclusion of 14.286% w/w furosemide into the formulations had a slightly detrimental effect on the crushing strength (± 34.79 N) when compared with chitosan minitables containing no active ingredient and compressed at the same compression setting. Interestingly, the formulation containing no binder produced tablets which were slightly harder than formulations containing Kollidon[®] VA64 or Methocel[®] K100M, regardless the method of production used (i.e. physically mixed filler-systems or granulated filler-systems) or the concentration in which the binders were included. However, no significant differences in crushing strength could otherwise be found between formulations containing either of the binders and all of these tablets portrayed acceptable friability (< 1.00%) despite the low crushing strengths. From table 6.2 it could be concluded that all of the formulations resulted in tablets that almost disintegrated immediately (within 1 minute).

However, no significant difference could be found between the different formulations when disintegration time was considered.

Table 6.2: *Physical properties of the different chitosan filler-systems at a compression setting of 50. %RSD is indicated in parentheses.*

Formulation	Mass variation (mg)	Thickness (mm)	Diameter (mm)	Crushing strength (N)	% Friability	Disintegration time (sec)
0% Binder	14.24 (1.94)	1.67 (0.70)	3.00 (0.24)	30.93 (24.20)	0.069	11.00
3% KVA64 (mixture)	14.03 (1.37)	1.78 (0.49)	2.99 (0.26)	25.70 (17.57)	0.000	53.00
3% KVA64 (granulated)	13.49 (1.98)	1.66 (0.49)	2.98 (0.14)	27.66 (22.96)	0.228	20.83
5% KVA64 (mixture)	13.87 (1.26)	1.73 (0.37)	2.99 (0.23)	23.41 (20.20)	0.217	28.00
5% KVA64 (granulated)	14.07 (1.80)	1.63 (0.86)	2.99 (0.18)	20.44 (30.38)	0.213	8.00
3% HPMC (mixture)	13.54 (1.54)	1.78 (0.59)	2.99 (0.27)	28.99 (24.01)	0.224	16.00
3% HPMC (granulated)	14.04 (1.92)	1.70 (0.44)	2.99 (0.39)	31.32 (30.04)	0.412	15.50
5% HPMC (mixture)	13.24 (1.65)	1.63 (0.50)	2.99 (0.19)	26.09 (18.97)	0.501	11.17
5% HPMC (granulated)	13.56 (3.11)	1.67 (0.73)	2.97 (0.66)	16.43 (55.63)	0.998	17.33

KVA64 = Kollidon[®] KVA64; HPMC = Methocel[®] K100M

When considering the physical properties of the tablet formulations, no conclusive decision concerning the inclusion of a binder could be made. Therefore, one should take the drug release properties of the different formulations into consideration.

6.2.2.2 Evaluation of formulation variables on drug dissolution from physically mixed and granulated filler-systems

The physicochemical properties of the excipients in directly compressed tablet formulations play an important role in the release (disintegration) and dissolution of the active ingredient, especially in formulations containing low drug doses where the filler comprises more than 80% of the tablet composition. Thus, the final success of a formulation study is measured by the pharmaceutical availability i.e. the process of dissolution, of the drug from the tablet.

Dissolution is defined as a process by which a solid substance enters into a solvent to yield a solution (Banakar, 1991:1). The dissolution rate is determined by the amount of active ingredient that passes into solution per unit time under standardised conditions of liquid/solid interface, temperature and solvent composition. The most common theory for dissolution, the film theory (or diffusion layer model), espouses the assumption that dissolution belongs to the type of heterogeneous reaction where the rate is determined by the transport process (Abdou, 1989:11).

Dissolution is the rate-limiting step during absorption (preceding appearance of the drug in the systemic circulation) for sparingly water-soluble drugs, like furosemide. Usually slow dissolution rates; a low extent of dissolution due to inherent poor solubility (C_s) and a low concentration gradient ($C_s - C_t$) are exhibited for these drugs. According to the general dissolution equation, the rate of dissolution depends primarily upon the surface-area in contact with the surrounding medium (the effective surface-area of the drug). It could therefore be assumed that any factor, which affects the establishment of rapid contact between drug particles and the surrounding medium, could influence both the rate and extent of drug dissolution. The aim during tablet formulation is to optimise drug release and dissolution through manipulation of formulation variables such as the choice of the excipient type and the concentration; and through manipulation of process variables, especially the compression force. These factors not only determine the physical properties of the tablet, but also contribute or govern the release and dissolution of the drug from the tablet matrix.

6.2.2.2.1 Factorial design

A factorial design (2^3) was devised to study the effects of the binders on the drug release properties and dissolution properties of chitosan. The purpose of factorial designs is to establish significant interactions and effects of various combinations of variables (factors) and levels of these factors. In the case of formulation it provides an unbiased, systematic guide to evaluation of various excipients and their concentrations resulting in optimisation of these variables. In summary it could be stated that the use of this statistical tool eradicates the guesswork from experimentation (Steyn *et al.*, 2000:526).

6.2.2.2.2 Full factorial design as employed in the study

In order to evaluate the results from the various chitosan formulations, a full factorial design was employed (Ahlneck & Waltersson, 1986:148-149). In this experiment 3 formulations variables were evaluated, namely binder type, binder concentration and the method of production of the filler-system. Letters of the alphabet were assigned to the factors, e.g. A, B and C. All factors were evaluated on two levels. The levels were assigned as either 0 or 1 and implicated either low/absent (0) or high/present (1). A letter indicated the variable and a number the level, e.g. A₀ means that factor A is evaluated at its lowest level and A₀B₁ indicates that factors A and B are evaluated in combination and B resides at its highest level. Table 6.3 is a representation of the factor and level designation that was followed and table 6.4 indicates the combinations that were evaluated.

Table 6.3: Factors and level designation.

Factor	Designation	Level	
		0	1
Binder type	A	Kollidon [®] VA64	Methocel [®] K100M
Binder concentration (% w/w)	B	3	5
Method of production of the filler-system	C	Physically mixed	Granulated

Table 6.4: The factorial design employed to evaluate formulation variables and levels.

		Variable A: Binder type			
		A ₀ : Kollidon® VA64		A ₁ : Methocel® K100M	
		Variable B: Binder concentration			
		B ₀ : 3.0%	B ₁ : 5.0%	B ₀ : 3.0%	B ₁ : 5.0%
Variable C: Method of production of the filler system	C ₀ : Mixed	A ₀ B ₀ C ₀	A ₀ B ₁ C ₀	A ₁ B ₀ C ₀	A ₁ B ₁ C ₀
	C ₁ : Granulated	A ₀ B ₀ C ₁	A ₀ B ₁ C ₁	A ₁ B ₀ C ₁	A ₁ B ₁ C ₁

6.2.2.3 Dissolution studies

The ultimate method to evaluate the effect of formulation variables and tablet properties is through its effect on the rate and extent of drug release. Dissolution studies were performed in four fold in 0.1 M HCl for 150 minutes at 75 rpm. Each dissolution basket contained 10 minitables of a specific formulation (i.e. total of 20 mg of furosemide per basket) and the dissolution properties were evaluated according to the procedures described in section 3.5.4. A total of 8 formulations were evaluated in a full factorial design to determine the effects of each factor on the dissolution profiles of furosemide and chitosan. For each effect measured (i.e. initial rate of drug dissolution, the extent of drug dissolution and the percentage drug dissolved), the average response at each level of the different variables was calculated and compared to determine which level of each variable provided the best results for that particular effect. The best results for a particular level of a variable were the highest average value.

Two of the dissolution parameters (area under the curve, i.e. AUC and initial dissolution rate, i.e. DR_i) that were researched were normalised. Normalisation of data allows comparison of the AUC's (extent of dissolution) and DR_i 's (initial rate of dissolution from $t_0 - t_n$) of the various formulations. These normalisations abstract the basic constituents of the tracer formulations from the dissolution parameters and allow evaluation of the contribution that auxiliaries made to these parameters. This method is an adaptation of the method utilised to compare AUC's of various formulations to that of a suspension of an analyte (Vadas *et al.*, 1984:782). Calculations of these values made it possible to rank AUC and the respective DR_i values to determine the dissolution efficiency of the formulations. Equations 6.1 and 6.2 shows the equations utilised to normalise the dissolution parameters.

$$(AUC)_n = \frac{AUC_{formula}}{AUC_{baseline}} \quad [6.1]$$

Where AUC_n = normalised AUC, $AUC_{formula}$ = AUC of the formulation and $AUC_{baseline}$ = AUC of the reference (suspension).

$$(DR_i)_n = \frac{(DR_i)_{formula}}{(DR_i)_{baseline}} \quad [6.2]$$

Where $(DR_i)_n$ = normalised DR_i , $(DR_i)_{\text{formula}} = DR_i$ of the formulation and $(DR_i)_{\text{baseline}} = DR_i$ of the reference.

The calculations of the average values for each level of the different variables for each response are presented in annexure B. Table 6.5 summarise the average value calculated for each level of each variable for the effect measured.

Table 6.5: Average responses calculated from the responses measured for each variable at each level.

Furosemide suspension		AUC (µg.min.cm ⁻³)			DR _i (µg.cm ⁻³ .min ⁻¹)		
		2319.43			2.002		
Measured response		Variables					
		A ₀	A ₁	B ₀	B ₁	C ₀	C ₁
Furosemide	AUC (µg.min.cm ⁻³)	1598.74	1202.23	1435.00	1519.73	1425.81	1375.17
	DR _i (µg.cm ⁻³ .min ⁻¹)	0.085	0.045	0.046	0.043	0.069	0.061
	% Dissolved	33.935	27.400	30.395	34.931	32.127	29.208
	AUC _n	0.689	0.518	0.619	0.655	0.615	0.593
	(DR _i) _n	0.042	0.022	0.023	0.021	0.034	0.030
Chitosan	AUC (µg.min.cm ⁻³)	9765.71	9373.30	9341.01	10186.6	9835.99	9303.02
	DR _i (µg.cm ⁻³ .min ⁻¹)	2.627	2.745	1.756	2.563	2.871	2.501
	% Dissolved	53.739	50.920	51.386	56.490	53.425	51.234

The inherent poor solubility of furosemide in the dissolution medium (pH $\approx$ 1) was reflected in the fact that only $\pm$ 30% of the available drug dose (20 mg) dissolved within 150 minutes from the different chitosan formulations, respectively. Comparison of the dissolution parameters (DR_i and AUC) in table 6.5 clearly indicated the dependency of the AUC on the DR_i, i.e. the higher the DR_i, the higher the AUC. The decreased DR_i-values of both furosemide and chitosan, however, were contradictory to the prodigious disintegration of all the chitosan formulations which suggested that tablet disintegration is no absolute guarantee for drug dissolution.

Disintegration studies were performed in distilled water ($\text{pH} \approx 7$) where chitosan was not able to form a gel or dissolve, but the tablets simply separated into small particles, which caused the tablets to disintegrate rapidly. In the dissolution medium, however, the tablets did not disintegrate at all, but they bundled together on the surface of the medium and formed a gel. This formation produced a gel-like matrix through which the furosemide first had to diffuse prior to dissolution, thus prohibiting a rapid initial dissolution rate.

Figure 6.1 illustrates the difference in dissolution profiles of furosemide from the various chitosan tablet formulations. It could clearly be seen that formulations containing Kollidon[®] VA64 as binder produced tablets with significant faster and higher drug dissolution ($p < 0.05$) than formulations containing only chitosan or formulations which contained Methocel[®] K100M, regardless of the concentration of the binder or the method of production of the filler-system.

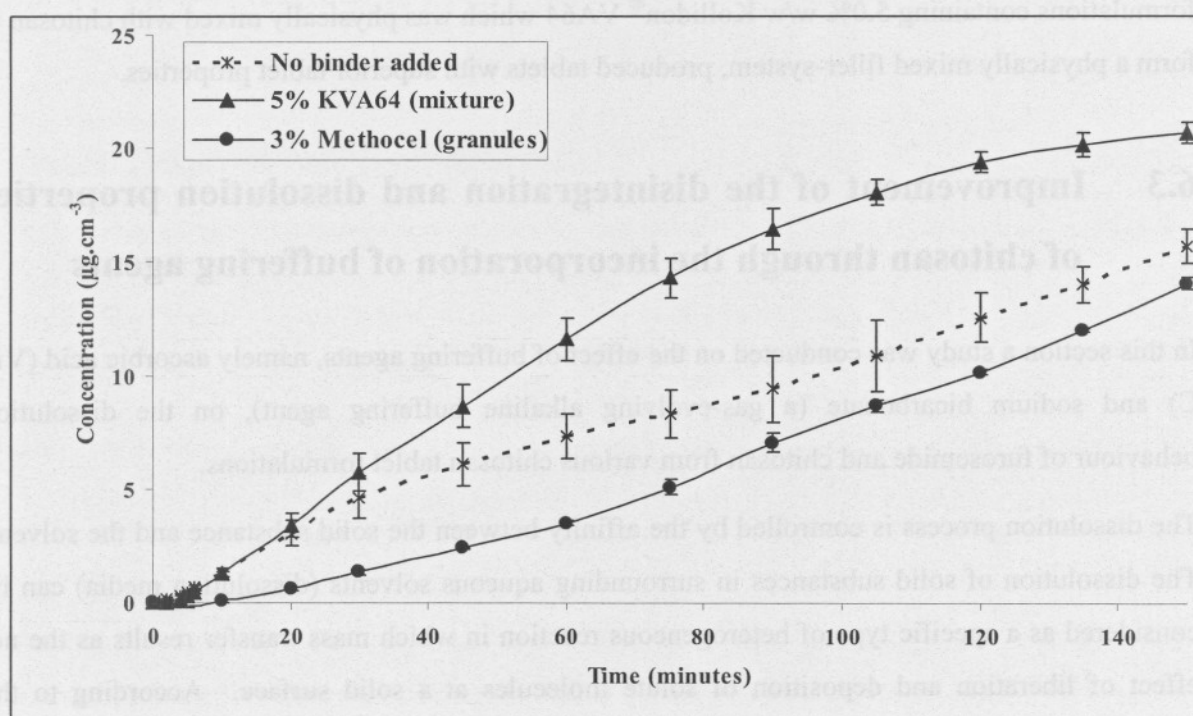


Figure 6.1: Dissolution profiles of furosemide in 0.1 M HCl at 75 rpm from chitosan minitab formulations containing only chitosan, 5.0% w/w Kollidon[®] VA64 or 3.0% w/w Methocel[®] K100M. Error bars indicate standard deviations.

From table 6.5 it was obvious that formulations containing Kollidon[®] VA64 performed more exceptionally compared to the Methocel[®] K100M formulations in terms of all the variables. This was probably due to the difference in the viscosity of the binders. According to Bühler (1993:93,

228) the dissolution rate of certain drugs was found to depend directly on the molecular weight and viscosity of the Kollidon[®]. The viscosity of Kollidon[®] VA64 is significantly lower (Bühler, 1993:22, 195) and as mentioned before Methocel[®] K100M is usually incorporated into formulations to control drug release. The initial dissolution rate of chitosan in formulations containing Kollidon[®] VA64 as binder was slightly retarded compared to the Methocel[®] K100M formulations; however, this difference was negligible.

Another observation was that only through physically mixing the binders with chitosan preceding the incorporation of the active ingredient, could formulations be produced that depicted enhanced dissolution properties when compared to formulations produced from granulated filler-systems. When considering the concentration in which the binders should be used, the higher level, i.e. 5.0% w/w seems to produce tablets with a more pronounced extent of and higher initial dissolution rate of both furosemide and chitosan. It could therefore be concluded that formulations containing 5.0% w/w Kollidon[®] VA64 which was physically mixed with chitosan to form a physically mixed filler-system, produced tablets with superior tablet properties.

6.3 Improvement of the disintegration and dissolution properties of chitosan through the incorporation of buffering agents

In this section a study was conducted on the effect of buffering agents, namely ascorbic acid (Vit. C) and sodium bicarbonate (a gas-evolving alkaline buffering agent), on the dissolution behaviour of furosemide and chitosan from various chitosan tablet formulations.

The dissolution process is controlled by the affinity between the solid substance and the solvent. The dissolution of solid substances in surrounding aqueous solvents (dissolution media) can be considered as a specific type of heterogeneous reaction in which mass transfer results as the net effect of liberation and deposition of solute molecules at a solid surface. According to the kinetics in heterogeneous reactions dissolution can be: diffusion-controlled, interface-controlled or intermediate-controlled (Habashi, 1991:53; Abdou, 1989:11).

Noyes and Whitney (1897:930-934) were the first to publish a law describing the diffusion layer formed during the dissolution process. The original Noyes-Whitney equation was expanded to eventually yield equation 6.3:

$$\frac{dC}{dt} = \frac{D}{Vh} A(C_s - C) \quad [6.3]$$

Where $\frac{dC}{dt}$ is the dissolution rate (change in concentration per unit time); A is the effective surface area of the solid, i.e., the surface area of the solid in contact with the surrounding liquid; C_s is the saturation solubility or concentration of solid in the boundary layer, C_t is the concentration of solid in surrounding medium at time, t; D is the diffusion coefficient of the solid, V is the volume of the dissolution medium and h is the thickness of the diffusion layer.

The dissolution process can be summarised as follows:

When dissolution of a solid in a liquid takes place under sink conditions, a thin stagnant liquid film, of thickness h , adheres to the surface of the solid (boundary layer). The liquid in direct contact with the solid, i.e., at the solid/liquid interface, is saturated with the solid in solution. As the distance from the dissolving solid surface increases, the concentration of the solid in the liquid drops. At the outermost boundary of the film, the concentration in the film is the same as in the surrounding medium, C_t . The driving force governing the movement of solute molecules through the boundary layer is the difference between the saturation concentration, C_s , and the concentration in the surrounding medium, C_t . The greater the difference between these concentrations, the faster the dissolution rate (Marais, 2000:26).

Biological studies of drug absorption showed that the drug concentration on both sides of the membrane in the intestinal wall approaches equilibrium in a short time. Drugs are absorbed almost as rapidly as they dissolve, i.e., the GI-tract acts as a natural sink where the drug is absorbed instantaneously the moment it dissolves. Accordingly, for drugs that are sparingly soluble, i.e. furosemide, the rate of dissolution of the drug particles plays a fundamental role in determining bioavailability. Also, because of the *in-vivo* sink conditions, dissolution-rate determinations should always be carried out under *in-vitro* sink conditions. This is usually achieved by using a large dissolution medium volume or by replenishing the solution constantly with fresh solvent at a specified rate (Abdou, 1989:13).

From various studies it became clear that the pH in the diffusion layer surrounding dissolving solid particles played an important part in the solubility and, hence, the dissolution rate of drugs. This suggested that the pH of this microenvironment could be utilised to control the dissolution rates of drugs. Since Nelson (1957:610) showed the effect of the pH in the diffusion layer on the

dissolution rate of drugs, several researchers have used this principle to improve drug dissolution through manipulation of the pH_d (pH of diffusion layer) either through salt formation or the addition of water-soluble alkaline excipients or buffers.

Several authors employed buffer systems to control the pH in the diffusion layer in order to control drug dissolution (Chakrabarti & Southard, 1997:467; Blasé & Peck, 1992:869; Doherty & York, 1989:227; Pagay, 1988:886). Doherty and York (1989:227) and Marais and Van der Watt (1991:1718) proposed the incorporation of a buffer system in furosemide formulations to modify the pH in the microenvironment surrounding the dissolving drug particles in order to control drug solubility in the diffusion layer. Doherty and York (1989:227) showed that the inclusion of various ratios of a phosphate/citric acid buffer system resulted in an increase in the dissolution rate of the drug depending on the pH in the diffusion layer, rather than the pH of the medium. The buffers caused a controlled change in the pH of the diffusion layer. They suggested that inclusion of buffers in tablet formulations provided a feasible method to control (enhances or retards) drug dissolution, independent of bulk pH. Marais and Van der Watt (1991:1718) found that sodium bicarbonate increased the pH_d (in 0.1 M HCl) to 8.16, while ascorbic acid decreased the pH_d to less than 1. This resulted in the initial dissolution rate of furosemide from the sodium bicarbonate formulation being almost 4 times higher compared to the ascorbic acid formulation. In buffered media, the magnitude of the effect of the excipients on the pH_d was influenced by the buffer capacity of the medium, with smaller changes in the pH_d observed at higher buffer capacities. In buffered media the dissolution rates of furosemide from the sodium bicarbonate and ascorbic acid formulations were much closer compared to the rates in unbuffered media and media with low buffer capacities. An increase in the buffer capacity of the medium decreased the magnitude of the differences between the pH_d 's of the different formulations, thus resulting in smaller differences between the dissolution rates of furosemide from the various formulations (Marais, 2000:47).

From a wide range of available alkaline and gas-evolving compounds, *sodium bicarbonate* was chosen to determine the relative contribution of alkalinity and gas evolution to the dissolution of furosemide and chitosan. This compound is known to enhance drug dissolution through the release of carbon dioxide in acidic media (Javaid & Cadwallader, 1972:1721) or through an increase in the pH of the diffusion layer surrounding individual drug particles (Marais & Van der Watt, 1991:1718) or a combination of both these processes. Sodium bicarbonate was used in low concentrations, as its buffering effect could have a detrimental effect on the chitosan dissolution.

Ascorbic acid was also evaluated to determine its effect on the dissolution process because it had been shown that ascorbic acid, at low concentrations at room temperature, already induces a comparatively rapid destruction of chitosan in acid solutions with the formation of water-soluble fractions, thus increasing its solubility. The air oxygen substantially accelerates this process. At high concentrations, this effect is, however, less pronounced than at low concentrations (Zoldners *et al.*, 2005:1-4; Park *et al.*, 2001:519; Muzzarelli *et al.*, 1984:150).

6.3.1 Effect of ascorbic acid and sodium bicarbonate on the physical and dissolution properties of the tablet formulations

This phase of the study comprised of the comparison of the properties of minitabets prepared from furosemide, physically mixed filler-systems or granulated filler-systems and a buffer (0.5% w/w ascorbic acid or 2 or 4% w/w sodium bicarbonate). The formulations were prepared and minitabets of approximately 14 mg were tableted at setting 50 as described in section 3.5.2. The compositions of the mixtures prepared are summarised in table 6.6. The physical properties and the dissolution of furosemide from the tablet formulations in 0.1 M HCl were determined as described in sections 3.5.3 and 3.5.4. The dissolution data of the tablets are tabulated in table 6.7 and the physical properties of the tablets are tabulated in table B.1 of annexure B.1.

Table 6.6: *Composition of chitosan minitabets.*

Component	Excipient	Filler mixture composition (% w/w)
Active ingredient	Furosemide	14.286
Filler	Chitosan	Qs to 100%
Binder	Kollidon [®] VA64	0, 3 or 5
Buffer	Ascorbic acid or	0.5
	Sodium bicarbonate (NaHCO ₃)	2 or 4

Table 6.7: *The dissolution properties of furosemide and chitosan from physically mixed and granulated formulations containing Kollidon® VA64 in 3.0 or 5.0% w/w and: either ascorbic acid or sodium bicarbonate as buffer in different concentrations.*

Filler system	Excipient concentration (% w/w)		Dissolution properties							
			Furosemide					Chitosan		
	Binder	Buffer	AUC	DR _i	% Dissolved	AUC _n	(DR _i) _n	AUC	DR _i	% Dissolved
	KVA64	Vit. C*								
Mixed	3.0	0.5	1473.63	0.091	44.22	0.635	0.045	11671.27	2.693	60.84
Granulated	3.0	0.5	1632.45	0.077	48.64	0.704	0.038	11906.26	4.380	61.64
Mixed	5.0	0.5	1550.34	0.065	46.36	0.668	0.032	10144.48	2.239	54.02
Granulated	5.0	0.5	1923.45	0.226	56.74	0.829	0.113	13124.96	3.189	68.96
	KVA64	NaHCO ₃ *								
Mixed	3.0	2.0	1492.60	0.083	43.08	0.644	0.041	12912.10	2.574	65.92
Granulated	3.0	2.0	2342.03	1.292	70.39	1.010	0.645	16614.16	9.444	88.37
Mixed	5.0	2.0	2287.07	0.860	68.09	0.986	0.430	15410.33	7.516	83.27
Granulated	5.0	2.0	1728.33	0.124	51.85	0.745	0.062	14420.79	4.090	78.517
	KVA64	NaHCO ₃								
Mixed	3.0	4.0	2265.34	0.452	67.26	0.977	0.226	14566.50	4.574	78.45
Granulated	3.0	4.0	1995.50	0.562	60.24	0.860	0.281	13827.93	4.963	75.76
Mixed	5.0	4.0	2091.90	0.715	62.51	0.902	0.357	12268.06	4.408	68.16
Granulated	5.0	4.0	2384.34	0.664	71.53	1.028	0.332	12069.31	4.640	67.43

* KVA64 = Kollidon® VA64; Vit. C = Ascorbic acid; NaHCO₃ = Sodium bicarbonate

The incorporation of either ascorbic acid or sodium bicarbonate into the chitosan minitabket formulations, regardless of their concentration or the type of filler-system included, resulted in the enhancement of some of the tablet properties. The formulations produced minitabkets of significantly higher mechanical strengths (an increase of approximately 1.9 times), faster disintegration times (± 7.1 seconds) and all of the tablets portrayed acceptable friability values ($< 1.00\%$). No significant differences were found in the other tablet properties. Formulations containing ascorbic acid produced the hardest tablets, whereas formulations containing sodium bicarbonate depicted the most significant alteration in the disintegration times. The formulations containing 2% w/w sodium bicarbonate presented the highest mechanical strengths, whereas the 4% w/w formulations produced tablets with the fastest disintegration times.

Comparison of the dissolution parameters (% dissolved, DR_i and AUC) in table 6.7 once again clearly indicated the dependency of the AUC on the DR_i , as indicated previously. Also, the decreased DR_i -values of both furosemide and chitosan were again contradictory to the prodigious disintegration of all the chitosan formulations which validated that tablet disintegration is no absolute prerequisite for drug dissolution. However, tablets from these formulations did not agglomerate as tablets from the previous formulations, but drifted apart while swelling considerably to form small individual matrices on the surface of the dissolution media. These matrices were not as thick and although the furosemide dissolution had again been preceded by diffusion, the initial dissolution rate increased in these formulations.

The incorporation of ascorbic acid proved valid as a significant increase in the percentage chitosan and furosemide dissolved was illustrated compared to the formulations containing 0% w/w ascorbic acid ($p < 0.05$). As described previously, ascorbic acid increases the solubility of chitosan in low concentrations. This increase in chitosan solubility led to an increased dispensation of furosemide from the chitosan minitabket formulations, thus also enhancing the dissolution properties of furosemide. It was also clear that the concentration in which ascorbic acid was used in the formulations, was low enough not to have a detrimental effect on the solubility of furosemide and it did not influence (change) the pH of the dissolution medium. However, there was not a considerable difference between the DR_i -values of the different formulations. This indicated that the ascorbic acid created an unfavourable microenvironment for the furosemide to be able to dissolve promptly (figure 6.2).

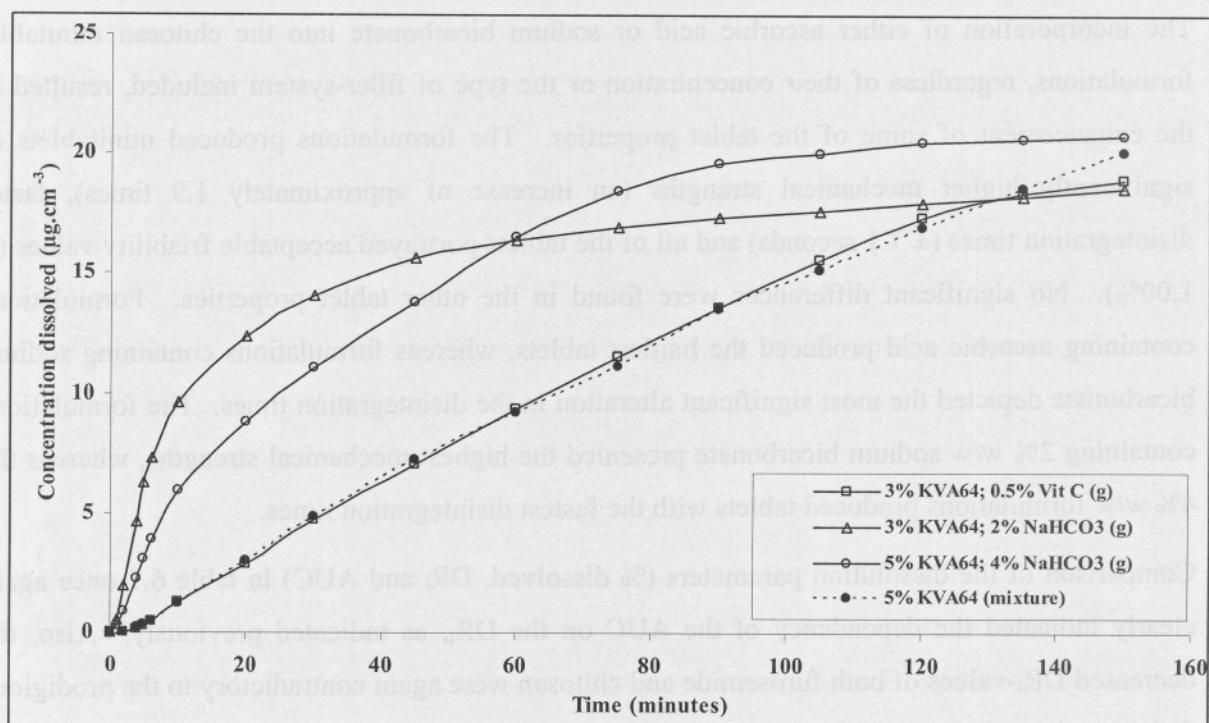


Figure 6.2: Examples of dissolution profiles of furosemide in 0.1 M HCl at 75 rpm from chitosan minitabket formulations containing Kollidon® VA64(KVA) as binder and a buffer (either 0.5% w/w ascorbic acid (Vit C) or sodium bicarbonate (NaHCO₃) in 2.0 or 4.0% w/w).

The incorporation of sodium bicarbonate into the formulations resulted in a more significant increase in dissolution of furosemide than the formulations which contained ascorbic acid as buffer ($p < 0.05$). Formulations containing up to 4% w/w sodium bicarbonate did not disintegrate in the dissolution medium although the evolution of small amounts of carbon dioxide from the tablet surfaces were observed during dissolution. The observed increase in the DR_i of furosemide from these tablets was attributed to a pH-increase in the microenvironment surrounding the dissolving furosemide particles caused by the dissolution of the highly soluble sodium bicarbonate. However, the concentrations in which sodium bicarbonate was included into the formulations did not affect the pH of the dissolution medium. Dissolution of sodium bicarbonate increased the pH of the aqueous medium inside the tablet which provided a favourable environment for the solubility of the drug ($pK_a = 3.6-3.9$). Marais (2000:47) showed that the diffusion layer, saturated with the highly water soluble sodium bicarbonate, could reach pH-values as high as 8.16. This increase in the pH level of the microenvironment resulted in an increase in the dissolution of furosemide; however, the dissolution of sodium bicarbonate had a slightly negative influence on the microenvironment of the chitosan which only dissolves in an

acidic environment. The microenvironment surrounding or adjacent to mixtures of dissolving solid particles is defined as a solution of the dissolving solid particles saturated with the components (Nelson, 1958:300). The pH of this microenvironment can be calculated (Serajuddin & Jarowski, 1985:153; Mooney *et al.*, 1981:19) or measured (Marais & Van der Watt, 1991:1716; Nelson, 1957:611). Research showed an excellent agreement between the pH-values obtained from the different methods.

In addition to its pH-effect the gas-evolving sodium bicarbonate also increased solvent penetration into the tablets due to the creation of capillaries in the tablet structure upon reaction with the acidic medium (Sallam *et al.*, 1998:505). Faster disintegration in the acidic medium was thus possible. The chitosan particles could therefore also be released into the acidic dissolution medium and was hence, able to slowly dissolve.

The DR_i of furosemide from all the formulations increased with an increase in the sodium bicarbonate concentrations (figure 6.2), whereas the DR_i of chitosan decreased (table 6.7). An increase in the concentration of the alkaline excipient enhanced the excipient/solvent reaction at the surface, which increased capillary creation and improved solvent penetration. At the higher concentration, the sodium bicarbonate available in the formulation acted as a reservoir for dissolved sodium bicarbonate particles at the surface and thus maintained the pH-increase at the surface and inside the capillaries for an extended time. Primary drug particles were exposed to the surrounding medium, resulting in an increase in the surface-area in contact with the medium, which caused a significant increase in the DR_i of furosemide.

The extent of furosemide dissolution, indicated by the AUC_n , from tablets containing 4% w/w sodium bicarbonate, was 0.977; 0.960; 0.902 and 1.028 respectively. The high AUC_n values of all of the formulations containing a buffer, regardless the concentration used, suggested dissolution conditions comparable and even superior to that of the suspension. This could be attributed to thorough dispersion of the primary drug particles throughout the medium before settling at the bottom of the dissolution vessel.

6.4 Improvement of disintegration and dissolution properties through the incorporation of super disintegrants

The results from the previous section showed the poor dissolution of furosemide from tablets that did not disintegrate in the dissolution medium, but rather formed individual matrices on the surface of the dissolution medium from which furosemide first had to diffuse. The restricted dissolution was caused by the limited surface-area of furosemide particles which were exposed to the dissolution medium. In order to facilitate the break-up of the tablet structure and release granules or primary drug particles, disintegrants are incorporated into tablet formulations. These excipients increase the surface-area of the drug exposed to the dissolution medium, therefore increasing the dissolution rate.

6.4.1 Incorporation of Ac-Di-Sol[®] and Kollidon[®] CL into chitosan minitablen formulations

Although several mechanisms of action for disintegration have been proposed, Ac-Di-Sol[®] (croscarmellose sodium) and Kollidon[®] CL (crospovidone) used in this study, act mainly through water uptake into the tablet structure and subsequent swelling resulting in the development of a disintegrating force that causes rapid and complete tablet disintegration. They proved to be superior to the traditional disintegrants in wet granulated and directly compressed tablets and in both soluble and insoluble formulations. They are highly effective in relatively low concentrations (compared to that of the starches) and do not usually negatively affect the process of direct compression or impart negative properties in tablets (List & Muazzam, 1979:21-24; Gissinger & Stamm, 1980:189).

Disintegrants differ in their rate and extent of swelling and water-uptake. Ac-Di-Sol[®] is known as a high swelling disintegrant, whereas Kollidon[®] CL is only moderately swelling and acts by capillarity. At higher compression forces this effect can be diminished, resulting in the important decrease of dissolution rate with tablets containing Kollidon[®] CL (Plaizier-Vercammen & van den Bossche, 1992:976-977; Caramella *et al.*, 1988:2167-2177; Caramella *et al.*, 1984:132-139). However, even amongst the other disintegrants, differences occurred in terms of their rate and extent of swelling and water-uptake. Various authors suggested that the amount and rate of

water-uptake do not necessarily correlate with the rate and extent of swelling (; Caramella *et al.*, 1988:2167-2177; Caramella *et al.*, 1984:132-139; Gissinger & Stamm, 1980:190).

6.4.1.1 Croscarmellose sodium (Ac-Di-Sol®)

Ac-Di-Sol® is produced from an internally cross-linked form of sodium carboxymethylcellulose. It exhibits consistent disintegrative functionality as a result of good water uptake (high capillary action), rapid swelling properties and relatively long fibre length which provides efficient fluid channelling. The fibrous nature of Ac-Di-Sol® provides for its outstanding water-wicking capability whilst its cross-linked chemical structure creates an insoluble hydrophilic, highly absorbent material that results in good swelling properties. This dual functionality translates into superior disintegration characteristics at very low use levels (< 5% w/w) compared to other super disintegrants. In addition, Ac-Di-Sol® has demonstrated good long-term stability in a variety of different tablet formulations (Rudnic & Rhodes, 1982:96; Gissinger & Stamm, 1980:190).

In pure disintegrant tablets, Ac-Di-Sol® exhibited a swelling capacity equal to sodium starch glycolate despite a lower rate of water-uptake and swelling and a smaller extent of water sorption (Gissinger & Stamm, 1980:190). In insoluble tablets containing dicalcium phosphate dihydrate, however, Ac-Di-Sol® proved to be superior to sodium starch glycolate in both the rate and extent of swelling. In concentrations of 1% w/w and lower the disintegrant caused rapid disintegration of the tablets and rendered the drug, phenacetin, in solution within 25 minutes at a 5% level. Rudnic and Rhodes (1982:96) found that in tablets containing dicalcium phosphate dihydrate/lactose (75/25), Ac-Di-Sol® caused rapid tablet disintegration within 4.6 – 5.3 seconds in a concentration of 0.25% w/w. An increase in the concentration to 2% w/w had no noticeable negative effect on either weight variation or friability of the tablets.

The solubility and/or hygroscopicity of the formulation in both wet-granulated and directly compressed tablets affect the efficiency of Ac-Di-Sol® (Johnson *et al.*, 1991:471; Gordon & Chowhan, 1987:909). An increase in the solubility of the other formulation ingredients, especially the drug and filler/binder, and an increase in overall tablet hygroscopicity decrease the efficiency of the disintegrant in promoting *in-vitro* dissolution (Chen *et al.*, 1997:510; Johnson *et al.*, 1991:471; Gordon & Chowhan, 1987:908). The observed decrease in the dissolution rate of the drug might have been due to an inhibition of the disintegrant by the soluble and hygroscopic component(s) competing for the available water.

Studying the mechanism of action of disintegrants, Bolhuis *et al.* (1982:112) found that the swelling capacity of Ac-Di-Sol[®] was unaffected by the presence of hydrophobic materials. To increase drug dissolution in direct compression formulations, Ac-Di-Sol[®] should be added to the other formulation components and dry blended to a homogenous mixture, prior to the addition of lubricants. This uniform distribution of Ac-Di-Sol[®] into the tablet matrix will enable it to wick water and swell, thereby breaking the tablets apart and exposing more surface-area of the drug to the gastric fluids.

In conclusion, it can be said that croscarmellose sodium is a highly efficient disintegrant with excellent disintegration properties at relative low concentrations with quick liberation of primary drug particles over a wide range of compression forces. Its mechanism of action is through water-uptake and swelling, and thus, a resultant development of a disintegrating force. It does not negatively affect other physical tablet properties such as friability and weight variation and it is relatively unaffected by the presence of hydrophobic materials.

6.4.1.2 Insoluble polyvinylpyrrolidone (Kollidon[®] CL)

The insoluble grades of Kollidon[®] are manufactured by a polymerisation process that produces mainly physically crosslinked insoluble polyvinylpyrrolidone in the form of a popcorn polymer. The disintegration effect of this insoluble polyvinylpyrrolidone in tablets i.e. swell in a predictable manner without forming a gel, its ability to hydrophylise insoluble active ingredients and to adsorb and form complexes are the main properties that make it useful as a pharmaceutical auxiliary (Bühler, 1993:129; Carli & Garbassi, 1985:963).

The particle size distribution and its effect on the flow and swelling properties of crospovidone are important factors. Kollidon[®] CL has a large surface-area and good flow properties, even though a relatively fine particle size (106-125 µm) was selected to minimise changes in the tablet surface as a result of atmospheric humidity and swelling, although, large particles with their greater swelling volume, would have given more rapid disintegration. Kollidon[®] CL also demonstrates excellent stability after processing into tablets, granules, capsules or suspensions, so that no changes are to be expected over many years. However, it should be born in mind that the products are hygroscopic, so that if there are any leaks in the packaging, through which atmospheric humidity can enter, the Kollidon[®] CL particles may swell with time (Bühler, 1993:133).

The optimum quantity of Kollidon[®] CL in a tablet or in granules is specific to each particular formulation and cannot be predicted accurately. It also depends on the desired disintegration time. In practice, the usual concentration in which Kollidon[®] CL is used varied in the 1-5% w/w range. If the proportion is increased beyond 5% w/w, the disintegration time is frequently no longer improved by a worthwhile amount. The solubility of the tableting mixture (drug and/or filler) in water also has a definite influence on the effectiveness of this disintegrant. It is generally more effective in insoluble mixtures. Thus, for instance, insoluble calcium phosphate placebo tablets with 4% w/w Kollidon[®] CL disintegrated significantly faster than corresponding tablets with soluble lactose (Bühler, 1993:157).

Compression force and the tablet hardness usually have little effect on the integration time of tablets containing Kollidon[®] CL. This means that it is possible to achieve short disintegration times with good tablet hardness and friability regardless of the formulation. Kollidon[®] CL normally improves the dissolution of the drug from the tablet. As with the integration time, the dissolution is usually not adversely affected over a certain compression force range (Bühler, 1993:162).

6.4.2 Effect of super disintegrants on the physical- and dissolution properties of chitosan minitabket formulations containing Kollidon[®] KVA64

The disintegrants incorporated represent two classes namely highly swelling (Ac-Di-Sol[®]) and moderately swelling, capillary (Kollidon[®] CL) disintegrants. A preliminary study was performed to determine the concentrations of the disintegrants that would be investigated. It was decided to use both types of disintegrants in 0.625 and 1.25% w/w concentrations. Mixtures of the different filler-systems were prepared and tableted as described in sections 3.5.1 and 3.5.2. To determine the effect of disintegration caused by swelling and the development of a disintegrating force on the dissolution properties of chitosan minitabket formulations, furosemide was again incorporated and the composition of these formulations are summarised in table 6.8. The mixtures were tableted at a compression setting of 50 producing tablets of approximately 14 mg.

Table 6.8: *Composition of chitosan minitablets.*

Component	Excipient	Filler mixture composition (% w/w)
Active ingredient	Furosemide	14.286
Filler	Chitosan	Qs 100
Binder	Kollidon [®] VA64	0, 3 or 5
Disintegrant	Ac-Di-Sol [®] (ADS) or Kollidon [®] CL (KCL)	0.625 or 1.25

Ac-Di-Sol[®] and Kollidon[®] CL were evaluated in various combinations with the selected binder to determine their positive or adverse contributions to the disintegration phenomenon and other physical tablets characteristics. Their effect on the dissolution properties were also determined and discussed. The use of another full factorial design on 16 formulations to evaluate the effects of the disintegrants on the tablet properties provided insight into the choice and concentration of the disintegrants that were incorporated into the different chitosan minitabulet formulations.

In this section 4 formulation variables (factors) were evaluated namely the method of production of the filler-system (i.e. chitosan and Kollidon[®] VA64 were either physically mixed or granulated), Kollidon[®] VA64 concentration, type of disintegrant incorporated and the concentration in which the disintegrant was incorporated (tables 6.9 and 6.10). Letters of the alphabet were again, assigned to the factors and all of these factors were evaluated on two levels (0 or 1, implicating either low or high or absent or present).

Table 6.9: *Factors and level designation.*

Factor	Designation	Level	
		0	1
Method of production of the filler-system	A	Physically mixed	Granulated
Kollidon [®] VA64 concentration (% w/w)	B	3	5
Disintegrant type	C	Ac-Di-Sol [®] (ADS)	Kollidon [®] CL (KCL)
Disintegrant concentration (% w/w)	D	0.625	1.250

Evaluation in terms of weight variation (an indirect evaluation of the flowability), crushing strength, thickness (tablet height), diameter, friability, and disintegration time was conducted (section 3.5.3). The results of the physical properties that were obtained for the different chitosan

minitab formulations are summarised in (table 6.11). The mean effect of each factor and level was calculated and compared to evaluate the contribution to the seen phenomena.

Table 6.10: *The factorial design employed to evaluate formulation variables and levels.*

				Variable A: Method of production of the filler system				
				A ₀ : Physically mixed		A ₁ : Granulated		
				Variable B: Kollidon® VA64 concentration				
				B ₀ : 3.0%	B ₁ : 5.0%	B ₀ : 3.0%	B ₁ : 5.0%	
Variable C: Disintegrant type	C ₀ : ADS	Variable D: Disintegrant concentration	D ₀ : 0.625%	A ₀ B ₀ C ₀ D ₀	A ₀ B ₁ C ₀ D ₀	A ₁ B ₀ C ₀ D ₀	A ₁ B ₁ C ₀ D ₀	
			D ₁ : 1.250%	A ₀ B ₀ C ₀ D ₁	A ₀ B ₁ C ₀ D ₁	A ₁ B ₀ C ₀ D ₁	A ₁ B ₁ C ₀ D ₁	
	C ₁ : KCL		D ₀ : 0.625%	A ₀ B ₀ C ₁ D ₀	A ₀ B ₁ C ₁ D ₀	A ₁ B ₀ C ₁ D ₀	A ₁ B ₁ C ₁ D ₀	
			D ₁ : 1.250%	A ₀ B ₀ C ₁ D ₁	A ₀ B ₁ C ₁ D ₁	A ₁ B ₀ C ₁ D ₁	A ₁ B ₁ C ₁ D ₁	

6.4.2.1 Physical characterisation

All of the formulations produced tablets which exhibited satisfactory appearances. No significant differences were found for the individual tablet dimensions ($p > 0.05$) of the different formulations and tablet weight did not vary discernibly, illustrating exceptional good flowability. The %RSD of each formulation was of relatively small magnitude ($< 3.00\%$), consequently of little concern (table 6.11). The presence of a disintegrant had, therefore, no discernible negative impact on flowability, however, it should be noted that as machinery heats up during manufacturing, the incorporation of Kollidon® CL in larger concentrations, could result in stunted flow due to melting of the Kollidon® particles to form a viscous mass. However, in this study formulations containing Kollidon® CL (C₁) indicated a slightly better flowability than Ac-Di-Sol®. In conclusion, however, none of the combinations stunted the flowability of the powder mixtures and all comply with the prerequisite for direct compression.

Table 6.11: Average responses calculated from the responses measured for each variable at each level.

Measured response	Variables							
	A ₀	A ₁	B ₀	B ₁	C ₀	C ₁	D ₀	D ₁
Mass variation (%RSD)	2.76	2.85	2.73	2.87	2.98	2.62	2.61	2.99
Crushing strength (N)	43.92	48.48	44.38	48.02	44.90	47.50	51.75	40.65
Disintegration time (sec)	11.92	129.06	12.58	128.40	22.19	118.79	121.40	19.58
% Friability	2.24	0.74	1.75	1.22	0.87	2.10	1.39	1.59
Tablet thickness (mm)	1.76	1.75	1.74	1.77	1.79	1.72	1.79	1.72
Tablet diameter (mm)	2.97	2.96	2.96	2.96	2.96	2.96	2.96	2.97

Crushing strength was evaluated according to the standard protocol. The incorporation of a binder in the higher concentration through granulation into the filler-system resulted in a slight enhancement of tablet strength and contradicted the findings of the previous sections to some degree. Additional inspection of the effects revealed that the presence of Ac-Di-Sol[®] in 0.625% w/w resulted in tablets of considerable strength (compared to the other formulations) and might suggest that Kollidon[®] VA64 could not be seen as the only contributor to tablet strength. However, the presence of a disintegrant could have detrimental effects on tablet stability even under normal storage conditions. This could be attributed to the degree of deformability and hygroscopicity of the disintegrant. The sorption of water would not only render the disintegrant ineffective, but also mechanically impaired due to the swelling force that develops from water sorption (section 6.4.1).

A significant difference was seen for the type of disintegrant (C) on tablet strength when the individual concentrations of the two disintegrants were taken into consideration. Kollidon[®] CL produced the weakest tablets compared to formulations which contained Ac-Di-Sol[®]. Since this disintegrant is a povidone, it was expected that the elastic recovery of the material might result in tablets of comparatively lesser strength. The concentration of Kollidon[®] CL had no marked effect on tablet strength and therefore, mechanical strength was proven insensitive to the inclusion of Kollidon[®] CL. On the other hand, the crushing strength of formulations containing

Ac-Di-Sol[®] increased with a decrease in its concentration, thus causing a detrimental effect on the mechanical tablet strength with an increase in concentration of this disintegrant.

Friability was negatively affected by the incorporation of a disintegrant, regardless the type or concentration. The results were in congruence with the results obtained for tablet strength. Formulations which consisted of filler-systems produced through granulation of chitosan and 5% w/w Kollidon[®] VA64 rendered tablets with acceptable friability (% friability < 1%). However, formulations containing Kollidon[®] CL produced tablets with friability values higher than the acceptable 1% due to the lack of adequate compaction and were expected as these formulations also produced the weakest tablets. Furthermore, an increase in Ac-Di-Sol[®] concentration generated an increase in tablet friability, whereas the friability of formulations containing Kollidon[®] CL decreased with an increase in concentration. The differences in friability tendency should therefore, be attributed to disintegrant type and concentration.

Disintegrants were incorporated with the major purpose of disintegrating tablets upon submersion in the disintegration and dissolution medium. Interestingly, formulations containing either ascorbic acid or sodium bicarbonate as a buffer, disintegrated faster than formulations where a disintegrant was incorporated. However, all of the formulations disintegrated within the set limit of 900 seconds (15 minutes). It was furthermore demonstrated that formulations containing Kollidon[®] CL disintegrated significantly slower ($p < 0.05$) than formulations containing Ac-Di-Sol[®]. The reason for this was that Kollidon[®] CL did not swell to the same degree as did Ac-Di-Sol[®], confirming the requirement of a higher concentrations of Kollidon[®] CL to effect disintegration.

The higher concentration binder proved detrimental to the disintegration process. Explanations for this phenomenon could relate to the enhancement in tablet strength afforded by the binder and an increase in the viscosity of the microenvironment of the disintegrant particles. Povidones are classified according to their grades of viscosity (K-value) and are known to be viscous in solution (Bühler, 1993:133, 191). The increase in viscosity could impair wetting of disintegrant particles and delay swelling.

The level of disintegrant determined the efficiency of disintegration to a large extent. The higher level (D₁) had the most significant effect of all factors on the disintegration time of the tablets. A six-fold decrease in disintegration time was illustrated by doubling the concentration of the disintegrant. It would seem that the type and concentration of the disintegrant imparted a more

significant enhancement of disintegration effectiveness than did any other variable. It would seem prerequisite to incorporate a disintegrant in a relatively high concentration in chitosan minitablen formulations or to combine a disintegrant with a buffer in the formulations.

6.4.2.2 Evaluation of the dissolution properties

The dissolution of tablets from each formulation was determined in 0.1 M HCl as described in section 3.5.4. The dissolution parameters of furosemide and chitosan (as determined from the factorial design) namely % dissolved, initial dissolution rate (DR_i), normalised initial dissolution rate ($DR_i)_n$, area under the dissolution curve (AUC) and normalised area under the dissolution curve (AUC_n) of the tablets from the formulations are summarised in table 6.12.

The incorporation of a disintegrant proved valid, for tablets from all of these formulations which included a disintegrant, disintegrated in the dissolution medium and none of the tablets drifted on the surface. Chitosan particles were, thus, immediately released into the acidic medium from which it could dissolve more rapidly due to increased exposure to the low pH-level of the medium and no matrices were formed from which furosemide first had to diffuse. This phenomenon resulted in an increase in the initial dissolution rate of both furosemide and chitosan, which then led to an increase in the AUC, AUC_n and the percentage furosemide and chitosan dissolved from the different chitosan minitablen formulations which contained a disintegrant. Therefore, comparison of the dissolution parameters of these formulations again clearly indicated the dependency of the AUC on the DR_i ; as well as that tablet disintegration is not an absolute prerequisite for drug dissolution.

When considering the formulation variables in table 6.12, it was clear that formulations containing physically mixed filler-systems and a disintegrant had significantly increased dissolution properties ($p < 0.05$). Furthermore, results of formulations containing higher concentrations of Kollidon[®] VA64 (i.e. 5% w/w) showed a slightly more pronounced increase in the initial dissolution rate as well as in the extent of drug and chitosan dissolved. This was contradictory to the disintegration times of the formulations, where the formulations containing 3% w/w binder disintegrated significantly faster (± 2 minutes).

Table 6.12: Average responses calculated from the responses measured for each variable at each level.

Measured response		Variables							
		A ₀	A ₁	B ₀	B ₁	C ₀	C ₁	D ₀	D ₁
Furosemide	AUC ($\mu\text{g} \cdot \text{min} \cdot \text{cm}^{-3}$)	2148.74	1839.76	1960.12	2028.38	2203.02	1785.48	1839.54	2148.96
	DR _i ($\mu\text{g} \cdot \text{cm}^{-3} \cdot \text{min}^{-1}$)	0.647	0.348	0.403	0.592	0.564	0.431	0.384	0.612
	AUC _n	0.926	0.793	0.845	0.875	0.950	0.770	0.793	0.927
	(DR _i) _n	0.323	0.174	0.201	0.296	0.282	0.215	0.192	0.306
	% Dissolved	64.23	54.94	58.70	60.47	65.89	53.28	55.00	64.17
Chitosan	AUC ($\mu\text{g} \cdot \text{min} \cdot \text{cm}^{-3}$)	14411.33	12360.99	13686.44	13085.88	15277.35	11494.96	12884.64	13887.67
	DR _i ($\mu\text{g} \cdot \text{cm}^{-3} \cdot \text{min}^{-1}$)	5.013	4.075	4.358	4.731	5.554	3.535	4.142	4.946
	% Dissolved	76.24	65.25	71.59	69.90	80.78	60.71	67.82	73.67

It should however be kept in mind that Kollidon[®] VA64 is universally soluble and would therefore, support dissolution, however, the crushing strength of tablets from these formulations might also have played a role during dissolution.

The incorporation of Ac-Di-Sol[®] as disintegrant into the chitosan minitabket formulations resulted in a more significant increase ($p < 0.05$) in the dissolution properties of furosemide and chitosan than the formulations which contained Kollidon[®] CL. The dissolution of chitosan was affected most. These findings were in accordance with the disintegration results where formulations containing Kollidon[®] CL depicted elongated disintegration times. The reason was again, that Kollidon[®] CL did not swell to the same degree as did Ac-Di-Sol[®] and therefore slightly inhibited the onset of dissolution. Furthermore, results showed a ($p < 0.05$) increase in the dissolution parameters of both furosemide and chitosan with an increase in disintegrant concentration. The disintegration times as well as the crushing strengths decreased with an increase in the concentration of these disintegrants. Two continuances regarding the dependence of $(AUC)_n$ on $(DRi)_n$ were found – one for formulations containing Kollidon[®] CL, the other for 0.625% w/w Ac-Di-Sol[®] formulations (figure 6.3).

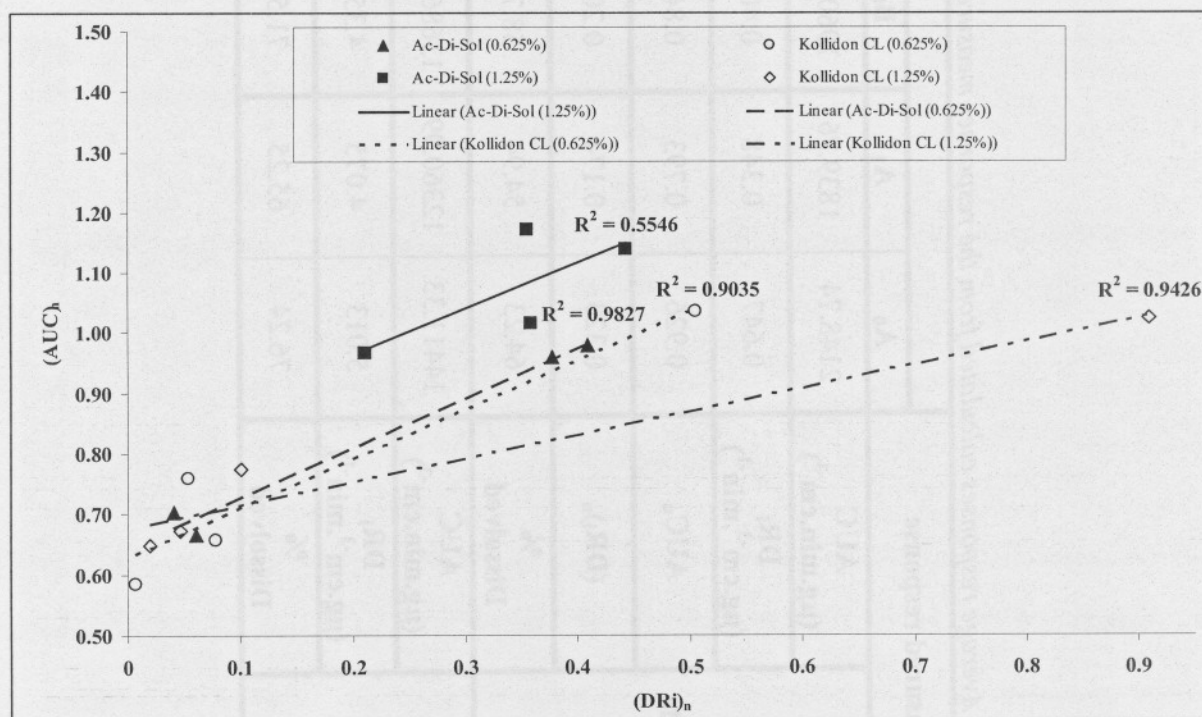


Figure 6.3: The dependence of the extent of dissolution $(AUC)_n$ on the initial rate of dissolution $(DRi)_n$. Real data points were utilised to construct linear regression trendlines.

The formulations containing 0.625% w/w Ac-Di-Sol[®] illustrated a significant positive linear dependence ($r^2 > 0.98$) of $(AUC)_n$ on $(DRI)_n$, whilst Kollidon[®] CL formulations illustrated a slightly less apparent dependence ($r^2 > 0.90$ and $r^2 > 0.94$ for concentrations of 0.625 and 1.25% w/w, respectively). However, an increase in concentration of Ac-Di-Sol[®] resulted in an inadequate correlation coefficient ($r^2 < 5.60$), thus, indicating that the $(AUC)_n$ were less dependant on the $(DRI)_n$ with increasing concentrations of this disintegrant.

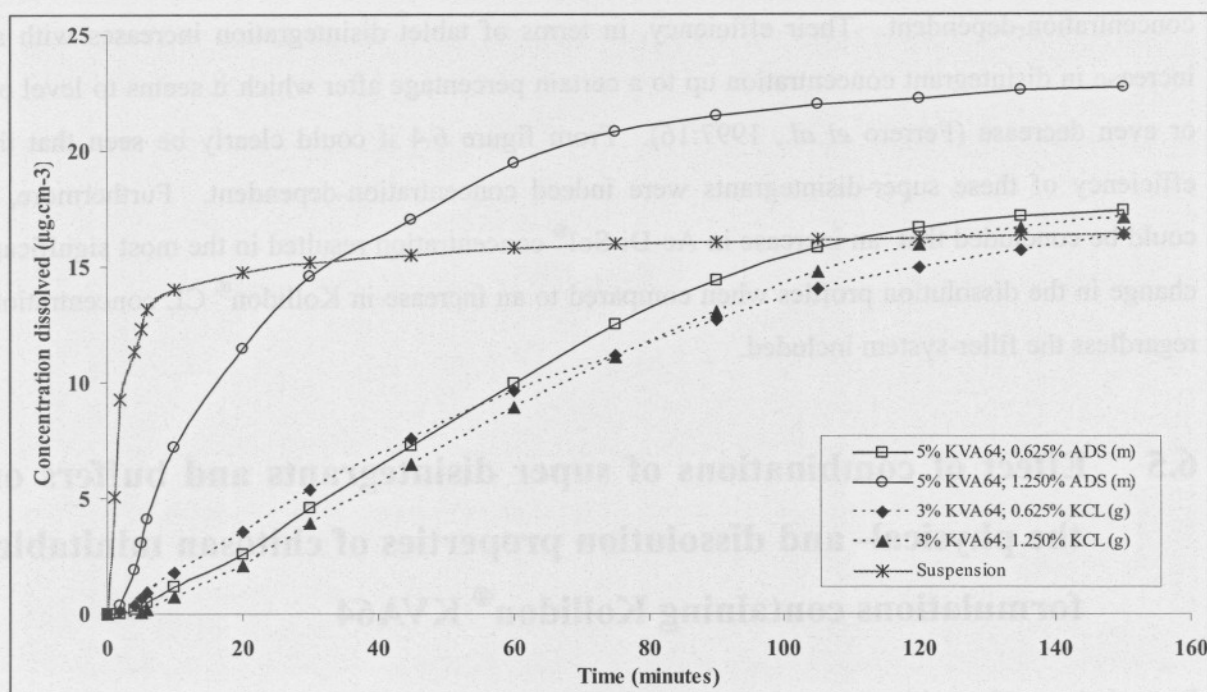


Figure 6.4: Dissolution profiles of formulations which vary in disintegrant concentration, compared with the dissolution profile of a furosemide suspension. ADS = Ac-Di-Sol[®]; KCL = Kollidon[®] CL; (m) = Formulation consisted of a physically mixed filler-system; (g) = Formulation consisted of a granulated filler-system.

The effect of different concentrations disintegrant on the dissolution of furosemide from chitosan minitables compared to a suspension is shown in figure 6.4. The suspension provided well-dispersed and well-wetted drug particles with a large surface-area in contact with the medium resulting in significantly improved dissolution of furosemide. The best dissolution from tablets was obtained from the formulation containing a physically mixed filler-system (chitosan and 5% w/w Kollidon[®] VA64) and 1.25% w/w Ac-Di-Sol[®], where 80.75% of furosemide dissolved. The AUC of furosemide from this formulation was approximately 1.171 times higher than that of the suspension. Therefore, an increase in Ac-Di-Sol[®] concentration improved the contact between

drug particles and solvent molecules. However, the $(DRI)_n$ -value was only approximately 0.355 indicating that the initial dissolution rate of this formulation was still inferior when compared to the DRI of the suspension. The formulation which illustrated the most ineffective dissolution profile was the formulation containing 0.625% w/w Kollidon[®] CL which was mixed with a granulated filler-system (chitosan + 5% w/w Kollidon[®] VA64). Only 40.26% furosemide dissolved from this formulation.

The efficiency of super disintegrants which act through water uptake and swelling is concentration-dependent. Their efficiency, in terms of tablet disintegration increases with an increase in disintegrant concentration up to a certain percentage after which it seems to level off or even decrease (Ferrero *et al.*, 1997:16). From figure 6.4 it could clearly be seen that the efficiency of these super-disintegrants were indeed concentration-dependent. Furthermore, it could be concluded that an increase in Ac-Di-Sol[®] concentration resulted in the most significant change in the dissolution profiles when compared to an increase in Kollidon[®] CL concentration, regardless the filler-system included.

6.5 Effect of combinations of super disintegrants and buffers on the physical- and dissolution properties of chitosan minitab^let formulations containing Kollidon[®] KVA64

Formulations of combinations of the two super disintegrants and the two different buffers at various concentrations were prepared to evaluate the effects of the excipients on the tablet properties. It was decided to continue using both types of disintegrants in the different combination formulations even though results obtained from formulations containing Kollidon[®] CL were not as favourable as results obtained from the Ac-Di-Sol[®] formulations. Ascorbic acid and sodium bicarbonate were included in the same concentrations as were previously used to confirm results found and conclusions made from the previous sections. Mixtures of the different filler-systems, disintegrants and buffers were prepared and mixed for 5 minutes. Furosemide was then added and mixed for an additional 5 minutes prior to tableting. These formulations are summarised in table 6.13.

Table 6.13: Composition of chitosan minitables.

Component	Excipient	Filler mixture composition (% w/w)
Active ingredient	Furosemide	14.286
Filler	Chitosan	Qs to 100%
Binder	Kollidon [®] VA64	3 or 5
Disintegrant	Ac-Di-Sol [®] (ADS) or Kollidon [®] CL (KCL)	0, 0.625 or 1.25
Buffer	Ascorbic acid (Vit C) or Sodium bicarbonate (NaHCO ₃)	0, 2 or 4

6.5.1 Effects of combinations of a disintegrant and ascorbic acid on the tablet properties of chitosan minitables

Furosemide/filler-system mixtures containing 0, 0.625 and 1.25% w/w Ac-Di-Sol[®] or Kollidon[®] CL, and 0 or 0.5% w/w ascorbic acid were prepared as described in section 3.5.1. Tablets of approximately 14 mg were produced at a compression setting of 50 (section 3.5.2). The dissolution of furosemide and chitosan in 0.1 M HCl at 75 rpm, and the physical properties of the tablets were determined as described in sections 3.5.3 and 3.5.4. Evaluation of the physical properties was conducted according to standard protocol. Two full factorial designs on 20 formulations each provided insight into the choice and concentration of the excipients that were incorporated into the different chitosan minitab formulations. The two disintegrants were not compared in these designs, but each design compared formulations containing either Ac-Di-Sol[®] or Kollidon[®] CL or ascorbic acid with combinations of one of the disintegrants and ascorbic acid.

Four formulation variables were evaluated for each factorial design namely method of production of the filler-system (i.e. chitosan and Kollidon[®] VA64 were either physically mixed or granulated), Kollidon[®] VA64 concentration, concentration in which ascorbic acid was incorporated and concentration in which the disintegrant was used. Letters of the alphabet were assigned to the factors. Factors A, B and C were evaluated on two levels, but factor D was evaluated on three levels (tables 6.14 and 6.15).

Table 6.14: *Factors and level designation.*

Factor	Designation	Level		
		0	1	2
Method of production of the filler-system	A	Physically mixed	Granulated	
Kollidon [®] VA64 concentration (% w/w)	B	3	5	
Ascorbic acid concentration (% w/w)	C	0	0.5	
Disintegrant concentration (% w/w)	D	0	0.625	1.250

Table 6.15: *The factorial design employed to evaluate formulation variables and levels.*

			Variable A: Method of production of the filler system				
			A ₀ : Physically mixed		A ₁ : Granulated		
			Variable B: Kollidon [®] VA64 concentration				
			B ₀ : 3.0%	B ₁ : 5.0%	B ₀ : 3.0%	B ₁ : 5.0%	
Variable C: Ascorbic acid concentration	C ₀ : 0%	Variable D: Disintegrant concentration	D ₀ : 0%	A ₀ B ₀ C ₀ D ₀	A ₀ B ₁ C ₀ D ₀	A ₁ B ₀ C ₀ D ₀	A ₁ B ₁ C ₀ D ₀
			D ₁ : 0.625%	A ₀ B ₀ C ₀ D ₁	A ₀ B ₁ C ₀ D ₁	A ₁ B ₀ C ₀ D ₁	A ₁ B ₁ C ₀ D ₁
			D ₂ : 1.250%	A ₀ B ₀ C ₀ D ₂	A ₀ B ₁ C ₀ D ₂	A ₁ B ₀ C ₀ D ₂	A ₁ B ₁ C ₀ D ₂
	C ₁ : 0.5%		D ₀ : 0%	A ₀ B ₀ C ₁ D ₀	A ₀ B ₁ C ₁ D ₀	A ₁ B ₀ C ₁ D ₀	A ₁ B ₁ C ₁ D ₀
			D ₁ : 0.625%	A ₀ B ₀ C ₁ D ₁	A ₀ B ₁ C ₁ D ₁	A ₁ B ₀ C ₁ D ₁	A ₁ B ₁ C ₁ D ₁
			D ₂ : 1.250%	A ₀ B ₀ C ₁ D ₂	A ₀ B ₁ C ₁ D ₂	A ₁ B ₀ C ₁ D ₂	A ₁ B ₁ C ₁ D ₂

All of the tablets from the different formulations complied with standards set for visual appearances. The tablet weight and tablet dimensions did not vary discernibly from previous tableted formulations and were thus irrelevant in this experiment. Therefore, only the results of crushing strength, friability, and disintegration times of the different formulations were tabulated in tables 6.16 and 6.17.

Table 6.16: Average responses calculated from the responses measured for each variable at each level of combination formulations containing Ac-Di-Sol® as disintegrant.

Measured response		Variables								
		A ₀	A ₁	B ₀	B ₁	C ₀	C ₁	D ₀	D ₁	D ₂
Crushing strength (N)		40.94	44.16	42.92	42.18	44.90	40.98	58.72	45.644	31.368
Disintegration time (sec)		18.18	16.67	18.78	16.07	22.19	14.25	7.67	9.13	30.60
% Friability		1.181	0.777	1.181	0.776	0.873	1.050	0.533	1.130	1.050
Furosemide	AUC (µg.min.cm ⁻³)	2213.74	2024.01	2096.41	2141.34	2203.02	2062.78	1644.97	2061.14	2413.55
	DR _i (µg.cm ⁻³ .min ⁻¹)	0.582	0.427	0.557	0.453	0.564	0.465	0.115	0.529	0.675
	AUC _n	0.954	0.873	0.904	0.923	0.950	0.889	0.709	0.889	1.041
	(DR _i) _n	0.291	0.213	0.278	0.226	0.282	0.232	0.057	0.264	0.337
	% Dissolved	66.34	60.52	62.78	64.08	65.89	61.79	48.99	61.94	72.14
Chitosan	AUC (µg.min.cm ⁻³)	14458.1	13770.6	14624.0	13604.7	15277.4	13339.0	11711.7	14124.9	15305.1
	DR _i (µg.cm ⁻³ .min ⁻¹)	5.496	4.886	5.657	4.725	5.554	4.950	3.125	5.217	6.198
	% Dissolved	76.74	72.89	76.59	73.04	80.78	70.84	61.37	74.88	81.48

Table 6.17: Average responses calculated from the responses measured for each variable at each level of combination formulations containing Kollidon® CL as disintegrant.

Measured response		Variables								
		A ₀	A ₁	B ₀	B ₁	C ₀	C ₁	D ₀	D ₁	D ₂
Crushing strength (N)		46.42	52.99	50.93	48.48	47.50	51.18	58.72	44.83	50.07
Disintegration time (sec)		4.87	108.82	4.67	109.02	118.79	15.54	7.67	121.44	16.83
% Friability		2.145	0.686	1.636	1.196	2.104	0.957	0.533	1.667	1.606
Furosemide	AUC (µg.min.cm ⁻³)	1880.01	1633.49	1713.16	1800.34	1785.48	1737.60	1644.97	1746.42	1822.98
	DR _i (µg.cm ⁻³ .min ⁻¹)	0.483	0.122	0.234	0.371	0.431	0.217	0.115	0.241	0.458
	AUC _n	0.811	0.704	0.739	0.776	0.770	0.749	0.709	0.753	0.786
	(DR _i) _n	0.241	0.061	0.117	0.185	0.215	0.108	0.057	0.120	0.229
	% Dissolved	56.29	48.47	51.15	53.61	53.28	51.78	48.99	52.11	54.34
Chitosan	AUC (µg.min.cm ⁻³)	12605.7	10692.4	11749.6	11548.5	11495.0	11751.8	11711.7	11080.1	12186.6
	DR _i (µg.cm ⁻³ .min ⁻¹)	4.042	2.703	3.479	3.265	3.535	3.264	3.125	2.899	3.969
	% Dissolved	66.88	56.18	61.40	61.67	60.71	62.08	61.37	58.45	64.70

The mean effect and level of each dissolution parameter of furosemide and chitosan (as determined from the factorial design) was calculated and compared to evaluate the contribution to the seen phenomena. The results of the % dissolved, initial dissolution rate (DR_i), normalised initial dissolution rate ($DR_i)_n$, area under the dissolution curve (AUC) and normalised area under the dissolution curve (AUC_n) of furosemide and chitosan from the tableted formulations are also summarised in tables 6.16 and 6.17.

The incorporation of 5% w/w Kollidon[®] VA64 through granulation of the filler-system into formulations containing Ac-Di-Sol[®] as disintegrant resulted in a slight enhancement of tablet strength and decrease in the percentage friability. Friability results were in congruence with the results obtained for tablet strength. However, neither the method of production of the filler-system used, nor the concentration in which the binder was incorporated into the filler-system had any significant effect ($p > 0.05$) on the disintegration of the tablets. The incorporation of ascorbic acid, Ac-Di-Sol[®] or a combination of these excipients had detrimental effects on tablet strength and friability. The inclusion of either one, or both of these excipients resulted in tablets which were more fragile and which pulverised more easily when friability was tested. Furthermore, as the concentrations of these excipients increased, tablet strength decreased extensively.

Prodigious disintegration (< 60 seconds) was illustrated for all formulations containing Ac-Di-Sol[®], ascorbic acid or a combination of these excipients. Kollidon[®] VA64 concentration or the method in which it was incorporated into the filler-system was omissible when disintegration was considered. The inclusion of ascorbic acid into chitosan minitablet formulations, on the other hand, proved to have a more prominent positive effect on disintegration times than did the inclusion of Ac-Di-Sol[®]. Interestingly, an increase in Ac-Di-Sol[®] concentration slightly prolonged the disintegration times of these formulations (± 20 seconds).

Adversatively, formulations containing Kollidon[®] CL produced tablets of higher mechanical strength, and an increase in concentration of the disintegrant resulted in an increase in tablet strength and a decrease in friability. This effect contradicted results from the previous section where the two disintegrants were compared and was probably due to a synergistic effect that ascorbic acid and Kollidon[®] CL had on the tablet strength. Even though friability results were in congruence with the results obtained for tablet strength, formulations containing Kollidon[®] CL produced tablets of higher friability probably due to inadequate compaction when compared to

formulations containing Ac-Di-Sol[®]. Furthermore, an increase in ascorbic acid concentration also produced tablets with enhanced mechanical tablet strength and reduced friability. This effect was, however, not as pronounced as the effect that the disintegrant had on these tablet properties. The incorporation of Kollidon[®] VA64 into the filler-systems of formulations containing Kollidon[®] CL regardless the method used, or the concentration in which it was incorporated, did not play a significant role in the improvement of the crushing strength ($p > 0.05$) of the different tablets. Formulations containing granulated filler-systems, where 5% w/w Kollidon[®] VA64 were incorporated, did however, produce tablets that were less prone to trituration.

All of the formulations containing Kollidon[®] CL, ascorbic acid or combinations of these two excipients disintegrated within 3 minutes which was well within the set limit of 900 seconds (15 minutes). Formulations which contained Ac-Di-Sol[®] as disintegrant, did however, disintegrate more rapidly, indicating that Ac-Di-Sol[®] is the preferred choice of disintegrant when used in these concentrations and combinations with ascorbic acid. It was evident that the production of a filler-system through granulation and the incorporation of the higher concentration binder significantly impaired the disintegration process (1.7 minutes respectively) in formulations containing Kollidon[®] CL. Povidones are known to be viscous in solution (Bühler, 1993:133, 191) and an increase in viscosity through an increase in binder concentration could impair wetting of disintegrant particles and thus, delay swelling. The inclusion of ascorbic acid into formulations containing Kollidon[®] CL proved to have a positive effect on disintegration times which was in concurrence with formulations containing Ac-Di-Sol[®]. However, contrary to formulations containing Ac-Di-Sol[®], the disintegration times of formulations containing Kollidon[®] CL improved with an increase in the concentration of this disintegrant.

During dissolution studies all of the tablets from the different formulations in each dissolution basket disintegrated simultaneously in the dissolution medium and none of the tablets drifted on the surface. The concentration of the ascorbic acid in the formulations in which it was included was too low to have any effect on the pH of the dissolution medium. It did however, lower the pH of the microenvironment creating an environment where increased dissolution of chitosan particles should be possible.

It is clear from figure 6.5 that formulations containing Ac-Di-Sol[®] depicted faster dissolution rates and more pronounced extent of dissolution than formulations containing Kollidon[®] CL. These results were in concurrence with the previous sections where it was clear that Kollidon[®]

CL is not the ideal disintegrant when used in these concentrations as it did not swell to the same degree when compared to Ac-Di-Sol[®] and therefore slightly inhibited the onset of dissolution.

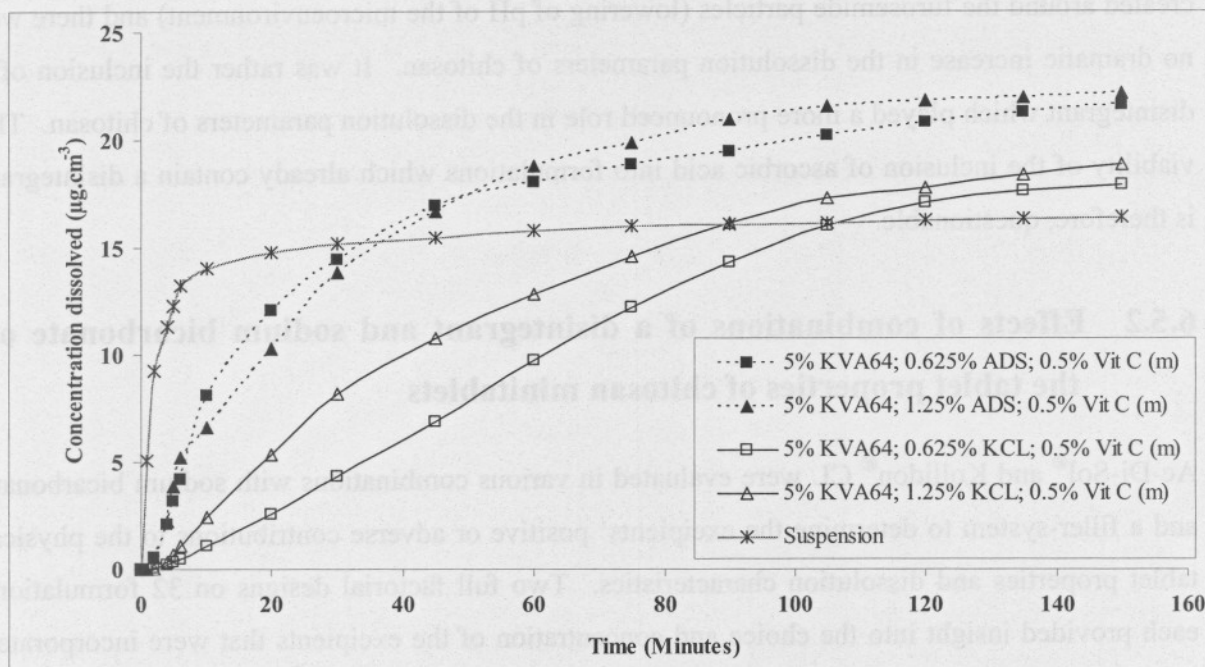


Figure 6.5: Examples of dissolution profiles of physically mixed chitosan minitab^let formulations containing 5% w/w Kollidon[®] VA64, 0.5% w/w ascorbic acid and either Ac-Di-Sol[®] or Kollidon[®] CL in two concentrations. These formulations were compared with the dissolution profile of a furosemide suspension. KVA = Kollidon[®] KVA64; ADS = Ac-Di-Sol[®]; KCL = Kollidon[®] CL; Vit C = ascorbic acid.

From tables 6.16 and 6.17 it could be concluded that formulations which contained physically mixed filler-systems regardless the other excipients included, depicted pronounced dissolution parameters. The concentration in which Kollidon[®] VA64 was included into the formulations did, however, not play an immense role during dissolution studies even though the 5% w/w concentration seemed more acceptable. Another important observation was that the higher concentration disintegrant included into the formulations regardless the type, produced tablets with more satisfactory dissolution parameters. Formulations containing 1.25% w/w Ac-Di-Sol[®] resulted in dissolution profiles of furosemide comparable to that from a suspension; in fact the inclusion of this disintegrant significantly improved the dissolution of the drug when considering the AUC_n-value (1.041). However, from figure 6.5 it could be seen that the different chitosan minitab^let formulations did not improve the initial dissolution rate of the furosemide suspension.

There was no discernable vindication for the inclusion of a combination of a disintegrant and ascorbic acid into the formulations (tables 6.16 and 6.17). The inclusion of ascorbic acid had a negative effect on the dissolution of furosemide because of the unfavourable microenvironment it created around the furosemide particles (lowering of pH of the microenvironment) and there was no dramatic increase in the dissolution parameters of chitosan. It was rather the inclusion of a disintegrant which played a more pronounced role in the dissolution parameters of chitosan. The viability of the inclusion of ascorbic acid into formulations which already contain a disintegrant is therefore, questionable.

6.5.2 Effects of combinations of a disintegrant and sodium bicarbonate on the tablet properties of chitosan minitables

Ac-Di-Sol[®] and Kollidon[®] CL were evaluated in various combinations with sodium bicarbonate and a filler-system to determine the excipients' positive or adverse contributions to the physical tablet properties and dissolution characteristics. Two full factorial designs on 32 formulations each provided insight into the choice and concentration of the excipients that were incorporated into the different chitosan minitab formulations. The two disintegrants were not compared in these designs, but each design compared combinations of either Ac-Di-Sol[®] or Kollidon[®] CL with different concentrations of sodium bicarbonate.

Table 6.18: *Factors and level designation.*

Factor	Designation	Level		
		0	1	2
Method of production of the filler-system	A	Physically mixed	Granulated	
Kollidon [®] VA64 concentration (% w/w)	B	3	5	
Disintegrant concentration (% w/w)	C	0	0.625	1.250
Sodium bicarbonate concentration (% w/w)	D	0	2	4

Four formulation variables were evaluated for each factorial design namely method of production of the filler-system (i.e. chitosan and Kollidon[®] VA64 were either physically mixed or granulated), Kollidon[®] VA64 concentration, concentration in which the disintegrant was

incorporated and concentration in which sodium bicarbonate was used in. Letters of the alphabet were again, assigned to the factors. Factors A and B were evaluated on two levels, but factors C and D were evaluated on three levels (tables 6.18 and 6.19).

Table 6.19: *The factorial design employed to evaluate formulation variables and levels.*

			Variable A: Method of production of the filler system				
			A ₀ : Physically mixed		A ₁ : Granulated		
			Variable B: Kollidon® VA64 concentration				
			B ₀ : 3.0%	B ₁ : 5.0%	B ₀ : 3.0%	B ₁ : 5.0%	
Variable C: Disintegrant concentration	C ₀ : 0%	Variable D: Sodium bicarbonate concentration	D ₀ : 0%	A ₀ B ₀ C ₀ D ₀	A ₀ B ₁ C ₀ D ₀	A ₁ B ₀ C ₀ D ₀	A ₁ B ₁ C ₀ D ₀
			D ₁ : 2%	A ₀ B ₀ C ₀ D ₁	A ₀ B ₁ C ₀ D ₁	A ₁ B ₀ C ₀ D ₁	A ₁ B ₁ C ₀ D ₁
			D ₂ : 4%	A ₀ B ₀ C ₀ D ₂	A ₀ B ₁ C ₀ D ₂	A ₁ B ₀ C ₀ D ₂	A ₁ B ₁ C ₀ D ₂
	C ₁ : 0.625%		D ₀ : 0%	A ₀ B ₀ C ₁ D ₀	A ₀ B ₁ C ₁ D ₀	A ₁ B ₀ C ₁ D ₀	A ₁ B ₁ C ₁ D ₀
			D ₁ : 2%	A ₀ B ₀ C ₁ D ₁	A ₀ B ₁ C ₁ D ₁	A ₁ B ₀ C ₁ D ₁	A ₁ B ₁ C ₁ D ₁
			D ₂ : 4%	A ₀ B ₀ C ₁ D ₂	A ₀ B ₁ C ₁ D ₂	A ₁ B ₀ C ₁ D ₂	A ₁ B ₁ C ₁ D ₂
	C ₂ : 1.25%		D ₀ : 0%	A ₀ B ₀ C ₂ D ₀	A ₀ B ₁ C ₂ D ₀	A ₁ B ₀ C ₂ D ₀	A ₁ B ₁ C ₂ D ₀
			D ₁ : 2%	A ₀ B ₀ C ₂ D ₁	A ₀ B ₁ C ₂ D ₁	A ₁ B ₀ C ₂ D ₁	A ₁ B ₁ C ₂ D ₁
			D ₂ : 4%	A ₀ B ₀ C ₂ D ₂	A ₀ B ₁ C ₂ D ₂	A ₁ B ₀ C ₂ D ₂	A ₁ B ₁ C ₂ D ₂

Evaluation of the physical properties was conducted according to standard protocol. However, only the results of crushing strength, friability, and disintegration time were tabulated in tables 6.20 and 6.21. The other physical properties did not vary discernibly from previous tableted formulations and were thus irrelevant in this experiment. All of the tablets from the different formulations complied with standards set for visual appearances. No significant differences were found for the individual tablet dimensions ($p > 0.05$) of the different formulations and tablet weight did not vary discernibly, illustrating exceptionally good flowability. Dissolution studies of tablets from each formulation were performed in four fold in 0.1 M HCl at 75 rpm as described in section 3.5.4. The mean effect and level of each dissolution parameter of furosemide and chitosan (as determined from the factorial design) was calculated and compared to evaluate the contribution to the seen phenomena. The results of the % dissolved, initial dissolution rate

(DR_i), normalised initial dissolution rate (DR_i)_n, area under the dissolution curve (AUC) and normalised area under the dissolution curve (AUC)_n of furosemide and chitosan from the tableted formulations are also summarised in tables 6.20 and 6.21.

The incorporation of Kollidon® VA64 into formulations containing either Ac-Di-Sol® or Kollidon® CL as disintegrant, in the higher concentration through granulation of the filler-system resulted in a slight enhancement of tablet strength. However, neither the method of production of the filler-system used, nor the concentration in which the binder was incorporated into the filler-system had any significant effect on the crushing strength ($p > 0.05$) and was thus, omissible. It was evident that Ac-Di-Sol® and sodium bicarbonate had detrimental effects on tablet strength. The inclusion of either one, or both of these excipients resulted in tablets with slightly higher mechanical strength, however, as the concentrations increased, tablet strength decreased extensively. Contrariwise, formulations containing Kollidon® CL produced tablets of higher mechanical strength and an increase in concentration of the disintegrant resulted in an increase in tablet strength. This effect contradicted results from the previous section where the two disintegrants were compared and was probably due to the synergistic effect that sodium bicarbonate and Kollidon® CL had on the tablet strength.

Friability results were in congruence with the results obtained for tablet strength. Even though friability does not play an important role in minitables as these tablets are encapsulated and are thus, not subjected to intensive handling; formulations which consisted of filler-systems produced through granulation of chitosan and 5% w/w Kollidon® VA64, still rendered tablets with acceptable results (% friability < 1%). Friability was adversely affected by the incorporation of Ac-Di-Sol®, Kollidon® CL and/or sodium bicarbonate. An increase in concentration of Ac-Di-Sol® or Kollidon® CL also had a detrimental effect on the friability, however, formulations containing Kollidon® CL produced tablets with friability values higher than formulations containing Ac-Di-Sol®. This was probably due to inadequate compaction. Interestingly, an increase in sodium bicarbonate concentration in formulations containing Kollidon® CL resulted in a decrease in friability, whereas in formulations containing Ac-Di-Sol®, friability results concurred with the corresponding crushing strength values.

Table 6.20: Average responses calculated from the responses measured for each variable at each level of formulations containing Ac-Di-Sol® as disintegrant.

Measured response		Variables									
		A ₀	A ₁	B ₀	B ₁	C ₀	C ₁	C ₂	D ₀	D ₁	D ₂
Crushing strength (N)		38.67	38.68	36.95	40.40	41.76	41.64	33.65	44.90	36.40	36.79
Disintegration time (sec)		15.13	105.18	16.35	103.95	6.77	59.08	96.81	22.19	9.86	135.75
% Friability		1.618	0.453	1.127	0.944	0.361	1.031	1.490	0.873	1.251	0.929
Furosemide	AUC (µg.min.cm ⁻³)	2184.30	2042.98	2104.78	2122.51	2073.39	1981.66	2272.46	2203.02	2310.24	1857.47
	DR _i (µg.cm ⁻³ .min ⁻¹)	0.803	0.594	0.727	0.670	0.594	0.514	0.953	0.564	0.947	0.540
	AUC _n	0.942	0.881	0.907	0.915	0.894	0.854	0.980	0.950	0.996	0.801
	(DR _i) _n	0.401	0.297	0.363	0.335	0.297	0.257	0.476	0.282	0.473	0.270
	% Dissolved	65.41	61.19	63.01	63.59	61.87	59.45	68.12	65.89	69.26	55.62
Chitosan	AUC (µg.min.cm ⁻³)	14415.3	13310.8	14386.0	13340.1	14011.1	13223.6	14403.8	15277.4	14960.6	11822.7
	DR _i (µg.cm ⁻³ .min ⁻¹)	6.808	5.667	6.651	5.824	5.276	5.106	8.011	5.554	8.235	4.696
	% Dissolved	78.20	72.10	76.98	73.32	75.74	71.44	78.47	80.78	81.05	65.50

Table 6.21: Average responses calculated from the responses measured for each variable at each level of formulations containing Kollidon® CL as disintegrant.

Measured response		Variables									
		A ₀	A ₁	B ₀	B ₁	C ₀	C ₁	C ₂	D ₀	D ₁	D ₂
Crushing strength (N)		46.22	49.20	46.81	48.60	41.76	47.60	51.78	47.50	51.42	44.13
Disintegration time (sec)		9.70	69.06	4.59	74.17	6.77	87.56	12.94	118.79	14.13	11.69
% Friability		2.061	0.668	1.732	0.997	0.361	1.779	1.619	2.104	1.417	0.819
Furosemide	AUC (µg.min.cm ⁻³)	1952.02	1712.24	1829.82	1834.44	2073.39	1779.98	1723.44	1780.23	1783.40	1915.46
	DR _i (µg.cm ⁻³ .min ⁻¹)	0.555	0.331	0.416	0.470	0.594	0.456	0.329	0.408	0.361	0.548
	AUC _n	0.842	0.738	0.789	0.791	0.894	0.767	0.743	0.768	0.769	0.826
	(DR _i) _n	0.277	0.165	0.208	0.235	0.297	0.228	0.164	0.204	0.180	0.274
	% Dissolved	58.17	51.16	54.52	54.82	61.87	53.06	51.47	53.12	53.16	57.21
Chitosan	AUC (µg.min.cm ⁻³)	12788.0	11181.7	12240.3	11729.4	14011.1	11400.9	11218.0	11495.0	12499.1	11797.2
	DR _i (µg.cm ⁻³ .min ⁻¹)	4.647	3.684	4.179	4.152	5.276	4.223	3.368	3.535	4.243	4.509
	% Dissolved	69.07	60.60	65.32	64.35	75.74	61.44	60.96	60.71	67.21	65.21

All of the formulations disintegrated within the set limit of 900 seconds (15 minutes). However, when taking all of the formulations into consideration, the formulations containing Kollidon[®] CL disintegrated slightly faster than formulations containing Ac-Di-Sol[®]. These findings were also contradictory to the previous section where the two disintegrants were compared. In section 6.4.2.1 it was concluded that formulations containing a buffer disintegrated faster than formulations where only a disintegrant was incorporated. Formulations containing combinations of Kollidon[®] CL and sodium bicarbonate could therefore, have had synergistic effects on the different disintegration times.

In both of the factorial designs, the production of the filler-system through granulation and the higher concentration binder incorporated, proved detrimental to the disintegration process. Explanations for this phenomenon could relate to the enhancement in tablet strength afforded by the granulated filler-system, the binder concentration and an increase in the viscosity of the microenvironment of the disintegrant particles. Povidones are known to be viscous in solution (Bühler, 1993:133, 191) and an increase in viscosity could impair wetting of disintegrant particles and delay swelling.

From tables 6.20 and 6.21 it seemed as though an increase in disintegrant concentrations negatively affected the disintegration process, however, it should be noted that these values included all of the combinations with sodium bicarbonate and formulations which only contained a disintegrant. These findings could therefore, be misleading. It should be noted that despite these results, all of the formulations disintegrated within the set time limit. On the other hand, the inclusion of sodium bicarbonate seemed to have a significant positive effect on the disintegration process ($p < 0.05$). Formulations which contained 2% w/w sodium bicarbonate disintegrated the fastest, but an increase up to 4% w/w affected this process negatively. Sodium bicarbonate is known to have a negative effect on the dissolution of chitosan because it creates an unpropitious microenvironment for dissolution around the chitosan particles. These particles, especially when granulated, are therefore, unable to rapidly break away from the tableted compacts to form individual particles and thus, prolong disintegration in the dissolution medium. It would seem that the inclusion of sodium bicarbonate imparted a more significant enhancement of disintegration effectiveness than did any other variable.

All of the tablets from the different formulations in each basket disintegrated in the dissolution medium and none of the tablets drifted on the surface. Therefore, most of the chitosan particles

were immediately released into the acidic medium from which it could dissolve more rapidly due to increased exposure to the low pH-level of the medium and no matrices were formed. Formulations containing Ac-Di-Sol[®] depicted significantly faster dissolution rates ($p < 0.05$) and more pronounced extent of dissolution than formulations containing Kollidon[®] CL and these results were thus, in concurrence with the previous section. The reason was again, that Kollidon[®] CL did not swell to the same degree as did Ac-Di-Sol[®] and therefore slightly inhibited the onset of dissolution. From tables 6.20 and 6.21 it was clear that formulations which contained physically mixed filler-systems regardless of the other excipients included, significantly ($p < 0.05$) increased the dissolution parameters. However, when variable B (binder concentration), C (disintegrant concentration) and D (sodium bicarbonate concentration) were taken into consideration there proved to be valid differences between formulations containing either one of the two disintegrants.

For formulations containing Ac-Di-Sol[®] there was no discernable vindication that either the lower or higher concentration of Kollidon[®] VA64 enhanced the dissolution properties. However, for formulations containing Kollidon[®] CL, a physically mixed filler-system comprising of chitosan and 5% w/w Kollidon[®] VA64, seemed to improve all of the dissolution properties. This was contradictive to the disintegration times of the formulations, where the formulations containing 3% w/w binder disintegrated significantly faster. It should however be kept in mind that Kollidon[®] VA64 is universally soluble and would therefore, support dissolution, however, the crushing strength of tablets from these formulations might also have played a role during dissolution. Furthermore, results showed a significant increase in the dissolution parameters of both furosemide and chitosan with an increase in Ac-Di-Sol[®] concentration ($p < 0.05$).

From figure 6.6 it is clear that an increase in all the dissolution parameters were primarily due to the positive influence the disintegrant concentration had on the initial dissolution rate (DR_i). However, when comparing figure 6.6 with figure 6.7 it could also be noted that an increase in the sodium bicarbonate concentration in formulations containing Ac-Di-Sol[®] resulted in decreased dissolution parameters ($\pm 14\%$ decrease in furosemide dissolved). From table 6.7 it is however evident that the inclusion of sodium bicarbonate into formulations containing Ac-Di-Sol[®] rendered tablets with exceptional dissolution properties. Ac-Di-Sol[®] and sodium bicarbonate had a synergistic effect on the dissolution of both furosemide and chitosan in an acidic medium. As discussed in the previous sections, an increase in sodium bicarbonate concentration had a

detrimental effect on the dissolution of chitosan for it created an unfavourable microenvironment into which the chitosan particles had to dissolve.

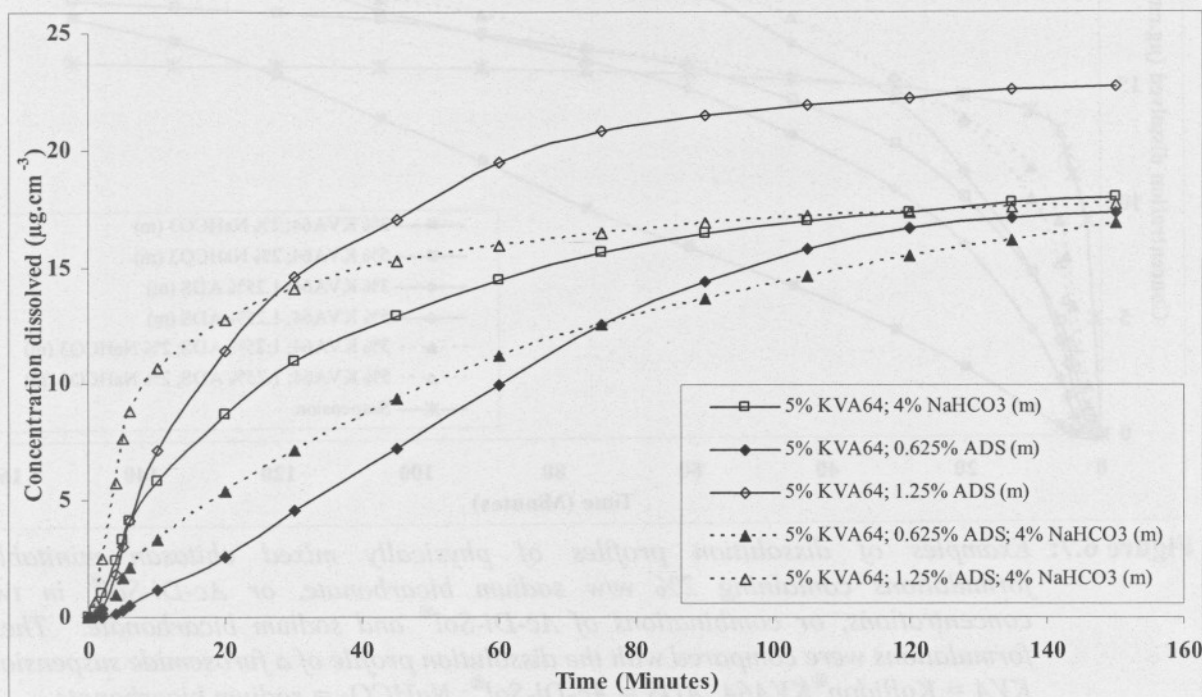


Figure 6.6: Examples of dissolution profiles of physically mixed chitosan minitablet formulations containing 5% w/w Kollidon[®] VA64 and either 4% w/w sodium bicarbonate, or Ac-Di-Sol[®] in two concentrations, or combinations of Ac-Di-Sol[®] and sodium bicarbonate. ADS = Ac-Di-Sol[®]; NaHCO₃ = sodium bicarbonate.

It was evident that formulations containing only Ac-Di-Sol[®] or formulations containing combinations of Ac-Di-Sol[®] and sodium bicarbonate (figure 6.7) resulted in dissolution profiles of furosemide comparable to that from a suspension. However, the initial dissolution rates of all of the formulations were still inferior to that of the suspension. The formulation which contained a physically mixed filler-system (consisting of chitosan and 5% w/w Kollidon[®] VA64) with 1.25% w/w Ac-Di-Sol[®] and 2% w/w sodium bicarbonate derived the most suitable dissolution properties (84.18% furosemide dissolved). Its DR_i-value was however, only 64.19% from that of the suspension, but the AUC-value was 20.97% higher compared to the AUC of the suspension.

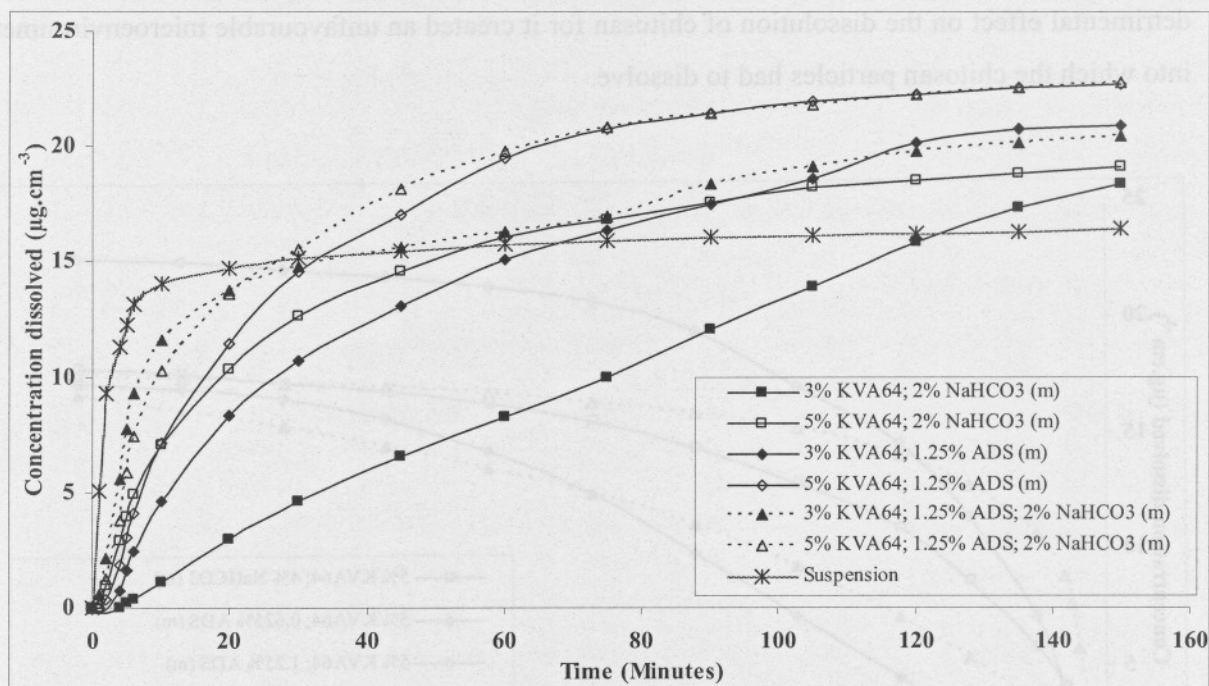


Figure 6.7: Examples of dissolution profiles of physically mixed chitosan minitab formulations containing 2% w/w sodium bicarbonate, or Ac-Di-Sol[®] in two concentrations, or combinations of Ac-Di-Sol[®] and sodium bicarbonate. These formulations were compared with the dissolution profile of a furosemide suspension. KVA = Kollidon[®] KVA64; ADS = Ac-Di-Sol[®]; NaHCO₃ = sodium bicarbonate.

Formulations which included Kollidon[®] CL into the combinations did not value the inclusion of a disintegrant, but the dissolution properties rather decreased with an increase in this disintegrants' concentration. When Kollidon[®] CL was combined with sodium bicarbonate; regardless the concentration in which it was used, the Kollidon[®] CL seemed to have an inhibitory effect on the dissolution properties of the formulations (table 6.21 and figure 6.8). It was further evident that combinations with this disintegrant and sodium bicarbonate necessitated the higher concentration sodium bicarbonate to slightly improve the dissolution of furosemide ($\pm 5\%$). The increase in sodium bicarbonate concentration did not impair the dissolution of chitosan unlike in the combinations which contained Ac-Di-Sol[®] as disintegrant. Furthermore, the dissolution properties of the combination formulations did not compare satisfactorily to the properties of the suspension.

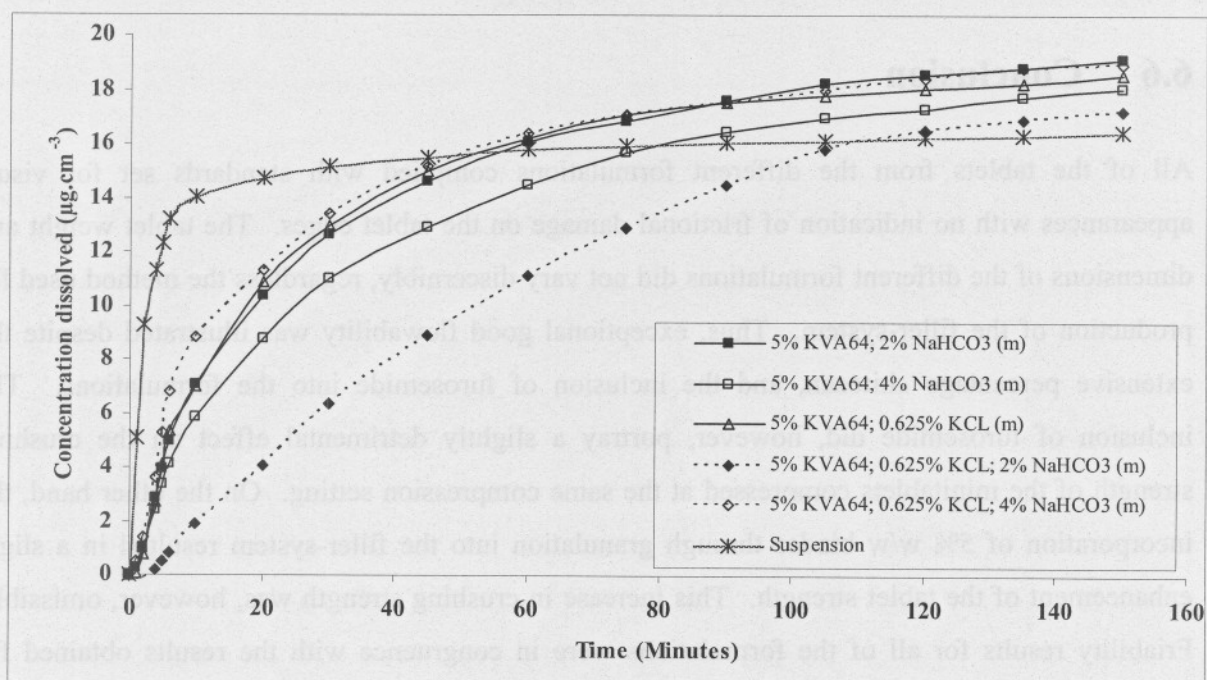


Figure 6.8: Examples of dissolution profiles of physically mixed chitosan minitabket formulations containing 5% w/w Kollidon® VA64 and either 4% w/w sodium bicarbonate, Kollidon® CL in two concentrations, or combinations of Kollidon® CL and sodium bicarbonate. These formulations were compared with the dissolution profile of a furosemide suspension. KVA = Kollidon® KVA64; KCL = Kollidon® CL; NaHCO₃ = sodium bicarbonate.

The best dissolution profile from tablets which consisted of a Kollidon® CL and sodium bicarbonate combination was obtained from the formulation containing a physically mixed filler-system (chitosan and 5% w/w Kollidon® VA64), 0.625% w/w Kollidon® CL and 4% w/w sodium bicarbonate, where 69.85% of furosemide dissolved (approximately 15% lower than the formulation containing Ac-Di-Sol® as disintegrant). The AUC of furosemide from this formulation was approximately equal to that of the suspension and the DR_i-value was 1.75 times slower than that of the suspension. It could therefore be concluded that formulations which consisted of combinations of Kollidon® CL and sodium bicarbonate was not recommended or feasible to enhance the dissolution of furosemide. From figure 6.8 it is evident that formulations containing only 0.625% w/w Kollidon® CL or only sodium bicarbonate regardless the concentration in which the buffer was included, produced tablets with dissolution properties equal to or higher than any of the combination formulations.

6.6 Conclusion

All of the tablets from the different formulations complied with standards set for visual appearances with no indication of frictional damage on the tablet edges. The tablet weight and dimensions of the different formulations did not vary discernibly, regardless the method used for production of the filler-system. Thus, exceptional good flowability was illustrated despite the extensive percentage chitosan, and the inclusion of furosemide into the formulations. The inclusion of furosemide did, however, portray a slightly detrimental effect on the crushing strength of the minitables compressed at the same compression setting. On the other hand, the incorporation of 5% w/w binder through granulation into the filler-system resulted in a slight enhancement of the tablet strength. This increase in crushing strength was, however, omissible. Friability results for all of the formulations were in congruence with the results obtained for tablet strength. Friability does, however, not play an important role in minitables as these tablets are encapsulated and are thus, not subjected to intensive handling.

The incorporation of additional excipients resulted in tablets of considerable strength; with formulations containing ascorbic acid producing the hardest tablets (with the least trituration). A significant difference was seen for the effect that the type of disintegrant had on tablet strength and friability. Kollidon[®] CL produced the weakest tablets compared to formulations containing Ac-Di-Sol[®]. The concentration of Kollidon[®] CL had no marked effect on tablet strength and therefore, mechanical strength was proven insensitive to the inclusion of Kollidon[®] CL. However, these formulations produced tablets with friability values higher than the acceptable 1% due inadequate compaction. When considering the inclusion of Ac-Di-Sol[®] and/or sodium bicarbonate it was evident that these excipients had detrimental effects on tablet strength and friability. The inclusion of either one, or both of these excipients resulted in tablets with slightly higher mechanical strength and lower friability, however, as the concentrations increased, tablet strength decreased extensively. Contrariwise, formulations containing Kollidon[®] CL and sodium bicarbonate produced tablets of higher mechanical strength with less pulverisation and an increase in concentration of the disintegrant resulted in an increase in tablet strength and decrease in percentage friability. This was probably due to a synergistic effect that sodium bicarbonate and Kollidon[®] CL had on the tablet strength. Therefore, it might suggest that Kollidon[®] VA64 could not be seen as the only contributor to tablet strength.

Most of the formulations resulted in tablets that disintegrated well within the set limit of 15 minutes. The production of a 5% w/w Kollidon[®] VA64 filler-system through granulation proved detrimental to the disintegration process for all the different formulations. Interestingly, formulations containing a buffer disintegrated faster than formulations where a disintegrant was incorporated. It was furthermore demonstrated that formulations containing ascorbic acid disintegrated slower than formulations containing sodium bicarbonate, whereas formulations containing Kollidon[®] CL disintegrated significantly slower than formulations containing Ac-Di-Sol[®]. These results indicated that Ac-Di-Sol[®] is the preferred choice of disintegrant when used in these concentrations and in combinations with various buffers. The reason for this was that Kollidon[®] CL did not swell to the same degree, confirming the requirement of higher concentrations Kollidon[®] CL to effect disintegration. Furthermore, the level of disintegrant or buffer determined the efficiency of disintegration to a large extent. The higher level imparted a more significant enhancement of disintegration effectiveness than did the type of excipient incorporated. When considering formulations where sodium bicarbonate and a disintegrant were combined, formulations which contained 2% w/w sodium bicarbonate disintegrated the fastest, but an increase up to 4% w/w affected this process negatively. It would seem that the inclusion of sodium bicarbonate imparted a more significant enhancement of disintegration effectiveness than did any other variable and that this buffer should be included in formulations containing Ac-Di-Sol[®] so as to enhance the disintegration process.

Comparison of the dissolution parameters of all of the formulations clearly indicated the dependency of the AUC on the DR_i. The decreased DR_i-values of both furosemide and chitosan, however, were contradictory to the prodigious disintegration of all the chitosan formulations which validated that tablet disintegration is no absolute guarantee for drug dissolution. It was further evident that formulation variables played a much more pronounced role during dissolution testing than with the physical properties.

The incorporation of Kollidon[®] VA64 into the different formulations provided tablets with enhanced dissolution properties, whereas Methocel[®] K100M inhibited the onset of dissolution. In general it could be concluded that formulations containing 5.0% w/w Kollidon[®] VA64, which was physically mixed with chitosan to form a physically mixed filler-system, produced tablets with superior dissolution properties for both furosemide and chitosan. The high AUC_n values of all of the formulations containing a buffer, regardless the concentration used, suggested dissolution conditions comparable and even superior to that of the suspension. This could be

attributed to thorough dispersion of the primary drug particles throughout the medium before settling at the bottom of the dissolution vessel. Incorporation of 0.5% w/w ascorbic acid led to an increased dispensation of furosemide due to increased chitosan solubility. However, the ascorbic acid created an unfavourable microenvironment for the furosemide to be able to dissolve promptly. A more significant increase in dissolution of furosemide was produced through the incorporation of sodium bicarbonate into the minitabiet formulations. The observed increase in the DR_i of furosemide from these tablets was attributed to a pH-increase in the microenvironment surrounding the dissolving furosemide particles caused by the dissolution of the highly soluble sodium bicarbonate. In addition it also increased solvent penetration into the tablets due to the creation of capillaries in the tablet structure upon reaction with the acidic medium. Faster disintegration in the acidic medium was thus possible. The chitosan particles could therefore also be released into the acidic dissolution medium and was hence, able to slowly dissolve; however, an increase in sodium bicarbonate concentration had a negative influence on the microenvironment of the chitosan which only dissolves in an acidic environment, thus inhibiting prompt dissolution.

It could clearly be seen that the incorporation of a disintegrant resulted in the most significant change in the dissolution profiles and the efficiency of these super-disintegrants were indeed concentration-dependent. Furthermore, it could be concluded that formulations containing Ac-Di-Sol[®] depicted faster dissolution rates and a more pronounced extent of dissolution than formulations containing Kollidon[®] CL. These results were in concurrence with the previous sections where it was clear that Kollidon[®] CL is not the ideal disintegrant when used in these concentrations. The formulations containing 0.625% w/w Ac-Di-Sol[®] illustrated a significant positive linear dependence of $(AUC)_n$ on $(DRI)_n$, whilst Kollidon[®] CL formulations illustrated a slightly less apparent dependence. However, an increase in concentration of Ac-Di-Sol[®] resulted in an inadequate correlation coefficient, thus, indicating that the $(AUC)_n$ were less dependant on the $(DRI)_n$ with increasing concentrations of this disintegrant.

There was no discernable vindication for the inclusion of a combination of a disintegrant and ascorbic acid into the formulations, as there was no dramatic increase in the dissolution parameters. Rather, it was the inclusion of a disintegrant which played a more pronounced role in the dissolution parameters. It was however clear that the inclusion of 2.0% w/w sodium bicarbonate into formulations containing Ac-Di-Sol[®] rendered tablets with exceptional dissolution properties. Ac-Di-Sol[®] and sodium bicarbonate had a synergistic effect on the

dissolution of both furosemide and chitosan in an acidic medium. Formulations consisting of combinations of Kollidon[®] CL and sodium bicarbonate were not recommended or feasible to enhance the dissolution of furosemide. It was evident that formulations containing only 0.625% w/w Kollidon[®] CL or only sodium bicarbonate, regardless the concentration in which the buffer was included, produced tablets with dissolution properties equal to or higher than any of the combination formulations. The formulation which contained a physically mixed filler-system (consisting of chitosan and 5% w/w Kollidon[®] VA64) with 1.25% w/w Ac-Di-Sol[®] and 2% w/w sodium bicarbonate, derived the most suitable dissolution properties (84.18% furosemide dissolved).

It is thus evident that the undesirable properties of chitosan could be modified to comply with the set standards of directly compressed minitables. The following phase of the study, therefore, focused on the validity of the incorporation of chitosan minitables into hard gelatin capsules and the comparison of drug release between chitosan minitables and commercially available products containing furosemide.

CHAPTER 7

COMPARISON OF DRUG RELEASE BETWEEN CHITOSAN MINITABLETS AND COMMERCIALLY AVAILABLE PRODUCTS CONTAINING FUROSEMIDE

7.1 Introduction

With the wide range of potential applications and the rate of scientific progress being made, chitosan is truly a multifunctional designer polymer for future biotechnology. The successful exploitation of chitosan as filler in the direct compression of tablets and especially minitables could result in phenomenal time saving and economical benefits in the pharmaceutical industry. Furthermore, the mucoadhesive and absorption enhancing properties of chitosan could also benefit and improve bioavailability of various poorly absorbed compounds or active ingredients. Various directly compressible fillers are commercially available, but no single excipient is known to meet all the prerequisites, including chitosan. From the previous chapters it is evident that the undesirable properties of chitosan could, however, be modified to comply with the set standards of directly compressed minitables.

In this chapter, a study was conducted to evaluate the influence of the incorporation of 10 minitables into hard gelatin capsules, on the dissolution properties of the chitosan minitab formulation of the most desirable tablet properties (i.e. a physically mixed filler system consisting of 5% w/w Kollidon[®] VA64 and chitosan, mixed for 5 minutes; which was then mixed with 1.25% w/w Ac-Di-Sol[®], 2% w/w sodium bicarbonate and 14.29% w/w furosemide for an additional 5 minutes). This formulation has been named CF/36 for ease of reference. The dissolution properties of CF/36 were also compared with reference tablets containing a familiar filler, as well as with commercially available furosemide tablets.

7.2 Chitosan minitabket formulation incorporated into hard gelatin capsules

A key property of a capsule or a tablet formulation is the in vitro dissolution rate of the drug substance. Before a drug is absorbed from the gastrointestinal tract, it has to be released and dissolved first. The in vitro dissolution test is an important step to assess the quality of a certain compound especially when incorporated into capsules, and to guide development of new formulations (von Orelli & Leuenberger, 2004:136).

It is not desirable to produce a capsule containing hydrophilic minimatrices because in vivo they would quickly form a single mass due to the hydration and to the swelling of the polymer and thus, would be emptied from the stomach. According to Rouge *et al.* (1997b:165-166) hydrophilic minimatrices introduced into a capsule did indeed exhibit a strong tendency to adhere to one another due to the presence of the gelatin capsule and to the earlier hydration of the minitabkets. Moreover, the incomplete dispersion of minitabkets decreases the surface of tablets in contact with the medium and this may decrease the drug release. They did, however, develop a method to prevent floating minitabkets from aggregating in vitro through the concomitant filling of the capsule with minitabkets and a protective filler. One of the fillers that the authors used due to its good flowability and affordability, was microcrystalline cellulose (Avicel®), which allowed an almost total separation of the tablets, thus assisting the dispersion of the minitabkets. For this reason, some of the capsules from this study were filled with Avicel® PH-101 to be able to achieve the same results.

7.2.1 Evaluation of the dissolution properties of the chitosan minitabket formulation incorporated into hard gelatin capsules

Dissolution tests were performed to evaluate the effect that chitosan mini-tablets, incorporated into hard gelatin capsules, would have on the rate and extent of the dissolution of furosemide. The capsules were filled with ten mini-tablets of approximately 14 mg each (i.e. total of 20 mg of furosemide per capsule), prior to the dissolution tests. Some of these capsules contained Avicel® PH-101 as filler, whereas the other capsules did not contain any filler. Dissolution studies were performed in four fold in 0.1 M HCl for 150 minutes at 75 rpm, as described in the sections 3.5.4.1 to 3.5.4.3.1. The standard USP-paddles were used in these analyses. The dissolution

parameters of furosemide and chitosan namely % dissolved, initial dissolution rate (DR_i), normalised initial dissolution rate (DR_i)_n, area under the dissolution curve (AUC) and normalised area under the dissolution curve (AUC)_n, are summarised in table 7.1.

Table 7.1: *The average dissolution properties of furosemide and chitosan from the CF/36 formulation; the CF/36 formulation incorporated into hard gelatin capsules and the CF/36 formulation incorporated into hard gelatin capsules filled with Avicel® PH-101.*

Furosemide suspension		AUC ($\mu\text{g} \cdot \text{min} \cdot \text{cm}^{-3}$)		DR _i ($\mu\text{g} \cdot \text{cm}^{-3} \cdot \text{min}^{-1}$)	
		2319.43		2.002	
		CF/36	CF/36 in hard gelatin capsules	CF/36 in capsules filled with Avicel® PH-101	
Furosemide	AUC ($\mu\text{g} \cdot \text{min} \cdot \text{cm}^{-3}$)	2805.856	2402.300	2175.255	
	DR _i ($\mu\text{g} \cdot \text{cm}^{-3} \cdot \text{min}^{-1}$)	1.468	1.071	0.613	
	% Dissolved	84.18	71.53	66.061	
	AUC _n	1.210	1.036	0.938	
	(DR _i) _n	0.733	0.535	0.306	
Chitosan	AUC ($\mu\text{g} \cdot \text{min} \cdot \text{cm}^{-3}$)	15107.863	14550.965	14282.839	
	DR _i ($\mu\text{g} \cdot \text{cm}^{-3} \cdot \text{min}^{-1}$)	9.472	5.215	4.317	
	% Dissolved	83.60	79.45	77.51	

The shell of all of the capsules opened in less than 1 minute. Interestingly, none of the minitables agglomerated in the capsules that did not contain any protective filler, but separated rather quickly and sank one after the other following the opening of the capsule. However, in less than 60 seconds most drifted to the surface where dissolution proceeded. Capsules containing CF/36 tablets and Avicel® PH-101 did not separate as promptly. From table 7.1 it was evident that all of the dissolution profiles were comparable and even superior to that of a furosemide suspension. The incorporation of 10 CF/36 tablets into a hard gelatin capsule, regardless if the capsule was filled with an additional filler, had however a predictable detrimental effect on especially the initial dissolution rate of furosemide and chitosan ($p < 0.05$)

as the presence of the gelatin of the capsules did not favour good dispersion, which was governed by the rate of opening of the capsule and the disintegration of the capsule shell (allowing lag time). The chitosan dissolution parameters were, however, not affected to the same extent as were the dissolution parameters of furosemide.

Contrary to the findings of Rouge *et al.* (1997b:165-171) the capsules which did not contain any protective filler depicted slightly more favourable dissolution profiles than capsules containing Avicel® PH-101 (figure 7.1).

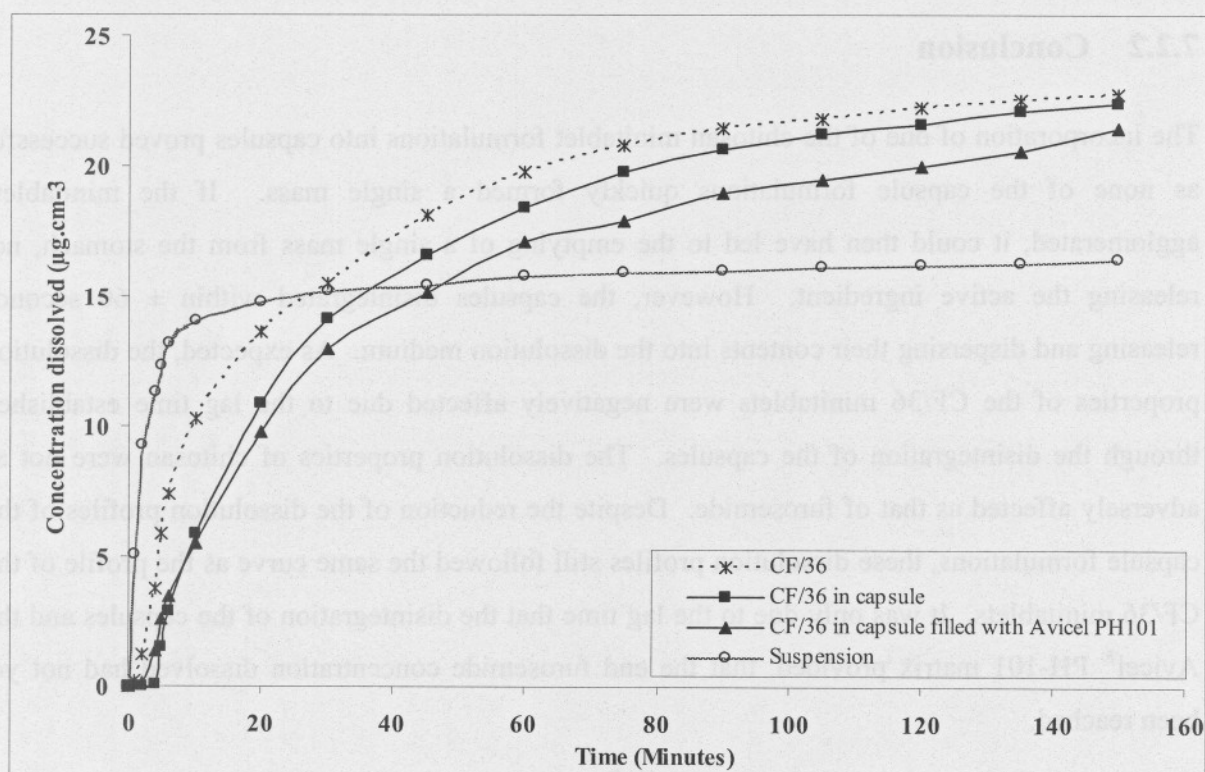


Figure 7.1: Dissolution profiles of CF/36 minitablets, CF/36 minitablets in capsules and CF/36 minitablets in capsules filled with Avicel® PH-101 compared with a dissolution profile of a furosemide suspension.

Avicel® PH-101 is insoluble, but relatively swellable (i.e. disintegrating properties) with an average particle size of 50 µm. During encapsulation these particles are not as closely compacted as when tableted. When dissolution is instigated, the dissolution medium penetrates through the capsule hydrating it and at the same time also hydrating the Avicel® PH-101 particles. The filler particles swell through the uptake of the medium, but due to the bulkiness of, and internal pores in the capsules between the particles, the disintegration properties are inhibited. A viscous mass

of microcrystalline cellulose was thus formed around the minitables and this formation inhibited instant disintegration of the minitables directly after the disintegration of the capsule, as the mass first needed to disintegrate. The lag time is therefore extended instead of eliminated or shortened. It is however clear, from figure 7.1, that both dissolution profiles of the capsules containing only CF/36 minitables and those containing CF/36 minitables and a protective filler, followed the same curve as the profile of the CF/36 minitables. It was only due to the lag time (± 60 seconds) that the disintegration of the capsules and the Avicel[®] PH-101 matrix provided that the final furosemide concentration dissolved (at t_{150}), had not yet been reached.

7.2.2 Conclusion

The incorporation of one of the chitosan minitab formulations into capsules proved successful as none of the capsule formulations quickly formed a single mass. If the minitables agglomerated, it could then have led to the emptying of a single mass from the stomach, not releasing the active ingredient. However, the capsules disintegrated within ± 60 seconds releasing and dispersing their contents into the dissolution medium. As expected, the dissolution properties of the CF/36 minitables were negatively affected due to the lag time established through the disintegration of the capsules. The dissolution properties of chitosan were not as adversely affected as that of furosemide. Despite the reduction of the dissolution profiles of the capsule formulations, these dissolution profiles still followed the same curve as the profile of the CF/36 minitables. It was only due to the lag time that the disintegration of the capsules and the Avicel[®] PH-101 matrix provided, that the end furosemide concentration dissolved had not yet been reached.

Capsules containing CF/36 tablets and Avicel[®] PH-101 did not separate as promptly as capsules which did not contain a protective filler and contrary to previous findings, these capsules depicted slightly less favourable dissolution profiles than capsules containing only the minitables. This was probably due to the formation of a rather viscous microcrystalline cellulose matrix around the minitables which first had to disintegrate preceding disintegration of the minitables. In future it may be favourable to rather incorporate an Avicel[®] grade with a coarser particle size (i.e. Avicel[®] PH-200) or to mix the Avicel[®] with Ac-Di-Sol[®]. Ac-Di-Sol[®] is a non free-flowing powder, while the flow rate of Avicel[®] PH-200, for example, is 2.4 g/s through an orifice of 15 mm (Rouge *et al.*, 1997b:168). As the ratio of Avicel[®] PH-200/Ac-D-Sol[®] does not have a great

influence on the dispersion factor, the combination of the two is recommended. Another protective filler that might be considered is sodium bicarbonate. The mechanism of this excipient is different from that of common disintegrating agents as previously discussed. The carbon dioxide generated from the sodium bicarbonate in contact with gastric juice or dissolution medium, forms bubbles around the minitables and this will keep the minitables separated from one another. However, sodium bicarbonate may also increase the disintegration of the minitables themselves prior to the disintegration of the capsule.

7.3 Comparison of drug release between chitosan minitables and commercially available products containing furosemide

The pharmaceutical availability of sparingly soluble and poorly wettable drugs still remain a major problem during the formulation of effective solid oral dosage forms. The rate and extent of the dissolution of these drugs are inherently poor, but may be manipulated through formulation variables and processing factors. Numerous formulations have been evaluated in the previous chapter regarding their physical and dissolution properties. These studies converged on the establishment of various optimised drug delivery systems in the form of directly compressed minitables. The drawbacks of chitosan minitablet formulations were circumvented and various mechanically strong, disintegrating minitables with exceptional dissolution properties were formulated. The suitability of these delivery systems, however, can only be measured once the formulations are compared with traditional tablets and commercially available products containing the same active ingredient. This section passes final judgement on the suitability of minitablet formulations; chitosan as a direct compression diluent as well as the suitability of the optimised excipients utilised to modify its undesirable characteristics.

7.3.1 Evaluation of the dissolution properties of the chitosan minitablet formulation and the commercially available products

Dissolution tests were performed to compare the drug release between CF/36 minitables; traditional tablets (10 mm diameter) and commercially available products, all containing furosemide. Dissolution studies were performed in four fold in 0.1 M HCl for 150 minutes at 75 rpm as described in the section 3.5.4. The standard USP-paddles were used in these analyses.

The compositions of the mixtures prepared and the commercial products are summarised in table 7.2. The two reference tablet formulations consisted of 20 mg furosemide per tablet, Avicel[®] PH-101 as filler and 1.25% w/w Ac-Di-Sol[®] as disintegrant. The second reference tablet formulation contained additional sodium bicarbonate to assist in the dissolution process, therefore, only differing in filler from the CF/36 formulation. Two commercial products namely 20 mg Lasix[®] and 40 mg Puresis[®] were also used to compare with the CF/36 minitab formulation.

Table 7.2: Reference tablet formulations.

Tablet content	Furosemide amount (mg)	Tablet weight (mg)
Furosemide, Avicel [®] PH101, 1.25% w/w Ac-Di-Sol (Formulation 1)	20	245 mg ± 1 mg
Furosemide, Avicel [®] PH101, 1.25% w/w Ac-Di-Sol, 2% w/w NaHCO ₃ (Formulation 2)	20	245 mg ± 1 mg
Lasix [®] (Aventis, batch nr. 22C)	20	80 mg ± 1 mg
Puresis [®] (Aspen Pharmacare, batch nr. E15302T)	40	170 mg ± 1 mg

The dissolution parameters of furosemide namely area under the dissolution curve (AUC), initial dissolution rate (DR_i), % furosemide dissolved, normalised area under the dissolution curve (AUC_n) and normalised initial dissolution rate (DR_i)_n, are summarised in table 7.3.

Table 7.3: The average dissolution properties of furosemide from the CF/36 minitab formulation; two reference tablet formulations and two commercial products.

	CF/36	Formulation 1	Formulation 2	20 mg Lasix [®]	40 mg Puresis [®]
AUC (µg.min.cm ⁻³)	2805.86	604.25	941.35	1570.51	2242.85
DR _i (µg.cm ⁻³ .min ⁻¹)	1.468	0.103	0.361	0.600	1.051
% Dissolved	84.18	18.20	28.20	46.91	33.49
AUC _n	1.210	0.261	0.406	0.677	0.967
(DR _i) _n	0.733	0.051	0.180	0.300	0.525

Tablets from all of the formulations (the reference tablets and commercial products) disintegrated instantly in the dissolution medium and none of the tablets drifted on the surface. Comparison of the dissolution parameters in table 7.3 again clearly indicated the dependency of the AUC on the DR_i . Also, the decreased DR_i -values of furosemide were again contradictory to the prodigious disintegration of all the formulations, which validated that tablet disintegration is no absolute prerequisite for drug dissolution.

The incorporation of sodium bicarbonate into formulation 2 again proved valid, as a significant ($p < 0.05$) increase in the percentage furosemide dissolved (increase of approximately 10%) was illustrated, compared to that of formulation 1 containing 0% w/w sodium bicarbonate. The evolution of small amounts of carbon dioxide from the tablet surfaces were observed during dissolution, however, the incorporation of 2% w/w sodium bicarbonate did not have any effect on the pH of the dissolution medium. The observed increase in the DR_i of furosemide from these tablets was attributed to a pH-increase in the microenvironment surrounding the dissolving furosemide particles, caused by the dissolution of the highly soluble sodium bicarbonate. However, none of these two formulations were comparable to a furosemide suspension or to the CF/36 minitab formulation when considering the rate and extent of furosemide dissolved. This could be attributed to insufficient dispersion of the primary drug particles throughout the medium before settling at the bottom of the dissolution vessel. The dissolution values of formulation 1 and 2 were almost 4 times lower than that of the CF/36 formulation. It is thus clear that the CF/36 formulation and various other chitosan minitab formulations (chapter 6) proved superior to formulations which were tableted into traditional 10 mm tablets. Furthermore, when comparing dissolution properties of formulation 2 with the CF/36 formulation (only the filler and tablet diameter differ), regardless whether CF/36 tablets were encapsulated or not (figure 7.2), the Avicel® PH-101 filler seemed inferior to chitosan, thus proving chitosan a directly compressible filler to be reckoned with.

Comparing the dissolution properties of the CF/36 formulation with commercial products provided interesting results. From table 7.3 it could be concluded that the CF/36 minitab formulations depicted dissolution values significantly higher than the Lasix® tablets containing the exact same amount furosemide. The AUC and AUC_n of the CF/36 minitab formulations were approximately 1.8 times higher than that of the 20 mg Lasix® tablets, whereas the DR_i and $(DR_i)_n$ were approximately 2.4 times higher. Furthermore, nearly 40.0% more furosemide dissolved from the minitab formulation compared to the 20 mg Lasix® tablets during the dissolution studies. From the

dissolution profiles (figure 7.2) it could clearly be seen that the end furosemide concentration dissolved from 10 CF/36 minitables, regardless whether the tablets were encapsulated or not, was almost double of the final concentration furosemide dissolved (at t_{150}) from the 20 mg Lasix[®] tablets.

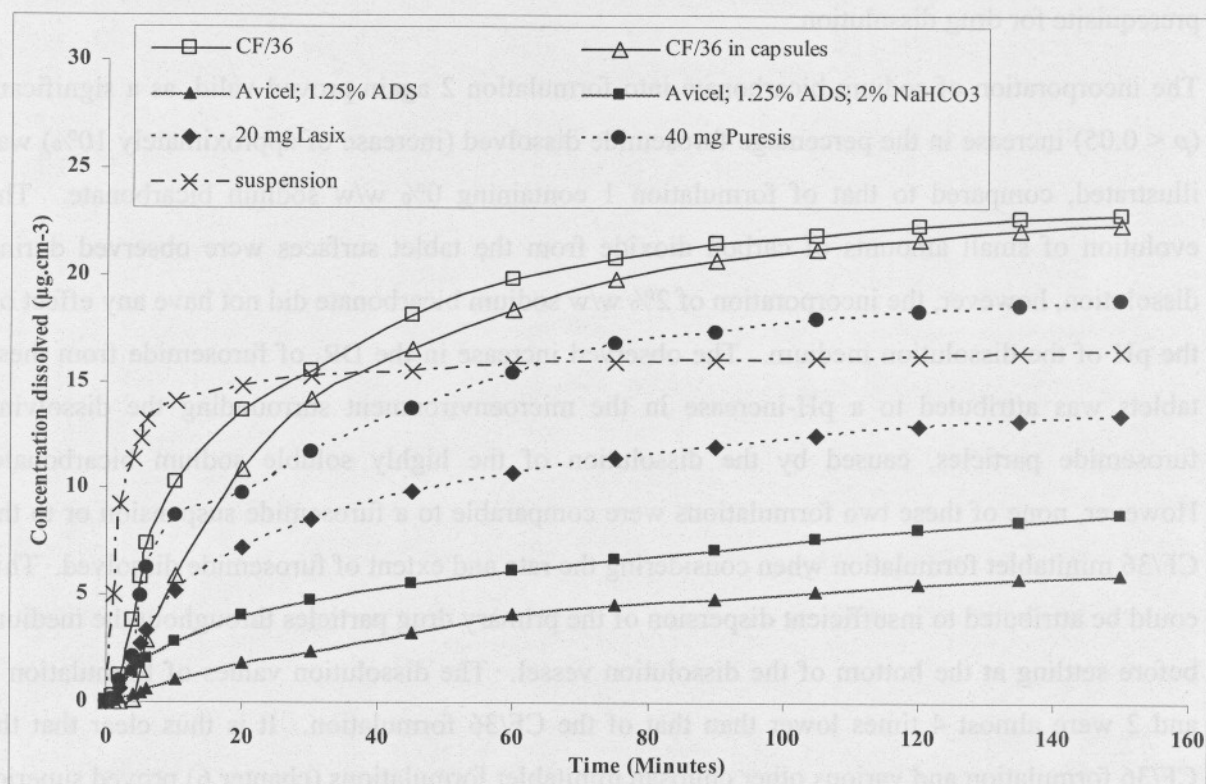


Figure 7.2: Dissolution profiles of CF/36 minitables, CF/36 minitables in capsules, formulation 1, formulation 2, 20 mg Lasix[®] tablets and 40 mg Puresis[®] tablets compared with a dissolution profile of a furosemide suspension.

Last but not least, the CF/36 minitab formulation was compared with 40 mg Puresis[®] tablets which contain double the furosemide concentration. Not even this commercial product could compete with the minitab formulation when considering the dissolution properties in table 7.3. The CF/36 formulation depicted AUC and AUC_n values of 1.3 times higher than that of the Puresis[®] product, whereas the DR_i and (DR_i)_n values of the minitab formulation were 1.4 times faster. Interestingly, only an average of 33.49% furosemide dissolved from 40 mg Puresis[®] tablets. This was approximately 51% lower than the average percentage furosemide which dissolved from 10 CF/36 minitables. Figure 7.2 clearly depicted these results which in turn

confirmed that chitosan minitabket formulations may be of value in future development of pharmaceutical oral dosage forms.

7.3.2 Comparison of drug release between chitosan minitabkets and commercially available products in conclusion

All of the tablets from the various formulations disintegrated instantly and a clear dependency of the AUC on the DR_i was illustrated. However, the decreased DR_i -values of furosemide were again contradictory to the prodigious disintegration of all the formulations which validated that tablet disintegration is no absolute prerequisite for drug dissolution.

None of the dissolution profiles of traditional 10 mm tablets or commercial products were comparable to a furosemide suspension or to the CF/36 minitabket formulation, regardless encapsulation or not. This could have been attributed to insufficient dispersion of the primary drug particles throughout the medium before settling at the bottom of the dissolution vessel. The end furosemide concentration that dissolved from 10 CF/36 minitabkets was almost double the end concentration furosemide that dissolved from the 20 mg Lasix[®] tablets and approximately 1.2 times higher than that of 40 mg Puresis[®] tablets. It is therefore clear that the CF/36 formulation and various other chitosan minitabket formulations proved superior to commercially available products. It was thus confirmed that chitosan minitabket formulations are of value for future development of oral dosage forms for poorly soluble and water wettable drugs.

7.4 Conclusion

Chitosan does not rank highly regarding its employment as a first choice filler. Undesirable properties that limit its utilisation include its hydration propensity influencing stability, problematic tablet strength characteristics, poor flowability and the availability of fillers that circumvent some of the undesirable properties of chitosan (Otto, 2002:125). This study, however, proved that chitosan could serve as a relatively cheap alternative to other direct compression fillers especially in the formulation of minitabkets, if the formulations were carefully optimised.

The optimisation of chitosan minitabket formulations seemed relatively challenging and was perplexed. The study proved that empirical observation played an important role in determining

the choice of excipient. It could be suggested that perceived advantages of certain excipients that are documented in literature, should not be considered absolute until proven. A systematic process of optimisation could ensure adequate performance of chitosan minitabiet formulations in direct compression. The utilisation of chitosan could certainly be advocated from this perspective and provide advantages of time saving and economical benefits compared to alternative fillers and tablet production methods. Furthermore, its mucoadhesive and absorption enhancing properties could also benefit and improve bioavailability of various poorly absorbed compounds or active ingredients. An interesting new development, which should be considered based on these results, would be a chitosan multifunctional excipient.

7.4 Conclusion

Chitosan does not rank highly regarding its employment as a first choice filler. Undesirable properties that limit its utilisation include its hygroscopicity, poor flowability and the availability of fillers that overcome some of the undesirable properties of chitosan (Olo, 2002, 125). This study, however, proved that chitosan could serve as a relatively cheap alternative to other direct compression fillers especially in the formulation of minitabiet. If the formulations were carefully

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CHITOSAN: A MULTIPURPOSE EXCIPIENT IN DIRECTLY COMPRESSED MINITABLET FORMULATIONS

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ABSTRACT

Purpose:

In recent years chitosan has attracted a great deal of attention and has become well known as a multifunctional biopolymer and a potential absorption enhancer across mucosal epithelia, with a wide range of applications both in the medical and pharmaceutical field. The main driving force in the development of new applications for chitosan lies in the fact that the polysaccharide is not only naturally abundant, but it is also non-toxic and biodegradable. Unlike oil and coal, chitosan is a naturally regenerating resource (e.g., crab and shrimp shells) that can be further enhanced by artificial culturing. The purpose of this study was to determine whether chitosan could be used as a directly compressed multipurpose excipient in mini-tablet formulations.

Methods:

Furosemide was chosen as a model drug representing a group of drugs, which exhibits poor dissolution due to low water-solubility and poor water-wettability. Due to the physical properties of chitosan, none of the formulations necessitated the inclusion of a lubricant such as magnesium stearate. Kollidon[®] VA-64 was included as a binder in 3 and 5 % (w/w) concentrations either through physical mixing or through wet granulation with chitosan. The furosemide and different disintegrants (Ac-Di-Sol[®] and Kollidon[®] CL) in various concentrations was incorporated into different formulations. Some of these formulations contained sodium bicarbonate (2 and 4 % w/w) to facilitate disintegration.

Results:

It was found that mixtures which were directly compressed with 5 % Kollidon® VA-64 produced mini-tablets which displayed negligible weight variation (i.e. good flowability of the mixtures), and excellent hardness, friability and disintegration properties. Furthermore, from the dissolution results it was evident that Kollidon® increased the solubility of furosemide in a discriminating dissolution medium. A disintegrant was, however, necessary to facilitate a fast initial dissolution rate, and sodium bicarbonate played a synergetic role in the disintegration and hardness of the tablets but it is debatable whether this excipient was actually necessary to include into the formulations. Other factors that played an important role in the properties of the formulations were the moisture content of the chitosan and the temperature and humidity at which the chitosan were stored.

Conclusions:

Chitosan could indeed be used as a multipurpose excipient in directly compressed mini-tablet formulations. These formulations produced tablets with good formulation properties and conducted immediate release of the active ingredient. The mucoadhesive properties of these chitosan formulations are to be considered because this could indeed have a marked increase in the absorption of the active ingredient.

INTRODUCTION

In recent years chitosan has attracted a great deal of attention and has become well known as a multifunctional biopolymer and a potential absorption enhancer across mucosal epithelia, with a wide range of applications both in the medical and pharmaceutical field.^{1,3, 4,5}

The main driving force in the development of new applications for chitosan lies in the fact that the polysaccharide is not only naturally abundant, but it is also non-toxic and biodegradable. Unlike oil and coal, chitosan is a naturally regenerating resource (e.g., crab and shrimp shells) that can be further enhanced by artificially culturing.^{1,3,4}

Chitosan was also shown to open tight junctions and increase the absorption of large hydrophilic drugs including peptides and proteins.²

OBJECTIVES

- To determine whether chitosan could be used as a directly compressible multipurpose excipient in minitab formulations, and
- To determine its effect on drug release.

METHODS

- A tracer drug (furosemide) was included to evaluate drug release properties from the tablets.
- Kollidon[®] VA64 was included as a binder in 3 and 5% w/w concentrations, either through physical mixing or through wet granulation with chitosan.
- Various concentrations of either Ac-Di-Sol[®], Kollidon[®] CL (0; 0.625; 1.25% w/w) or sodium bicarbonate (2.0; 4.0% w/w), or combinations thereof were included into the formulations tested.
- Some of these formulations also contained ascorbic acid (0.5% w/w) to improve the dissolution of chitosan.
- The mixtures were prepared and mixed in a Turbula[®]-mixer, for 10 minutes at 69 rpm.
- Slightly biconcave tablets (3 mm diameter and weighing approximately 14 mg) were prepared from each mixture at a constant die fill volume on a Cadmach[®] single press at a constant compaction load.
- Tablet evaluation:

The crushing strength, friability, disintegration time and mass variation were determined, using standard BP methods.

- Dissolution studies:

Dissolution studies were performed in a six-station Erweka[®] dissolution apparatus at 50 rpm, using the standard USP method. However, for the analysis of the minitab, the standard USP-paddles were removed from the rods and replaced with stainless steel rotating baskets (United States Pharmacopoeia, 2002:194-195). The initial slope of the dissolution curve (DR_i) and the area under the curve (AUC) were determined.

RESULTS

- All of the formulas tested, displayed negligible weight variation (< 3% standard deviation), which indicated good flowability of the mixtures.
- Despite the fact that the tablet hardness of all of the formulations was less than 80 Newton, these formulations depicted acceptable friability and excellent disintegration properties.
- From the factorial design (table 1) it could be seen that although formulations containing Kollidon[®] CL disintegrated faster than formulations containing Ac-Di-Sol[®], these formulations did not display the same excellent dissolution profiles.
- Formulations that included super disintegrants in the highest concentration level (1.25% w/w) and sodium bicarbonate (NaHCO₃) at a 2% w/w level depicted the fastest disintegration properties.

Table 1: A summary of the results according to a factorial design.

Variables	AUC (µg/ml/min)	DR _i (µg.min/ml)	% Dissolved (%)	Disintegration (sec)
Granules	1820.134	0.360	53.537	76.967
Mixtures	2075.945	0.640	61.423	12.221
3% w/w Kollidon [®] VA64	1941.400	0.514	57.257	12.775
5% w/w Kollidon [®] VA64	1954.678	0.485	57.703	76.413
Ac-Di-Sol [®]	2163.216	0.710	64.881	62.843
Kollidon [®] CL	1761.072	0.367	52.533	42.558
NaHCO ₃	1945.290	0.600	58.200	50.075
0.5% w/w Ascorbic acid	1951.230	0.386	58.341	16.342
0.625% w/w Ac-Di-Sol [®]	2037.490	0.539	61.196	45.271
1.250% w/w Ac-Di-Sol [®]	2288.941	0.881	68.566	80.416
0.625% w/w Kollidon [®] CL	1767.752	0.382	52.712	69.292
1.250% w/w Kollidon [®] CL	1754.393	0.353	52.351	15.824

- Mixtures containing 5% w/w Kollidon[®] VA64 produced minitables that displayed excellent dissolution properties.

- The presence of Ac-Di-Sol[®], especially in a higher concentration (1.25% w/w) as well as sodium bicarbonate (2% w/w) seemed to have the greatest influence on the dissolution properties.
- The inclusion of ascorbic acid did not seem to have a positive influence on the dissolution properties of both the tracer drug and the chitosan.
- The formulation containing 5% w/w Kollidon[®] VA64, 1.25% w/w Ac-Di-Sol[®] and 2% w/w sodium bicarbonate (figure 1) depicted the highest percentage dissolved (84.18%).

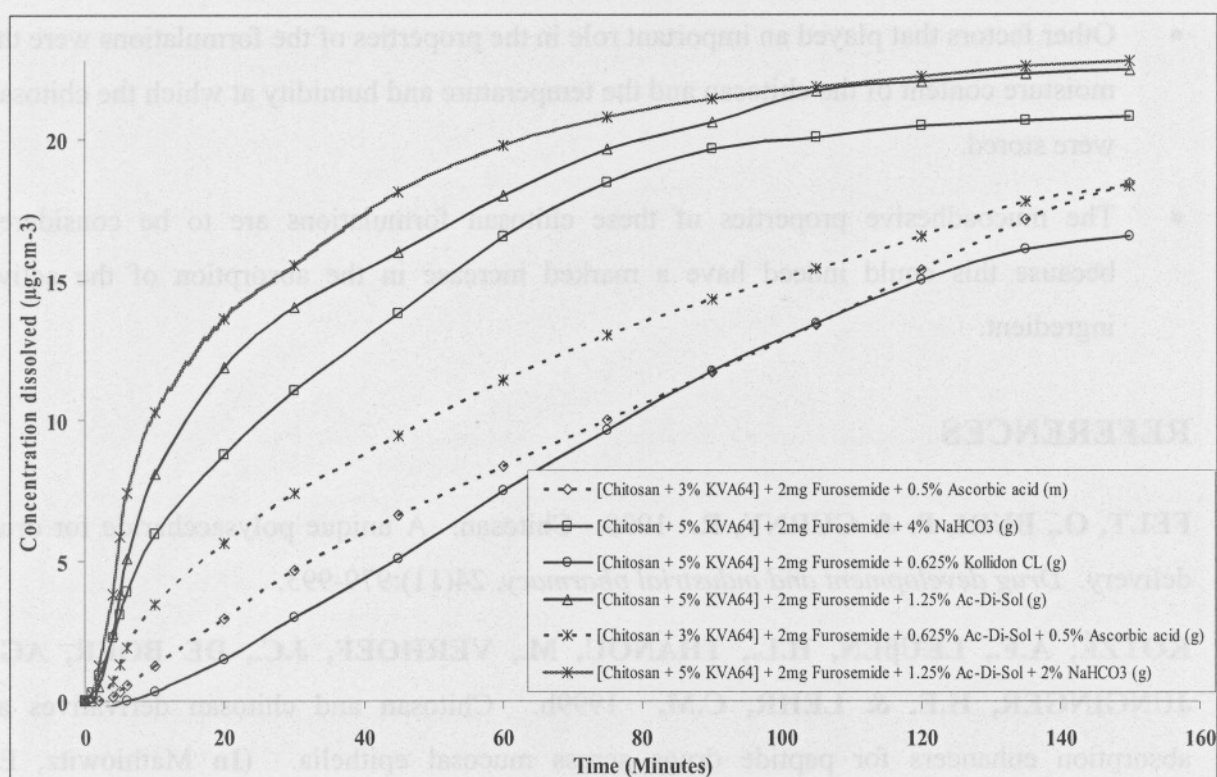


Figure 1: Dissolution curve of the different tablet formulations.

CONCLUSIONS

- Chitosan could indeed be used as a multipurpose excipient in directly compressed minitabiet formulations. These formulations produced tablets with good formulation properties and conducted immediate release of the active ingredient.
- The reason for the delayed disintegration times of the formulations which contained Ac-Di-Sol[®] could have been due to the insolubility of chitosan in the disintegration medium (distilled water). Chitosan only dissolves in acid media.

- A disintegrant (Ac-Di-Sol[®]) was, however, necessary to facilitate a fast initial dissolution rate, and sodium bicarbonate in a concentration of 2% w/w played a synergetic role in the disintegration (during dissolution studies) and hardness of the tablets.
- From the dissolution results it was evident that the binder increased the solubility of furosemide in a discriminating dissolution medium.
- It is however, debatable whether the inclusion of ascorbic acid was actually necessary, as it hindered the initial dissolution rate.
- Other factors that played an important role in the properties of the formulations were the moisture content of the chitosan and the temperature and humidity at which the chitosan were stored.
- The mucoadhesive properties of these chitosan formulations are to be considered because this could indeed have a marked increase in the absorption of the active ingredient.

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ANNEXURE A: CHARACTERISATION OF CHITOSAN POWDER

A.1 MOLECULAR WEIGHT:

ASTRA 4.72.03 SUMMARY REPORT:		
File	C:\ASTRA\HE-NE\FARMASIE\DEWALD\2003\JOE1.ADF	
Sample ID	Joe, chitosan 20 cp	
Operator	Dewald Snyman	
COLLECTION INFORMATION:		
Collection time	Fri Mar 14, 2003 12:40 PM	
Instrument type	DAWN DSP	
Cell type	K5	
Laser wavelength	632.8 nm	
Solvent name	Water	
Solvent RI	1.332	
Calibration constants		
DAWN	2.0850e-05	
» AUX1	3.0000e-05	
Flow rate	0.800 ml/min	
PROCESSING INFORMATION:		
Processing time	Fri Mar 14, 2003 02:25 PM	
DAWN/AUX1 delay	0.220 ml	
Fit method / model	Zimm	
Calculation method	dn/dc + AUX Constant	
Detectors used	7 8 9 10 11 12 13 14 15	
RESULTS: Peak #1		
Time (min)	17.021 – 28.425	
Slices	2738	
A2 (mol ml/g ²)	0.000e+00	
Fit degree	1	
Injected Mass (g)	5.0000e-04	
Calc. Mass (g)	7.6383e-04	
dn/dc (ml/g)	0.170	
Polydispersity (M _w /M _n)	1.920 ±0.095 (5%)	
Polydispersity (M _z /M _n)	4.372 ±0.512 (12%)	
Molar Mass Moments (g/mol)		
M _n	6.543e+04 (4%)	
M _w	1.256e+05 (2.0%)	
M _z	2.861e+05 (10%)	
R.M.S. Radius Moments (nm)		
R _n	36.1 (12%)	
R _w	41.2 (5%)	
R _z	58.8 (2.4%)	

A.2 CERTIFICATE OF ANALYSIS FOR CHITOSAN:

XIAMEN JIANGYUAN IMPORT AND EXPORT COMPANY

4/F, NO.168 QIXING ROAD XIAMEN, FUJIAN CHINA

TEL: 86-592-5911378

FAX: 86-592-5911318

CERTIFICATE OF ANALYSIS FOR CHITOSAN

Original

Report No.: 021009

Batch No.: 021009	Quantity: 1005kgs	Report Date : NOV. 05, 2002	
Expiry date		Before NOV. 04, 2004.	
ITEMS	TESTED	INSPECTION	RESULTS
Appearance		Off white	
Moisture		6.82%	
Ash		0.81%	
Deacetylation		91.23%	
Viscosity (0.5%)		20cps	
Mesh		80mesh	

ABSA BANK LIMITED IB CENTRE CAPE TOWN
LETTER OF CREDIT N JMBER 827-01-0074500-G

*Drum's No.: 1-67



WCI 7109

XIAMEN JIANGYUAN IMPORT AND EXPORT COMPANY

4/F, NO.168 QIXING ROAD XIAMEN, FUJIAN CHINA

TEL: 86-592-5911378

FAX: 86-592-5911318

CERTIFICATE OF ANALYSIS FOR CHITOSAN

Original

Report No.: 021010

Batch No.: 021010	Quantity: 1005kgs	Report Date : NOV. 05, 2002
Expiry date	Before NOV. 04, 2004.	
ITEMS TESTED	INSPECTION	RESULTS
Appearance	Off white	
Moisture	5.93%	
Ash	0.89%	
Deacetylation	91.46%	
Viscosity (0.5%)	21cps	
Mesh	80mesh	

AESA BANK LIMITED 1B CENTRE CAPE TOWN

LETTER OF CREDIT NUMBER 827-01-0074500-G

*Drum's No.: 68-134



WCI 7109

**A.3 CALCULATED RELATIVE, REDUCED AND INHERENT
VISCOSITIES OF CHITOSAN:**

		η_{rel}	η_{red}	η_{inh}
0.025	%	1.98	39.20	27.32
0.050	%	3.36	47.28	24.26
0.100	%	7.48	64.80	20.12
0.200	%	16.19	75.95	13.92

A.4 KARL-FISHER ANALYSIS OF CHITOSAN:

RESEARCH INSTITUTE FOR INDUSTRIAL PHARMACY

POTCHEFSTROOM UNIVERSITY FOR CHRISTIAN HIGHER EDUCATION

MOIST01B

MOISTURE DETERMINATION REPORT

FILE CODE:	-	REF: Book no:	-	Page no:	-
PRODUCT:	Chitosan	DATE TESTED:	02/09/2003		
COMPANY:	Private	AGE:	1 Day		
BATCH NO:	1	REF:	BP, 1993, VOL. II, A144, IXC		
DOSAGE FORM:	Powder	APPARATUS:	Karl fischer titrator		
CONTAINER:	Glass	ANALYST:	M. le Roux		
TIME SPENT:	-	CONTROL ANALYST:	-		

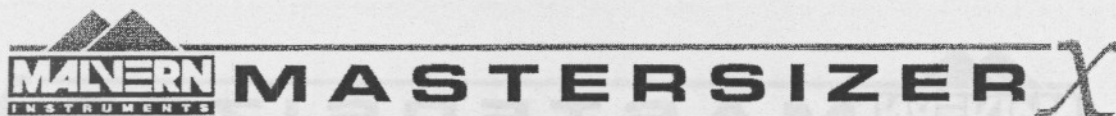
SAMPLE MASS: 0.2500 g $\pm$ 0.0004 g **STIRRING TIME:** 300 seconds (5 min)

	$^{\circ}\text{C}$	%RH
Date:	2003-09-02	Time: 15:23:45
Smpl size:	0.2499 g	ident.
KFR volume:	2.014 ml	
Drift man.:	0.0 $\mu\text{l}/\text{min}$	
(-d) time:	2:05	
Water:	3.9897 %	
COMMENT ON SAMPLE:		

RESULTS	
Temp.	$^{\circ}\text{C}$
n:	2
% Mean:	4.00745
% SD:	0.02510229
% CV:	0.6264

A.5 POWDER CHARACTERISTICS:

MEAN PARTICLE SIZE DETERMINATIONS:



Version 1.2a

Tue, Sep 16, 2003 9:32AM

Chitosan :Run Number 1

Batch nr.: 021010 Drum: 116
Dispersant: 15 ml Ethanol in MSX1

Sample 1

Sample File Name: JOE_02 , Record: 1

Source: Analysed

Measured on: Fri, Jan 04, 1980 7:01PM Last saved on: Fri, Jan 04, 1980 7:01PM

Presentation: 2THD

Very Polydisperse model

Volume Result

Focus = 300 mm.

Residual = 1.082 %

d (0.5) = 214.99 µm

D [4, 3] = 224.03 µm

Sauter Mean (D[3,2]) = 138.50 µm

Specific Surface Area = 0.0433 sq. m. / gm

Concentration = 0.060 %

d (0.1) = 91.80 µm

Span = 1.25

Obscuration = 17.16 %

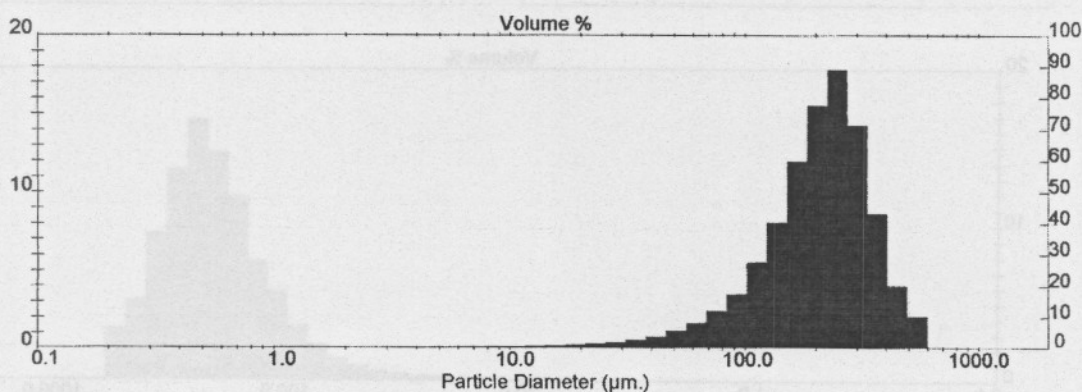
d (0.9) = 361.56 µm

Mode = 239.76 µm

Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.50	0.00	1.32	0.00
1.32	0.00	1.60	0.00
1.60	0.00	1.95	0.00
1.95	0.00	2.38	0.00
2.38	0.01	2.90	0.01
2.90	0.02	3.53	0.03
3.53	0.05	4.30	0.08
4.30	0.07	5.24	0.15
5.24	0.08	6.39	0.23
6.39	0.08	7.78	0.31
7.78	0.10	9.48	0.41
9.48	0.12	11.55	0.53
11.55	0.15	14.08	0.68
14.08	0.19	17.15	0.87
17.15	0.24	20.90	1.11
20.90	0.31	25.46	1.42

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
25.46	0.42	31.01	1.84
31.01	0.57	37.79	2.42
37.79	0.82	46.03	3.23
46.03	1.15	56.09	4.39
56.09	1.65	68.33	6.04
68.33	2.42	83.26	8.46
83.26	3.49	101.44	11.95
101.44	5.51	123.59	17.46
123.59	8.29	150.57	25.75
150.57	11.67	183.44	37.42
183.44	15.95	223.51	53.37
223.51	17.32	272.31	70.69
272.31	14.75	331.77	85.43
331.77	8.32	404.21	93.76
404.21	4.18	492.47	97.94
492.47	2.06	600.00	100.00



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MasterSizer X Ver. 1.2a
Serial No.

p. 3
16 Sep 03 09:32

MASTERSIZER^X

Version 1.2a

Tue, Sep 16, 2003 9:32AM

Chitosan :Run Number 3

Batch nr.: 021010 Drum: 116
Dispersant: 15 ml Ethanol in MSX1

Sample 2

Sample File Name: JOE_02 , Record: 3

Source: Analysed

Measured on: Fri, Jan 04, 1980 7:05PM Last saved on: Fri, Jan 04, 1980 7:05PM

Presentation: 2THD

Very Polydisperse model

Volume Result

Focus = 300 mm.

Residual = 1.855 %

Concentration = 0.056 %

Obscuration = 16.42 %

d (0.5) = 216.80 μ m

d (0.1) = 93.52 μ m

d (0.9) = 375.02 μ m

D [4, 3] = 228.39 μ m

Span = 1.30

Sauter Mean (D[3,2]) = 137.81 μ m

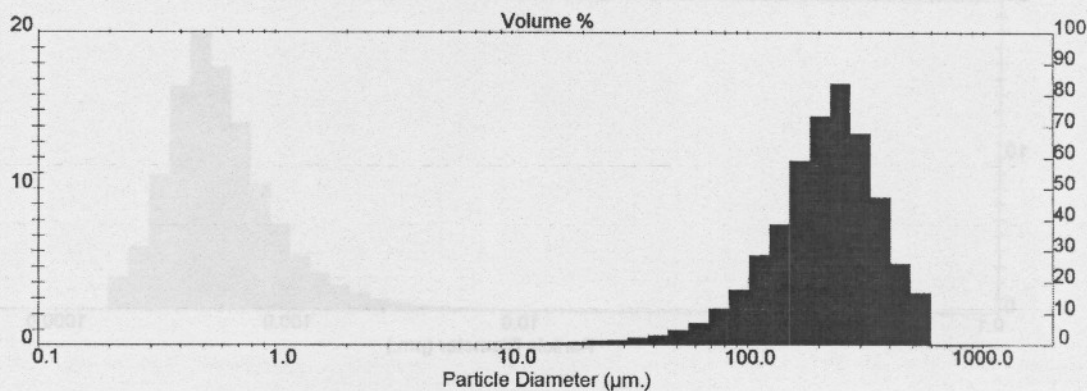
Mode = 238.49 μ m

Specific Surface Area = 0.0435 sq. m. / gm

Density = 1.00 gm. / c.c.

Size (Lo) μ m	Result In %	Size (Hi) μ m	Result Below %
0.50	0.00	1.32	0.00
1.32	0.00	1.60	0.01
1.60	0.01	1.95	0.01
1.95	0.01	2.38	0.02
2.38	0.01	2.90	0.03
2.90	0.03	3.53	0.06
3.53	0.05	4.30	0.11
4.30	0.06	5.24	0.18
5.24	0.07	6.39	0.24
6.39	0.07	7.78	0.32
7.78	0.10	9.48	0.42
9.48	0.12	11.55	0.54
11.55	0.15	14.08	0.68
14.08	0.19	17.15	0.87
17.15	0.24	20.90	1.11
20.90	0.31	25.46	1.42

Size (Lo) μ m	Result In %	Size (Hi) μ m	Result Below %
25.46	0.40	31.01	1.82
31.01	0.53	37.79	2.34
37.79	0.76	46.03	3.10
46.03	1.07	56.09	4.17
56.09	1.56	68.33	5.73
68.33	2.39	83.26	8.12
83.26	3.53	101.44	11.65
101.44	5.60	123.59	17.25
123.59	8.17	150.57	25.42
150.57	11.54	183.44	36.96
183.44	15.60	223.51	52.57
223.51	16.74	272.31	69.31
272.31	14.46	331.77	83.77
331.77	8.72	404.21	92.49
404.21	4.79	492.47	97.28
492.47	2.72	600.00	100.00



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Malvern, U.K.

MasterSizer X Ver. 1.2a
Serial No.

p. 4
16 Sep 03 09:32



MASTERSIZER X

Version 1.2a

Tue, Sep 16, 2003 9:29AM

Chitosan :Run Number 1

Batch nr.: 021010 Drum: 115
Dispersant: 15 ml Ethanol in MSX1

Sample 1

Sample File Name: JOE_01 , Record: 1

Source: Analysed

Measured on: Fri, Jan 04, 1980 6:51PM Last saved on: Fri, Jan 04, 1980 6:51PM

Presentation: 2THD

Very Polydisperse model

Volume Result

Focus = 300 mm.

Residual = 2.162 %

d (0.5) = 193.31 μ m

D [4, 3] = 212.56 μ m

Sauter Mean (D[3,2]) = 124.48 μ m

Specific Surface Area = 0.0482 sq. m. / gm

Concentration = 0.075 %

d (0.1) = 77.21 μ m

Span = 1.54

Obscuration = 23.18 %

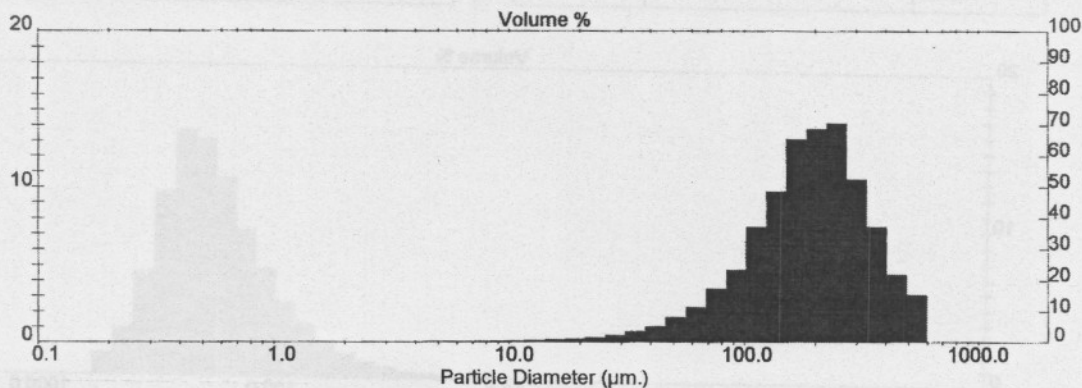
d (0.9) = 375.78 μ m

Mode = 208.64 μ m

Density = 1.00 gm. / c.c.

Size (Lo) μ m	Result In %	Size (Hi) μ m	Result Below %
0.50	0.00	1.32	0.00
1.32	0.00	1.60	0.00
1.60	0.00	1.95	0.00
1.95	0.00	2.38	0.01
2.38	0.01	2.90	0.02
2.90	0.02	3.53	0.04
3.53	0.04	4.30	0.08
4.30	0.07	5.24	0.15
5.24	0.09	6.39	0.24
6.39	0.11	7.78	0.36
7.78	0.12	9.48	0.48
9.48	0.14	11.55	0.61
11.55	0.17	14.08	0.78
14.08	0.21	17.15	0.99
17.15	0.27	20.90	1.26
20.90	0.36	25.46	1.62

Size (Lo) μ m	Result In %	Size (Hi) μ m	Result Below %
25.46	0.53	31.01	2.14
31.01	0.74	37.79	2.88
37.79	1.13	46.03	4.01
46.03	1.61	56.09	5.62
56.09	2.34	68.33	7.96
68.33	3.47	83.26	11.43
83.26	4.73	101.44	16.17
101.44	7.41	123.59	23.57
123.59	9.92	150.57	33.49
150.57	12.77	183.44	46.26
183.44	14.24	223.51	60.50
223.51	13.74	272.31	74.23
272.31	10.68	331.77	84.91
331.77	7.46	404.21	92.37
404.21	4.49	492.47	96.86
492.47	3.14	600.00	100.00



Malvern Instruments Ltd.
Malvern, U.K.

MasterSizer X Ver. 1.2a
Serial No.

p. 1
16 Sep 03 09:25



MASTERSIZER X

Version 1.2a

Tue, Sep 16, 2003 9:29AM

Chitosan :Run Number 3

Batch nr.: 021010 Drum: 115
Dispersant: 15 ml Ethanol in MSX1

Sample 2

Sample File Name: JOE_01 , Record: 3

Source: Analysed

Measured on: Fri, Jan 04, 1980 6:56PM Last saved on: Fri, Jan 04, 1980 6:57PM

Presentation: 2THD

Very Polydisperse model

Volume Result

Focus = 300 mm.

Residual = 0.818 %

d (0.5) = 193.99 μm

D [4, 3] = 208.86 μm

Sauter Mean (D[3,2]) = 123.69 μm

Specific Surface Area = 0.0485 sq. m. / gm

Concentration = 0.062 %

d (0.1) = 77.22 μm

Span = 1.46

Obscuration = 19.72 %

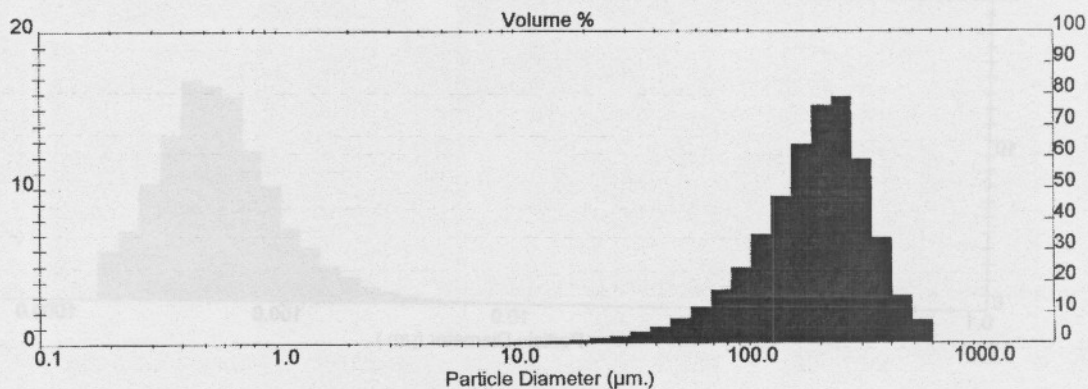
d (0.9) = 359.54 μm

Mode = 229.54 μm

Density = 1.00 gm. / c.c.

Size (Lo) μm	Result In %	Size (Hi) μm	Result Below %
0.50	0.00	1.32	0.00
1.32	0.00	1.60	0.00
1.60	0.00	1.95	0.00
1.95	0.00	2.38	0.01
2.38	0.01	2.90	0.02
2.90	0.02	3.53	0.04
3.53	0.05	4.30	0.09
4.30	0.08	5.24	0.17
5.24	0.09	6.39	0.26
6.39	0.10	7.78	0.36
7.78	0.12	9.48	0.48
9.48	0.14	11.55	0.61
11.55	0.17	14.08	0.78
14.08	0.21	17.15	0.99
17.15	0.27	20.90	1.26
20.90	0.37	25.46	1.63

Size (Lo) μm	Result In %	Size (Hi) μm	Result Below %
25.46	0.52	31.01	2.15
31.01	0.74	37.79	2.89
37.79	1.11	46.03	4.00
46.03	1.61	56.09	5.61
56.09	2.36	68.33	7.97
68.33	3.47	83.26	11.45
83.26	4.80	101.44	16.25
101.44	7.22	123.59	23.47
123.59	9.72	150.57	33.19
150.57	12.74	183.44	45.93
183.44	14.76	223.51	60.70
223.51	14.85	272.31	75.54
272.31	11.08	331.77	86.63
331.77	7.23	404.21	93.86
404.21	3.83	492.47	97.69
492.47	2.31	600.00	100.00



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MasterSizer X Ver. 1.2a
Serial No.

p. 2
16 Sep 03 09:29

Table A.5.1: Flow properties of chitosan powder (Angle of repose).

Mass (g)	D (cm)	H (cm)	Φ (°)	Flow time (s)	Mass flowed (g)	Flow rate (g.s ⁻¹)
100.00	14.3	5.30	36.55	10.30	66.33	6.440
100.00	15.8	5.80	36.29	10.27	65.89	6.416
100.00	16.4	6.00	36.19	9.140	65.02	7.114
Average	15.5	5.70	36.34	9.903	65.75	6.656

D = diameter; H = height; Φ = calculated angle of repose

Table A.5.2: Hopper flow rate of chitosan powder.

Mass (g)	Flow time (s)	Flow rate (g.s ⁻¹)
100.00	11.90	8.403
100.00	13.60	7.353
100.00	12.50	8.000
Average	12.67	7.919

Table A.5.3: Density measurements of chitosan powder samples (± 100 g; amplitude of 10 A).

Time (minutes)	V_b (cm ³)	P_b (g.cm ⁻³)	V_t (cm ³)	P_t (g.cm ⁻³)	ϵ (%)	V_v (cm ⁻³)
5	621.00	0.1614	394.00	0.2544	36.554	227.00
	618.00	0.1618	393.00	0.2545	36.408	225.00
	622.00	0.1608	394.00	0.2538	36.656	228.00
Average	620.33	0.1613	393.67	0.2542	36.539	226.67
10	551.00	0.1819	389.00	0.2576	29.401	162.00
	548.00	0.1825	391.00	0.2558	28.650	157.00
	548.00	0.1825	392.00	0.2551	28.467	156.00
Average	549.00	0.1823	390.67	0.2562	28.839	158.33
15	550.00	0.1822	387.00	0.2590	29.636	163.00
	540.00	0.1852	385.00	0.2597	28.704	155.00
	546.00	0.1832	386.00	0.2591	29.304	160.00
Average	545.33	0.1835	386.00	0.2593	29.215	159.33

V_b = bulk volume; ρ_b = bulk density; V_t = tapped volume; ρ_t = tapped density; ϵ = porosity; V_v = void volume.

TRUE DENSITY MEASUREMENTS:

Cell used:	Large
Total sample mass:	15.700 g
Tare mass:	0.0000 g
Real mass:	15.700 g

Table A.5.4: Volume measurements.

Repetition	1	2	3
Sample 1:			
V_{out}	17.093	16.719	16.251
V_{in}	11.168	10.933	10.624
$P_{true} (g \cdot cm^{-3})$			
Sample 2:			
V_{out}	16.959	16.345	17.823
V_{in}	10.982	10.590	11.552
$P_{true} (g \cdot cm^{-3})$			

Table A.5.5: Pressure measurements.

Repetition	1	2	3
Sample 1:			
V_1	17.093	16.719	16.251
V_2	11.168	10.933	10.624
Sample 2:			
V_1	16.959	16.345	17.823
V_2	10.982	10.590	11.552

Table A.5.6: Density results.

Results	Sample 1	Sample 2	Average
Sample mass (g)	15.700	21.100	18.400
Sample volume (cm^3)	10.871	14.656	12.764
$\rho_{true} (g \cdot cm^{-1})$	1.4440	1.4400	1.4420

Table A.6.1: Physical properties of chitosan/Avicel® PH 200; chitosan/Prosolv® 50 and Chitosan/Prosolv® 90 tablets at various settings and mixing times. The mixtures were mixed in a 30:70 ratio. %RSD is indicated in parentheses.

		Avicel					Prosolv 50					Prosolv 90				
		30	35	40	45	50	30	35	40	45	50	30	35	40	45	50
5	WV	97.41 (0.54)	97.05 (0.47)	95.11 (1.09)	95.49 (0.44)	96.13 (0.94)	114.43 (1.32)	112.91 (0.55)	112.30 (0.36)	112.67 (0.63)	116.63 (1.90)	85.30 (1.32)	84.47 (1.14)	82.26 (0.97)	81.94 (1.49)	81.84 (1.12)
	CS	31.71 (5.53)	108.97 (2.36)	207.83 (0.98)	275.58 (1.58)	291.21 (1.86)	54.64 (8.37)	144.52 (3.30)	289.92 (3.31)	320.64 (0.30)	324.53 (1.16)	18.32 (4.22)	75.19 (4.24)	150.80 (4.85)	235.24 (3.87)	252.89 (3.32)
	H	5.34 (0.25)	4.00 (0.18)	3.38 (0.15)	3.22 (0.57)	3.21 (0.31)	5.42 (0.30)	4.28 (0.31)	3.70 (0.29)	3.61 (0.32)	3.56 (0.40)	5.24 (0.22)	3.83 (0.31)	3.15 (0.67)	2.89 (0.71)	2.86 (0.79)
	D	5.61 (0.11)	5.58 (0.10)	5.57 (0.14)	5.56 (0.13)	5.57 (0.12)	5.58 (0.06)	5.56 (0.09)	5.54 (0.14)	5.54 (0.17)	5.55 (0.13)	5.59 (0.12)	5.59 (0.13)	5.57 (0.14)	5.56 (0.17)	5.56 (0.15)
	F	0.533	0.010	0.000	0.000	0.031	0.184	0.000	0.009	0.000	0.000	1.614	0.035	0.000	0.000	0.061
	DT	179.50	N/d	N/d	N/d	N/d	61.00	N/d	N/d	N/d	N/d	50.00	309.67	N/d	N/d	N/d
10	WV	96.00 (0.47)	96.59 (0.40)	95.95 (0.62)	95.65 (0.39)	95.46 (0.49)	115.25 (1.00)	113.57 (0.86)	112.49 (0.52)	113.97 (0.69)	113.29 (1.83)	85.84 (1.25)	84.83 (1.24)	82.92 (1.05)	81.81 (1.02)	81.44 (0.77)
	CS	30.35 (5.72)	101.19 (2.56)	208.80 (2.61)	275.57 (2.13)	284.47 (0.98)	54.83 (5.06)	148.78 (2.63)	293.51 (2.23)	325.02 (0.65)	330.17 (1.33)	19.49 (8.03)	77.20 (4.12)	157.16 (4.45)	236.36 (2.65)	250.19 (4.42)
	H	5.31 (0.43)	4.03 (0.25)	3.40 (0.48)	3.23 (0.49)	3.19 (0.29)	5.44 (0.24)	4.28 (0.39)	3.71 (0.43)	3.64 (0.41)	3.60 (0.67)	5.25 (0.25)	3.82 (0.31)	3.15 (0.81)	2.88 (0.29)	2.83 (0.73)
	D	5.60 (0.29)	5.59 (0.18)	5.57 (0.21)	5.56 (0.10)	5.57 (0.09)	5.58 (0.15)	5.55 (0.10)	5.53 (0.13)	5.54 (0.10)	5.54 (0.15)	5.59 (0.06)	5.58 (0.14)	5.57 (0.27)	5.56 (0.10)	5.55 (0.09)
	F	0.552	0.072	0.062	0.010	0.084	0.131	0.000	0.000	0.000	0.027	1.529	0.012	0.000	0.049	0.000
	DT	233.67	N/d	N/d	N/d	N/d	82.00	685.83	N/d	N/d	N/d	68.00	335.67	N/d	N/d	N/d
20	WV	98.99 (0.72)	99.78 (0.50)	99.67 (0.43)	99.72 (0.49)	99.96 (0.51)	116.47 (0.92)	116.70 (0.81)	115.22 (0.74)	115.86 (0.69)	114.07 (0.40)	85.73 (0.63)	85.49 (0.83)	85.51 (1.41)	83.85 (0.92)	82.90 (0.98)
	CS	32.19 (6.85)	109.18 (2.16)	225.46 (1.75)	295.34 (2.41)	293.43 (2.67)	58.34 (3.18)	167.89 (4.50)	310.42 (0.94)	330.76 (1.40)	334.50 (0.53)	18.94 (6.83)	81.66 (4.61)	184.09 (5.90)	251.40 (2.39)	256.68 (2.28)
	H	5.32 (0.17)	4.04 (0.29)	3.48 (0.16)	3.34 (0.40)	3.33 (0.32)	5.45 (0.19)	4.33 (0.20)	3.78 (0.34)	3.68 (0.24)	3.63 (0.56)	5.25 (0.15)	3.81 (0.57)	3.25 (0.60)	2.95 (0.40)	2.88 (0.36)
	D	5.57 (0.17)	5.55 (0.15)	5.57 (0.14)	5.57 (0.170)	5.57 (0.17)	5.57 (0.09)	5.55 (0.11)	5.54 (0.15)	5.54 (0.12)	5.54 (0.24)	5.59 (0.100)	5.59 (0.01)	5.57 (0.01)	5.55 (0.13)	5.57 (0.10)
	F	0.111	0.100	0.010	0.080	0.050	0.103	0.000	0.000	0.000	0.000	1.678	0.035	0.000	0.000	0.000
	DT	271.17	N/d	N/d	N/d	N/d	91.00	N/d	N/d	N/d	N/d	95.00	355.83	N/d	N/d	N/d

* WV = weight variation (mg), CS = crushing strength (N), H = height (thickness) (mm), D = diameter (mm), F = friability (%), DT = disintegration time (s), N/d = no data.

Table A.6.2: Physical properties of chitosan/*Avicel*[®] PH 200; chitosan/*Prosolv*[®] 50 and Chitosan/*Prosolv*[®] 90 tablets at various settings and mixing times. The mixtures were mixed in a 70:30 ratio. %RSD is indicated in parentheses.

		Avicel				Prosolv 50				Prosolv 90			
		35	40	45	50	35	40	45	50	35	40	45	50
5	WV	82.41 (0.71)	82.97 (0.66)	81.89 (0.78)	83.03 (0.94)	87.27 (1.41)	87.28 (1.72)	89.40 (1.31)	86.34 (1.27)	78.42 (1.37)	77.43 (1.03)	78.47 (0.97)	76.68 (1.88)
	CS	9.77 (10.70)	45.77 (5.60)	76.83 (6.10)	105.83 (6.54)	5.41 (19.20)	16.89 (11.58)	29.89 (7.16)	45.89 (6.30)	8.63 (13.38)	45.82 (7.40)	79.58 (5.09)	95.69 (8.10)
	H	5.00 (0.10)	3.81 (0.19)	3.37 (0.45)	3.27 (0.19)	4.99 (0.64)	4.02 (0.29)	3.46 (0.51)	3.38 (0.24)	4.92 (0.29)	3.71 (0.34)	3.30 (0.74)	3.12 (0.30)
	D	5.56 (0.16)	5.56 (0.10)	5.55 (0.10)	5.55 (0.17)	5.57 (0.23)	5.57 (0.18)	5.59 (0.06)	5.59 (0.090)	5.52 (0.18)	5.56 (0.08)	5.55 (0.14)	5.55 (0.16)
	F	8.736	0.000	0.000	0.000	0.490	0.000	0.000	0.000	9.609	0.000	0.000	0.000
	DT	N/d	N/d	N/d	N/d	23.33	31.83	60.83	80.50	149.00	N/d	N/d	N/d
10	WV	82.10 (0.76)	82.72 (1.26)	81.32 (0.40)	82.81 (0.94)	87.49 (1.06)	87.45 (1.90)	87.52 (1.61)	86.19 (0.80)	78.62 (1.79)	79.81 (1.43)	77.51 (1.62)	77.29 (1.44)
	CS	9.76 (9.50)	47.44 (4.76)	79.90 (4.77)	105.83 (6.54)	14.09 (15.55)	59.06 (5.61)	102.02 (4.86)	123.46 (3.92)	9.98 (32.24)	47.93 (17.82)	80.09 (7.17)	98.16 (3.41)
	H	5.03 (0.28)	3.84 (0.41)	3.38 (0.45)	3.27 (0.82)	4.97 (0.22)	3.92 (0.61)	3.46 (0.39)	3.31 (0.47)	4.91 (0.19)	3.69 (0.23)	3.27 (0.26)	3.15 (0.54)
	D	5.55 (0.35)	5.56 (0.09)	5.54 (0.13)	5.54 (0.12)	5.58 (0.19)	5.57 (0.09)	5.56 (0.09)	5.55 (0.11)	5.55 (0.22)	5.56 (0.10)	5.56 (0.18)	5.54 (0.06)
	F	10.545	0.000	0.000	0.000	5.481	0.000	0.000	0.000	7.072	0.000	0.000	0.000
	DT	643.50	N/d	N/d	N/d	39.67	N/d	N/d	N/d	398.33	N/d	N/d	N/d
20	WV	83.52 (0.93)	82.04 (0.69)	82.08 (0.69)	81.84 (0.87)	87.87 (0.92)	88.02 (1.83)	86.59 (1.12)	87.25 (1.04)	79.43 (1.11)	78.02 (1.83)	77.75 (1.53)	78.36 (1.03)
	CS	10.59 (18.85)	45.39 (4.68)	73.01 (35.15)	102.27 (4.71)	14.84 (11.80)	62.31 (4.35)	109.81 (2.42)	130.79 (5.61)	11.60 (19.60)	48.42 (7.00)	81.93 (5.26)	103.63 (4.90)
	H	5.02 (0.22)	3.85 (0.42)	3.40 (0.22)	3.26 (0.16)	4.97 (0.22)	3.92 (0.47)	3.47 (0.53)	3.33 (0.63)	4.92 (0.37)	3.75 (90.52)	3.33 (0.24)	3.17 (0.25)
	D	5.55 (0.32)	5.56 (0.11)	5.54 (0.09)	5.54 (0.10)	5.58 (0.12)	5.57 (0.090)	5.56 (0.150)	5.55 (0.32)	5.55 (0.210)	5.56 (0.19)	5.55 (0.12)	5.54 (0.14)
	F	11.333	0.000	0.000	0.000	4.826	0.000	0.000	0.000	6.933	0.000	0.000	0.000
	DT	N/d	N/d	N/d	N/d	N/d	N/d	N/d	N/d	430.67	N/d	N/d	N/d

* WV = weight variation (mg), CS = crushing strength (N), H = height (thickness) (mm), D = diameter (mm), F = friability (%), DT = disintegration time (s), N/d = no data

ANNEXURE B:
FORMULATION PROPERTIES

B.1 TABLET PROPERTIES OF THE CHITOSAN MINITABLET FORMULATIONS:

Table B.1:	Results of crushing strength (N) of the different formulations.
Table B.2:	Results of disintegration time (sec) of the different formulations.
Table B.3:	Results of tablet thickness (mm) of the different formulations.
Table B.4:	Results of tablet diameter (mm) of the different formulations.
Table B.5:	Results of mass variation (% RSD) of the different formulations.
Table B.6:	Results of percentage friability (%) of the different formulations.
Table B.7:	Results of AUC ($\mu\text{g} \cdot \text{min} \cdot \text{cm}^{-3}$) of furosemide from the different formulations.
Table B.8:	Results of AUC _n of furosemide from the different formulations.
Table B.9:	Results of DR _i ($\mu\text{g} \cdot \text{cm}^{-3} \cdot \text{min}^{-1}$) of furosemide from the different formulations.
Table B.10:	Results of (DR _i) _n of furosemide from the different formulations.
Table B.11:	Results of AUC ($\mu\text{g} \cdot \text{min} \cdot \text{cm}^{-3}$) of chitosan from the different formulations.
Table B.12:	Results of DR _i ($\mu\text{g} \cdot \text{cm}^{-3} \cdot \text{min}^{-1}$) of chitosan from the different formulations.
Table B.13:	Results of percentage furosemide dissolved (%) from the different formulations.
Table B.14:	Results of percentage chitosan dissolved (%) from the different formulations.

Table B.1: Physical properties of minitablets comprising of different chitosan filler-systems containing either ascorbic acid or sodium bicarbonate in varying concentrations. %RSD is indicated in parentheses.

Formulation	Mass variation (mg)	Thickness (mm)	Diameter (mm)	Crushing strength (N)	% Friability	Disintegration time (sec)
3% KVA64; 0.5% Vit C (m)	14.01 (1.52)	1.85 (0.64)	2.97 (0.27)	60.79 (34.20)	0.360	5.67
3% KVA64; 0.5% Vit C (g)	13.40 (2.22)	1.85 (0.93)	2.94 (0.46)	64.92 (22.46)	0.720	4.00
5% KVA64; 0.5% Vit C (m)	14.22 (1.93)	1.81 (1.52)	2.96 (0.30)	51.04 (36.05)	0.560	11.50
5% KVA64; 0.5% Vit C (g)	14.03 (2.73)	1.87 (1.33)	2.97 (0.25)	58.13 (47.16)	0.493	9.50
3% KVA64; 2% NaHCO ₃ (m)	13.91 (3.06)	1.79 (0.77)	2.95 (0.17)	61.00 (42.68)	0.432	7.50
3% KVA64; 2% NaHCO ₃ (g)	14.19 (1.97)	1.85 (1.28)	2.99 (0.24)	49.99 (42.67)	0.212	6.50
5% KVA64; 2% NaHCO ₃ (m)	14.20 (1.90)	1.83 (1.21)	2.95 (0.17)	61.41 (32.42)	0.927	11.33
5% KVA64; 2% NaHCO ₃ (g)	14.53 (1.99)	1.87 (1.04)	2.96 (0.16)	47.2 (58.97)	0.535	9.50
3% KVA64; 4% NaHCO ₃ (m)	12.62 (3.31)	1.73 (0.81)	2.90 (0.40)	24.36 (32.53)	0.290	5.17
3% KVA64; 4% NaHCO ₃ (g)	14.00 (2.46)	1.66 (0.66)	2.98 (0.28)	24.98 (29.64)	0.075	3.00
5% KVA64; 4% NaHCO ₃ (m)	13.10 (2.59)	1.82 (0.89)	2.98 (0.25)	32.71 (19.75)	0.273	8.50
5% KVA64; 4% NaHCO ₃ (g)	13.87 (2.68)	1.77 (0.55)	2.96 (0.28)	32.40 (32.10)	0.144	2.67

KVA64 = Kollidon[®] KVA64; Vit C = ascorbic acid; NaHCO₃ = sodium bicarbonate

B.2 EXAMPLE OF THE CALCULATIONS OF THE AVERAGE RESPONSES IN THE FACTORIAL DESIGN:

For all the responses (initial rate of drug dissolution, the extent of drug dissolution and the percentage drug dissolved) the following applied:

- A₀:** equals the sum of the average values of all the combinations containing Kollidon[®] VA64 as binder.
- A₁:** equals the sum of the average values of all the combinations containing Methocel[®] K100M as binder.
- B₀:** equals the sum of the average values of all the combinations containing 3.0% binder.
- B₁:** equals the sum of the average values of all the combinations containing 5.0% binder.
- C₀:** equals the sum of the average values of all the combinations of filler-systems that were physically mixed before the active ingredient was added.
- C₁:** equals the sum of the average values of all the combinations of filler-systems that were granulated before the active ingredient was added.

ANNEXURE C:

TWO EXAMPLES OF ANOVA: TWO-FACTOR WITH REPLICATION:

C.1 RESULTS OF TABLET THICKNESS OF THE DIFFERENT FORMULATIONS:

	3% Mixtures	5% Mixtures	3% Granules	5% Granules
Ac-Di-Sol®	1.779	1.732	1.660	1.632
	1.791	1.827	1.846	1.873
	1.728	1.818	1.661	1.766
	1.806	1.824	1.854	1.901
	1.878	1.749	1.649	1.762
	1.657	1.638	1.644	1.685
	1.721	1.779	1.723	1.737
	1.873	1.858	1.884	1.764
	1.753	1.741	1.713	1.771
	1.846	1.808	1.851	1.868
	1.76	1.767	1.763	1.771
	1.864	1.708	1.776	1.775
Kollidon® CL	1.779	1.732	1.660	1.632
	1.791	1.827	1.846	1.873
	1.728	1.818	1.661	1.766
	1.739	1.727	1.692	1.755
	1.738	1.745	1.711	1.713
	1.733	1.728	1.751	1.864
	1.729	1.719	1.683	1.864
	1.736	1.764	1.720	1.715
	1.802	1.761	1.741	1.778
	1.846	1.808	1.851	1.868
	1.748	1.762	1.743	1.761
	1.791	1.755	1.827	1.740

SUMMARY:

Ac-Di-Sol®:

	3% Mixtures	5% Mixtures	3% Granules	5% Granules	Total
Count	12	12	12	12	48
Sum	21.456	21.249	21.024	21.305	85.034
Average	1.788	1.77075	1.752	1.775417	1.771542
Variance	0.00474	0.003794	0.008049	0.005866	0.005425

Kollidon® CL:

	3% Mixtures	5% Mixtures	3% Granules	5% Granules	Total
Count	12	12	12	12	48
Sum	21.16	21.146	20.886	21.149	84.341
Average	1.76333	1.7621667	1.7405	1.762417	1.757104
Variance	0.00142	0.0013591	0.004608	0.005669	0.003151

Total:

	3% Mixtures	5% Mixtures	3% Granules	5% Granules
Count	24	24	24	24
Sum	42.616	42.395	41.91	42.454
Average	1.77567	1.7664583	1.74625	1.768917
Variance	0.00311	0.0024837	0.006088	0.005561

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Sample	0.005	1	0.005003	1.127047	0.291311	3.949324
Columns	0.01154	3	0.003848	0.866878	0.461465	2.708191
Interaction	0.0009	3	0.000299	0.067409	0.977081	2.708191
Within	0.3906	88	0.004439			
Total	0.40805	95				

C.2 AUC'S OF FUROSEMIDE IN DIFFERENT FORMULATIONS:

	No disintegrant	Ac-Di-Sol®
Mixture	1628.499	2269.388
	1708.129	1628.376
	1628.499	2239.761
	1708.129	2715.775
Granule	1860.747	2228.054
	1901.595	1543.431
	1860.747	2354.95
	1901.595	2644.408

SUMMARY:

Mixtures:

	No disintegrant	Ac-Di-Sol®	Total
Count	4	4	8
Sum	6673.256	8853.3	15526.6
Average	1668.314	2213.325	1940.82
Variance	2113.645633	199487.75	171268

Granules:

	No disintegrant	Ac-Di-Sol®	Total
Count	4	4	8
Sum	7524.684	8770.843	16295.5
Average	1881.171	2192.7108	2036.94
Variance	556.186368	217721.77	121278

Total:

	No disintegrant	Ac-Di-Sol®
Count	8	8
Sum	14197.94	17624.143
Average	1774.7425	2203.0179
Variance	14089.38584	178925.5

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Sample	36957.27493	1	36957.3	0.35208	0.56396	4.74722
Columns	733679.1873	1	733679	6.98943	0.02142	4.74722
Interaction	54508.82458	1	54508.8	0.51928	0.48495	4.74722
Within	1259638.072	12	104970			

ANNEXURE D:

D.1 SHORT-TERM STABILITY EVALUATION OF CHITOSAN:

Table D.1: *Physical properties of chitosan tablets compressed from different chitosan samples exposed to elevated temperatures and various time intervals.*

Temperature (°C)	Physical properties	Time (hours)			
		1	2	3	4
30 °C	Moisture content (%)	4.9	5.1	5.1	5.2
	Crushing strength (N)	80.7	84.0	81.7	79.4
	Friability (%)	0.033	0.014	0.027	0.038
	Hardness-friability ratio (N.% ⁻¹)	2445.46	6000.00	3025.93	2089.47
	Disintegration time (sec)	59.8	52.7	64.8	66.5
40 °C	Moisture content (%)	4.5	4.0	4.6	4.0
	Crushing strength (N)	78.5	77.4	83.3	80.9
	Friability (%)	0.018	0.055	0.036	0.020
	Hardness-friability ratio (N.% ⁻¹)	4361.11	1407.27	2313.89	4045.00
	Disintegration time (sec)	70.0	71.4	64.2	66.8
50 °C	Moisture content (%)	3.5	3.3	2.9	2.6
	Crushing strength (N)	81.8	67.7	51.9	38.5
	Friability (%)	0.054	0.321	1.003	1.413
	Hardness-friability ratio (N.% ⁻¹)	1514.82	210.90	51.74	27.25
	Disintegration time (sec)	144.0	170.1	243.8	257.1
60 °C	Moisture content (%)	2.7	3.6	3.2	2.8
	Crushing strength (N)	95.6	64.2	49.6	30.6
	Friability (%)	0.322	0.434	0.521	1.667
	Hardness-friability ratio (N.% ⁻¹)	296.89	147.93	95.20	18.36
	Disintegration time (sec)	157.6	178.9	287.8	301.4

D.2 LONG-TERM STABILITY EVALUATION OF CHITOSAN:

Table D.2.1: Physical properties of chitosan tablets compressed from chitosan powder after exposure to 25 °C / 60% RH for 6 months. %RSD included in parenthesis.

Physical property	Time (Months)					
	1	2	3	4	5	6
Average weight (mg)	62.75 (2.07)	60.18 (1.30)	59.98 (1.51)	59.35 (2.07)	55.38 (1.38)	53.56 (2.41)
Crushing strength (N)	48.7 (7.31)	37.8 (13.05)	26.9 (9.28)	22.8 (5.49)	11.7 (16.33)	8.9 (8.79)
Friability (%)	0.069	0.258	0.477	0.488	0.533	0.674
Disintegration time (sec)	*N/D	N/D	N/D	N/D	N/D	N/D

*No disintegration

Table D.2.2: Physical properties of chitosan tablets compressed from chitosan powder after exposure to 40 °C / 75% RH for 6 months. %RSD included in parenthesis.

Physical property	Time (Months)					
	1	2	3	4	5	6
Average weight (mg)	62.78 (2.26)	60.37 (1.57)	58.35 (1.67)	57.52 (1.17)	52.97 (2.33)	46.75 (1.45)
Crushing strength (N)	40.3 (11.29)	29.8 (9.88)	20.7 (13.82)	11.9 (11.41)	8.9 (7.88)	5.9 (12.31)
Friability (%)	0.098	0.521	0.747	0.997	1.035	1.065
Disintegration time (sec)	N/D	N/D	N/D	N/D	N/D	N/D

Table D.2.3: Physical properties of chitosan tablets after exposure to 25 °C / 60% RH for 6 months. %RSD included in parenthesis.

Physical property	Time (Months)					
	1	2	3	4	5	6
Average weight (mg)	65.14 (1.14)	65.07 (1.57)	58.35 (1.67)	57.52 (1.17)	52.97 (2.33)	46.75 (1.45)
Crushing strength (N)	51.42 (5.31)	40.0 (10.22)	33.6 (12.07)	22.15 (9.47)	15.7 (6.72)	12.4 (8.55)
Friability (%)	0.000	0.004	0.067	0.100	0.289	0.398
Disintegration time (sec)	N/D	N/D	N/D	N/D	N/D	N/D

Table D.2.4: Physical properties of chitosan tablets after exposure to 40 °C / 75% RH for 6 months. %RSD included in parenthesis.

Physical property	Time (Months)					
	1	2	3	4	5	6
Average weight (mg)	65.18 (1.05)	63.24 (1.05)	61.12 (1.12)	60.54 (1.27)	58.66 (1.38)	53.81 (1.11)
Crushing strength (N)	44.2 (10.21)	34.12 (6.79)	21.1 (11.34)	13.82 (12.30)	7.45 (5.29)	4.11 (15.08)
Friability (%)	0.031	0.069	0.136	0.428	0.677	0.844
Disintegration time (sec)	N/D	N/D	N/D	N/D	N/D	N/D

Table D.2.5: Physical properties of tablets containing a mixture of chitosan and 5% w/w Kollidon® VA64 after exposure to 25 °C / 60% RH for 6 months. %RSD included in parenthesis.

Physical property	Time (Months)					
	1	2	3	4	5	6
Average weight (mg)	59.97 (5.37)	60.37 (3.78)	58.35 (4.00)	59.97 (3.97)	49.62 (5.23)	47.45 (6.07)
Crushing strength (N)	33.1 (8.78)	35.7 (10.12)	27.2 (11.24)	25.7 (9.55)	19.4 (7.36)	15.9 (7.42)
Friability (%)	0.092	0.113	1.484	3.289	5.052	8.742
Disintegration time (sec)	11.0	22.0	52.1	46.7	61.9	81.7

Table D.2.6: Physical properties of tablets containing a mixture of chitosan and 5% w/w Kollidon® VA64 after exposure to 40 °C / 75% RH for 6 months. %RSD included in parenthesis.

Physical property	Time (Months)					
	1	2	3	4	5	6
Average weight (mg)	59.35 (6.55)	59.74 (7.67)	59.98 (6.40)	60.18 (8.97)	54.38 (5.99)	46.75 (9.14)
Crushing strength (N)	30.9 (8.79)	20.7 (9.28)	18.7 (11.11)	17.8 (8.77)	8.5 (10.44)	7.7 (9.15)
Friability (%)	0.346	2.453	6.481	10.215	11.141	14.017
Disintegration time (sec)	23.0	24.2	26.9	38.2	94.1	102.0

Table D.2.7: *Physical properties of chitosan tablets containing a 5% w/w Kollidon® VA64 granulate after exposure to 25 °C / 60% RH for 6 months. %RSD included in parenthesis.*

Physical property	Time (Months)					
	1	2	3	4	5	6
Average weight (mg)	65.07 (3.29)	65.14 (2.94)	62.86 (2.58)	60.79 (4.02)	60.55 (3.74)	60.47 (4.01)
Crushing strength (N)	46.3 (3.48)	45.7 (2.19)	36.3 (4.01)	33.0 (3.49)	31.5 (5.01)	30.0 (4.75)
Friability (%)	0.180	0.182	0.441	0.897	0.942	0.715
Disintegration time (sec)	6.0	6.0	11.0	14.2	15.1	15.0

Table D.2.8: *Physical properties of chitosan tablets containing a 5% w/w Kollidon® VA64 granulate after exposure to 40 °C / 75% RH for 6 months. %RSD included in parenthesis.*

Physical property	Time (Months)					
	1	2	3	4	5	6
Average weight (mg)	60.25 (5.55)	63.96 (7.03)	61.18 (9.28)	60.54 (6.15)	61.12 (6.00)	53.12 (9.24)
Crushing strength (N)	37.7 (4.54)	33.0 (5.25)	27.5 (6.87)	26.5 (4.98)	21.0 (9.78)	17.5 (8.17)
Friability (%)	0.175	0.285	0.333	0.527	0.699	0.834
Disintegration time (sec)	14.5	17.8	23.8	32.3	33.4	37.8