A COMPARISON ON THE RELEASE MODIFYING BEHAVIOUR OF CHITOSAN AND KOLLIDON® SR

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INTRODUCTION AND AIM OF STUDY

Controlled release formulations aim at achieving an economical, effective and patient friendly dosage form. These dosage forms offer many advantages over conventional dosage forms as they reduce dosing intervals, result in constant drug levels in the blood and decrease adverse effects of drugs. The goal is to manipulate the drug delivery to target the intestines or colon as the site of drug delivery and thus minimizing the side effects of the drug. Controlled release formulations often require the addition of a polymer to the formulation to modify the release of the drug from the formulation.

Chitosan is a polymer synthesised from chitin, which is abundant in nature. Chitosan has various properties which are of value to the pharmaceutical sciences. These properties include absorption promoting properties, as well as properties like the ability to modify the release of a drug from a dosage form. Chitosan has been shown to produce sustained release of ketoprofen, prednisolone and indomethacin over an extended period of time. Besides modifying the release of drugs chitosan also has been shown to improve the dissolution behaviour of drugs.

Beads and granules have been researched extensively for their ability as controlled release dosage forms. These dosage forms show various advantages over single unit dosage forms and they are flexible in dosage form development, because they disperse freely in the gastrointestinal tract, maximize drug absorption, reduce peak plasma fluctuation and minimize side effects without lowering drug bioavailability.

In this study ketoprofen loaded chitosan beads and granules were prepared. Kollidon® SR was added to investigate the influence of this polymer on the release characteristics of the formulations. Besides release characteristics the dosage forms were compared with respect to drug loading, surface and internal structure, swelling behaviour (beads only) and friability (beads only).

This study aimed to achieve effective controlled release of ketoprofen over an extended period of time and it included the following main objectives:

- A literature review on the development, effects and mechanism of drug release from controlled release formulations. The importance and effectiveness of chitosan as a polymer were investigated and documented.

- To formulate and prepare chitosan beads and granules, and to investigate the effect of a pharmaceutical excipient (Kollidon® SR) on the properties of the beads and granules.

- Evaluation of the prepared dosage with respect to the following:

  Beads: drug loading, surface and internal structure, friability, swelling and drug release.

  Granules: drug loading, surface and internal structure and drug release.

- To determine the effectiveness of these formulations as controlled drug delivery systems by conducting dissolution studies on the formulations and comparing the release rate of ketoprofen from the formulations.

ABSTRACT

Controlled release formulations deliver an active ingredient over an extended period of time. It is an ideal dosage form for an active ingredient with a short elimination half-life. An active ingredient with a short elimination half-life would be released in small portions over an extended period of time and thus less frequent administration is necessary and this improve patient compliance. Other advantages of these formulations include: decreased side effects, constant drug levels in the blood, improvement in treatment efficiency and reduction in cost of administration.

Controlled release beads are formulated in such a way that the active ingredient is embedded in a matrix of insoluble substance like chitosan; the dissolving drug then has to find its way through the pores of the matrix into the surrounding medium. The chitosan matrix swells to form a gel, the drug then has to first dissolve in the matrix and diffuse through the outer surface into the surrounding medium.

Chitosan is a biocompatible, biodegradable polymer of natural origin. It has mucoadhesive properties as well as the ability to manipulate the tight junctions in the epithelium membrane and these properties have qualified chitosan as an effective drug carrier in controlled release dosage forms. The effect of a modern controlled release polymer namely Kollidon® SR in combination with chitosan on drug release was investigated. Ketoprofen was chosen as model drug. Ketoprofen is an anti-inflammatory drug that causes gastrointestinal side effects in conventional dosage forms. Ketoprofen has a short elimination half-life of 2.05 ± 0.58 h and this characteristic makes it an ideal candidate for use in a controlled release formulation. The aim of this study was to achieve controlled release and minimize gastrointestinal effects of ketoprofen with chitosan particles. Kollidon® SR was used as polymer because it exhibits pH independent release characteristics and previous studies have shown potential for this combination.

Chitosan beads and chitosan-Kollidon® SR beads, as well as chitosan granules and chitosan-Kollidon® SR granules, were prepared and investigated as potential controlled release formulations. Chitosan beads were prepared through the inotropic gelation method using

tripolyphosphate as a cross linking agent. Granules were prepared through wet granulation using 2% v/v acetic acid as the granulating fluid or by dissolving ketoprofen in ethanol and Kollidon® SR in 2-pyrrolidinone and using the solution as granulating fluid. Kollidon® SR was added in concentrations of 0.25, 0.5 and 1% (w/v) in the bead formulations and concentrations of 1, 5 and 10% (w/w) in the granule formulations. The beads and granules were characterised by evaluating the following properties: morphology, drug loading and drug release. Additionally swelling and friability tests were also conducted on the bead formulations.

The cross linking times of the bead formulations were varied to investigate the effect of cross linking time on the characteristics of the beads. Chitosan-Kollidon® SR beads showed promising results for controlled release formulations and ketoprofen were released over an extended period of time. Drug loading of the plain chitosan beads was 74.65 ± 0.71% and it was noted that the inclusion of Kollidon® SR in the beads resulted in an increase in drug loading and the formulation containing 1% (w/v) Kollidon® SR, cross linked for 30 minutes had a drug loading of 77.38 ± 0.01%. Drug loading of the beads that were cross linked for a longer time were slightly lower which is an indication that some of the drug might have leached out during cross linking. The degree of swelling was promising with some beads swelling to a degree of 2.5 in phosphate buffer solution pH 5.6. Granules had a drug loading between 81.73 ± 1.53% and 93.30 ± 0.50%.

Ketoprofen release from the beads and the granules in PBS pH 7.40 at 37 °C over a period of 6 hours were investigated. The bead formulations were more effective in achieving controlled release and it was noted that the bead formulations that was cross linked for a longer period was more efficient in achieving controlled release. The granules did not form a matrix and were not effective in achieving controlled release. Controlled release of ketoprofen were achieved and the results show potential for chitosan-Kollidon® SR formulations in the future.

Key words: Beads; Granules; Chitosan; Controlled release; Ketoprofen; Kollidon® SR; Inotropic gelation

UITTREKSEL

Gekontroleerde vrystellingsdoseervorme stel 'n aktiewe bestandeel vry oor 'n verlengde tyd. Dit is 'n ideale dosseervorm vir 'n aktiewe bestandeel wat 'n kort eliminasiehalfleeftyd het. 'n Aktiewe bestandeel met 'n kort eliminasiehalfleeftyd word dan in klein hoeveelhede oor 'n verlengde periode vrygestel en dus kan die geneesmiddel minder gereeld toegedien word en dit bevorder pasiëntsamewerking. Ander voordele van hierdie doseervorme sluit in: minder newe-effekte, konstante geneesmiddelvlakke in die bloed, verbetering in behandelingseffektiwiteit en 'n verlaging in toedieningskostes.

Gekontroleerde vrystellingskrale word so geformuleer dat die aktiewe bestandeel vasgevang word in 'n matriks van 'n onoplosbare bestandeel, byvoorbeeld kitosaan. Die geneesmiddel los op en moet dan sy weg vind deur die porieë van die matriks om in die omliggende medium vrygestel te word. Die kitosaanmatriks swel en vorm 'n gel, die geneesmiddel moet eers in die matriks oplos en moet dan deur die buitenste oppervlak in die omliggende medium vrygestel word.

Kitosaan is 'n bioverenigbare, bio-afbreekbare polimeer van natuurlike oorsprong. Dit besit mukoklewende eienskappe en het ook die vermoë om die digsluitende hegtingskomplekse ("tight junctions") in die epiteelmembrane te open en hierdie eienskappe maak kitosaan geskik as 'n effektiewe geneesmiddeldraer in gekontroleerde vrystellingsdoseervorme. Die effek van 'n gekontroleerde vrystellingspolimeer naamlik Kollidon® SR in kombinasie met kitosaan op geneesmiddelvrystelling is ondersoek. Ketoprofen is gekies as modelgeneesmiddel. Ketoprofen is 'n anti-inflamatoriese geneesmiddel wat dikwels gastroïntestinale newe-effekte veroorsaak in konvensionele dosseervorme. Ketoprofen het 'n kort eliminasiehalfleeftyd van 2.05 ± 0.58 h en hierdie eienskap maak dit 'n ideale kandidaat vir gekontroleerde vrystellingsdoseervorme.

Die doel van die studie was om gekontroleerde vrystelling met minimale gastro-intestinale newe-effekte te verkry deur middel van kitosaandeeltjies. Kollidon® SR is gebruik as polimeer omdat dit pH-onafhanklike vrystellingseienskappe vertoon en vorige studies het bewys dat daar potensiaal is vir so 'n kombinasie.

Kitosaankrale en kitosaan-Kollidon® SR krale sowel as kitosaangranules en kitosaan-Kollidon® SR granules is voorberei en die kombinasie se potentiaal as gekontroleerde vrystellingsdoseervorme is ondersoek. Kitosaankrale is deur middel van inotropiese jelering voorberei waartydens tripolifosfaat (TPP) as kruisbindingsmiddel gebruik is. Granules is berei deur middel van natgranulering waartydens 'n 2% (v/v) asynsuuroplossing gebruik is as granuleervloeistof of deur middel van 'n alternatiewe metode waartydens ketoprofen opgelos is in etanol en Kollidon® SR opgelos is in 2-pyrrolidinone en die oplossing as granuleringsvloeistof te gebruik. Kollidon® SR is bygevoeg in konsentrasies van 0.25, 0.5 en 1% (m/v) in die kitosaankrale en in konsentrasies van 1, 5 en 10% (m/v) in die granule formulerings. Die krale en granules is gekarakteriseer deur middel van die volgende eienskappe: morfologie, geneesmiddellading en geneesmiddelvrystelling uit die doseervorme. Swelling en afsplytingstoetse is ook uitgevoer op die krale om te bepaal watter doseervorm die mees effektiewe gekontoleerde vrystellingsformulering is.

Die kruisbindingstyd van die kraalformulerings is gewissel om die effek van die kruisbindingstyd op die formulering se einskappe te ondersoek. Kitosaan-Kollidon® SR krale het belowende resultate getoon as 'n gekontroleerde vrystellingsformulering en die ketoprofen is oor 'n verlengde periode vrygestel. Die geneesmiddellading van die skoon kitosaankrale was 74.65 ± 0.71% en daar was gevind dat die geneesmiddellading van die krale verhoog het met die insluiting van Kollidon® SR in die doseervorm en die formulering wat 1% (m/v) Kollidon® SR bevat het en vir 30 minute gekruisbind is, het 'n geneesmiddellading van 77.38 ± 0.01% gehad. Geneesmiddelladings van die krale wat vir 'n langer periode kruisbinding ondergaan het was effens laer. Dit kan 'n aanduiding wees dat daar van die geneesmiddel uit die krale uitgelek het gedurende kruisbinding. Die graad van swelling het ook belowende resultate vertoon en sommige krale het tot 'n graad van 2.5 geswel in 'n fosfaatbufferoplossing (pH 5.6). Granules het 'n minder komplekse metode van bereiding en die granules se geneesmiddellading het gewissel tussen 81.73 ± 1.53% en 93.30 ± 0.50%.

Ketoprofenvrystelling van die krale en die granules is in fosfaatbufferoplossing (PBS) pH 7.4 by 37 °C oor 'n periode van 6 ure bepaal. Die kraalformulerings was meer effektief in gekontroleerde vrystelling en daar is gevind dat die krale wat vir 'n langer tydperk kruisbinding ondergaan het meer effektief was om gekontroleerde vrystelling te lewer. Die granules het nie 'n matriks gevorm nie en was nie 'n effektiewe gekontroleerde vrystellingsformulering nie. Gekontroleerde vrystelling van ketoprofen is wel verkry met die

kraalformulerings en die resultate vertoon belowende potensiaal vir die chitosan-Kollidon® SR kombinasie in die toekoms.

Sleutel woorde: Krale; Granules; Kitosaan; Gekontroleerde vrystelling; Ketoprofen; Kollidon® SR; Inotropiese Jelering.

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CHAPTER 1

1 FORMULATION OF A CONTROLLED RELEASE DOSAGE FORM

1.1 Introduction

Judging by the proliferation of published papers in recent years, there has been an increasing interest in the development and marketing of controlled release drug delivery systems. Many factors are responsible for this interest.

There is a substantial body of literature attesting to the problem of patient compliance and its considerable effect on drug therapy. Minimization of patient compliance problems through prolonged action drugs or dosage forms are very desirable.

In recent years there has been extensive research directed toward a better understanding of the mechanisms of drug absorption from various routes of drug administration. Parallel research has explored some of the negative aspects of drugs at absorption sites, for example, the causes of possible injury to subcutaneous and intramuscular sites, and approaches to minimize such injury. Such knowledge has led, in many cases, to a more rational design of prolonged action dosage forms.

Meanwhile, our understanding of the mechanisms of drug action and the relationships between tissue drug and metabolite levels and drug action have improved significantly in the last quarter of a century. Part of this improvement is due to the accessibility and utilization of computers for pharmacokinetic simulation and modelling. These advances in turn have led to improvements in dosage from design and evaluation (Ballard, 1978:2).

Basically, all slow and extended release dosage forms are designed to release the drug in small amounts at predefined rates, thus influencing the rate of absorption. The release of the drug may only be controlled accurately if the release mechanism and the influence of the excipients are known. This knowledge permits the scientific development of individual

dosage forms, the use of relevant control tests, the assessment and improvement of behaviour *in vivo*, including the determination of the food effects, and found *in vitro/in vivo* correlations (Lippold & Düsseldorf, 1991:15).

Controlled drug release may be achieved by mechanical pumps, osmotic pumps, chemically controlled mechanisms involving biodegradation, and by diffusional systems with specially prepared polymeric membranes (Bruck, 1983:6).

From the therapeutic point of view the following reasons can be given to justify the development of a controlled release form:

- better compliance,
- prevention of unwanted effects and
- the maintenance of a therapeutic effect over an extended dosage interval without producing large fluctuations.

Contrasted with these advantages are the following possible disadvantages:

- reduction in the amount of drug that is absorbed/systemically available,
- dose dumping,
- higher variability of the amount absorbed/systemically available and
- the possible development of tolerance (Grundert-Remy, 1990:13).

The main advantages of controlled drug delivery systems is maintaining therapeutically optimum drug concentration in the plasma through zero-order release without significant fluctuations and eliminating the need for frequent single dose administrations.

1.2 Controlled release

1.2.1 Introduction

Over the years, there were several attempts to classify long-acting oral dosage forms. One classification of such products proposes that there are three basic types namely sustained release, prolonged action, and repeat action dosage forms.

- Ideally, a sustained release oral dosage form is designed to release rapidly some predetermined fraction of the total dose into the gastrointestinal tract (GI tract). This fraction (loading dose) is an amount of drug which will produce the desired pharmacological response as promptly as is consistent with the drug's intrinsic availability for absorption from gastrointestinal absorption sites. The remaining fraction of the total dose (maintenance dose) is then released as rapidly as is required to maintain constant maximum intensity of pharmacological activity. Thus the rate of drug absorption from the maintenance dose into the body should be equal to the rate of drug removal from the body by all processes over the time desired intensity of pharmacological response is required.

- Prolonged action oral dosage forms initially make the drug available to the body in amounts sufficient to produce the desired pharmacological response. Such dosage forms also provide for replenishing the supply of drug to the body at some rate which extends the length of time the pharmacological response could be maintained when compared to the usual single dose of the drug. Note that with prolonged release systems, constant drug levels are maintained.

- A repeat action oral dosage form is designed to release initially the equivalent of a usual single dose of drug, and then another single dose of the drug at some later time (Ballard, 1978:3).

Controlled drug delivery is aimed at providing not only sustained, but also constant action. That is ideally, zero-order release rates in which the amount of drug released to the absorption site remains reasonably constant over prolonged periods of time. Only some drug delivery systems can fulfil the latter requirement, although, some repository preparations can remain

therapeutically effective up to several days even without exhibiting zero-order kinetic release behaviour (Bruck, 1983:2).

It should be emphasized that the rate at which a drug is delivered to absorption sites does not necessarily reflect its concentration in the blood plasma. In other words, constant rate of release of a drug into tissues (other than directly into blood by intravenous administration) should not be equated necessarily with its concentration in blood plasma. The latter depends on factors such as the oil/water partition coefficients of lipid-soluble drugs, molecular size of lipid-insoluble drugs and local blood flow. These important points sometimes receive little attention in various articles dealing with controlled drug delivery, especially those which emphasize primarily the engineering and physicochemical aspects of materials and devices (Bruck, 1983:2).

According to Lippold (1990:42) the following principles must be taken into account in the development of controlled release dosage forms:

- it must be a multiparticulate system,
- slow release over a period of about 5 hours,
- release independent of the hydrodynamics (stress of the test system, motility of the GI tract),
- release as far as possible independent of conditions in the environment of the stomach and the small intestine such as food that has been shown to affect the release and pH.
- reproducibility in manufacture (release) within batch and batch-to-batch (homogeneity and conformity) and
- stability of release characteristics.

If the above mentioned principles are fulfilled, the dosage forms produced should provide long-lasting, reproducible blood levels exhibiting little fluctuation and relative high bioavailability.

1.2.2 Advantages and disadvantages of controlled release products

The advantages of controlled release are such that the appropriate applications of this principle could revolutionize drug therapy. However extensive investigation is required to define its scope and clinical limitations.

Theoretical advantages of oral controlled release include (Prescott, 1981:51):

- ability to regulate drug delivery at the absorptive site to give virtually ideal absorption kinetics with early sustained therapeutic drug concentrations,
- prolongation of the action and duration of the drug,
- reduction in the frequency of dosing and better patient compliance,
- reduction in GI toxicity by control of the rate and site of drug release and by avoidance of very high drug concentrations at the GI mucosa,
- reduction in systemic toxicity by attenuation of peak drug concentrations,
- reduction in local and systemic side-effects,
- reduction in costs of administration and specialized hospital personnel,
- improvement of treatment efficiency and
- decreased blood level fluctuations.

Contrasted with these advantages are the following possible disadvantages (Grundert-Remy, 1990:17):

- reduction in the amount absorbed/systemically available,
- dose dumping,
- higher variability of the amount absorbed/systemically available and
- possible development of tolerance.

Controlled release seems most appropriate for drugs that have clearly defined minimum therapeutic and maximum toxic levels, especially those with a short half life, low therapeutic index and potential for serious toxicity (Grundert-Remy, 1990:18).

1.2.3 Mechanism of release

Basically, all controlled release formulations are designed to release the drug in small amounts at predefined rates thus influencing the rate of absorption. The release of the drug may only be controlled accurately if the release mechanism and the influence of the excipients are known. Slow and extended release dosage forms may be divided into the following groups (Lippold, 1991:15):

- Coated dosage formulation
- Matrices
- Drugs embedded in hydrophilic polymers
- Ion exchangers
- Dissolution controlled dosage formulation
- Erosion dosage formulation
- Osmotic systems

The above mentioned groups will be discussed briefly.

Coated dosage forms:

This is the most important of the controlled release dosage forms. The drug is surrounded by a barrier. The slow rate of diffusion through this barrier determines the rate of release and, consequently, the rate of absorption. Basically the drug release follows the pattern described below (Lippold, 1991:16):

- water/gastric or intestinal juice permeates the coating,

- the drug dissolves; if the core has a sufficient amount of drug and the solubility concentration is obtained,
- the drug diffuses through the coating,

As long as drug concentration (Cs) is maintained in the core, the rate of release remains constant. If Cs is no longer sustained, the release rate decreases exponentially. The constant release rate Q/t is described as:

$$Q/t = P \times A \times Cs/d$$

Where: Q/t = drug released per unit time

t = time

P = permeability of the coating

A = area

d = thickness of the coating.

The mechanism and kinetics of drug delivery depend on the nature of the film. The kinetics is usually zero order. Granules, pellets, microcapsules, and film coated tablets are examples of coated dosage forms (Martin, 1993:532).

Matrix controlled release:

Matrix dosage forms are characterized by their insoluble, possibly porous "skeleton" of indigestible fats and waxes, thermoplastics or inorganic matrix formers such as gypsum. This framework includes the drug and, if necessary, soluble additives. The drug is released by diffusion; however, the excipients do not play a role in this procedure and is left behind as bare framework (Lippold, 1991:18).

Lee and Robinson (1978:146) studied water soluble drugs in hydrophilic matrices. The results, using chlorpheniramine maleate dispersed in methylcellulose, showed that the release rate was controlled mostly by drug diffusion rather than polymeric dissolution. Thus, even

when drugs are placed in a water-soluble matrix which will be subject to erosion, the rate-limiting step is diffusion of a drug out of the matrix.

Matrix tablets for oral use are generally quite safe. However, for certain patients with reduced GI motility caused by disease, the polymeric matrix tablet should be avoided, because accumulation of obstruction of the GI tract by matrix tablets has been reported. As an oral sustained-release product, the matrix tablet has not been popular. In contrast the use of the matrix tablet in implantation has been more popular (Shargel & Yu, 1999:187).

Drugs embedded in hydrophilic polymers:

Gel-forming substances which swell readily, such as cellulose derivates and synthetic polymers, have been in use since the 1960's. These substances are not to be confused with hydrogel matrices which consist of cross-linked polymers like beads which swell readily but are insoluble. Hydrophilic polymers may be divided into two groups depending on their release mechanism namely those which swell up on contact with water resulting in highly viscous, poorly soluble gel and those which swell slowly, with a low level of viscosity (these polymers tend to dissolve faster) (Lippold, 1991:22).

Ion exchangers:

Ion exchange preparations usually involve an insoluble resin capable of reacting with either an anionic or cationic drug. An anionic resin is negatively (-) charged so that a positively (+) charged cationic drug may react with the resin to form an insoluble nonabsorbable resin-drug complex (Shargel & Yu, 1999:184). Those polymers used most frequently are cross-linked polymers with acid end groups containing bonded basic drugs which are released gradually after forming a hydrogel by swelling. Proper first order release through control of the film, (because of the liquid adhering to the surface of the ion exchanges particles) occurs less frequently (Lippold, 1991:24).

Dissolution controlled slow release:

Controlled oral products employing dissolution as the rate-limiting step are in principle the simplest to prepare. A drug with a slow dissolution rate is inherently sustained, and of those drugs with high water-solubility, one can decrease solubility through appropriate salt or derivative formation (Lee and Robinson, 1978:150). The active ingredient dissolves slowly and therefore cannot sufficiently be absorbed because the dissolution kinetics determines the

subsequent absorption. According to the release mechanism, the release rate of dissolution controlled slow release products is influenced by the stirring rate of the formulation and the GI-motility (Lippold, 1991:26).

Erosion Controlled slow release:

Erosive controlled release dosage form may be produced by combining inert lipophilic substances with high dose, soluble binding and filling agents in the absence of disintegrants. Instead of decomposing, they erode following the dissolution/swelling of certain additives on the surface. The most important parameter is the ratio between the non-soluble and the soluble excipients. The disadvantage of this type of dosage form is that release is heavily dependent on hydrodynamics (Lippold, 1991:28).

Osmotic systems:

The osmotic pump (see figure 1.1) represents a significant concept in controlled release preparations. Drug delivery is precisely controlled by the use of an osmotically controlled device that pumps a constant amount of water through the system, dissolving and releasing a constant amount of drug per unit time. This device consists of an outside layer of semipermeable membrane filled with a mixture of drug and osmotic agent. When the device is placed in water, osmotic pressure is generated by the osmotic agent within the core which causes water to move into the device, which forces the dissolved drug to move out of the delivery orifice. The process continues until all the drug is released (Shargell & Yu, 1999:187).

An important aspect to the success of this type of delivery system, aside from the polymeric coat and core formulation, is the size of the delivery orifice. Two conditions must be met in order for the system to be effective:

- The orifice must be smaller than a theoretical maximum size to minimize the contribution to the delivery rate made by solute diffusion through the orifice.
- The orifice must be sufficiently large enough to minimize hydrostatic pressure inside the system.

Too small an orifice will depress the delivery rate whereas too large an opening will increase delivery rate over and above the desired constant delivery (Lee & Robbinson, 1978:172).

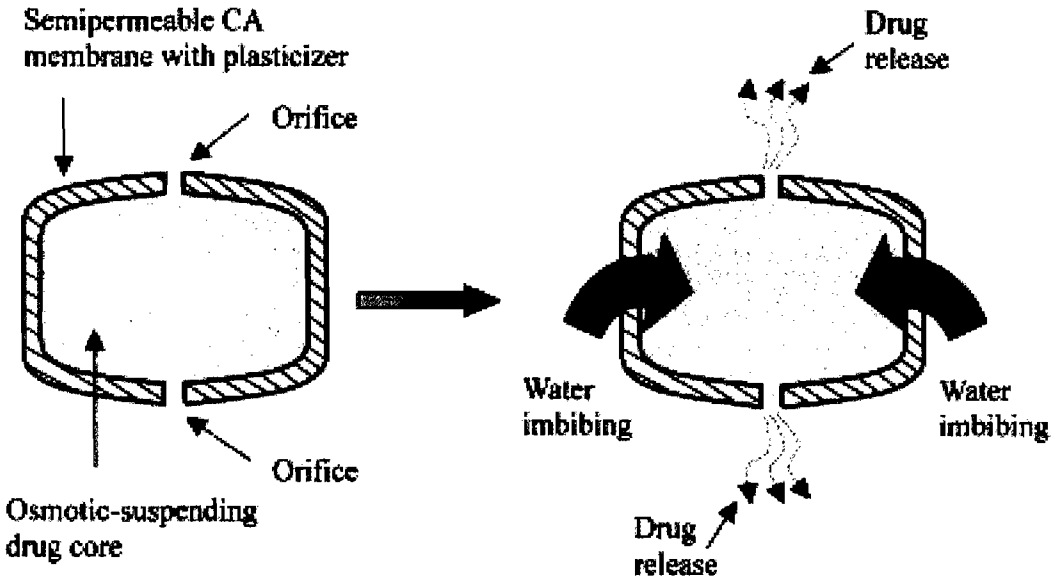


Figure 1.1: Schematic diagram of the monolithic osmotic tablet system composed of a monolithic tablet surrounded by a semipermeable membrane with two orifices (Adapted from Liu *et al.*, 2000:312).

1.3 Beads

1.3.1 Uses of beads

Beads are spherical loaded drug gel particles. It is prepared by dropping a biocompatible polymer into a solution containing a cross-linking agent. An example is the preparation of dropping a positively charged polysaccharide, chitosan into a tripolyphosphate (TPP) solution which resulted in spherical chitosan beads (Bodmeier *et al.*, 1989:1475). Beads range in size between 0.8 – 1.5 mm (Shu & Zu, 2000:53). The surface and cross-section morphology varies according to the method of drying, either freeze dried or oven dried, with freeze-dried beads being more porous (Bodmeier *et al.*, 1989:1478). The cross-linking time has a significant influence on the strength and porosity of the bead (Shu & Zu, 2000:55).

Beads are frequently used in the pharmaceutical industry as controlled release products. They offer flexibility in single dosage form development and offer therapeutic advantages over single unit dosage forms with their controlled release capabilities. They reduce variations in

gastric emptying time and reduce peak plasma fluctuation (Ghebre-Sellassie, 1989:6). A number of studies have been conducted to study chitosan beads as a potential system for controlled drug delivery (Gupta & Ravi Kumar, 2000:1115; Mi *et al.*, 2002:61). The drug release from chitosan beads depended on the penetration of the dissolution medium into the beads, the eventual swelling and dissolution of the chitosan matrix, and the dissolution and subsequent diffusion of the drug through the swollen or unswollen matrix. The swelling of beads was dependent on the pH of the dissolution medium. The beads, when wetted by the acidic dissolution medium, swelled extensively and formed a hydrogel matrix before they dissolved completely. They did not swell or dissolve in simulated intestinal fluid (Bodmeier *et al.*, 1989:1488).

Gupta & Ravi Kumar (2000:1116) found that the release rate of beads were slower in comparison to microgranules. They also found that a burst effect occurs at a pH of 2 and that the release rates of drugs from chitosan beads were much higher in an acidic environment than in alkaline environment. Fattah *et al.* (1998:541) found that the drug release was dependant on the ionic properties of the polymers and the pH of the release media. In acidic pH, chitosan beads showed a rapid drug release and a sustained drug release in an alkaline pH.

Drug release characteristics from beads of different microcrystalline cellulose products (MCC) have been reported (Goskonda & Upadrashta 1993:916). The main aim of bead formulation is to achieve controlled drug delivery (Bodmeier *et al.*, 1989:1488); Aydin & Akbuga (1996:916) achieved controlled drug delivery of salmon calcitonin from chitosan beads and Anal *et al.* (2003:713) prepared beads for the sustained release of ampicillin.

1.3.2 Advantages of beads

One of the main advantages of beads is the ability to incorporate polymers and drug particles into the bead matrix without complicated procedures. Chitosan beads can easily be modified physically and chemically and this opens up avenues for manufacturing a wide range of catalysts for applications in the fields of hydrogenation, oxidation, and fine chemical synthesis reactions (Guibal, 2005:71).

Sodium alginate (a polyanion) can interact with cationic chitosan on the surface of TPP:chitosan beads to form polyelectrolyte complex film for the improvement of the drug sustained release performances. The loading efficiency of model drugs in these beads was very high (more than 90%) (Shu & Zu, 2000:51).

Beads also have the following advantages which make this formulation ideal to achieve controlled release:

- beads disperse freely in the gastro-intestinal tract,
- maximize absorption of drug,
- drug delivery at different absorption areas can be achieved,
- reduce peak plasma fluctuation and
- good bio-availability can be achieved.

(Bodmeier *et al.*, 1989:1475; Ghebre-Sellasie, 1989:7; Gupta & Ravi Kumar, 2000:1115).

1.4 Chitosan as a pharmaceutical excipient

1.4.1 Introduction

During the past 20 years, a substantial amount of work has been published on this polymer and its potential use in various applications. Recently, chitosan has been considered for pharmaceutical formulation and drug delivery applications in which attention has been focused on its absorption-enhancing, controlled release and bioadhesive properties. Synthesized from a naturally occurring source, this polymer has been shown to be both biocompatible and biodegradable. Chitosan is a linear copolymer of β(1-4) linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glycopyranose (see figure 1.2). It is easily obtained by deacetylation of chitin, a polysaccharide widely distributed in nature. The intriguing properties of chitosan have been known for many years and the

polymer has been used in the fields of agriculture, industry and medicine (Dodane & Vivivalam, 1998:246).

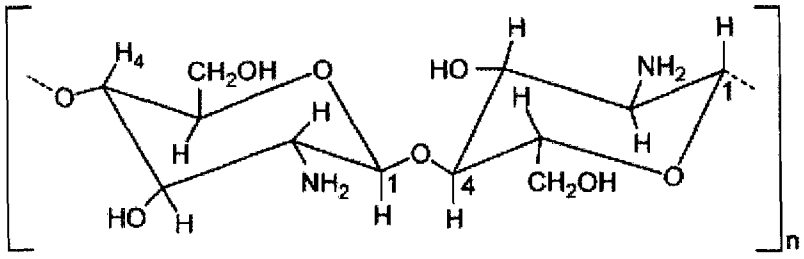


Figure 1.2: Structure of chitosan (Adapted from Van der Merwe *et al.*, 2004:226).

1.4.2 Synthesis of chitosan from chitin

Chitin is easily obtained from crab or shrimp cells and fungal mycelia. Chitin production is associated with food industries such as shrimp canning. The processing of crustacean shells mainly involves the removal of proteins and the dissolution of calcium carbonate which is present in crab shells in high concentrations (Ravi Kumar, 2000:2). However applications of chitin are limited compared to chitosan because chitin is structurally similar to cellulose but chemically inert. The acetamide group of chitin can be converted into an amino group to give chitosan (Agnihotri *et al.*, 2004:6). The production of chitosan-glucan complexes (see figure 1.3) is associated with fermentation processes, similar to those for the production of citric acid from Aspergillus niger, Mucor rouxii, and Streptomyces, which involves alkali treatment yielding chitosan-glucan complexes. The alkali removes the protein and deacetylates chitin simultaneously. The resulting chitin is deacetylated in 40% sodium hydroxide at 120°C for 1-3 hours. This treatment produces 70% deacetylated chitosan (Ravi Kumar, 2000:2). Commercially, chitosan is available in the form of dry flakes, solution and fine powder (Ravi Kumar, 2000:2). Comercialy available chitosan has an average molecular weight ranging between 3800 and 20000 daltons and is 66% to 95% deacetylated (Agnihotri *et al.*, 2004:6).

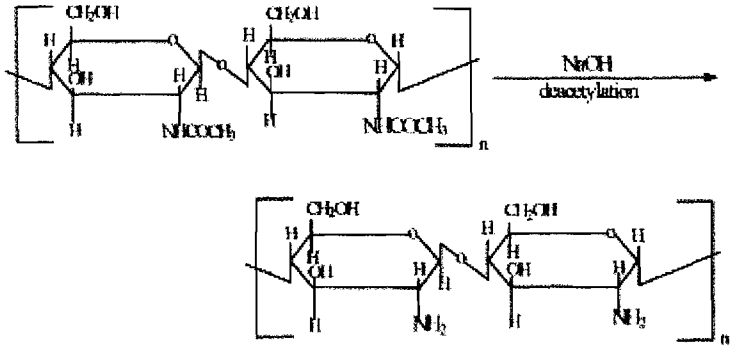


Figure 1.3: The deacetylation process of chitin to produce chitosan (Adapted from Ravi Kumar, 2000:2).

1.4.3 Chitosan mechanism of action

Chitosan is regarded as a biocompatible, biodegradable polymer of natural origin and is widely used in the food industry. Chitosan is known to improve peptide transport across the epithelial barrier, however, this polymers is only soluble in an acidic environment. N-Trimethyl chitosan chloride (TMC) is a derivate of chitosan and is soluble in a low pH range and has proven to be a potent absorption enhancer of peptide drugs by opening the tight junctions between epithelial cells, thereby facilitating the paracellular transport of hydrophilic compounds. TMC proved to be a potent absorption enhancer in for the paracellular transport of hydrophilic marker molecules and peptide drugs *in vitro* in Caco-2 cell monolayer, as well as *in vivo* after intestinal administration both in rats and pigs (Van der Merwe *et al.*, 2004:85).

Chitosan is a bioadhesive material which is able to decrease the clearance of formulations from the nasal cavity both in animal models and in humans. Both the bioadhesive characteristics of the material and its transient effect on the tight junction could lead to an improved immune response. More recent investigations on immune responses to chitosan would suggest that chitosan can also act as an adjuvant for antigens such as CRM197 after systemic administration. Chitosan has been shown to elicit the production of cytokines when applied to the surface of cells (Illum *et al.*, 2001:93)

Chitosan appears to increase cell permeability by affecting paracellular and intracellular pathways. Chitosan causes relatively mild and reversible effects on epithelial function and

morphology, which makes it a promising absorption-enhancing compound for the mucosal delivery of drugs (Dodane & Vilivalam, 1998:251).

1.4.4 Pharmaceutical applications of chitosan

Chitosan is a hydrophilic and positively charged polysaccharide which is biodegradable and non-toxic, it can be used for controlled release purposes in the form of gels and films, it also has bioadhesive properties and can be used in the formation of complex coacervates, microspheres and microcapsules (Dodane & Vilivalam, 1998:246).

In the pharmaceutical field the use of chitosan offers many advantages as an excipient for increasing dissolution rate of poorly soluble drugs, as an auxiliary substance in compressed tableting, as a binder and lubricant in wet granulated tablets and as a stabilizing agent in emulsions. This biopolymer and its derivates are employed in the preparation of modified drug delivery systems such as implants, granules, pellets, xerogels and micro- and nanoparticles (Muzzarelli, 2000:22). During the last few years, glucosamine salts and chitosan have been made available to the public and sold as over-the-counter dietary supplements without medical prescription. Glucosamine is recommenced in osteoarthritis prevention and management, while chitosan is recommended for in weight control and hypercholesterolemia treatment (Muzzarelli, 2000:3).

In general, chitosan administration is associated with a diet. There is a general agreement on the fact that orally administered chitosan lowers blood pressure and cholesterol in volunteers. Reportedly, chitosan exhibits anticholesterolemic, antiulcer, antiarthritic and antiuricemic properties (Muzzarelli, 2000:15). These properties are related to the capacity to bind bile acids, with consequent reduction of their enterohepatic recycling, phospholipids and uric acid. Chitosan is able to form complex salts that bind triglycerides, fatty and bile acids, cholesterol and other sterols and a great portion of these bound lipids are excreted (Muzzarelli, 2000:15).

In vitro and in vivo application of chitosan

Oral drug delivery

Chitosan is a promising polymer for colon drug delivery since it can be biodegraded by the colonic bacterial flora, and it has mucoadhesive properties (Agnihotri *et al.*, 2004:18). The bioavailability of drugs has been improved by the use of mucoadhesive dosage forms. By prolonging the residence time of drug carriers at the absorption site, sustained release and improved bioavailability of drugs can be achieved. Among chitosans of various ranges of molecular-weight better mucoadhesion was observed for higher-molecular weight (approximately 1400kDa) compared to lower-molecular weight chitosans (500 to 800 kDa). This mucoadhesive property makes chitosan an ideal candidate for buccal delivery. Cross linked chitosan disks have been reported to control the *in vitro* release of a model drug, nifedipine (Dodane & Vilivalam, 1998:247).

Due to its specific properties chitosan has also been exploited with promising results in novel gastro-retentive formulations where the chitosan acts as a bioadhesive coating on the surface of small floating controlled release microspheres. Such systems are able to remain in the fasted stomach for extended periods of time. Chitosan and chitosan derivatives have also been shown to promote the absorption of polar drugs such as peptides across the intestinal membrane (Davis, 2000:138).

Parenteral drug delivery

In controlled-release technology, biodegradable polymeric carriers offer potential advantages for the prolonged release of low-molecular weight compounds to macromolecular drugs. The susceptibility of chitosan to lysozyme makes it biodegradable and an ideal drug carrier. The use of chitosan in injectable preparations has received recent attention. Pharmacokinetic and tissue-distribution studies were performed in mice using fluorescent glycolchitosan and N-succinyl-chitosan. Both chitosans demonstrated a good retention in the blood circulation and a slight accumulation in tissues, suggesting that chitosan is an effective carrier for drugs that are excreted rapidly (Dodane & Vilivalam, 1998:251). Chitosan microspheres are successfully used for drug delivery via the parenteral route. Drugs i.e. furosemide, indomethacin, methotrexate and theophylline may be entrapped in the chitosan microspheres. These microspheres has the ability to localize to the target site and since chitosan is

biodegradable and non-toxic to living tissues, it is a safe and effective method to deliver a drug to a specific site (Felt *et al.*, 1998:982).

Ocular drug delivery

Use of chitosan-based colloidal suspensions in *in vivo* studies showed a significant increase in ocular drug bioavailability (Dodane & Vilivalam, 1998:251). The antibacterial and wound healing properties of chitosan along with an excellent film capability make chitosan suitable for development of ocular bandage lenses (Ravi Kumar, 2000:11).

Gene delivery

Gene therapy is a challenging task in the treatment of genetic disorders. In the case of gene delivery, the plasmid DNA has to be introduced into the target cells, which should be translated into the corresponding protein (Agnihotri *et al.*, 2004:20). Chitosan forms polyelectrolyte complexes with DNA and therefore, chitosan and chitosan derivatives may represent potentially safe and efficient cationic carriers for gene delivery (Borchard, 2001:146). The development of new carrier systems for gene delivery represents an enabling technology for treating many genetic disorders. However, a critical barrier to successful gene therapy remains the formulation of an efficient and safe delivery vehicle. Promising results were reported in the formation of complexes between chitosan and DNA. Although chitosan increases transformation efficiency, the addition of appropriate ligands to the DNA–chitosan complex seems to achieve a more efficient gene delivery via receptor mediated endocytosis. Furthermore, incubation of cells with chitosan demonstrated low cytotoxic activity. These results suggest that chitosan has comparable efficacy without the associated toxicity of other synthetic polymers and can, therefore, be an effective gene-delivery vehicle *in vivo* (Dodane & Vilivalam, 1998:249).

Nasal delivery

There is a growing interest in the development of nasal delivery systems for many drugs, including peptides and proteins. Nasal mucosa has high permeability and easy access of drug to the absorption site. Chitosan has been found to enhance the drug absorption through the mucosa without damaging the biological system (Agnihotri *et al.*, 2004:19). Several studies have reported the use of chitosan as a safe nasal-delivery system for proteins. Illum *et al.* (2001:94) have shown that glutamate chitosan can enhance the transport of insulin across the

nasal mucosa of sheep and rats. Chitosan appears to be a safe and effective absorption enhancer for the nasal delivery of drugs.

1.4.5 Safety of chitosan

Chitosan has been used as a safe excipient in drug formulations over the last two decades. This polymer also attracted the attention of pharmaceutical scientists as a mucoadhesive polymer. Chitosan in the swollen state has been shown to be an excellent mucoadhesive and as a natural bioadhesive polymer that can adhere to hard and soft tissues and has been used in dentistry, orthopedics, ophthalmology and in surgical procedures. It adheres to epithelial tissues and to the mucus coat present on the surface of the tissues. A variety of chitosan-based colloidal delivery systems have been described in the literature for the mucosal delivery of polar drugs, peptides, proteins, vaccines and DNA. Clinical tests carried out in order to promote chitosan-based biomaterials do not report any inflammatory or allergic reactions following implantation, injection, topical application or ingestion in the human body (Senel, 2004:1469). The oral LD 50% of chitosan in mice has been reported to be over 16g/kg (Singla, 2001:1050).

Chitosan ingestion effectively lowers serum cholesterol. Chitosan at a dose of 3-6 g/day ingested as biscuits by 8 adult healthy males for two weeks induced a significant decrease in the total serum cholesterol (188 mg/dl to 177 mg/dl) and an increase in serum HDL-cholesterol (51 mg/dl to 56 mg/dl). The net result is a significant decrease in the atherogenic index (Koide, 1998:1091).

Almost all functional properties of chitosan depend on the chain length, charge density and charge distribution. Numerous studies have demonstrated that the salt-form, molecular weight, degree of deacetylation as well as the pH at which chitosan is used influence the properties of this polymer in drug delivery systems. Therefore, these factors must be considered carefully during formulation optimization of dosage forms. In addition, regulatory requirements concerning the use of chitosan in humans will be far more demanding. It has been reported that the purity of chitosan influences its toxicological profile. Dodane & Vivalam (1998:250) have demonstrated the safety of an ultra pure grade of chitosan salts in various biological and physiological systems. Therefore, it would stand to reason that only

the highest purity of chitosan would satisfy the standards set by regulatory agencies (Dodane & Vivalam, 1998:250).

1.5 Polymers for sustained drug delivery

Controlled drug delivery occurs when a natural or synthetic polymer is sensibly combined with a drug or other active agent in such a way that the active agent is released from the material in a predetermined manner (Brannon-Peppas, 1995:1). A factor that has sparked interest in prolonged action dosage forms has been the rapid growth of polymer technology and its application to the solution of some biomedical problems. Biocompatibility of polymers, polymers as biomaterials and the use of polymers in prosthetic devices can influence the release of the active ingredient from the dosage form (Ballard, 1978:2).

Recently natural polysaccharides have shown to be very useful for drug entrapment and sustained release of drug. The natural polymers used as carrier materials in the encapsulation technology have the great advantage of being nontoxic, biocompatible and biodegradable (Anal *et al.*, 2003:714).

Natural and synthetic polymers are generally used as important ingredients for both controlled release and conventional drug delivery systems. These polymers may function on a variety of mechanisms including (Narasimhan & Peppas, 1997:297):

- Porosity and non-porosity,
- swelling and non-swelling,
- degradability and non-degradability,
- semi-permeability,
- bio-adhesion.

In the past few decades, polymeric controlled drug delivery systems have emerged as important pharmaceutical dosage forms. This success can be attributed to the following major contributing factors (Thombre, 1991:159):

- It was recognized that controlled drug delivery has many potential medical and commercial advantages.
- Novel concepts and designs for drug delivery devices were developed through a concentrated effort by interdisciplinary research teams.
- Advances made in the fields of polymer science and engineering were successfully applied to drug delivery.

The polymers that have been studied for drug delivery applications can be classified into the following four categories (Thombre, 1991:164):

- inert, non-bioerodible hydrophobic polymers, e.g., ethylene vinylacetate, poly(dimethyl siloxane), poly(ether urethane), poly(vinyl chloride) and poly(ethylene);
- cross linked hydrogels which swell but do not dissolve in water, e.g., poly(ethylene oxide), and crosslinked poly(vinyl pyrrolidone);
- bio-erodible polymers, e.g., poly(lactic acid), poly(glycolic acid), poly(caprolactone), poly(hydroxyburyrate), poly(amino acids) labile poly(esters) and poly(anhydrides);
- water soluble polymers, e.g., hydroxypropyl methylcellulose, and poly(vinyl pyrrolidone). Some polymers have a pH-dependent aqueous solubility, e.g., cellulose acetate phthalate.

The release of medications from either category of polymer device traditionally has been diffusion-controlled. Currently, however, modern research is aimed at investigating biodegradable polymer systems. These drug delivery systems degrade into biologically acceptable compounds, often through the process of hydrolysis, which subsequently leave their incorporated medications behind. This erosion process occurs either in bulk (wherein the matrix degrades uniformly) or at the polymer's surface (whereby release rates are related to the polymer's surface area). The degradation process itself involves the breakdown of

polymers into lactic and glycolic acids. These acids are eventually reduced by the Kreb's cycle to carbon dioxide and water, which the body can easily expel (Vogelson, 2001:50).

Specific physical properties which contribute to the rate of degradation are summarized below (Pitt & Schindler, 1983:57):

- The water permeability and water solubility. These properties, a reflection of the free volume of the polymer and its hydrophilicity, will determine the rate of hydrolysis and whether bulk or surface hydrolytic degradation occurs. Autocatalysis of the degradation process is possible if acidic or basic groups are produced by the polymer breakdown, as in the case of polyesters and orthoesters.

- The crystallinity of the polymer. Only the amorphous phase of the polymer is accessible to permeants (specifically water and drug), and to enzymatic attack.

- The glass transition temperature. The glassy or rubbery nature of the polymer will be reflected in its permeability and molecular chain mobility. The chain mobility appears to be an important factor in determining the susceptibility to enzymatic attack. Also, the inability of cleaved fragments to diffuse out of a glassy polymer will magnify an autocatalytic hydrolytic process. This may contribute to the rates of degradation of polymers such as polylactic and polyglycolic acid.

- The physical dimensions, for example size and surface to volume ratio. These appear to become significant in the advanced stages of biodegradation, when phagocytosis may come into play.

The future technical challenges for the field of polymeric drug delivery include (Thombre, 1991:167):

- the understanding and control of absorption and metabolism factors which may ultimately lead to the oral delivery of proteins and peptides;

- the design of intelligent delivery systems with biofeedback, an example of such a systems which regulate drug release based on a biological marker, the release of insulin depending on the blood sugar levels;

- tailored release rates including increasing release rates with time and pulsatile release;

- targeted and site-specific delivery.

Polymers used as drug carriers need to comply with a wide array of requirements. Firstly these polymers need to be biocompatible. The chemicals employed in the polymer manufacturing must thus be carefully selected to meet regulatory requirements. Secondly the polymer must posses the necessary mechanical properties required for dosage from design. Furthermore the polymer needs to posses certain pharmacokinetic properties. The polymer should not undergo degradation, and if degradation does occur, the by-products must be biocompatible, non-toxic, non-carcinogenic and non-immunogenic (Passl, 1996:629).

1.6 Polyvinylpyrrolidone as a pharmaceutical exicipient

1.6.1 Introduction

Polymer therapeutics is rapidly emerging as an enabling technology for the development of a significant number of therapeutic agents. The term polymer therapeutics includes polymeric drugs, polymer-protein conjugates, polymer-drug conjugates and polymeric non-viral vectors for gene delivery (D'souza *et al.*, 2003:91). Polyvinylpyrrolidone is a synthetic, water-soluble neutral polymer that is generally recognized as a safe excipient and is used in pharmaceutical formulations. Recently, efforts have been devoted to developing PVP conjugates of proteins and other low molecular weight compounds (D'souza *et al.*, 2003:98).

Synonyms for polyvinylpyrrolidone is PVP; povidone; 1-vinyl-2-pyrrolidinone polymer. The chemical name of polyvinylpyrrolidone is 1-Ethenyl-2-pyrrolidinone homopolymer. For the remainder of this study polyvinylpyrrolidone will be referred to as povidone. The trade name of povidone is Kollidon and is manufactured by the BASF corporation.

1.6.2 Properties of Povidone

Povidone is a fine, white to creamy colored, odorless or almost odorless, hygroscopic powder. The chemical structure of Povidone is depicted in figure 1.4. The degree of polymerization results in polymers with various molecular weight, this is characterized by its viscosity in

aqueous solution relative to that of water and is expressed as a K-value. Povidone with K-values equal to or lower than 30 are manufactured by spray drying and exist as spheres (see table 1.1). Povidone K-90 and higher K-value povidones are manufactured by drum drying and exist as plates (Walkling, 1994:392).

Povidone is freely soluble in acids, chloroform, ethanol, ketones, methanol and water; but is practically insoluble in ether hydrocarbons and mineral oil. In water the concentration of a solution is limited only by the viscosity of the resulting solution which is a function of the K-value (Walkling, 1994:393).

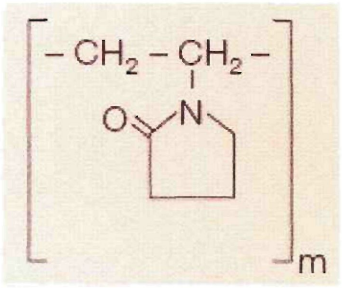


Figure 1.4: Structure of povidone (Adapted from Walkling, 1994:393).

Table 1.1: Approximate molecular weights for different povidone grades are shown below (Addapted from Walkling, 1994:394).

K-value	**Approximate molecular weight**
12	2500
15	8000
17	10000
25	30000
30	50000
60	400000
90	1000000
120	3000000

The viscosity of aqueous povidone solutions depends on the concentration and molecular weight of the polymer employed. Povidone darkens to some extent on heating at 150 °C, with a reduction in aqueous solubility. It is stable to a short cycle of heat exposure around 110-130°C and steam sterilization of an aqueous solution does not alter its properties. Povidone is hygroscopic and should be stored in an airtight container in a cool, dry, place (Walkling, 1994:393).

Polyvinylpyrrolidone constitutes a part of the synthetic polymers utilized as binding agent. Since is a versatile material, it is one of the most commonly used binders (Khankari, 2001:64).

1.6.3 Manufacturing

Povidone is manufactured by the Reppe process (figure 1.5). Acetylene and formaldehyde are reacted in the presence of a highly active copper acetylide catalyst to form butynediol which is hydrogenated to butanediol (and then cyclodenhydrogenated to form butyrolactone). Pyrrolidone is produced by reacting butyrolactone with ammonia. This is followed by a vinylation reaction in which pyrrolidone and acetylene are reacted under pressure. The monomer, vinylpyrrolidone, is then polymerized in the presence of a combination of catalysts to produce povidone (Walkling, 1994:398).

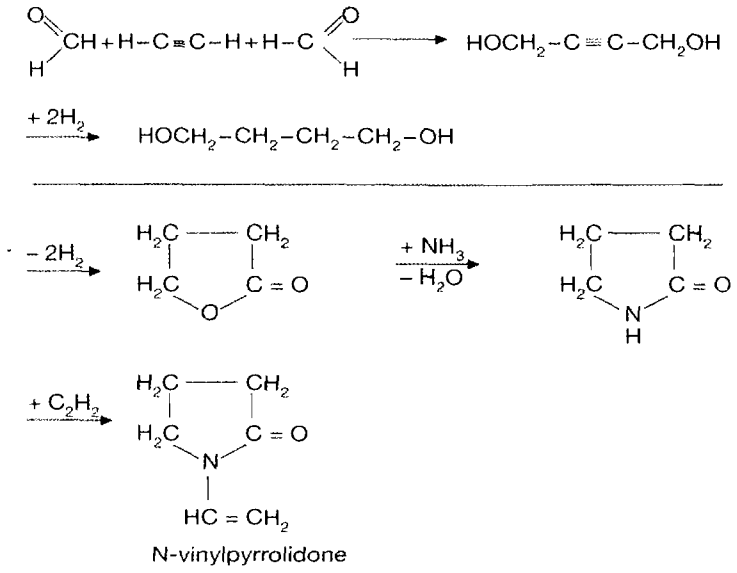


Figure 1.5: Reppe's synthesis of N-vinylpyrrolidone (C_6H_9NO; Mr 111.1) (Adapted from Bühler, 2003:9).

1.6.4 Pharmaceutical applications of Povidone

According to Walkling (1994:392) povidone is used in a variety of pharmaceutical formulations. It is primarily used in solid dosage forms. In tableting, povidone solutions are used as binders in wet granulation processes. Povidone is also added to powder blends in the dry form and granulated in situ by the addition of water, alcohol or hydroalcoholic solutions. Povidone solutions may also be used as coating agents. Povidone is additionally used as a suspending, stabilizing of viscosity-increasing agent in a number of topical and oral suspensions and solutions. The solubility of a number of poorly soluble active drugs may be increased by mixing with povidone. The uses of povidone are set apart in the table 1.2 below.

Table 1.2: Pharmaceutical applications of povidone.

Use	Concentration %
Carrier for drugs	10-25
Dispersing agent	Up to 5
Eye-drops	2-10
Suspending agent	Up to 5
Tablet binder, tablet diluent or coating agent	0.5-5

1.6.5 Toxicity

Povidone has been used in pharmaceutical formulations for many years, being first used in the 1940s as a plasma expander, although is has been superseded for this purpose by dextran. Povidone was widely used as an excipient, particularly in oral tablets and solutions. When consumed orally, povidone may be regarded as essentially non-toxic since it is not absorbed from the gastrointestinal tract or mucous membranes. Povidone additionally has no irritant effects on the skin and causes no sensitization. Reports of adverse effects to povidone primarily concern the formation of subcutaneous granulomas at the injection site off intramuscular injections formulated with povidone. Evidence also exists that povidone may accumulate in the organs of the body following intramuscular injection (Walkling, 1994:399). A temporary acceptable daily intake for povidone has been set by the WHO at up to 25 mg/kg body-weight. The following LD50 values are reported: LD_{50} (mouse, IP): 12g/kg; LD_{50} (mouse, IV): > 11g/kg; LD_{50} (rat, oral):8.25 g/kg.

1.7 Ketoprofen as active ingredient

Ketoprofen is a well known nonsteriodal anti-inflammatory agent requiring a high dosage for efficacy in arthritis. Ketoprofen also has analgesic and antipyretic properties; it possesses

antibradykinin activity as well as lysosomal membrane-stabilizing activity (Frust & Munster, 2001:605).

Ketoprofen is a propionic acid derivative that inhibits both cyclooxygenase (nonselectively) and lipoxygenase. The racemic drug is rapidly absorbed. It is metabolized completely in the liver, primarily to the glucuronide, which can undergo reactivation after enterohepatic circulation. The effectiveness of ketoprofen at dosages of 100-300 mg/d is equivalent to that of other NSAIDs in the treatment of rheumatoid arthritis, osteoarthritis, gout, dysmenorrhoea, and painful conditions (Frust & Munster, 2001:605).

Ketoprofen is a white or almost white, crystalline powder, practically insoluble in water, freely soluble in acetone, in ethanol (96%) and in methylene chloride. Its empirical formula is $C_{16}H_{14}O_3$ (see figure 1.5) with a molecular weight of 254.3 g/mol. Ketoprofen has a melting point between 94°C to 97°C (British Pharmacopoeia, Electronic Edition, 2005). The chemical name for ketoprofen is (2-3-benzoylphenyl-propionic acid) (Rx list, 2006). Ketoprofen is a weak acid and has a pK_a of 5.94 (El-Kamel *et al.*, 2001:14).

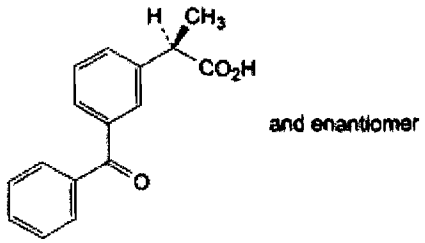


Figure 1.6: Structure of ketoprofen (British Pharmacopoeia, 2005).

The elimination half-life of ketoprofen has been reported to be 2.05 ± 0.58 h. The plasma clearance of ketoprofen is approximately 0.08 l/kg/h with a Vd of 0.1 L/kg after IV administration. The elimination half-life is longer in elderly patients and patients with renal failure (Rx list, 2006).

Ketoprofen was chosen for formulation in a controlled release dosage form because of its low solubility in acidic environments (Verwey, 2005:28). Ketoprofen has a short half life of 2.05 ± 0.58 h which make this drug an ideal drug for controlled release formulations. Its major adverse effects are on the gastrointestinal tract and the central nervous system (Frust & Munster, 2001:605). Formulations of ketoprofen in controlled release dosage forms can reduce the adverse effects, especially with chitosan due to chitosans anti-acid and anti-ulcer

activities. Therefore it would be favourable to produce a dosage form with enteric release properties as well as controlled drug delivery in the alkaline environments of the small intestine (Verwey, 2005:28).

1.8 Conclusion

Judging by the proliferation of published papers in recent years, there has been increased interest in the development and marketing of prolonged action or controlled release drug delivery systems. Controlled release dosage forms have a significant potential to enhance clinical efficacy and decrease total treatment costs, thereby providing economic value compared with immediate-release dosage forms, even when initial acquisition costs are higher.

The mechanism of drug release for controlled release systems has been shown to be primarily divided into two types, the first being a reservoir device in which the drug forms a core surrounded by an inert diffusion barrier and the second being a monolithic device in which the drug is dispersed in a polymer matrix. There are many formulations in which the above mentioned mechanism may be divided for example: coated dosage forms, osmotic systems, dissolution controlled dosage forms and erosion dosage formulation.

Natural and synthetic polymers are generally used as important ingredients for both controlled release and conventional drug delivery systems. Biocompatibility of polymers, polymers as biomaterials, and the use of polymers in prosthetic devices can influence the release of the active ingredient from the dosage form.

Povidone and chitosan are both polymers of natural origin and have been found by numerous studies to be safe and bio-compatible polymers suitable for use in controlled release formulations.

The production of relatively simple formulation consisting of a combination of chitosan and povidone, and the effect that these polymers will have on the release of ketoprofen, will be evaluated in this study. The preparations of such formulations are discussed in the following chapter as well as means for characterization of the samples.

CHAPTER 2

2 BEADS AND GRANULES FOR CONTROLLED DRUG DELIVERY: PREPARATION AND CHARACTERISATION

2.1 Introduction

Advances in polymer science have led to the development of several novel drug delivery systems. A proper consideration of surface and bulk properties can aid in the designing of polymers for various drug delivery applications. Biodegradable polymers such as chitosan find widespread use in drug delivery as they can be degraded to non-toxic monomers inside the body (Pillai & Panchagnula, 2001:447).

Biodegradable polymers have been used extensively in biomedical areas in the form of sutures, wound covering materials and as artificial skin. Polymer-based drug delivery systems have been considered for many applications to supplement standard means of medical therapeutics. These delivery systems are less complicated and smaller than mechanical pumps because the drug can be stored as a dry powder within the polymer matrix (Ravi Kumar, 2000:1115).

A combination of chitosan and PVP may have beneficial effects on the biological characteristics of complex materials because of their good biological activities. In particular, the addition of PVP may improve the hydrophilicity of chitosan without impairing its excellent biocompatibility (Xi, 2005:440).

The applications of polymers in controlled drug delivery systems have become important in the pharmaceutical industry. As discussed in chapter 1 polymers have several advantages which make them ideal candidates for controlled drug delivery systems. Research has demonstrated the use of beads and granules in controlled drug delivery systems, and the advantages of these dosage forms have been discussed in chapter 1. In this chapter different experimental methods for the development of controlled release dosage forms of ketoprofen

based on chitosan and povidone as polymers will be outlined. Two methods will be studied, one based on bead formulation and the other based on a granulation technique.

2.2 Cross linking of chitosan

Cross linking is the technique most frequently used to improve the chemical properties of chitosan. It renders chitosan less soluble in acid solutions (Milot *et al.*, 1998:571). The cross linking of chitosan chains with tripolyphosphate (TPP) occurs by the reaction of aldehyde groups (-CHO) of the tripolyphosphate with the amine groups ($-NH_2$) of the chitosan polymer chain (see Figure 2.1). The cross linking of chitosan beads is not a homogeneous reaction and occurs mainly near the surface of the biopolymer.

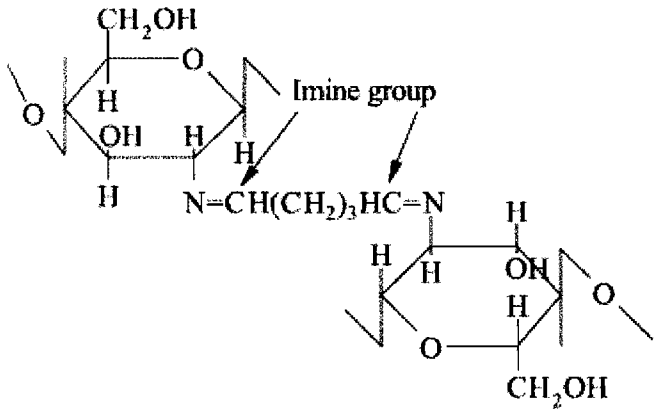


Figure 2.1: Structure of cross linked chitosan (Adapted from Agnihotri, 2004:7).

According to Mi *et al.* (2002:758), chitosan-TPP complexes were formed only by the ionic interaction between the positively charged amino groups and negatively charged counter ions. In acid solutions, this interaction between chitosan and TPP is more effective resulting in a higher cross-linking, due to the high contents of the positively charged amino group. However, in strong basic solution, the $-NH_3$ groups are deprotonated and the ionic interactions between chitosan and TPP disappear (Chiou & Li, 2003:1097).

2.3 Preparation of beads

Different methods have been used to prepare beads. Selection of any method depends upon factors such as particle size requirements, thermal and chemical stability of the active agent, reproducibility of the release profiles, stability of the final product and residual toxicity associated with the final product (Agnihotri *et al.*, 2004:8).

Methods for preparing micro/nanoparticles of chitosan include (Agnihotri *et al.*, 2004:8):

- emulsion cross-linking,
- coacervation/precipitation,
- spray-drying,
- emulsion-droplet coalescence,
- inotropic gelation,
- reverse micellar and
- sieving.

Of all the above mentioned methods, inotropic gelation is one of the simplest and most used methods for preparing beads with favourable characteristics, and was therefore used in the preparation of the chitosan beads used in this study. Inotropic gelation will be discussed in more detail in the following paragraph.

Ionotropic gelation method

The ionotropic gelation method (illustrated in Figure 2.2) is used for the preparation of beads and is one of the most trusted methods that has been used widely since the introduction of the method by Bodmeier (1989:1478). This method is based upon the ionic interactions between two opposite charges; this can be depicted between the positively charged amino groups of chitosan and the negatively charged counterion, tripolyphosphate (Bodmeier, 1989:1479). Briefly, chitosan is dissolved in aqueous acidic solution to obtain the cation of chitosan. This solution is then added drop wise under constant stirring to polyanionic tripolyphosphate solution. Due to complexation between oppositely charged species, chitosan undergoes ionic

gelation and precipates to form spherical particles (Agnihotri *et al.*, 2004:12). The bead forms on contact of the chitosan solution or dispersion with the TPP solution, entrapping the drug within a three-dimensional network of ionically linked polymer (Lubbe *et al.*, 2002:3). Benefits of bead formation, apart from ease of handling, include the expansion of the chitosan polymer network, providing easier diffusion to internal absorption sites (Grant, 2002:13).

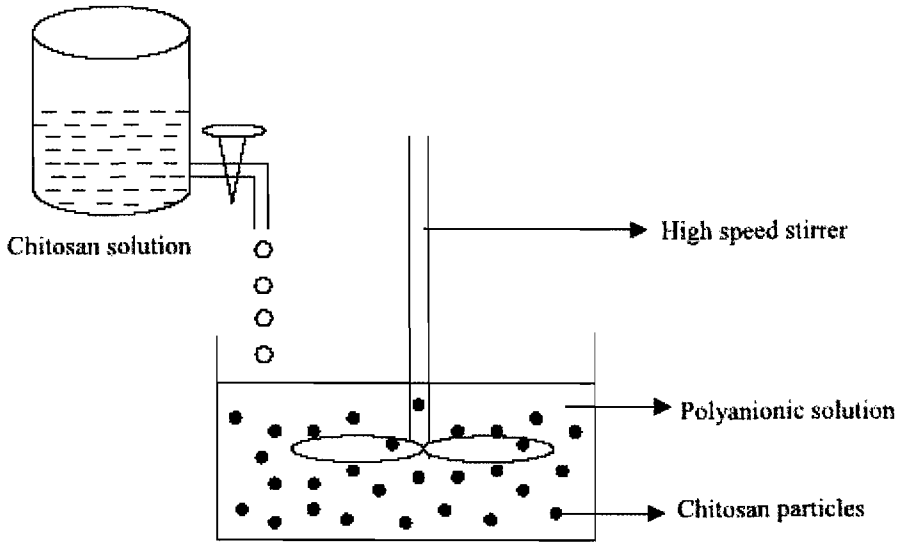


Figure 2.2: Illustration of the ionotropic gelation method (adapted from Agnothori *et al.*, 2004:13).

2.4 Preparation of beads for the study

2.4.1 Materials

The following materials were used during this study for the preparation of chitosan drug loaded beads. Chitosan (91,4% deacetylated)(Xiamen, South Africa), glacial acetic acid 99% (Merck, South Africa), tripolyphosphate (Sigma, South Africa), Kollidon® SR (BASF, South Africa) and ketoprofen (Boehringer Ingelheim Bidachem, Italy) [For COA see Annexure A].

2.4.2 Method

2.4.2.1 Optimisation of the method

The method used for the preparation of beads in this study was the method already established by Verwey (2005:34). According to Verwey (2005:31) chitosan concentration of 1 and 2% (w/v) had irregular shapes and inadequate resistance to mechanical force, and concentrations of 4% (w/v) and higher had a very high viscosity and the needle became clogged. Thus a concentration of 3% (w/v) chitosan was selected for this method (Verwey, 2005:31).

The optimal pH for preparing the beads also had to be determined. TPP solutions generally have a pH of between 8.9 and 9.1. The model drug ketoprofen has a pK_a-value of 5.94. Therefore most of the drug leached out of the beads into the TPP solution. It was determined that the optimal drug loading would be achieved at a pH below the pK_a value of ketoprofen. The optimal drug loading of ketoprofen was achieved at a pH of 5 and hence this pH was used in this study (Verwey, 2005:31).

The effect of the ratio of chitosan-drug dispersion to external TPP-phase on the percentage drug loading was also determined, because the ratio of chitosan-drug mixture to TPP-phase could affect the drug loading of the beads. An optimal drug loading was achieved at a chitosan-drug solution:TPP-phase ratio of 1:5 (Verwey, 2005:34). Therefore the chitosan-drug solution:TPP-phase ratio of 1:5 was selected for the preparation method.

2.4.2.2 Experimental method

Chitosan drug loaded beads were produced by preparing a 3% (w/v) dispersion of ketoprofen into a 3% (w/v) chitosan, prepared in a 2% (v/v) aqueous solution of acetic acid. The solution was sonicated (Julabo Labortechnik, Swarzwald, Germany) until it was free of any bubbles. Drug loaded chitosan-polymer beads were prepared using low concentrations of Kollidon® SR. The Kollidon® SR was added in concentrations of 0.25%, 0.5% and 1% (w/v).

The beads were formed by dropping the chitosan-drug-polymer dispersion through a 21 gauge needle into a gently agitated solution of TPP using a peristaltic pump (Watson-Marlow 205S, Watson-Marlow Ltd., England). The pH of the TPP solution was adjusted to pH 5.0 and an amount of 75 mg ketoprofen was added to 50 ml of the TPP solution prior to the bead preparation. The ratio of chitosan-drug-dispersion to TPP solution was kept steady at 1:5 to ensure optimal drug loading. A stirring time of 30 min for one batch and 60 min for the other

batch was allowed to study the effect of the stirring time on the cross linking of beads. The beads were removed from the solution by filtration, washed twice with deionised water and freeze dried for 24 hours (Verwey, 2005:35).

2.5 Granules

Granulation may be considered a size enlargement process during which small particles are formed into larger, physically strong agglomerates in which the original particles can still be identified. In modern times granulation has been used in a wide range of industries ranging from the pharmaceutical industry to the fertilizer and minerals processing industries. The main objective of granulation is to improve the flow properties and characteristics of the mix and prevent segregation of the constituents (Augsburger & Vuppala, 1993:8).

Advantages of granulation include the following (Iveson *et al.*, 2001:4):

- reduced dustiness which minimizes losses, inhalation and explosion risks,
- improved flow and handling which facilitates controlled metering,
- increased bulk density,
- reduced pressure loss for fluid flow through a packed bed, which is useful in blast furnaces and leach heaps,
- controlled dissolution rates; and the co-mixing of particles which would otherwise segregate during handling.

Granulated products often maintain a high proportion of the surface area of the original particles, which is useful in applications involving catalysts or requiring rapid dissolution.

The principle methods of granulating pharmaceuticals may be divided into three main categories: wet processes, dry processes and other processes. In the dry methods no liquid is employed and wet granulation is when a liquid is employed in the granulation process (Augsburger & Vuppala, 1993:8).

2.5.1 Wet granulation

Wet granulation is a process widely used in the pharmaceutical industry. It has not been replaced by direct compression technology, partly because of development cost considerations and habits, and partly because it remains in some cases an attractive technique. It provides better control of drug content uniformity at low drug concentrations, as well as control of product bulk density and ultimately compactibility (brittle fracture), even for high drug contents (Faure *et al.*, 2001:269).

Wet granulation involves the massing of a mix of dry primary powder particles using a granulating fluid. The fluid contains a solvent which must be volatile so that it can be removed by drying and be non-toxic. Typical liquids include water, ethanol and isopropanol, either alone or, more usually, as a solvent containing a dissolved adhesive (also referred to as a binder or binding agent) which is used to ensure particle adhesion once the granule is dry (Summers & Aulton, 2002:366).

In the traditional wet granulation method, the wet mass is forced through a sieve to produce wet granules which are then dried. A subsequent screening stage then breaks agglomerates of granules and removes the fine material, which can then be recycled. Variations of this traditional method depend on the equipment used, but the general principle of initial particle aggregation using a liquid remains in all of the processes (Summers & Aulton, 2002:367).

2.5.2 Dry granulation

It is possible to form granulates without the addition of a granulating fluid, by techniques generically referred to as dry granulation. These methods are useful for materials that are sensitive to heat and moisture but which may not be suitable for direct compression (Davies, 2004:431). In dry methods, of granulation the primary powder particles are aggregated under high pressure. There are two main processes. Either a large tablet (known as a slug) is produced in a heavy-duty tableting press (a process known as slugging) or the powder is squeezed between two rollers to produce a sheet of material (roller compaction). In both cases these intermediate products are broken using a suitable milling technique to produce granular material, which is usually sieved to separate the desired size fraction. The unused fine material may be reworked to avoid waste (Summers & Aulton, 2002:366).

2.6 Preparation of granules for study

2.6.1 Materials

The following raw materials were used during this study for the preparation of chitosan drug loaded granules. Chitosan (91,4% deacetylated)(Xiamen, South Africa), glacial acetic acid 99% (Merck, South Africa), tripolyphosphate (Sigma, South Africa), 2-Pyrrolidinone® (Fluka, South Africa) Kollidon® SR (BASF, South Africa) and ketoprofen (Boehringer Ingelheim Bidachem, Italy) [For COA see Annexure A].

2.6.2 Method

The granules were prepared with the same raw materials used in the preparation of the beads, and in such a way that it could resemble the composition of the beads. Granules were prepared with different methods to see which method will render the optimum drug release.

As mentioned, granules were made to resemble the composition of the beads; therefore the exact same materials had to be used to make a comparison between the beads and the granules. In the case of the beads, the cross linked chitosan acts as a binder, thus in the granules the chitosan also had to act as a binder.

Sakkinen *et al.* (2003:228) prepared granules with a similar method using chitosan as a gel-forming excipient in matrix granules. The granules were moistened with 2.5% v/v acetic acid and different concentrations of polymers were integrated in the matrices of the granules to achieve slow release from the granules.

As mentioned four different methods for granule preparation were prepared. In the first method, the chitosan and ketoprofen were weighed in a 50:50 ratio and added together in glass jar, the Kollidon® SR was then added in different concentrations (see table 2.1) and the powders were mixed together in a Turbula mixer (Model T2C, Wily A. Bachofen Maschinefabrik, Basel, Switzerland) for 5 minutes. The powder was then wetted with a 2% v/v acetic acid solution while it was stirred in a Kenwood mixer (KM300, Kenwood Ltd. Britian). The wet mass was granulated through a 2 mm sieve. The granules were dried in an oven (Term-O-Mat oven, Labotec, South Africa) at 37 °C for 6 hours and then granulated

through a fine sieve (Fritsch analysette, Germany), only granules between 710 μm and 800 μm were used since this was the sieve fraction.

In the second method, the method of drying was changed to see if it had any effect on the drug loading, hardness and drug release from the granules. Weighed amounts of chitosan and ketoprofen were added to a glass jar. Kollidon® SR was subsequently added in a concentration of 10% w/w and the powders were mixed in a Turbula mixer (Model T2C, Wily A. Bachofen Maschinefabrik, Basel, Switzerland) for 5 minutes. The mixture was then wetted with a 2% v/v acetic acid solution whiles it was stirred in a Kenwood mixer (KM300, Kenwood Ltd. Britian). The wet mass was granulated through a 2 mm sieve. The granules were dried in a freeze drier (Virtis, USA) for 24 hours at -59 °C and 100 mTorr. The granules were then granulated through a fine sieve (Fritsch analysette, Germany) and granules between 710 μm and 800 μm were used. A 10% w/w concentration of Kollidon® SR was used as it was postulated that if there would be a difference it would be more prominent with a high concentration.

In the third method, chitosan and ketoprofen were weighed in a 50:50 ratio and added together to a glass jar, the powders were then mixed in a Turbula mixer (Model T2C, Wily A. Bachofen Maschinefabrik, Basel, Switzerland) for 5 minutes. A 10% w/w concentration of Kollidon® SR was subsequently added to 20 ml 2-Pyrrolidinone® and stirred for 12 hours to dissolve. The chitosan-ketoprofen mixture was wetted with the Kollidon® SR/2-Pyrrolidinone® solution. The wet mass was then granulated through a 2 mm sieve and the granules were dried in an oven (Term-O-Mat oven, Labotec, South Africa) at 37 °C for 7 days. 2-Pyrrolidinone® has an oil base and the granules therefore took 7 days to dry. The granules were then granulated through a fine sieve (Fritsch analysette, Germany) and granules between 710 μm and 800 μm were used.

In the fourth and final method ketoprofen and chitosan were weighed in a 50:50 ratio. The ketoprofen was dissolved in 40 ml of ethanol and the solution was sonicated (Julabo Labortechnik, Swarzwald, Germany) for 1 hour to ensure complete solution. A 5% w/w concentration of Kollidon® SR was added in 10 ml 2-Pyrrolidinone® and the mixture was left to stir for 12 hours. The two mixtures were subsequently added together and stirred for 2 hours. The mixture was then sprayed over the chitosan to coat the entire surface of the powder. The resulting wet mass was mixed in a Kenwood® mixture (KM300, Kenwood Ltd. Britian) and thereafter granulated through a 2 mm sieve. The granules were dried in an oven

(Term-O-Mat oven, Labotec, South Africa) at 37 °C for 7 days. The dry granules were then granulated through a fine sieve (Fritsch analysette, Germany) and only granules between 710 µm and 800 µm were used.

Table 2.1: Composition of granules in study.

Granules	Composition		
	Chitosan (m/m)	Ketoprofen (m/m)	Kollidon® SR (m/m)
Chitosan granule	50%	50%	0%
1% Kollidon® SR granule	49.5%	49.5%	1%
5% Kollidon® SR granule	47.5%	47.5%	5%
10% Kollidon® SR granule	45%	45%	10%

2.7 Methods used for the characterization of beads and granules

To characterize the beads and granules the following characteristics was investigated: morphology, drug loading capacity, dissolution and drug release, mechanical strength, solubility and swelling behaviour.

2.7.1 Morphology: Scanning electron microscopy

Morphology of the beads and granules are an important characterization method to differentiate between the different behaviour patterns of the beads in various experiments. Scanning electron microscopy was used to investigate the surface morphology and cross sections of the beads and granules to see if the polymer was incorporated in the bead and granule structure. Shu & Zu (2000:53) conducted scanning electron microscopy on chitosan beads to investigate the surface and shape of beads.

Experimental method

The samples were set down on aluminium sample clasp and sprayed with gold palladium alloy to minimize the surface charging. The gold palladium alloy was sprayed on and a Philips XL30DX4i scanning electron microscope was used to investigate the surface and cross sections of the beads and granules.

2.7.2 Drug loading capacity

Drug loading capacity is the amount of drug incorporated in the granules and beads. It is of critical interest to determine the amount of drug in the beads and granules since it will determine how effective the drug is incorporated in the polymer. The drug loading is also important when calculating dosages.

Experimental method

A sample of the freeze dried beads were taken and crushed into powder. A weighed amount of the powder (20 mg) were then dissolved in a 100 ml volumetric flask and made up to volume with PBS pH 7.4 and left to stir for 24 hours. The solution was filtered through a Millipore® prefilter and 10 ml was pippeted to a 100 ml volumetric flask and made up to volume. The solution was made up to volume with PBS pH 7.4 and sonicated for 30 minutes. The absorbencies of the dilutions were measured with a spectrophotometer (Unicam, Cambridge, UK) at a wavelength of 258 nm. The drug concentrations were determined from the standard curve using linear regression. The drug loading capacity of the beads and granules were then calculated using the following equation.

$$\%\,Drug\ loading\ capacity = \frac{Experimental\ concentration\ \mu g/ml}{Theoretical\ concentration\ \mu g/ml} \times \frac{100}{1} \qquad \text{(equation 2-1)}$$

2.7.3 Dissolution and drug release

In light of the FDA's recent guidelines regarding oral modified release dosage forms, there is an increased awareness of the potential relevance of dissolution tests. What was at one time a test to differentiate a good batch of product from a bad one, is now developing into a tool for predicting bioavailability, and in some cases, replace clinical studies to determine bioequivalence (Crison, 2007). Gupta and Ravi Kumar (2000:1116) conducted drug release studies on beads in a glass apparatus at 37 °C in an acidic and basic solution. A known amount of drug was added in the dissolution medium and samples were withdrawn at predicted intervals and assayed spectrophotometrically.

Experimental method

Dissolution studies were preformed in a six station dissolution apparatus (Erweka® Apparatebau GmbH, Germany). The temperature was regulated at 37 °C by a thermostat and the standardised USP basket stirring element was used in all of the dissolution studies. Dissolution studies were preformed in 1000 ml of phosphate buffer solution (PBS pH 7.4) and the baskets were stirred at a constant speed of 50 revolutions per minute (rpm).

A sample amount of 25 mg of the beads or granules were weighed (Precisa 240A, Precisa balances, Zürich, Switzerland) and placed in the basket. The dissolution vessels were filled with the dissolution medium and heated to a temperature of 37 °C, the baskets were then pushed down into the vessels to a depth of 25 mm from the bottom. The rotation was kept constant at 50 rpm revolutions per minute and samples of 10 ml were withdrawn through a Millipore® prefilter. Samples were withdrawn at a constant height and the withdrawn volume was immediately replaced with fresh preheated PBS, a correction was made for the dilution caused by the replacement (equation 2.2). Samples were withdrawn after 2, 5, 10, 15, 20, 30, 45 and 60 minutes and thereafter every 60 minutes up to 360 minutes.

The samples were subsequently analysed spectrophotometrically with a Unicam® spectrophotometer (Unicam, Cambridge, UK) at a wavelength of 258 nm. The following equation was used to compromise for the dilution after replacing each sample with PBS.

$$Y_n^* = Y_n \pm \frac{V_s}{V_m} \bullet \sum^{n-1} Y^* \qquad \text{(equation 2.2)}$$

Where:

Y_n^* = corrected absorbency of the nth sample; Y_n = the measured absorbency of the nth sample; V_m = the dissolution medium volume and $\sum^{n-1} Y^*$ = is the sum of the corrected absorbencies to the nth sample.

The area under the dissolution curve (AUC) is the parameter used to indicate the extent of the dissolution of the active ingredient. The AUC was calculated from t_0 up to the completion of the dissolution test at 360 minutes, and the percentage of drug dissolved was determined using the trapezoidal rule (equation 2.3).

$$AUC = 0.5 \times \sum_{t=n}^{t=0} (t_n - t_{n-1})(c_n - c_{n-1}) \qquad \text{(equation 2.3)}$$

Where:

$t_n - t_{n-1}$ is the time difference between two consecutive sampling intervals and c_n and c_{n-1} is the corresponding drug concentrations at the sample times t_n and t_{n-1}.

2.7.4 Standard curve

A standard curve is a quantitive research tool, a method of plotting assay data that is used to determine the concentration of a substance. A standard curve was constructed before each dissolution study and drug loading capacity test. Two stock solutions were prepared by dissolving 25 mg and 50 mg of ketoprofen in phosphate buffer solution (PBS) pH 7.4, each solution were made up to volume (250 ml) in a volumetric flask. These solutions were sonicated for 1 hour to ensure complete solution of the drug. Standard solutions were then prepared by diluting an exact volume of the stock solution with PBS to prepare standard solutions with concentration of between 4 $\mu g.cm^{-3}$ and 20 $\mu g.cm^{-3}$. The standard solutions were then sonicated for another 30 minutes. The UV-absorbencies of the standard solution

were analysed with a spectrophotometer (Unicam, Cambridge, UK) and measured against a blank of pure PBS pH 7.4 solution at a wave length of 258 nm.

The standard curve was constructed by plotting the absorbencies against the concentrations on a straight line using linear regression (see figure 2.3). The concentrations obeyed Beer's law and only standard curves with a correlating coefficient (r^2) $\geq$ 0.999 were used. To analyze the data, one locates the measurement of the unknown substance and follows a line to intersect the standard curve. The corresponding value on the X-axis is the concentration of substance in the unknown sample.

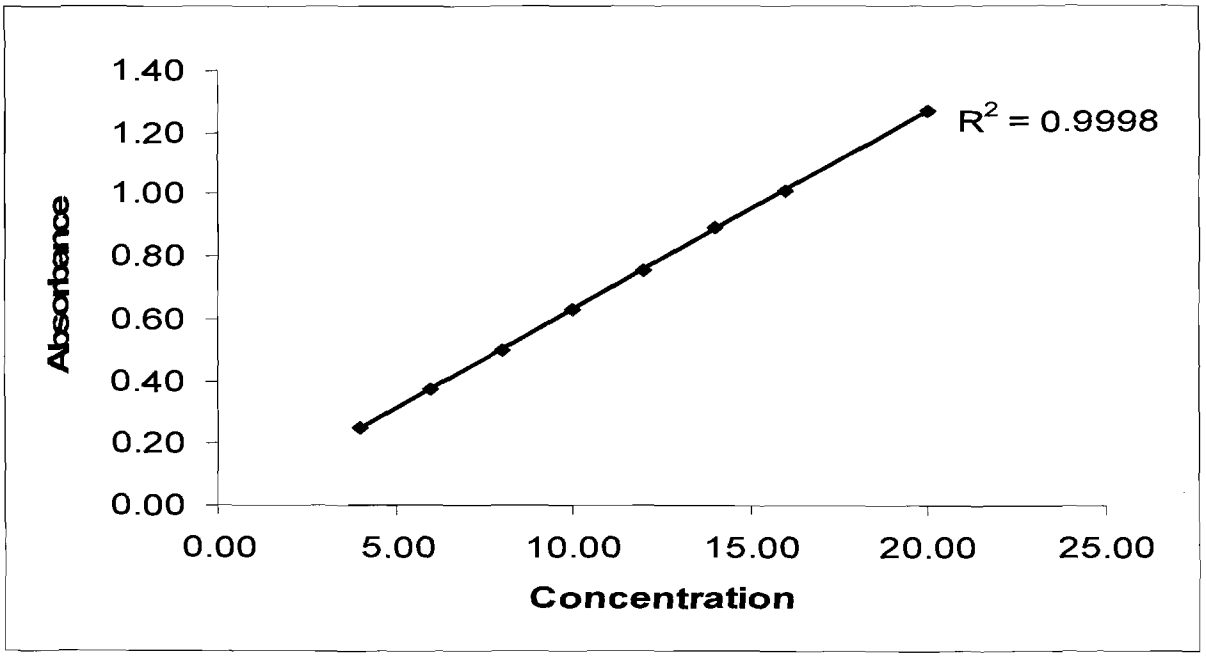


Figure 2.3: Example of a standard curve.

2.7.5 Friability

Friability tests were done on the beads to test their mechanical strength and establish the effect of the cross linking times on the hardness of the beads. Friability is determined as the percentage weight loss of the beads after subjecting the samples in a plastic chamber of a friabilator for a determined amount of time at a certain number of revolutions per minute (rpm) (Alsarra *et al.*, 2002:3639).

Samples of beads were dusted using compressed air and accurately weight on an analytical balance (Precisa 240A, Precisa balances, Zurich, Switzerland). The beads were then placed in a plastic chamber of a friabilator (Roche®) for 4 minutes at 25 revolutions per minute, thus a total number of 100 revolutions. The samples were dusted and weighed again and the percentage of friability was determined using equation 2.4.

$$\%\,Percentage friability = [(W_0 - W_t)/W_0] \times 100 \qquad \text{(equation 2.4)}$$

Where:

W_0 is the weight quantity of beads before placed in the friabilator and W_t is the weight quantity of beads after 100 revolutions in the friabilator.

The tests were done in triplicate and the average value was taken as the percentage friability.

2.7.6 Swelling and degradation

A swelling test was done on the beads according to Mi *et al.* (2003:6523). A known weight (100 mg) of beads was placed in 10 ml of the media (PBS pH 5.6 and pH 7.4) and collected at time intervals of 10, 60 and 360 minutes. The swollen beads were collected and the wet beads were first blotted down on filter paper to remove the excess water on the surface of the beads then weighed immediately on an analytical balance (Precisa 240A, Precisa balances, Zurich, Switzerland).

The swelling ratio of the cross linked chitosan beads in the media were then calculated with the following formula (equation 2.5).

$$E_{sw} = [(W_e - W_0)/W_0] \times 100 \qquad \text{(equation 2.5)}$$

Where:

E_{sw} is the swelling ratio of the co-cross linked chitosan beads at equilibrium. W_e is the weight of the beads at equilibrium of swelling after a certain time interval. W_o is the initial weight of the sample beads before swelling.

The swelling tests were done in triplicate and the average value was taken as the percentage of swelling.

Gupta and Ravi Kumar (2001:641) described the swelling value as the degree of swelling of the beads in their studies on semi-interpenetrating polymer network beads of chitosan-polyetheyleneglycol for the controlled release of drugs, the degree of swelling was calculated using the following equation (equation 2.6):

$$Degree\,of\,Swelling\,(E_{sw}) = (W_t - W_0)/W_0 \qquad \text{(equation 2.6)}$$

Where:

W_t is the optimum weight of the beads at time t, and W_0 is the initial weight of the beads.

2.7.7 Calculations

All calculations during this study were calculated by means of Microsoft Excel® 2003 for Windows (Microsoft® Corporation, Seattle, Washington, USA).

2.8 Conclusion

Ketoprofen was incorporated in chitosan beads and granules to achieve controlled release of the drug and minimizing side effects such as gastrointestinal discomfort. Chitosan is widely researched and has the ability to form a matrix it is thus an ideal formulation to achieve controlled release. With the addition of an enteric polymer such as Kollidon® SR the controlled release could even be more effective.

The method of preparation for the beads and granules in this study were altered to achieve optimum drug loading and effective drug release. Beads were made according to the method used by Bodmeier *et al.* (1998:1478) but were altered for the purpose of this study to achieve optimum incorporation of ketoprofen in the chitosan matrix as established Verwey (2005:34). Granules were made to compare the release characteristics of the two formulations and to

achieve effective controlled release. The method of preparation was altered and each sample was compared to each other to achieve an effective controlled release formulation.

Characterization of the chemical and physical properties of the beads and granules is an important step in the development of a dosage form. The samples can be characterized by means of swelling and degradation, morphology, friability, drug dissolution profile and drug loading capacity studies.

CHAPTER 3

3 BEADS AND GRANULES: CHARACTERIZATION, RESULTS AND DISCUSION

3.1 Introduction

Chitosan beads and granules have been widely studied and successfully used as a controlled drug delivery formulation (Shu & Zhu, 2000:52). The methods of preparation and advantages of these formulations were discussed in chapters 1 and 2. This chapter will focus on the characterization tests that were conducted on these formulations to evaluate their physical and chemical properties and the effect that a pharmaceutical excipient namely Kollidon® SR had on their physical and chemical properties.

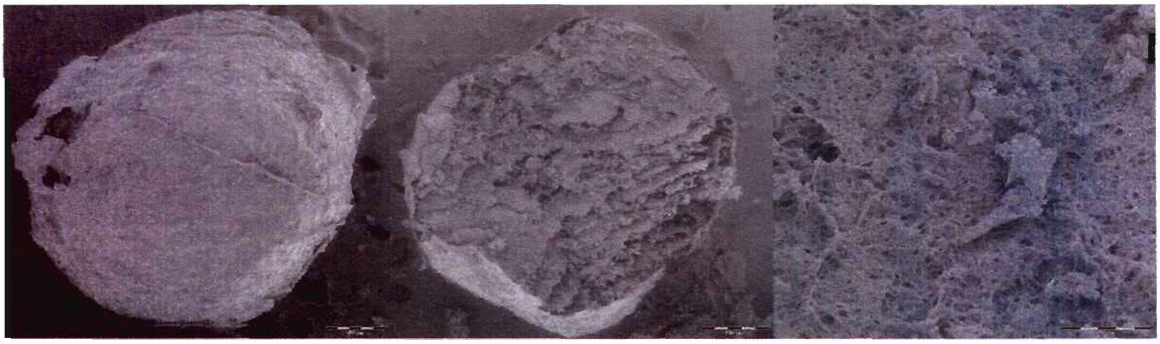
Characterization tests are an extremely important step in the development of a dosage form and are necessary to optimize the physical and chemical properties of a dosage form. The effect of various concentrations of Kollidon® SR on the beads and granules were evaluated. The results and discussion of these tests are presented in this chapter.

3.2 Morphology

3.2.1 Results

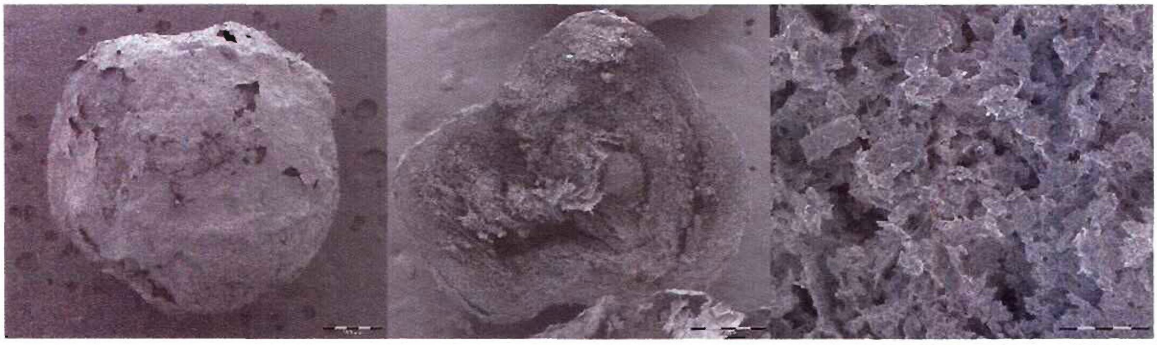
The morphology of the beads and granules was investigated using a scanning electron microscope as described in section 2.7.1. Images were captured and are presented in figures 3.1 to figure 3.21.

Morphology of beads



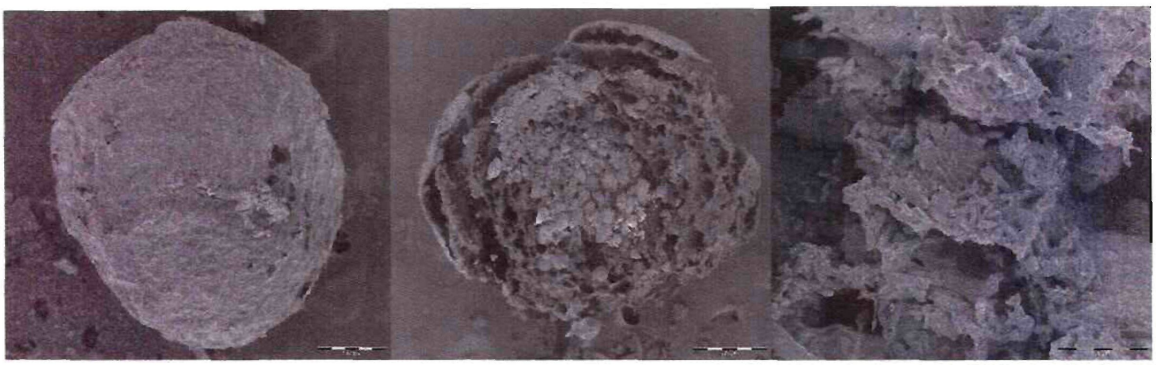
a b c

Figure 3.1: A SEM image of a ketoprofen loaded chitosan bead, cross linked for 60 minutes. **(a)** Full view of bead, **(b)** Cross-sectional view of bead, **(c)** Magnified view of the matrix of the bead.



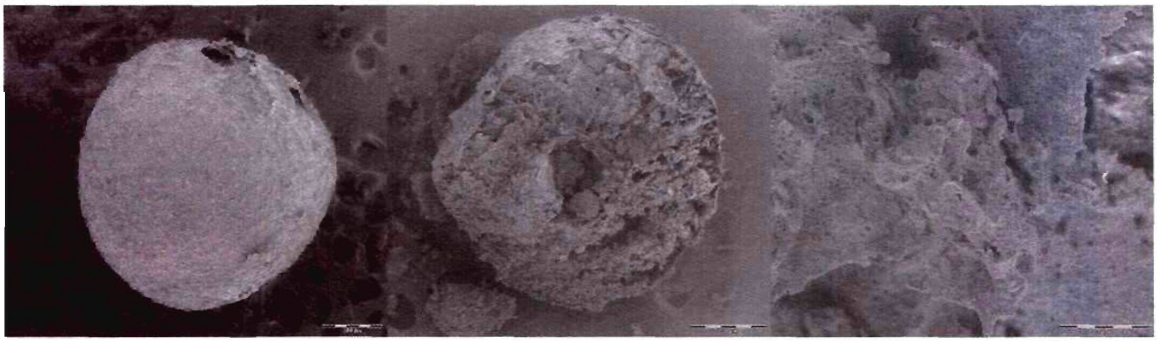
a b c

Figure 3.2: A SEM image of a ketoprofen loaded chitosan bead, cross linked for 30 minutes. **(a)** Full view of bead, **(b)** Cross-sectional view of bead, **(c)** Magnified view of the matrix of the bead.



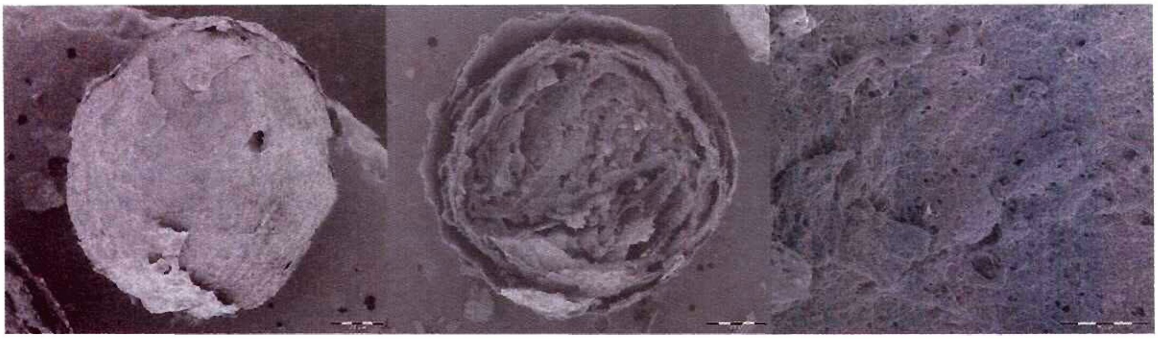
a b c

Figure 3.3: A SEM image of a ketoprofen loaded Kollidon/chitosan 0.25% (w/v) bead, cross linked for 60 minutes. **(a)** Full view of bead, **(b)** Cross-sectional view of bead, **(c)** Magnified view of the matrix of the bead.



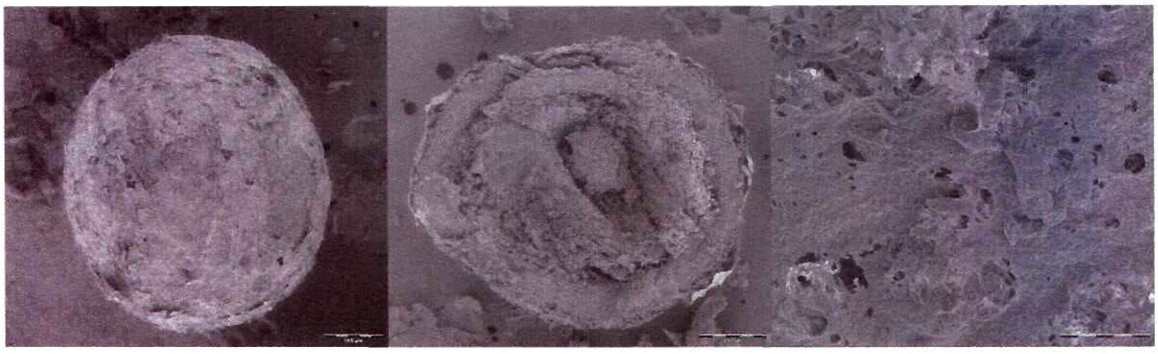
a b c

Figure 3.4: A SEM image of a ketoprofen loaded Kollidon/chitosan 0.25% (w/v) bead, cross linked for 30 minutes. **(a)** Full view of bead, **(b)** Cross-sectional view of bead, **(c)** Magnified view of the matrix of the bead.



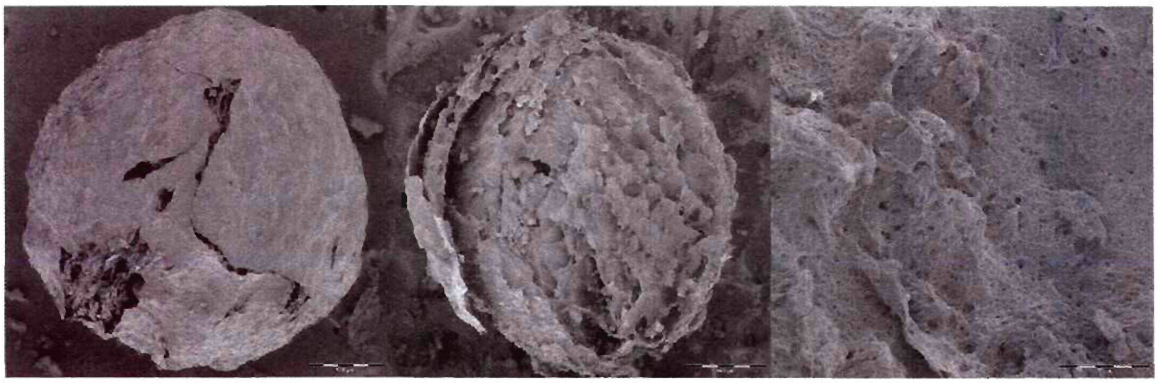
a b c

Figure 3.5: A SEM image of a ketoprofen loaded Kollidon/chitosan 0.5% w/v bead, cross linked for 60 minutes. **(a)** Full view of bead, **(b)** Cross-sectional view of bead, **(c)** Magnified view of the matrix of the bead.



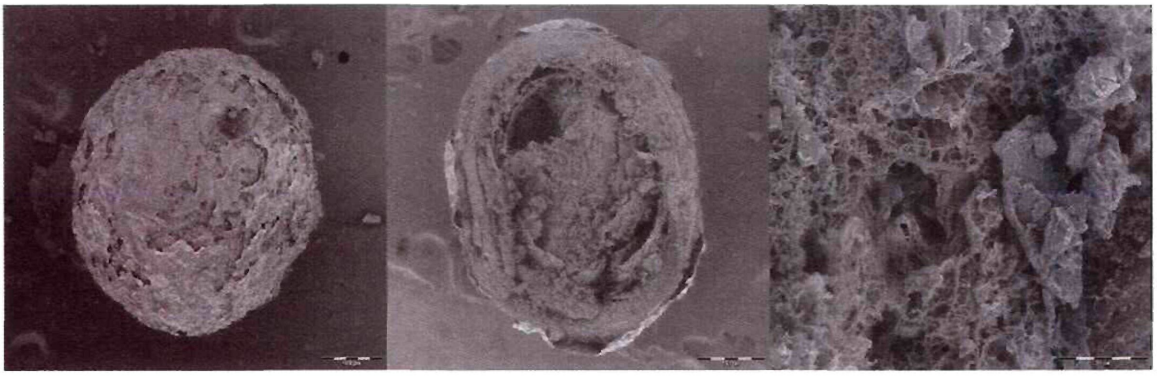
a b c

Figure 3.6: A SEM image of a ketoprofen loaded Kollidon/chitosan 0.5% (w/v) bead, cross linked for 30 minutes. **(a)** Full view of bead, **(b)** Cross-sectional view of bead, **(c)** Magnified view of the matrix of the bead.



a b c

Figure 3.7: A SEM image of a ketoprofen loaded Kollidon/chitosan 1% (w/v) bead, cross linked for 60 minutes. **(a)** Full view of bead, **(b)** Cross-sectional view of bead, **(c)** Magnified view of the matrix of the bead.



a b c

Figure 3.8: A SEM image of a ketoprofen loaded Kollidon/chitosan 1% (w/v) bead, cross linked for 30 minutes. **(a)** Full view of bead, **(b)** Cross-sectional view of bead, **(c)** Magnified view of the matrix of the bead.

Morphology of granules

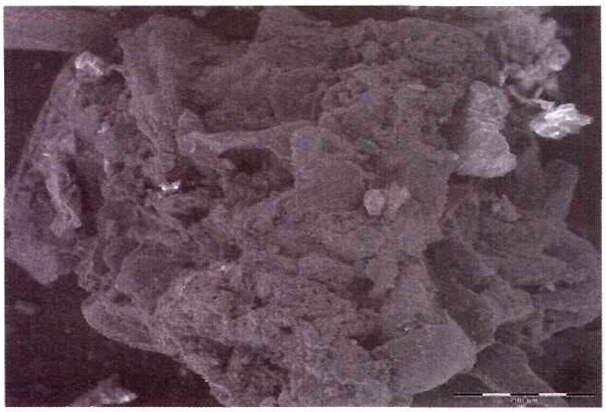


Figure 3.9: Full view of a chitosan granule loaded with ketoprofen.

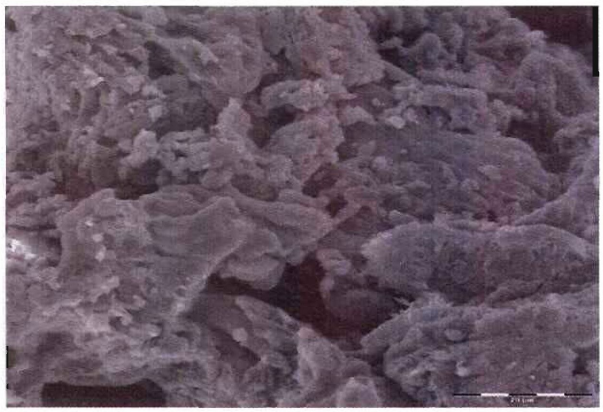


Figure 3.10: Magnified view of a chitosan granule loaded with ketoprofen.

Figure 3.11: Full view of a 1% (w/w) Kollidon® SR chitosan granule loaded with ketoprofen.

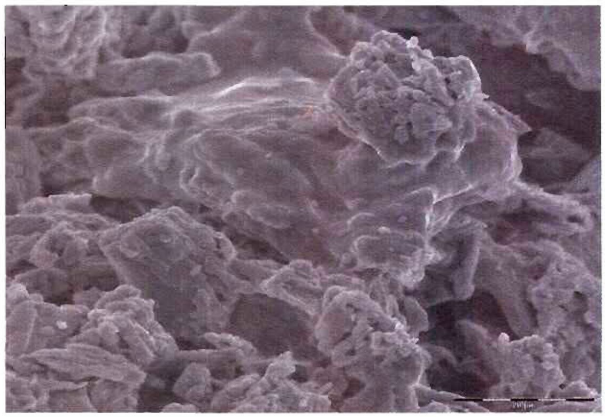


Figure 3.12: Magnified view of a 1% (w/w) Kollidon® SR chitosan granule loaded with ketoprofen.

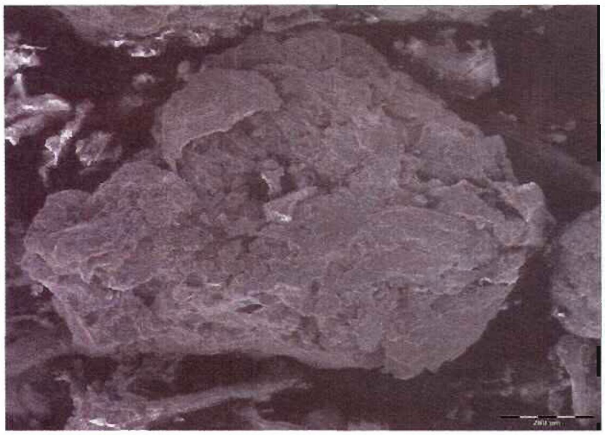


Figure 3.13: Full view of a 5 % (w/w) Kollidon® SR chitosan granule loaded with ketoprofen.

Figure 3.14: Magnified view of a 5 % (w/w) Kollidon® SR chitosan granule loaded with ketoprofen.

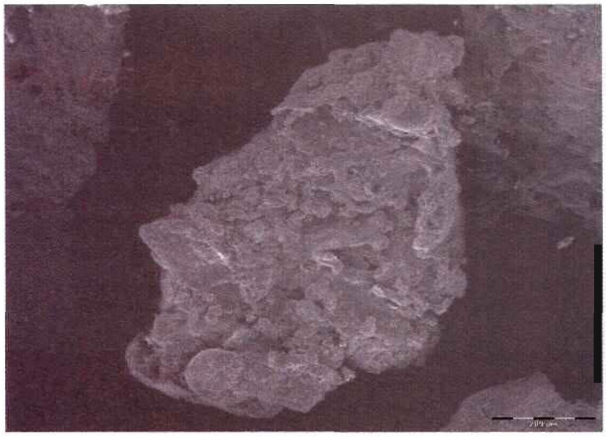


Figure 3.15: Full view of a 10% (w/w) Kollidon® SR chitosan granule loaded with ketoprofen.

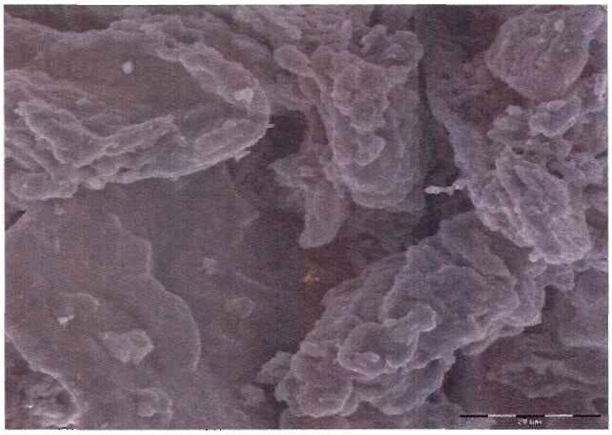


Figure 3.16: Magnified view of a 10% (w/w) Kollidon® SR chitosan granule loaded with ketoprofen.

Figure 3.17: Magnified view of a 10% (w/w) Kollidon® SR chitosan granule. The granule was freeze dried for a period of 24 hours.

Figure 3.18: Magnified view of a 10% (w/w) Kollidon® SR chitosan granule (Kollidon® SR was dissolved in 2-Pyrrolidinone® prior to granulation).

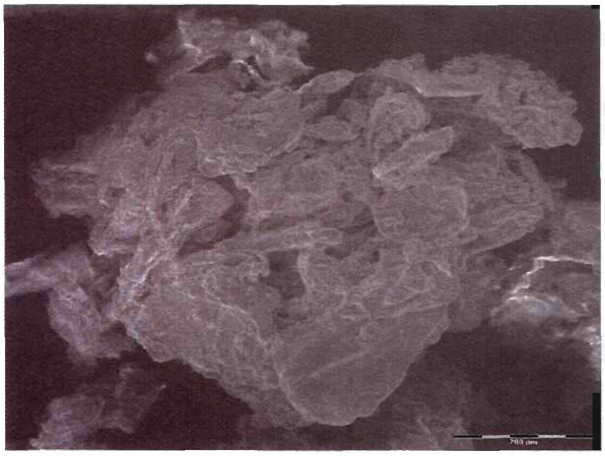


Figure 3.19: Full view of a 10% (w/w) Kollidon® SR chitosan granule (Kollidon® SR was dissolved in 2-Pyrrolidinone® prior to granulation).

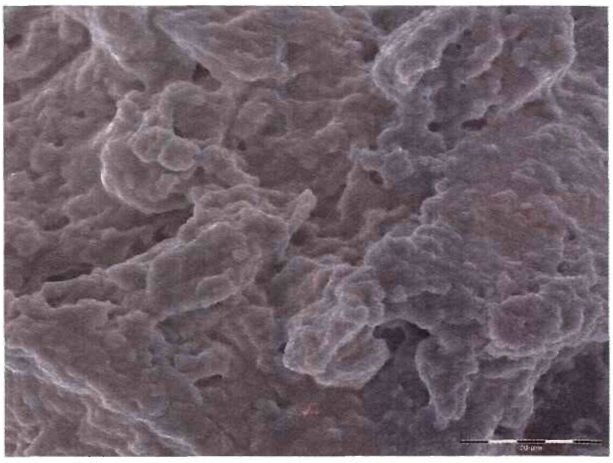


Figure 3.20: Magnified view a 5% (w/w) Kollidon® SR chitosan granule (Kollidon® SR was dissolved in 2-Pyrrolidinone® and ketoprofen dissolved in ethanol prior to granulation).

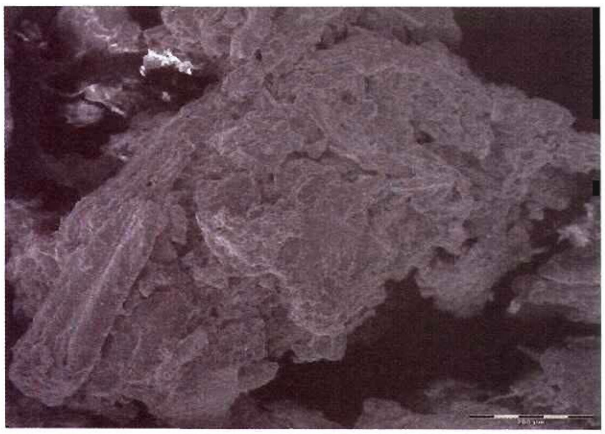


Figure 3.21: Full view of a 5% (w/w) Kollidon® SR granule (Kollidon® SR was dissolved in 2-Pyrrolidinone® and ketoprofen dissolved in ethanol prior to granulation).

3.2.2 Discussion

Beads

A view of the whole bead as well as a cross-sectional view and a view of the chitosan matrix of each formulation were used to investigate the structure of the bead. Beads were spherical in shape although beads that were cross linked for 60 minutes were a bit more irregular in shape, the diameter was between 1 and 2 mm. The circular cracks visible in figure 3.5 and 3.7 (a) are likely to be caused by the method used for preparing the beads for SEM photos. The beads were handled using a tweezer during the method and the circular cracks were likely to be caused by the tweezer. The surface of all the bead formulations appeared flaky (as seen in figure 3.6 and 3.8) and it seems that beads that were cross linked for a longer period had a smoother surface (figure 3.3, 3.5 and 3.7) indicating the possibility of better cross linking. The cross linking times had a obvious effect on the porosity of the beads, and beads that were cross linked for a longer period of time were less porous indicating better matrix forming. Although Kollidon® SR was only increased in small concentrations it is evident that it had an effect on the structure of the bead. Ketoprofen is crystalline in nature, and the crystalline pieces of the ketoprofen is still visible in figure 3.3 and 3.4 (c) but with an increase in the Kollidon® SR concentration the crystals were covered with the polymer and not visible

anymore. The Kollidon® SR were visible in the internal structure of the beads and looked spread throughout the entire matrix especially in the 1% w/v Kollidon® SR beads. This indicates that these formulations might be well suited for controlled release of ketoprofen.

Granules

Granules were prepared according to different methods as described in chapter 2, section 2.6.2. It is clearly visible that the different methods used, had an effect on the structure of the granules. The granules were investigated as a whole, and close up photos of the surface were taken to get a better indication of the structure.

In figure 3.10 the loose ketoprofen crystals on chitosan particles is clearly visible. The acetic acid which was used as granulating fluid was added in small volumes. It was postulated that if the chitosan dissolved in the acetic acid it could serve as a binder and could cover the ketoprofen and delay the release of the ketoprofen. The granules were uniform in size due to the fact that the granules were sieved through the same fine sieve but the shape of the granules was still irregular.

In figures 3.12, 3.14, 3.16 and 3.18 – 3.21 the effect of Kollidon® SR can be seen. With higher concentrations of Kollidon® SR, the ketoprofen crystals are less visible, the reason therefore is that the Kollidon® SR surrounds the ketoprofen and the crystals have a smoother surface due to the Kollidon® SR. In figure 3.12 depicting a 1% w/w Kollidon® SR granule, it can be seen that only a few crystals were encapsulated with the polymer but a large amount of ketoprofen was still attached to the chitosan without any polymer surrounding the crystals. In figure 3.16 depicting the effect of 10% w/w Kollidon® SR, the surface of the granules appears smoother, indicating that the polymer was thoroughly spread all over the granules and most of the ketoprofen were encapsulated with the polymer.

Figure 3.17 shows the effect of the freeze drying method. The granule is filled with holes and much more porous due to the fact that all the water and air were removed from the granule with the method of drying. It is possible that this drying method can have an effect on the release behaviour of the granule; because it increases the porosity of the granule. It is postulated that the higher porosity of the freeze dried granule might cause a faster drug release compared to oven dried granules.

Figures 3.19 and 3.21 show what a difference the method of preparation can have on the physical characteristics of the granule. There are no crystals visible in this figures and the granule surface has an uneven bumpy appearance. The Kollidon® SR is thoroughly spread all over the granule and the surface had a smoother appearance compared to granules where the Kollidon® SR was not dissolved in 2-pyrrolidinone prior to granulation.

3.3 Drug loading

3.3.1 Beads

3.3.1.1 Results

Drug loading of the beads was determined according to the method described in chapter 2, section 2.7.3. A standard curve was constructed before every drug loading experiment and all the experiments were run in triplicate. The percentage drug loading values of the different ketoprofen loaded bead formulations are shown in table 3.1. The theoretical and experimental amounts of ketoprofen in each bead formulation were calculated and the drug loading capacity was compared at cross linking times of 30 and 60 minutes. A graphical presentation of the drug loading capacities is depicted in figure 3.22.

Table 3.1: Drug loading capacity of bead formulations used in this study.

Bead formulation	Theoretical amount of ketoprofen in 100 mg beads (mg)		% Drug loading ± SD*		Experimental amount of ketoprofen in 100 mg beads (mg) ± SD*	
	30 min Cross linked	60 min Cross linked	30 min Cross linked	60 min Cross linked	30 min Cross linked	60 min Cross linked
Pure Chitosan/Ketoprofen (CB)	50	50	74.65 ± 0.71	74.65 ± 0.99	37.33 ± 0.39	37.33 ± 0.81
Chitosan/Ketoprofen/1% Kollidon® SR (K1B)	42.85	42.85	77.38 ± 0.01	76.34 ± 1.47	33.16 ± 0.01	32.71 ± 0.63
Chitosan/Ketoprofen/0.5% Kollidon® SR (K0.5B)	46.15	46.15	76.74 ± 0.25	63.97 ± 0.56	35.42 ± 0.11	29.52 ± 0.91
Chitosan/Ketoprofen/0.25% Kollidon® SR (K0.25B)	48	48	72.69 ± 0.35	66.05 ± 2.13	34.89 ± 0.17	31.70 ± 1.03

*SD = Standard deviation

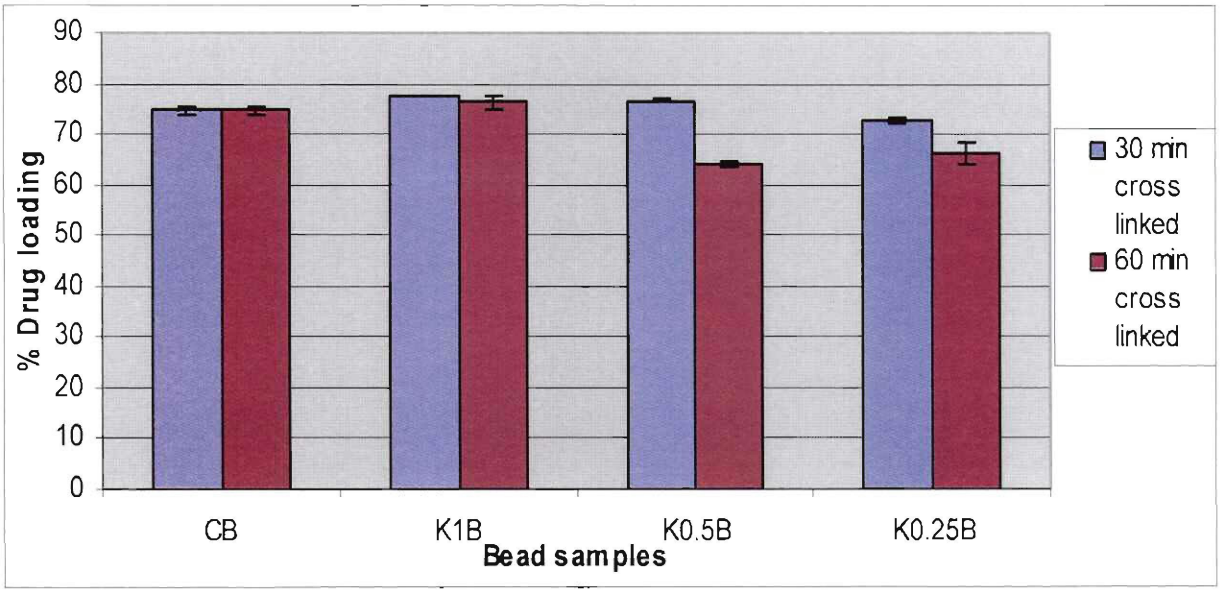


Figure 3.22: Graphic presentation of drug loading capacity of bead formulations and the effect of cross linking time on the drug loading.

3.3.1.2 Discussion

Beads were prepared at a 3% (w/v) chitosan, 3% (w/v) ketoprofen ratio and different concentrations of Kollidon® SR were added to investigate the effect of the polymer on the physical and chemical properties of the beads.

The cross linking time had a definite effect on the drug loading of the beads, and although the TPP solution in which the beads were cross linked, were saturated with 75 mg of ketoprofen, there was still some of the drug that leached out of the beads when the beads were cross linked for a longer period. This is evident in the results of the K0.5B formulation that was cross linked for 30 minutes and had an average drug loading of 76.75 ± 0.25% and the exact same formulation that was cross linked for 60 minutes had an average drug loading of 63.98 ± 0.56%. This difference was statistically significant (Tukey test, $p < 0.05$).

An increase in Kollidon® SR concentration tended to increase the drug loading of the beads. This effect was especially evident at the 1% (w/w) Kollidon® SR concentration for both cross linking times.

Bead formulations cross linked for 60 minutes had a lower drug loading compared to bead formulations cross linked for 30 minutes for both the 0.25 and 0.5% (w/w) Kollidon® SR concentrations. This might indicate that the Kollidon® SR contributed to the matrix forming of the beads which prevented the leaching of ketoprofen from the bead formulations containing 1% (w/w) Kollidon® SR.

The concentration of Kollidon® SR which was added had an effect on the drug loading of the beads. Optimum concentration was achieved with the K1B formulation where 1% (w/v) Kollidon® SR was incorporated and a cross linking time of 30 minutes was employed (77.38 ± 0.01%).

Verwey (2005:56) recognized that drug loading efficiency may be due to the matrix forming properties of polymers added. The polymers form a matrix during the production of the beads, and this matrix may decrease the drug release from the internal chitosan-polymer-drug dispersion into the external TPP-phase. This result is confirmed with the K1B-formulation.

3.3.2 Granules

3.3.2.1 Results

Drug loading of the granules were determined according to the method described in chapter 2, section 2.7.3. Every sample of granules had a unique method of preparation and it was therefore important to investigate their drug loading capacity. A standard curve was constructed before every drug loading experiment and all the experiments were run in triplicate. The results of the drug loading capacity are given in table 3.2 and a graphical presentation of the results is presented in figure 3.23.

Table 3.2: Drug loading capacity of granule formulations used in this study.

Granule formulation	Theoretical amount of ketoprofen in 100 mg granules (mg)	%Drug loading ± SD*	Experimental amount of ketoprofen in 100 mg beads (mg) ± SD*
Pure chitosan/ketoprofen granule (CG)	50	93.30 ± 0.50	46.65 ± 0.24
Chitosan/ketoprofen/1% Kollidon® SR (K1G)	49.5	89.85 ± 0.54	44.48 ± 0.28
Chitosan/ketoprofen/5% Kollidon® SR (K5G)	47.5	90.05 ± 1.84	42.77 ± 1.68
Chitosan/ketoprofen/10% Kollidon® SR (K10G)	45	91.94 ± 1.20	44.37 ± 0.55
Chitosan/ketoprofen/10% Kollidon® SR freeze dried (K10Gfd)	45	91.67 ± 0.49	41.25 ± 0.22
Chitosan/ketoprofen/10% Kollidon® SR dissolved in 2-Pyrrolidinone (10KPG)	45	81.73 ± 1.53	36.78 ± 0.68
Chitosan/ketoprofen-ethanol/5% Kollidon®-SR-2-Pyrrolidinone (5KPG)	47.5	86.52 ± 6.60	41.10 ± 3.13

*SD = Standard deviation

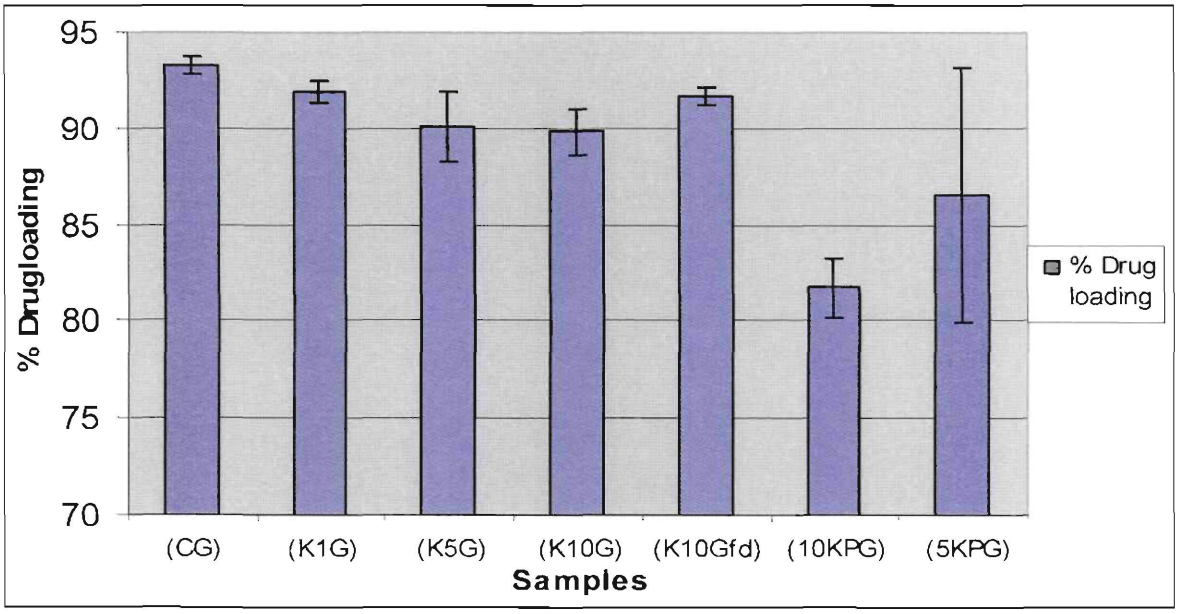


Figure 3.23: Graphic presentation of drug loading capacity of granule formulations used in this study.

3.3.2.2 Discussion

As mentioned previously granules were prepared according to the method described in chapter 2, section 2.6.2. Kollidon® SR was dissolved in 2-Pyrrolidinone® since this is the only solvent which dissolves Kollidon® SR. In all the samples ketoprofen and chitosan were added in the same quantity to ensure that the granules can be compared to each other. Kollidon® SR was added in different concentrations to investigate the effect of the polymer on the physical and chemical properties of the granules.

The drug loading of the granules was expected to be high since the method of preparation is less complex than the method used to prepare the beads. Formulations with a higher concentration Kollidon® SR had a drug loading that was slightly lower than the other formulations but still had an average drug loading above 90%. This difference in drug loading was not statistically significant (Kruskal-Wallis, $p > 0.05$).

Granules that were prepared with Kollidon® SR dissolved in 2-pyrrolidinone had a lower drug loading than the other formulations (Kruskal-Wallis, $p < 0.05$). This could be due to the fact that the granules were oven dried for seven days (time needed to dry granules sufficiently).

The extended period of time in the oven may have caused some drug degradation, evident by the lower drug loading. The 10KPG granule had a drug loading of 81.75 ± 1.53%. The 5KPG formulation where 5% w/w Kollidon® SR was dissolved in 2-pyrrolidinone, and the ketoprofen was dissolved in ethanol where after the solutions were mixed together (and used as the granulating solution), had a drug loading of 86.52 ± 6.61%.

It can thus be said that the method of preparation had an effect on the drug loading capacity of the granules, and external factors such as drying time can cause lower drug loading. In general the granules had a high drug loading and the addition of a polymer had little effect on the drug loading capacity of the granules.

3.4 Friability

3.4.1 Results

Friability tests were conducted to test the mechanical strength of the beads. The tests were conducted according to the method described in chapter 2, section 2.7.5 and the friability values determined using equation 2.4. Friability results were compared at cross linking times of 30 and 60 minutes to investigate the effect of cross linking time on the mechanical strength of the beads. The friability results are presented in figure 3.24.

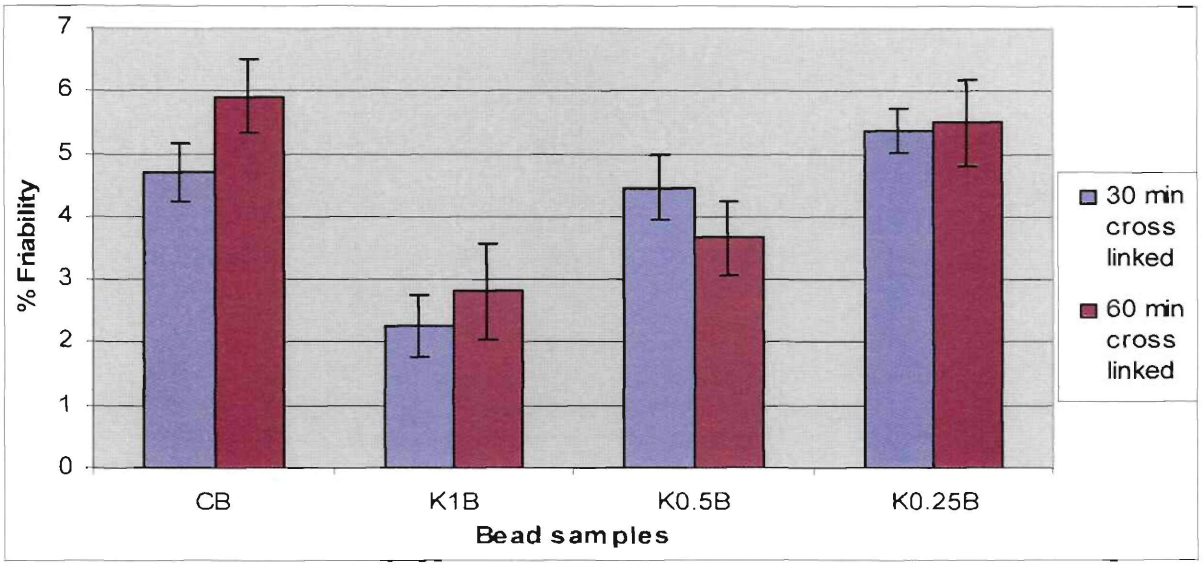


Figure 3.24: Percentage friability of bead samples.

3.4.2 Discussion

Percentage friability is a clear indication of the mechanical strength of the bead formulations and will give an indication on how much of the formulation will be lost during packaging and transport (Shu & Zu, 2000:55). There are no set values for the percentage friability of bead formulations since no requirements have been set by pharmacopoeias.

Plain chitosan beads that were cross linked for 60 minutes tended to be slightly more brittle and had a higher percentage friability than the chitosan beads that were cross linked for 30 minutes. The inclusion of Kollidon® SR in the bead structure had a varying effect on the percentage friability depending on the concentration and the time cross linked. K1B and K0.25B had a higher friability when the formulation was cross linked for 60 minutes than it had when it was cross linked for 30 minutes, but K0.5B had a slightly lower percentage friability when the formulation were cross linked for 60 minutes.

In general it can be said that the addition of Kollidon® SR caused a noticeable decrease in the percentage friability (Tukey test, $p < 0.5$) and the lowest friability was achieved when 1% w/v Kollidon® SR was added to the formulation. This formulation had a friability percentage of $2.26 \pm 0.50\%$ when it was cross linked for 30 minutes, while the chitosan bead formulation without Kollidon® SR had a friability percentage of $4.70 \pm 0.47\%$ when it was cross linked for 30 minutes. The results indicate a positive influence on the matrix formation if Kollidon® SR is added to the bead formulations.

3.5 Swelling behaviour

3.5.1 Results

Swelling tests were conducted on the beads to determine the percentage of aqueous solution, absorbed by the bead formulations and the degree that the beads swelled at a certain time interval. Studies were conducted in a PBS solution at pH 7.4 and pH 5.6 as described in chapter 2, section 2.7.6. Granule samples have a very high solubility as established by Verwey (20005:63) and were not cross linked therefore swelling tests were only conducted on the bead samples. The influence of the cross linking time on the degree of swelling were also

studied and the results are presented in table 3.3. A graphical presentation of the swelling behaviour is depicted in figure 3.25 to figure 3.28.

Table 3.3: Degree of swelling (Esw) of bead samples in PBS 7.4 and PBS 5.6.

Bead Formulation	Degree of Swelling (Esw) ± SD*					
	10min		60min		360min	
	pH 5.6	pH 7.4	pH 5.6	pH 7.4	pH 5.6	pH 7.4
Chit/Ket - 30 min (CB)	2.04 ± 0.06	2.45 ± 0.20	2.08 ± 0.07	2.25 ± 0.14	2.25 ± 0.06	2.23 ± 0.17
Chit/Ket - 60 min (CB)	2.69 ± 0.09	2.04 ± 0.02	2.70 ± 0.07	1.87 ± 0.07	2.75 ± 0.05	2.03 ± 0.12
Chit/Kol 1% - 30 min (K1B)	1.91 ± 0.12	1.86 ± 0.14	2.01 ± 0.13	1.96 ± 0.14	2.39 ± 0.27	2.25 ± 0.07
Chit/Kol 1% - 60 min (K1B)	1.67 ± 0.10	1.66 ± 0.08	1.73 ± 0.08	1.59 ± 0.05	1.94 ± 0.08	1.87 ± 0.06
Chit/Kol 0.5% - 30 min (K0.5B)	2.45 ± 0.07	2.18 ± 0.06	2.55 ± 0.13	2.16 ± 0.05	2.69 ± 0.07	2.17 ± 0.11
Chit/Kol 0.5% - 60 min (K0.5B0)	1.71 ± 0.07	1.60 ± 0.10	1.76 ± 0.04	1.72 ± 0.15	2.07 ± 0.06	1.98 ± 0.07
Chit/Kol 0.25% - 30 min (K0.25B)	2.51 ± 0.06	2.21 ± 0.06	2.51 ± 0.05	2.18 ± 0.07	2.70 ± 0.07	2.29 ± 0.12
Chit/Kol 0.25% - 60 min (K0.25B)	1.57 ± 0.03	1.55 ± 0.07	1.61 ± 0.05	1.42 ± 0.07	1.83 ± 0.08	1.72 ± 0.13

*SD = Standard deviation

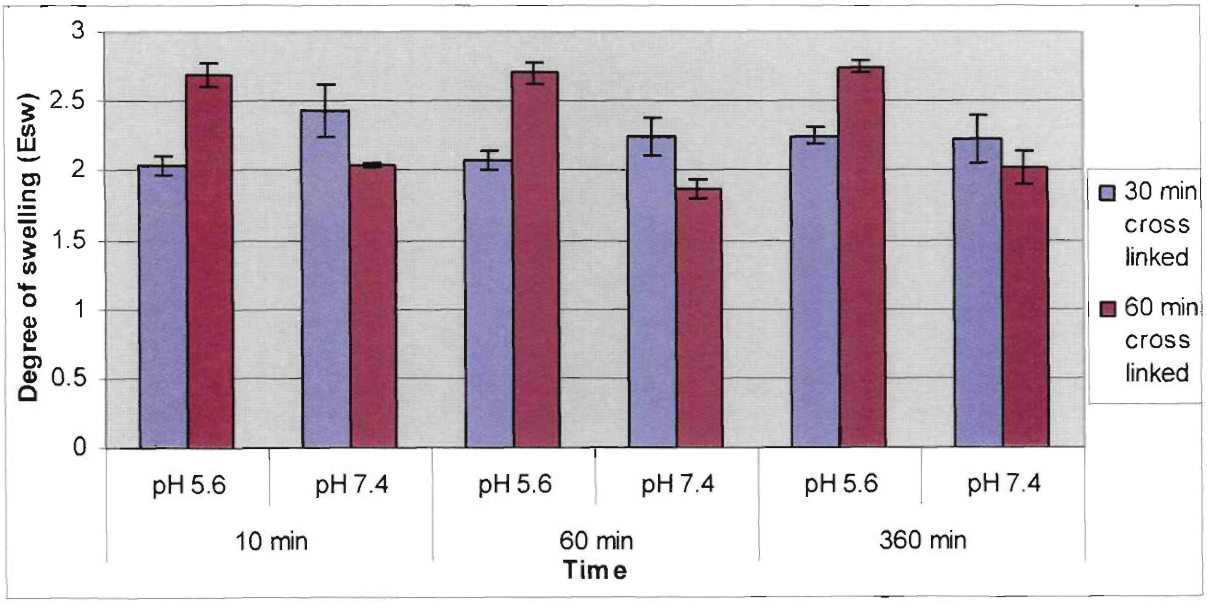


Figure 3.25: Degree of swelling for pure chitosan/ketoprofen bead formulation.

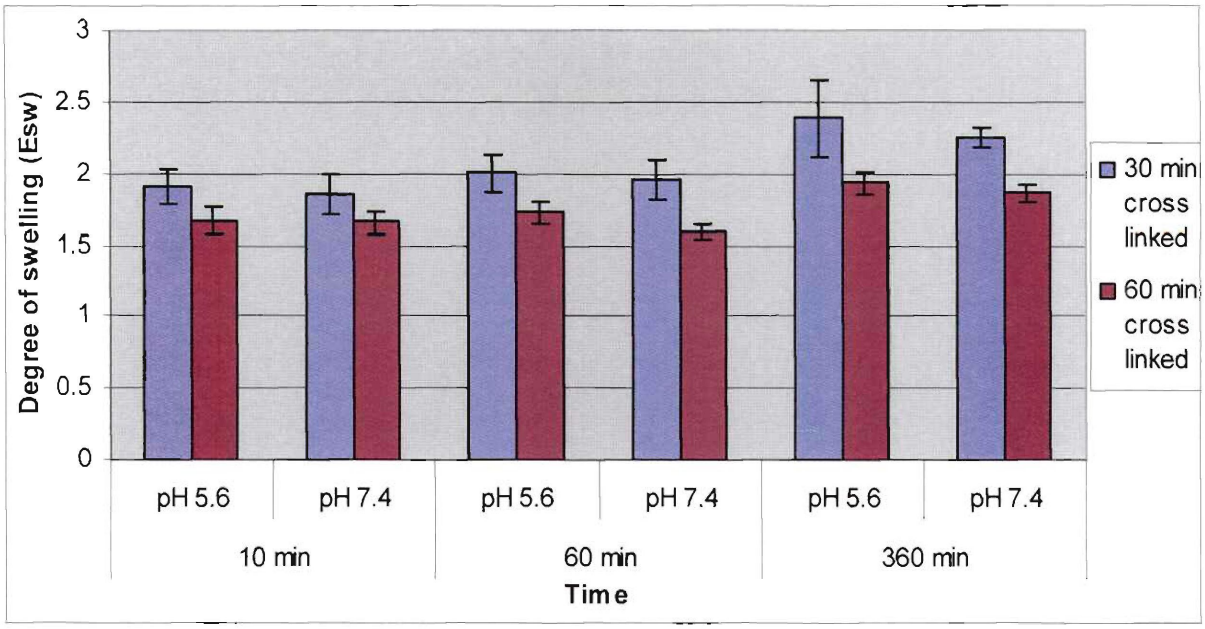


Figure 3.26: Degree of swelling for 1% (w/v) Kollidon® SR chitosan/ketoprofen bead formulation.

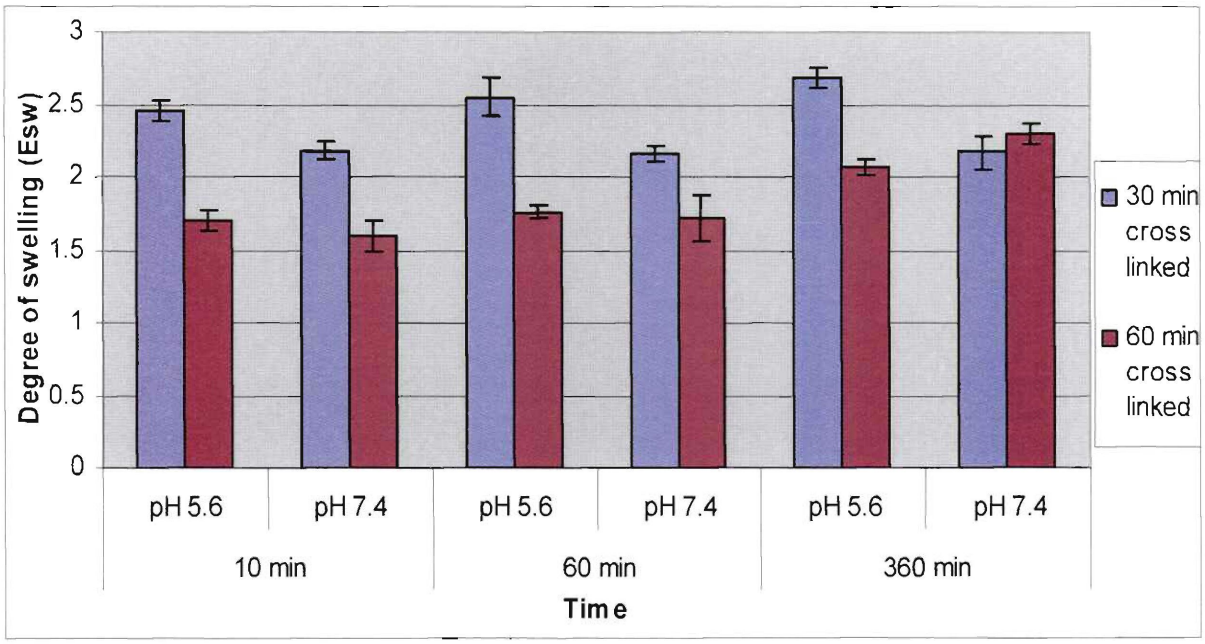


Figure 3.27: Degree of swelling for 0.5% (w/v) Kollidon® SR chitosan/ketoprofen bead formulation.

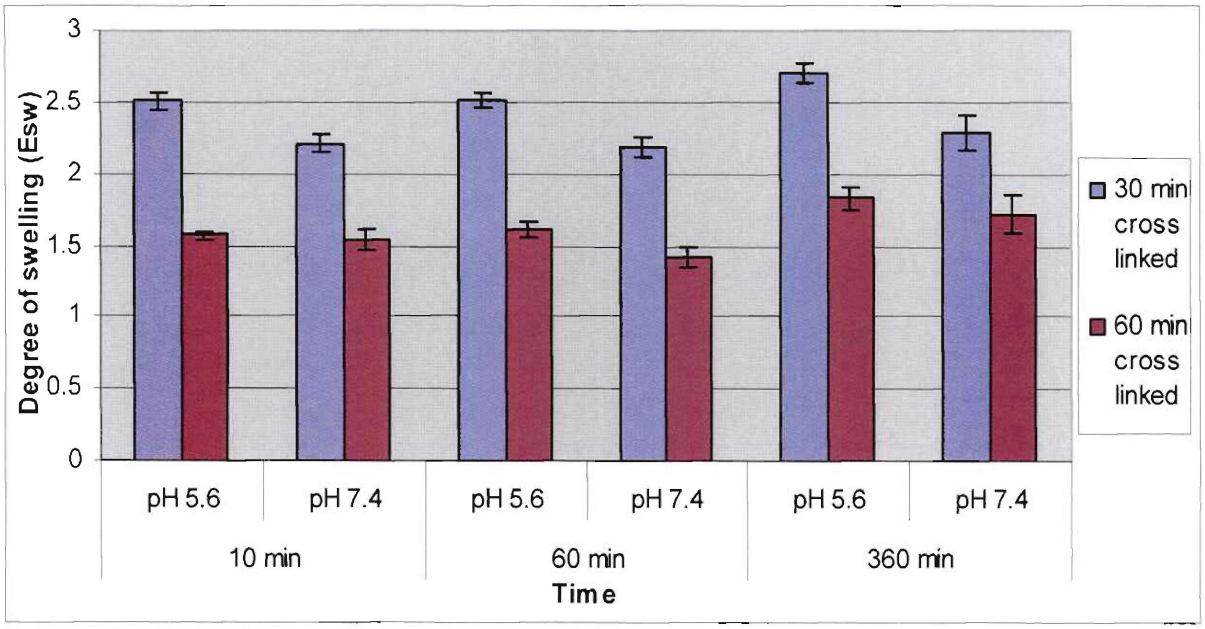


Figure 3.28: Degree of swelling for 0.25% (w/v) Kollidon® SR chitosan/ketoprofen bead formulation.

3.5.2 Discussion

Degree of swelling was determined since it is the method most commonly used in the literature to determine the amount of water absorbed by beads (Anal *et al.*, 2003:716, Mi *et al.*, 2003:6523). Mi *et al.* (2003:6526) determined that chitosan gel beads prepared in a more acidic TPP solution had a lower swelling ratio due to the less free NH_3^+ ions in the solution, this might be a factor since beads used in this study were prepared in a TPP solution at pH 5, to achieve optimum drug loading.

The pure chitosan ketoprofen bead samples (figure 3.25) exhibited good swelling during the first 10 minutes and swelling increased over the following time intervals, achieving a maximum swelling of 2.75 ± 0.05 after 360 minutes at a pH of 5.6. The beads were still intact and did not seem to loose any form after 360 minutes. The cross linking time did not play a significant role in the chitosan bead results and good cross linking occurred at both pH-values.

In figure 3.26 the Kollidon® SR 1% w/v bead formulation showed a decrease in the swelling capacity of the chitosan beads. The time of cross linking also played a part in the results and

beads that were cross linked for 60 minutes did not swell to the same degree than the samples that were cross linked for 30 minutes. This difference was statistically significant (Tukey test, $p < 0.05$). The pH of the swelling solution had a definite effect on the swelling capacity and the bead samples showed a increase in swelling over each time interval. The optimum degree of swelling for the K1B formulations were achieved with the formulation that was cross linked for 30 minutes in a pH of 5.6 for a period of 360 minutes (Esw = 2.54 ± 0.27).

The results for the Kollidon® SR 0.5% w/v and Kollidon® SR 0.25% w/v bead formulations are presented in figures 3.27 and 3.28. These results emphasize the effect of cross linking on the degree of swelling. All the samples that were cross linked for 30 minutes had a higher degree of swelling than the samples that were cross linked for 60 minutes and this was statistically significant (Tukey test, $p < 0.05$). Optimum swelling was achieved at a pH of 5.6 after a time interval of 360 minutes for both formulations.

The incorporation of Kollidon® SR into the bead formulations had an impact on the degree of swelling and reduced the impact of the swelling mediums on the beads. Throughout all the swelling tests the beads stayed intact and didn't start to dissolve. The reason for the lower swelling in the Kollidon® SR-containing formulations is not clear and need further investigation.

3.6 Conclusion

Bead and granule formulations were characterized and their physical and chemical properties were investigated. Morphology, drug loading capacity, friability and swelling tests were used to investigate the formulations and results showed that Kollidon® SR can be incorporated into chitosan bead and granule formulations. Kollidon® SR was used as polymer for this study because the release characteristics of this polymer is pH-independent.

Bead samples were prepared and the addition of low concentrations Kollidon® SR were investigated. Two cross linking times were used to investigate its effect on the bead formulations. The morphology showed that the Kollidon® SR was spread throughout the bead and incorporated into the matrix of the bead. The morphology of the granules showed that each method of preparation as described in chapter 2, produced a granule with a unique

surface appearance. Methods where the Kollidon® SR was dissolved in 2-Pyrrolidinone® had a smoother surface than the conventional method.

The drug loading of all the formulations was excellent and bead formulations consistently presented with results above 70% except for K0.25B and K0.5B due to the longer cross linking time. Cross linking time had a definite effect on the drug loading capacity of bead formulations and formulations that were cross linked for a longer period had a slightly lower drug loading, which can be an indication that there is still some drug that leached out of the bead matrix into the TPP solution during preparation. Granules had a less complex method of preparation and samples consistently presented with drug loading results above 89% except for 10KPG and 5KPG due to their extended drying period in the oven.

Friability tests showed that the beads were brittle but the addition of Kollidon® SR lowered the friability of the samples. Swelling tests increased over time and beads swelled to a positive degree. The pH of the swelling medium played a significant role in the test results and beads swelled to a higher degree in a pH of 5.6 than it did in a pH of 7.4.

Characterization tests showed promising results for bead and granule formulations and the method of preparation as well as the concentration of the polymer added made a significant difference on the characteristics of the beads and granules. The release behaviour of these formulations will be discussed in chapter 4.

CHAPTER 4

4 BEADS AND GRANULES: DRUG RELEASE

4.1 Introduction

Drug dissolution (or release) testing is an analytical technique used to assess release profiles of drugs from pharmaceutical products, generally solid oral products such as tablets and capsules. This test gains its significance from the fact that if a drug from a product is to produce its effect; it must be released from the product and should generally be dissolved in the fluids of the gastrointestinal (GI) tract. Thus, a drug dissolution test may be considered as an indicator of potential drug release and absorption characteristics of a product in humans as well as in animals (Qureshi, 2006:18).

In vitro dissolution has been recognized as an important parameter of drug products because of its association with drug bioavailability. Dissolution experiments can also be employed as a surrogate for assessment of bioequivalence under certain conditions. In view of the significance of dissolution, it is therefore essential to investigate the drug release characteristics of preparations (Peh & Wong, 2000:724).

Beads and granules as carriers have important potential applications for the administration of therapeutic molecules (Gupta & Ravi Kumar, 2000:1115). These particles have been widely used in controlled release formulations and offer advantages over larger, non-disintegrating delivery systems. Beads and granules can disperse throughout the GI tract; can be formulated to have multiple types of coatings to achieve a variety of release profiles and in most cases show dose proportionality (Crison, 2007).

Grassi *et al.* (2000:98) studied the drug release from a cross linked polymer matrix similar to beads, and found that the drug can be molecularly dispersed inside the polymeric network by means of solvent swelling or cogrinding techniques, so that an amorphous drug state is eventually attained. The polymer interacts with the drug and hinders the recombination of the drug molecules; this interaction causes controlled release (see in figure 4.1).

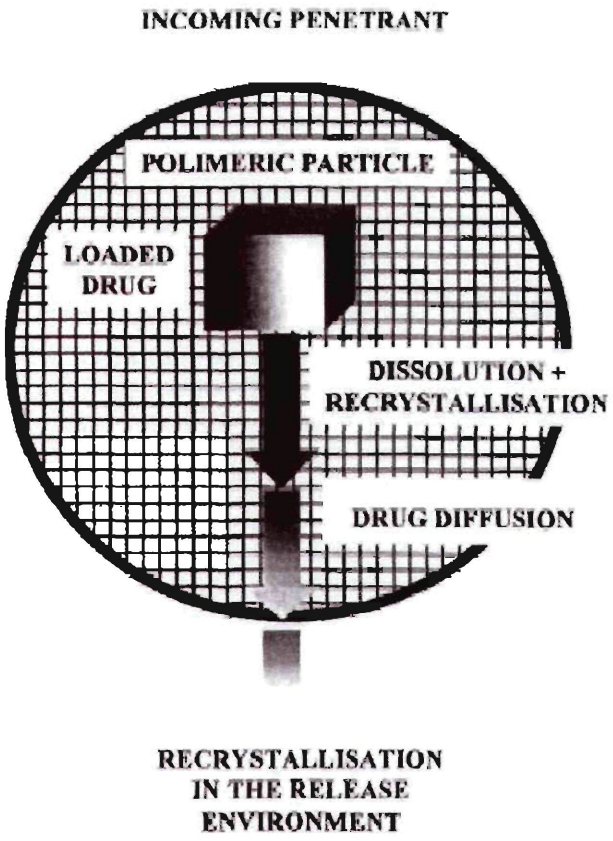


Figure 4.1: The dissolved drug molecules diffuse through the polymeric network to reach the release environment where new crystallization can take place (Grassi *et al.*, 2000:97).

The preparation and characterization of chitosan beads and granules were described in chapter 2 and 3. This chapter will focus on the ketoprofen release from the bead and granule formulations. The drug loading of both formulation types were determined in chapter 3 and these results were used in studying the release behaviour of ketoprofen from these formulations.

4.2 Burst effect

Burst effect is an initial increase in drug release from a large bolus of drug in the dosage form into the dissolution medium. The release rate reaches a stable profile after the initial release as depicted in figure 4.2 (Huang & Brazel, 2001:121).

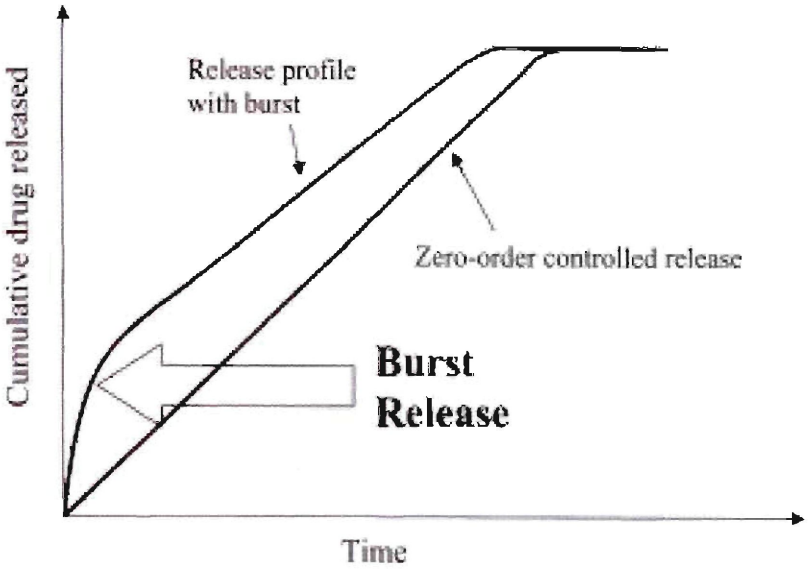


Figure 4.2: Graphic representation of the burst effect in a zero-order drug delivery system (Huang & Brazel, 2001:122).

During zero order release, the drug is released at a constant rate over an extended period of time and is an ideal release profile for controlled release. The burst effect can be unpredictable and cause pharmacologically negative effects but also has the ability to be the optimal mechanism of delivery in several instances. The burst effect is especially valuable in wound treatment, (burst release followed by a diminishing need for drug), encapsulated flavours, targeted delivery (triggered burst release) and pulsatile release. To achieve these effects it is important to study the burst effect and control the amount of burst effect (Huang & Brazel, 2001:122).

4.3 Dissolution profiles and parameters

The comparison of dissolution profiles has extensive applications throughout the product development process and can be used to develop *in vitro/in vivo* correlations, which can help to reduce costs, speed up product development and reduce the need to perform costly bioavailability/bioequivalence human volunteer studies. It can also establish final dissolution specifications for pharmaceutical dosage forms (O'Hara *et al.*, 1998:214).

There are many mathematical models describing drug dissolution in the literature, for example the zero order model, mean dissolution time, Higuchi model, similarity and dissimilarity factors and the Weibull model to mention a few (Costa & Sousa Lobo, 2001:123). For the purpose of this study a few of these models were used to compare the dissolution profiles of the bead and granule formulations and to determine if controlled release was achieved. These models will be discussed in sections 4.3.1 and 4.3.2.

4.3.1 Similarity factor

Moore and Flanner (1996:64) proposed a simple model-independent approach to compare the dissolution profile of a formulation against that of a reference formulation. These researchers used a parameter, the similarity factor (f_2) to compare dissolution profiles. The similarity factor appears to be simple and can be easily adopted by the industry. Application of the similarity factor has been exemplified and reported in the literature (Peh & Wong, 2000:724).

The higher the value of f_2 the more similar are the dissolution profiles. The FDA defines similarity of the dissolution profiles between two drug products as when f_2 has a value between 50 and 100. As such the dissolution profile of the test preparation is considered similar to that of the reference product if the f_2 value obtained is above 50 (Peh & Wong, 2000:724). The similarity factor can be calculated by the following equation:

$$f_2 = 50 \log\left[\left(1 + \frac{1}{n}\sum_{t=1}^{n} wt(Rt - Tt)^2\right)^{-0.5} \times 100\right] \qquad \text{(equation 4.1)}$$

Where R_t is the reference assay at time point t, T_t is the test assay at time point t, n is the number of pull points and w_t is an optional weight factor. The equation is a logarithmic transformation of the sum of squared error; it fits the average sums of the dissolution profile and reference profile and fit the result between 0 and 100.

4.3.2 Mean dissolution time

It is also possible to characterize dissolution profiles with statistical moments such as mean dissolution time (MDT), its relative dispersion (RD) and the variance associated with the

MDT (Costa *et al.*, 2003:206). Rigter & Peppas (1987:31) defined the MDT as the mean time for the drug to dissolve under *in vitro* dissolution conditions.

MDT can be calculated from the cumulative mass of drug dissolved using equation (4.2):

$$MDT = \frac{\sum_{i=1}^{n} t_{mid} \Delta X_d}{\sum_{i=1}^{n} \Delta X_d} \qquad \text{(equation 4.2)}$$

Where i is the sample number, n is the total number of sample times, t_{mid} is the time at the midpoint between sample times and ΔX_d is the additional mass of drug dissolved, between *i* and *i*-1 (Costa *et al.*, 2003:204).

4.3.3 Area under curve (AUC)

The total area under the curve in dissolution profiles gives the cumulative amount of drug released during the dissolution experiment. AUC is also used in bioavailability comparisons. The extent of absorption or relative extent of absorption of a drug from a product can be estimated by comparing the total area under the drug concentration in plasma versus time curve or the total amount of unchanged drug excreted in the urine after administration of a standard (Gibaldi, 1991:8)

There are several methods for estimating the area under a drug concentration-time curve. An estimate of area is required to determine bioavailability, clearance, apparent volume of distribution and other pharmacokinetic parameters. The most common method of estimating area is the use of the trapezoidal rule. The area bounded by the trapezoids approximates the area under the curve. The greater the number of data points, the closer is the approximation (Gibaldi, 1991:377). The area under a drug concentration vs time curve is approximated by the following equation:

$$Area = (\frac{1}{2})(C_{n-1} + C_n)(t_n - t_{n-1}) \qquad \text{(equation 4.3)}$$

Where C is the drug concentration, t is time at which the sample was taken, and n refers to the sample number (Gibaldi, 1991:377).

4.4 Ketoprofen release from chitosan granules and beads

Beads were prepared according to the method described in chapter 2, section 2.4.2 in a TPP solution (pH 5.0). A control formulation of the beads was made with the same method but no Kollidon® SR was added in this sample. The control formulation was compared with the other formulations to get a clear indication of the polymer's (Kollidon® SR) effect on the release profiles of the beads. Granules were prepared according to the method described in chapter 2, section 2.6.2. A control formulation was also made for the granules to study the effect of Kollidon® SR on the release profiles of the granules. Dissolution studies were conducted in a phosphate buffer solution pH 7.4 using the method described in chapter 2, section 2.7.3. A standard curve was conducted before every dissolution experiment.

4.5 Method

As mentioned in section 4.4 dissolution experiments were conducted according to the method described in chapter 2, section 2.7.3. Although the same quantity of each formulation was used the amount of ketoprofen in the formulations differed slightly due to the difference in drug loading and table 3.1 and 3.2 were used to calculate the amount of ketoprofen in each sample of beads and granules before the dissolution experiment. UV-absorbencies of the withdrawn samples were determined using a Unicam-spectrophotometer (Heliosα, Unicam Ltd, Cambridge, UK). The drug concentrations corresponding to the measured absorbance values were determined from a standard curve using linear regression.

4.6 Results and Discussion

4.6.1 Ketoprofen release from Chitosan/Kollidon® SR beads cross linked for 30 minutes

4.6.1.1 Results

The release of ketoprofen from chitosan bead formulations containing Kollidon® SR was determined as described in section 2.7.3 and the results are depicted in figure 4.3 and 4.4. The percentage ketoprofen that was released into the dissolution medium after 60 minutes of the dissolution experiment are presented in table 4.1. The mean dissolution times (MDT) for all of the bead formulations were calculated and the values are presented in table 4.2. The dissolution profile of bead samples cross linked for 30 minutes containing Kollidon® SR were compared with the dissolution profile of the plain chitosan/ketoprofen bead formulation cross linked for 30 minutes to get a better understanding of the effect of the polymer on the dissolution profile. The similarity factor values for this comparison are presented in table 4.3. Area under curve (AUC) values were calculated after 60 minutes and again after 360 minutes to compare the dissolution profiles of the bead formulations at these separate times and the results are presented in table 4.4 and 4.5.

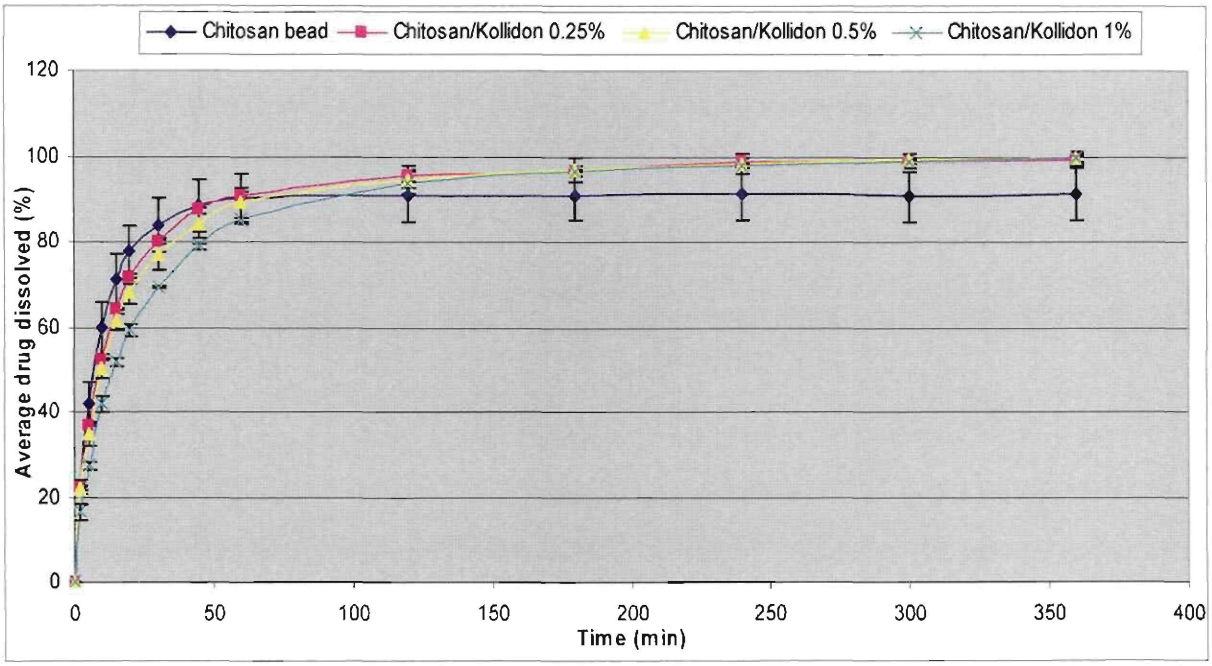


Figure 4.3: Ketoprofen release from bead formulations cross linked for 30 minutes in PBS pH 7.4 over 360 minutes.

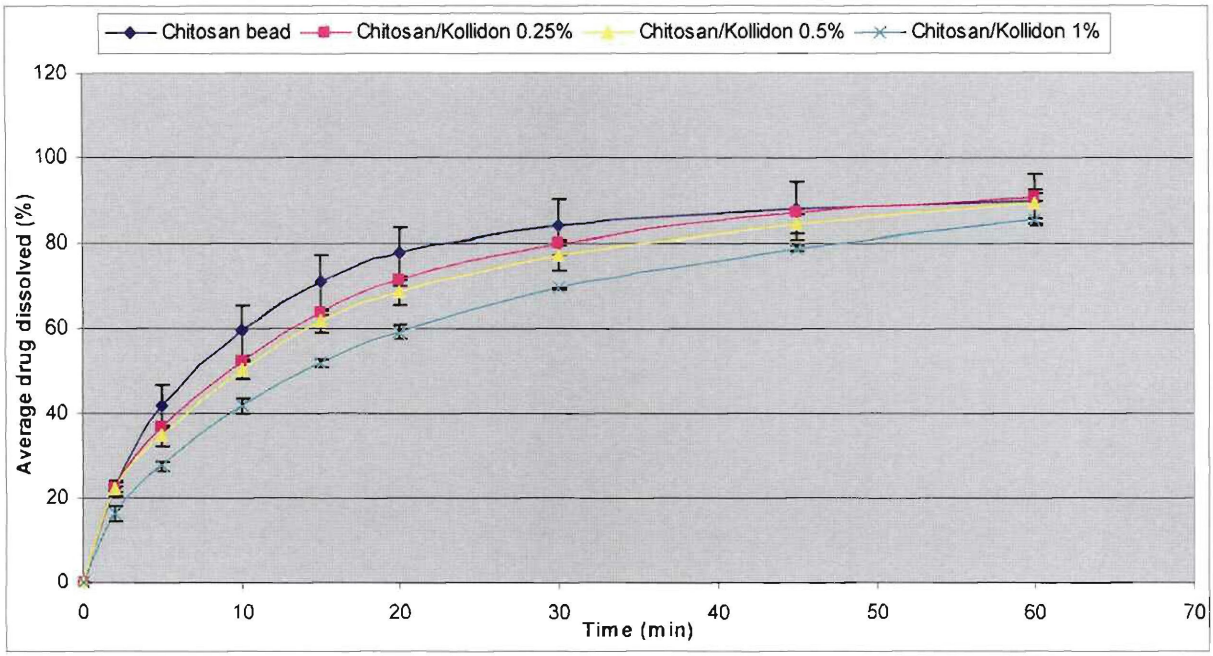


Figure 4.4: Ketoprofen release from bead formulations cross linked for 30 minutes in PBS pH 7.4 over the first 60 minutes of the dissolution experiment.

Table 4.1: Percentage ketoprofen (%) dissolved in the dissolution medium after 60 minutes in PBS pH 7.4.

Bead samples – 30 minutes cross linked	% Ketoprofen dissolved after 60 minutes ± SD*
Chitosan bead (CB)	90.14 ± 6.08
Chitosan/Kollidon 0.25% bead (K0.25B)	90.99 ± 0.80
Chitosan/Kollidon 0.5% bead (K0.5B)	89.34 ± 3.57
Chitosan/Kollidon 1% bead (K1B)	85.26 ± 0.55

*SD = Standard deviation

Table 4.2: Calculated mean dissolution time (MDT) values and average mean dissolution time (Ave MDT) for bead formulations cross linked for 30 minutes in PBS 7.4 for time 0 – 360 minutes.

Bead samples – 30 minutes cross linked	MDT 1	MDT 2	MDT 3	Ave MDT ± SD*
Chitosan bead (CB)	10.74	12.79	12.22	11.92 ± 1.06
Chitosan/Kollidon 0.25% bead (K0.25B)	23.97	24.40	22.98	23.78 ± 0.73
Chitosan/Kollidon 0.5% bead (K0.5B)	21.90	25.15	34.29	27.11 ± 6.41
Chitosan/Kollidon 1% bead (K1B)	31.68	31.41	36.99	33.36 ± 3.14

*SD = standard deviation

Table 4.3: Similarity factor values for bead formulations vs plain chitosan formulation in PBS 7.4.

Samples	Similarity factor (f_2)
	(f_2) vs Chitosan bead ± SD* 30 minutes cross linked beads
Chitosan/Kollidon 0.25% bead (K0.25B)	58.75 ± 4.35
Chitosan/Kollidon 0.5% bead (K0.5B)	56.30 ± 5.80
Chitosan/Kollidon 1% bead (K1B)	45.90 ± 3.59

*SD = Standard deviation

Table 4.4: Average surface area under the curve (AUC) after 360 minutes in PBS pH 7.4 for bead formulations cross linked for 30 minutes.

Bead samples – 30 minutes cross linked	Average AUC after 360 min ± SD* (%.min^{-1})
Chitosan bead (CB)	31781.37 ± 2107.129
Chitosan/Kollidon 0.25% bead (K0.25B)	33408.72 ± 354.71
Chitosan/Kollidon 0.5% bead (K0.5B)	33182.63 ± 902.67
Chitosan/Kollidon 1% bead (K1B)	32528.23 ± 141.07

*SD = Standard deviation

Table 4.5: Average surface area under the curve (AUC) after 60 minutes for bead formulations cross linked for 30 minutes in PBS pH 7.4.

Bead samples – 30 minutes cross linked	Average AUC after 60 min ± SD* (%.min^{-1})
Chitosan bead (CB)	1803.26 ± 164.27
Chitosan/Kollidon 0.25% bead (K0.25B)	1581.45 ± 14.69
Chitosan/Kollidon 0.5% bead (K0.5B)	1486.64 ± 79.17
Chitosan/Kollidon 1% bead (K1B)	1195.49 ± 31.43

*SD = Standard deviation

4.6.1.2 Discussion

In figure 4.4 it can be seen that a burst effect occurs during the first 5 minutes in PBS solution, pH 7.4 thereafter all the formulations showed a steady increase in drug release during the first hour. The burst effect could be an indication of the drug that was on the surface of the beads. The drug on the surface of the beads was not entrapped in a matrix and as a result dissolved quickly in the alkaline environment. As seen in figure 4.4 the addition of Kollidon® SR had a significant effect on the release of the ketoprofen from the chitosan beads. In figure 4.3 it can be seen that all of the ketoprofen was released within 6 hours and the curve reached its plateau. The pure chitosan beads released 91.302 ± 6.0% of its content in 360

minutes, but the profile reached a plateau after 60 minutes (90.14 ± 6.08% released after 60 minutes) of the dissolution experiment and it is unlikely that the batch will release more ketoprofen over an extended period of time. All the formulations that contained Kollidon® SR reached 100% dissolution within the 360 minutes of the dissolution experiment. In general a large amount of drug was dissolved over the first 60 minute period of the dissolution experiment, thereafter the average percentage drug dissolved increased constantly, until approximately 240 minutes of the dissolution experiment has elapsed.

The similarity factor values (f_2) for the bead formulations containing 0.25% and 0.5% of Kollidon® SR are greater than 50 ($f_2 > 50$), but the sample containing 1% Kollidon® SR has a value less than 50 ($f_2 = 45.90 \pm 3.59$) in comparison with the plain chitosan/ketoprofen bead formulation cross linked for 30 minutes. For the release profiles to be considered similar, the similarity factor must be greater than 50 ($f_2 > 50$). Thus the release profiles of formulations containing 0.25% and 0.5% Kollidon® SR can be considered similar to the plain chitosan/ketoprofen formulation, but it is visible that the release profile of the 1% Kollidon® SR formulation can not be considered similar to the profile of the plain chitosan/ketoprofen formulation.

Although all the average MDT-values for the different formulations were relatively short (less than 40 minutes) the 1% Kollidon® SR sample exhibited the longest mean dissolution time (33.36 ± 3.14 minutes) of all the bead formulations cross linked for 30 minutes. The average MDT values increased with an increase in Kollidon® SR concentration and the plain chitosan/ketoprofen formulation only had a MDT value of 14.17 ± 0.48 minutes, indicating that the inclusion of Kollidon® SR resulted in controlled release.

The AUC values confirm the effect of the Kollidon® SR on the bead formulation, and the 1% Kollidon® SR bead formulation (K1B) only had an AUC value of 1195.49 ± 31.43 after the first 60 minutes of the dissolution experiment while the pure chitosan bead (CB) had an AUC value of 1803.26 ± 164.27. This difference was statistically significant ($p < 0.05$). The pure chitosan bead did have a lower AUC value than the K1B after 360 minutes but this is due to the fact the chitosan bead did not release all the ketoprofen it contained. This difference however was not statistically significant ($p > 0.05$).

All the formulations that contained Kollidon® SR showed good swelling results, the swelling causes an increase in viscosity and this could cause a longer time for the ketoprofen to be released from the beads. It is thus evident that controlled release did take place.

4.6.2 Ketoprofen release from Chitosan/Kollidon® SR beads cross linked for 60 minutes

4.6.2.1 Results

The release of ketoprofen from chitosan bead formulations containing Kollidon® SR was determined as described in section 2.7.3 and the results are depicted in figure 4.5 and 4.6. The percentage ketoprofen that was released into the dissolution medium after 60 minutes of the dissolution experiment are presented in table 4.6. The mean dissolution time (MDT) values were calculated for all of the bead formulations and the values are presented in table 4.7. The dissolution profiles of the formulations cross linked for 60 minutes containing Kollidon® SR were compared with the dissolution profile of the plain chitosan/ketoprofen formulation cross linked for 60 minutes to get a better understanding of the effect of the polymer on the dissolution profile. The similarity factor values for this comparison are presented in table 4.8. Area under curve (AUC) values were calculated after 60 minutes and again after 360 minutes to compare the dissolution profiles of the bead formulations at these separate times, the results are presented in table 4.9 and 4.10.

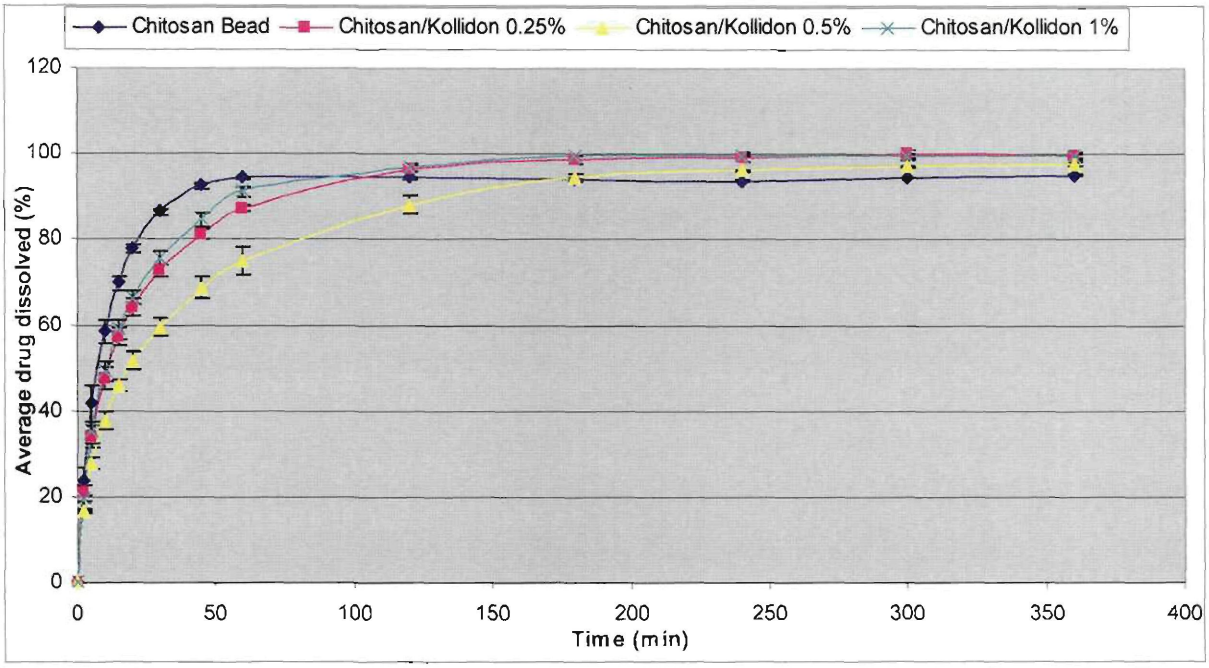


Figure 4.5: Ketoprofen release from bead formulations cross linked for 60 minutes in PBS pH 7.4 over 360 minutes.

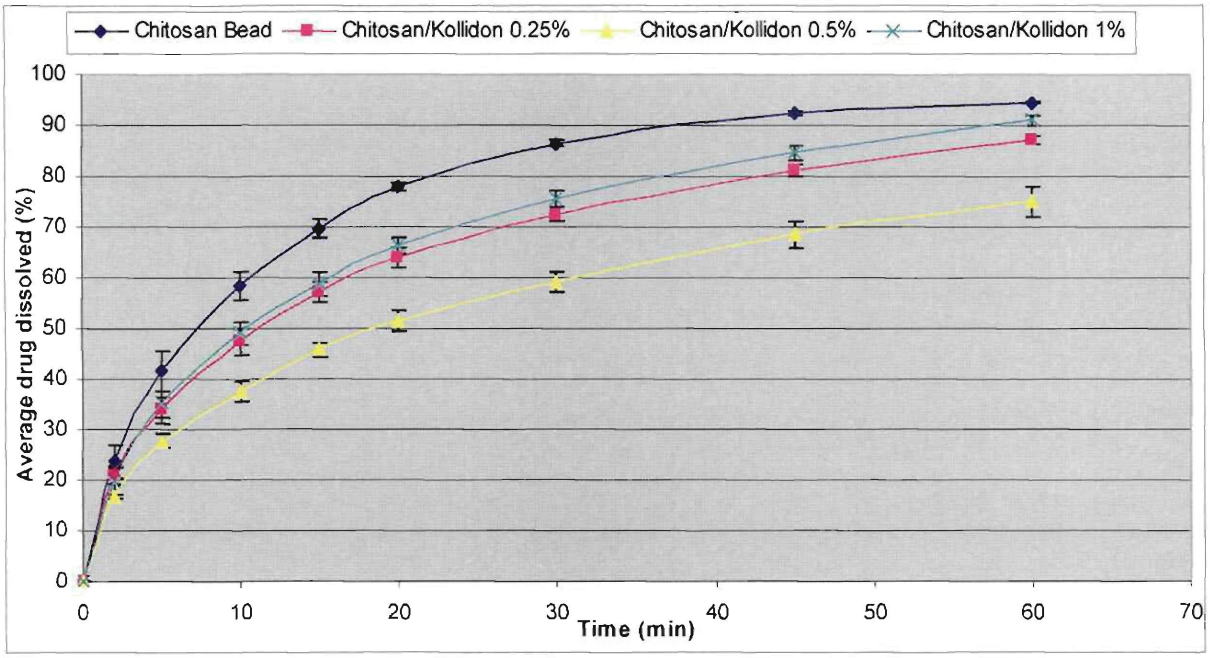


Figure 4.6: Ketoprofen release from bead formulations cross linked for 60 minutes in PBS pH 7.4 over the first 60 minutes of the dissolution experiment.

Table 4.6: Percentage ketoprofen (%) dissolved in the dissolution medium after 60 minutes in PBS pH 7.4.

Bead samples – 60 minutes cross linked	% Ketoprofen dissolved after 60 minutes ± SD*
Chitosan bead (CB)	94.43 ± 0.18
Chitosan/Kollidon 0.25% bead (K0.25B)	87.26 ± 0.80
Chitosan/Kollidon 0.5% bead (K0.5B)	75.07 ± 3.04
Chitosan/Kollidon 1% bead (K1B)	91.09 ± 1.10

*SD = Standard deviation

Table 4.7: Calculated mean dissolution time (MDT) values and average mean dissolution time (Ave MDT) for bead formulations cross linked for 60 minutes in PBS 7.4 for time 0 – 360 minutes.

Bead samples – 60 minutes cross linked	MDT 1	MDT 2	MDT 3	Ave MDT ± SD*
Chitosan bead (CB)	13.96	13.83	14.72	14.17 ± 0.48
Chitosan/Kollidon 0.25% bead (K0.25B)	25.83	27.13	25.84	26.27 ± 0.75
Chitosan/Kollidon 0.5% bead (K0.5B)	45.87	49.22	43.00	42.70 ± 3.34
Chitosan/Kollidon 1% bead (K1B)	24.66	23.14	20.90	22.90 ± 1.89

*SD = standard deviation

Table 4.8: Similarity factor values for bead formulations vs plain chitosan formulations in PBS 7.4.

Samples	Similarity factor (f_2)
	(f_2) vs Chitosan bead ± SD* 60 minutes cross linked beads
Chitosan/Kollidon 0.25% bead (K0.25B)	53.13 ± 1.71
Chitosan/Kollidon 0.5% bead (K0.5B)	39.52 ± 2.77
Chitosan/Kollidon 1% bead (K1B)	56.81 ± 5.44

*SD = standard deviation

Table 4.9: Average surface area under the curve (AUC) after 360 minutes for bead formulations cross linked for 60 minutes in PBS pH 7.4.

Bead samples – 60 minutes cross linked	Average AUC ± SD* (%.min^{-1})
Chitosan bead (CB)	32920.34 ± 112.19
Chitosan/Kollidon 0.25% bead (K0.25B)	33251.94 ± 266.54
Chitosan/Kollidon 0.5% bead (K0.5B)	31046.71 ± 471.90
Chitosan/Kollidon 1% bead (K1B)	33602.27 ± 182.21

*SD = Standard deviation

Table 4.10: Average surface area under the curve (AUC) after 60 minutes for bead formulations cross linked for 60 minutes in PBS pH 7.4.

Bead samples – 60 minutes cross linked	Average AUC ± SD*(%.min^{-1})
Chitosan bead (CB)	1795.01 ± 37.67
Chitosan/Kollidon 0.25% bead (K0.25B)	1353.70 ± 67.12
Chitosan/Kollidon 0.5% bead (K0.5B)	1040.71 ± 32.39
Chitosan/Kollidon 1% bead (K1B)	1391.41 ± 75.18

*SD = Standard deviation

4.6.2.2 Discussion

The dissolution experiment of the bead formulations cross linked for 60 minutes showed the same tendency as the formulations that were cross linked for 30 minutes. On closer inspection of the dissolution curves it can be seen that controlled release was more effective with the samples that were cross linked for 60 minutes. A burst effect occurred during the first 5 minutes of the dissolution experiment followed by a steady increase in average drug dissolved over the first 60 minutes (figure 4.6). The increase though was steadier and at a slower rate than the formulations that were cross linked for 30 minutes. The remaining drug

content of the formulations was steadily released over the last 300 minutes and the dissolution curves only reached a plateau after approximately 250 minutes (figure 4.5).

The longest mean dissolution time of all the formulations was achieved with the 0.5% Kollidon® SR bead formulation cross linked for 60 minutes. The formulation had a MDT-value of 42.70 ± 3.34 minutes. The MDT values of the bead formulations cross linked for 60 minutes, in general are higher than those for the formulations cross linked for 30 minutes except for the 1% Kollidon® SR sample, indicating that the cross linking time had an effect on the release characteristics of the bead formulations.

The AUC values for the bead formulations cross linked for 60 minutes where lower than it was for the bead formulations cross linked for 30 minutes, after 60 minutes of the dissolution experiment, indicating more effective controlled release probably because of a stronger matrix structure. This difference was statistically significant for the K0.5B formulation (1040.71 ± 32.39 vs 1486.64 ± 7.17) (Tukey test, $p < 0.05$). The fact that there is more drug left in the bead formulations cross linked for 60 minutes compared to the bead formulations cross linked for 30 minutes after 60 minutes of dissolution time means that there is more drug left for later release in these bead formulations, confirming better controlled release.

The similarity factor (f_2) results were very similar to the results of the samples cross linked for 30 minutes. The 0.25% and 1% Kollidon® SR samples had values greater than 50 ($f_2 > 50$), while the 0.5% Kollidon® SR sample had a value of 39.52 ± 2.77 which means the release profile can not be considered similar to the release profile of the plain chitosan/ketoprofen formulation cross linked for 60 minutes. This is due to the fact that controlled release was more efficient with this specific formulation.

The 0.5% Kollidon® SR formulation cross linked for 60 minutes had a significant higher swelling value than the other samples cross linked for 60 minutes and this could contribute to the better controlled release achieved with this specific formulation. The formulation had a degree of swelling of 2.29 ± 0.07 in PBS pH 7.4 after 360 minutes while the other formulations all had values below 2.10. The exact reason for the better swelling with this specific sample is unclear and remains to be elucidated.

The rate of release of the active from the bead formulations appear to decline with passing time but all the samples released the entire amount of drug entrapped in the matrix of the bead over a certain period of time.

4.6.3 Ketoprofen release form chitosan/Kollidon® SR granules

4.6.3.1 Results

The release of ketoprofen from chitosan granule formulations containing Kollidon® SR was determined as described in section 2.7.3 and the results are depicted in figure 4.7 to figure 4.10. The calculated percentage of ketoprofen released in the dissolution medium after 60 minutes of the dissolution experiment are presented in table 4.11. The mean dissolution time (MDT) values were calculated for all the granule formulations and the results are presented in table 4.12. The dissolution profiles of all the granule formulations containing Kollidon® SR were compared with the dissolution profile of the pure/chitosan granule formulation to get a better understanding of the effect of the polymer on the dissolution profile. The similarity factor values for this comparison are presented in table 4.13. The area under curve AUC values were calculated at 60 minutes and again at 360 minutes, the results are presented in table 4.14 and 4.15.

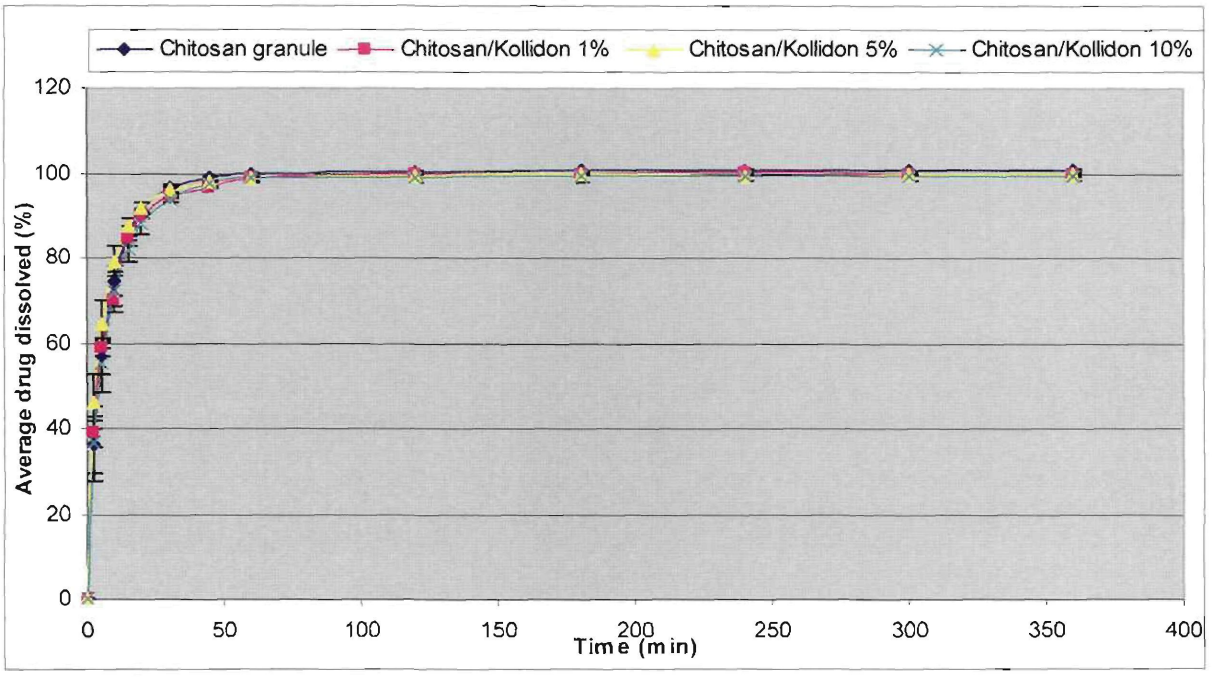


Figure 4.7: Ketoprofen release from chitosan granule formulations containing 0-10% Kollidon® SR in PBS pH 7.4 over 360 minutes.

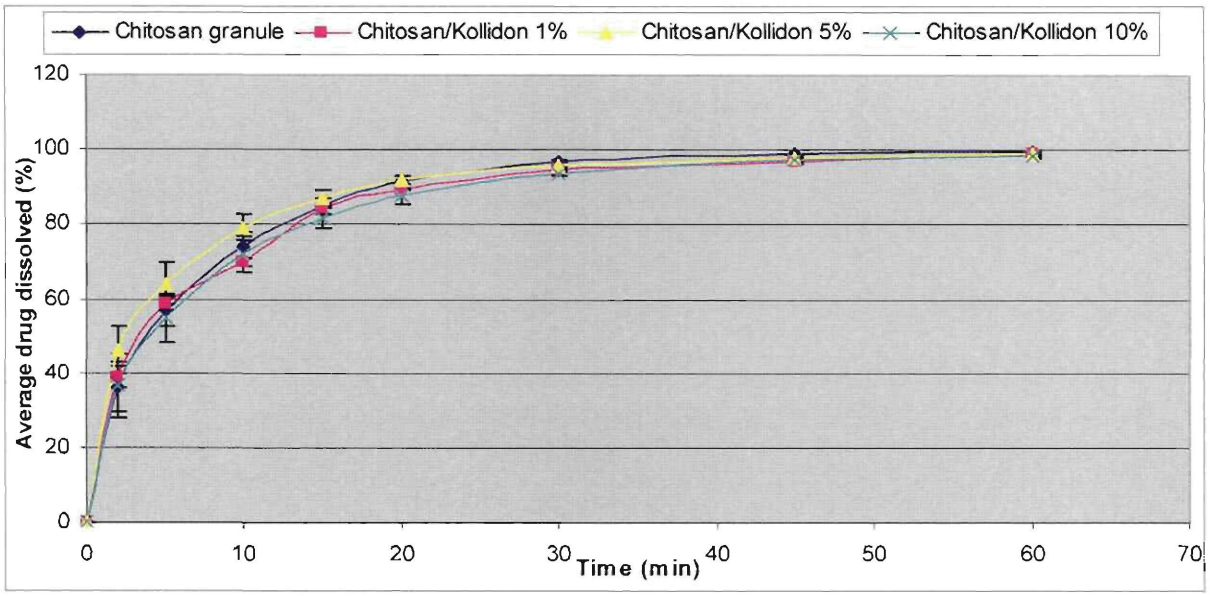


Figure 4.8: Ketoprofen release from chitosan granule formulations containing 0-10% Kollidon® SR in PBS pH 7.4 over the first 60 minutes of the dissolution experiment.

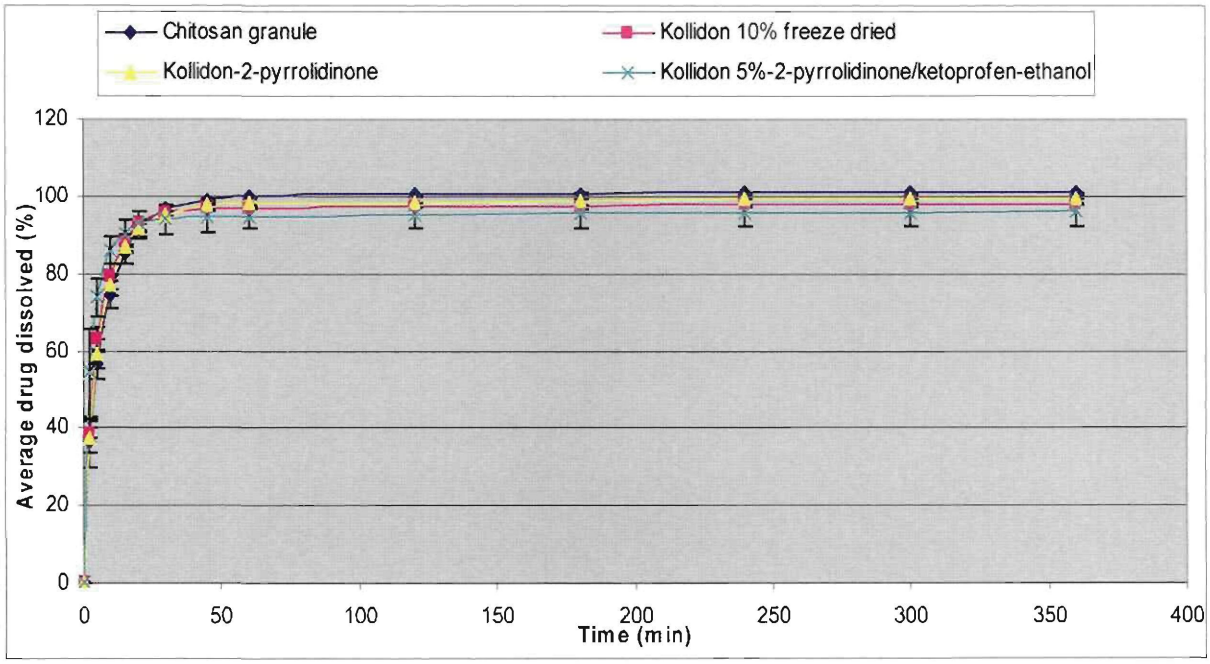


Figure 4.9: Ketoprofen release from chitosan granule formulations prepared according to alternative methods in PBS pH 7.4 over 360 minutes.

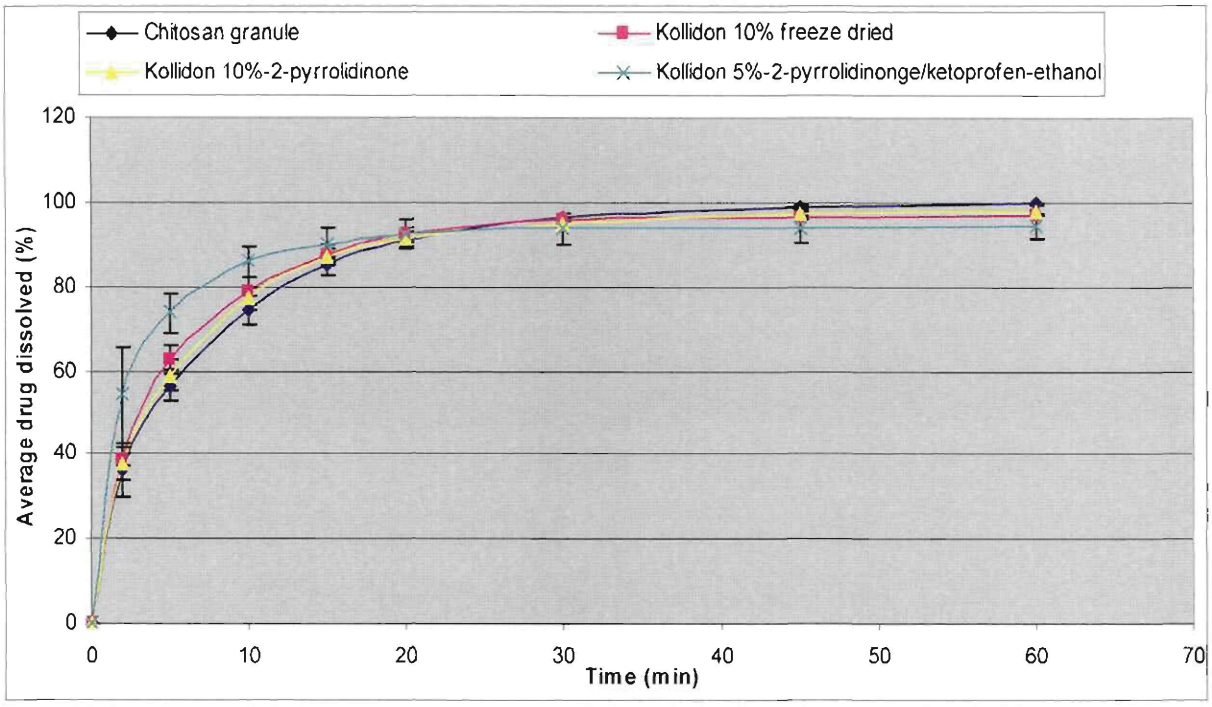


Figure 4.10: Ketoprofen release from chitosan granule formulations prepared according to alternative methods in PBS pH 7.4 over the first 60 minutes of the dissolution experiment.

Table 4.11: Percentage ketoprofen (%) dissolved in the dissolution medium after 60 minutes in PBS pH 7.4.

Granule samples	% Ketoprofen dissolved after 60 minutes ± SD*
Chitosan granule (CG)	99.79 ± 0.25
Chitosan/Kollidon 1% (K1G)	99.02 ± 0.47
Chitosan/Kollidon 5% (K5G)	98.81 ± 0.44
Chitosan/Kollidon 10% (K10G)	98.71 ± 0.96
Chitosan/Kollidon 10% - freeze dried (K10Gfd)	96.87 ± 0.09
Chitosan/Kollidon-10%-2-pyrrolidinone (10KPG)	98.17 ± 1.40
Chitosan/Kollidon-5%-2-pyrrolidinone/ketoprofen-ethanol (5KPG)	94.64 ± 2.96

*SD = standard deviation

Table 4.12: Calculated mean dissolution time (MDT) values and average mean dissolution time (Ave MDT) for granule formulations in PBS 7.4 for time 0 - 360 minutes.

Granule samples	MDT 1	MDT 2	MDT 3	Ave MDT ± SD*
Chitosan granule (CG)	7.67	10.35	8.35	8.82 ± 1.36
Chitosan/Kollidon 1% (K1G)	8.29	8.15	8.79	8.41 ± 0.34
Chitosan/Kollidon 5% (K5G)	7.78	6.50	8.07	7.45 ± 0.83
Chitosan/Kollidon 10% (K10G)	7.71	10.40	10.16	9.42 ± 1.49
Chitosan/Kollidon 10% - freeze dried (K10Gfd)	8.42	6.80	6.70	7.31 ± 0.96
Chitosan/Kollidon-10%-2-pyrrolidinone (10KPG)	8.44	8.87	8.31	8.54 ± 0.29
Chitosan/Kollidon-5%-2-pyrrolidinone/ketoprofen-ethanol (5KPG)	6.59	6.38	7.14	6.70 ± 0.39

*SD = standard deviation

Table 4.13: Similarity factor values for granule formulations vs plain chitosan granules in PBS 7.4.

Samples	Similarity factor (f_2)
	(f_2) vs Chitosan granule ± SD*
Chitosan/Kollidon 1% (K1G)	74.40 ± 3.67
Chitosan/Kollidon 5% (K5G)	72.23 ± 15.99
Chitosan/Kollidon 10% (K10G)	80.49 ± 1.87
Chitosan/Kollidon 10% - freeze dried (K10Gfd)	69.33 ± 7.47
Chitosan/Kollidon-10%-2-pyrrolidinone (10KPG)	81.44 ± 3.95
Chitosan/Kollidon-5%-2-pyrrolidinone/ketoprofen-ethanol (5KPG)	52.39 ± 2.79

*SD = Standard deviation

Table 4.14: Average surface area under the curve (AUC) after 360 minutes for granule formulations in PBS pH 7.4.

Granule samples	Average AUC ± SD* (%.min^{-1})
Chitosan granule (CG)	35403.51 ± 102.08
Chitosan/Kollidon 1% (K1G)	35091.79 ± 150.04
Chitosan/Kollidon 5% (K5G)	35147.13 ± 164.91
Chitosan/Kollidon 10% (K10G)	34872.60 ± 257.80
Chitosan/Kollidon 10% - freeze dried (K10Gfd)	34451.53 ± 24.90
Chitosan/Kollidon-10%-2-pyrrolidinone (10KPG)	34935.06 ± 410.13
Chitosan/Kollidon-5%-2-pyrrolidinone/ketoprofen-ethanol (5KPG)	33936.18 ± 1269.43

*SD = Standard deviation

Table 4.15: Average surface area under the curve (AUC) after 60 minutes for granule formulations in PBS pH 7.4.

Granule samples	Average AUC ± SD*(%.min^{-1})
Chitosan granule (CG)	2244.41 ± 84.15
Chitosan/Kollidon 1% (K1G)	22182.56 ± 19.39
Chitosan/Kollidon 5% (K5G)	2343.93 ± 69.83
Chitosan/Kollidon 10% (K10G)	2150.60 ± 119.33
Chitosan/Kollidon 10% - freeze dried (K10Gfd)	2346.07 ± 62.67
Chitosan/Kollidon-10%-2-pyrrolidinone (10KPG)	2283.74 ± 62.89
Chitosan/Kollidon-5%-2-pyrrolidinone/ketoprofen-ethanol (5KPG)	2465.40 ± 139.71

*SD = Standard deviation

4.6.3.2 Discussion

From the release profiles of figure 4.7 - 4.10 it is clear that granules proofed unsuccessful in achieving controlled release. The granules presented with a very high burst effect, especially over the first 10 minutes the burst effect is clearly visible from figure 4.8 and 4.10. The difference between the dissolution profiles of the beads and the granules could be attributed to the difference in surface structure and surface area. The granules had a flaky and cracked appearance and were a lot smaller than the beads. The smaller particles mean that there are bigger surface areas in contact with the dissolution medium and the ketoprofen could easily dissolve in the medium. In addition to the surface characteristics, the beads formed a matrix which entrapped the ketoprofen inside the bead and slowed down the release of the ketoprofen in the dissolution medium, while the granule formulations did not form a matrix and was not effective in achieving controlled release. Although it was expected that the Kollidon® SR which covered the drug especially in the K10G would modify release.

The dissolution profiles all look similar as seen in figure 4.7 and 4.9, therefore it can be concluded that the Kollidon® SR had very little if any effect on the release profiles of the granule formulations. The similarity values of all the granule formulations were above 50. This indicates that the dissolution profiles are similar to the dissolution profile of the plain granule formulation, with the 5KPG granule presenting with the lowest f_2-value (52.39 ± 2.79) and the 10KPG granule formulation with the highest (f_2) value (81.44 ± 3.95) (table 4.5).

The chitosan granule formulations released all their drug content within 60 minutes and the dissolution profiles reached a plateau after the initial 60 minutes. The release profiles were similar for all the dissolution profiles and the mean dissolution time (MDT-values) of the granules are significantly lower in comparison to the bead formulations. The MDT-values differ little from each other and the highest value of 9.42 ± 1.49 was achieved with the granule formulation containing 10% Kollidon® SR (K10G).

The AUC values where all higher than the bead formulations after 60 minutes of dissolution and this emphasize the lack of controlled release achieved with the granule formulations. The 5KPG granule formulation had the highest average AUC value of 2465.40 ± 139.71 after 60 minutes. The AUC values were similar for all the granule formulations and this indicates that the ketoprofen were released at the same rate and extent for all of the granule formulations. There were no statistically significant differences between the AUC values of the granules after 60 minutes of dissolution.

Kollidon® SR is a pH independent polymer and although the SEM photos in section 2.3.1 showed the polymer covering the ketoprofen crystals, the granules did not form a matrix and the ketoprofen was released at a high rate. Ketoprofen is a weak acid and has a pK_a-value of 5.94. The dissolution medium (PBS) had a favourable pH of 7.4 for the ketoprofen to dissolve in and could contribute to the fast dissolution rate of the ketoprofen in the granule formulations.

4.7 Conclusion

The results for both the bead and granule formulations were not as promising as expected and especially the granules were disappointing. Although all the formulations released almost all the ketoprofen it contained the formulations had a high burst effect and the ketoprofen were released at a fast rate.

The beads presented with far better results than the granules and the addition of Kollidon® SR had a definite effect on the release profiles of the bead formulations. The effect of the polymer on the formulations is visible in the similarity (f_2) values. The K0.5B formulation cross linked for 60 minutes and K1B cross linked for 30 minutes had f_2-values < 50, while the other formulations containing Kollidon® SR had f_2-values just above 50. The MDT-values of the bead formulations are considerably higher than those of the granules indicating controlled release in the case of the bead formulations.

The beads were especially promising during the first 60 minutes as there was still drug left for release after 60 minutes of dissolution and it is thus evident from the dissolution profiles that controlled release did indeed take place. For example the K0.5B cross linked for 60 minutes still had 24.93% ketoprofen left for release after 60 minutes of dissolution. The dissolution rate decreased with the addition of Kollidon® SR to the bead formulations. The granules had a fast dissolution rate and all the ketoprofen were released within 60 minutes; therefore it is evident that the granules used in this study are not suitable for a controlled release formulation.

The conclusion can be made that the granules did not form a matrix and the ketoprofen dissolved easily in the dissolution medium, this is visible in figure 4.8 and 4.10 where all the ketoprofen in the formulations dissolved within 60 minutes. The Kollidon® SR had little

effect on the granule formulations and all the profiles had a high similarity ($f_2 \geq 50$) value. The increase in Kollidon® SR also had little effect on the MDT-values and the granules all presented with low MDT-values with a small difference between the formulations containing Kollidon® SR and the formulation that did not contain Kollidon® SR.

CHAPTER 5

5 SUMMARY AND FUTURE PROSPECTS

5.1 Summary

The ways in which drugs are administered have gained increasing attention in the past three decades. Usually a drug is administered at a high dose at a given time only to have to repeat that dose several hours later. This is not economical and sometimes results in damaging side effects. As a consequence, increasing attention has been focused on methods of administering drugs continually for prolonged time periods and in a controlled fashion. The primary method of accomplishing this controlled release has been through incorporating polymers in drug formulations.

Chitosan could be ideal for use in formulations intended to release drugs slowly, since the gel formation by cationic chitosan, which is pronounced at acidic pH values results in marked retardant effects on drug release. Orally administered formulations are initially exposed to the acidic milieu of the stomach, especially if they have been administered to subjects in the fasted states when gastric pH is likely to range from approximately 1 to 2. Polymers commonly used in preparing slow release formulations do not form gels very efficiently in such environments. In contrast gel formation by chitosan takes place in an acidic environment (Säkkinen, *2003:11*).

To enhance the effect of the chitosan, Kollidon® SR was incorporated with chitosan in the bead and granule formulations prepared in this study. This polymer is unique due to the fact that its release properties are pH independent and previous studies have shown potential for this combination. The effect of the polymer on the drug release and morphology of the beads and granules were investigated and compared with plain chitosan beads and granules.

Ketoprofen were chosen as model drug for this study. Ketoprofen is a well known nonsteriodal anti-inflammatory drug and used in various conditions including rheumatoid arthritis and osteoarthritis. Ketoprofen has a short elimination half-life of 2.05 ± 0.58 h and

this characteristic makes it an ideal candidate for formulation in a chitosan bead matrix. By controlling the release of ketoprofen there will be less gastric irritation and better patient compliance.

Chitosan beads and granules were investigated during this study, and the release characteristics of these formulations were compared. A polymer namely Kollidon® SR was added to further delay the release of ketoprofen from beads and granules. The beads and granules were prepared according to various methods described in chapter 2, section 2.4.2 and 2.6.2. A spherical bead formed on contact with the TPP-solution and scanning electron microscopy was used to investigate the surface and matrix of the beads. It was found that beads were porous and had a flaky appearance. Beads that were cross linked for a longer period of time had a smoother surface indicating the possibility of better cross linking. The inclusion of the polymer was visible at higher concentrations. The polymer covered the crystalline drug and gave the bead matrix a smoother appearance. Granules had a less complex method of preparation and the conventional method was altered to investigate the effect of method of preparation on the characteristics of the granules. Granules were smaller in size and had a flaky and cracked appearance. The inclusion of the polymer was evident on close inspection, especially at higher concentrations and it gave the granules a smoother appearance since the polymer covered the crystalline drug.

The beads had a high drug loading and an increase in Kollidon® SR concentration tended to increase the drug loading of the beads, this was especially evident for the 1% (w/v) Kollidon® SR bead formulation. The cross linking time had a definite effect on the drug loading of the beads and beads that were cross linked for 60 minutes had lower drug loading than the same formulations that were cross linked for 30 minutes. This could be an indication that there was some of the drug that leached out into the TPP-solution during cross linking. The granules had a less complex method of preparation and all the formulations had a drug loading above 90% except for the 10KPG and 5KPG granule formulation which could be due to their extended time period in the oven. These two formulations contained 2-Pyrrolidinone which has an oily character, and it took the formulations 7 days to dry in the oven.

Friability tests were conducted on the bead formulations, and the beads were porous and offered poor resistance to mechanical force. It was evident that the inclusion of Kollidon® SR made a difference and the beads had a lower friability percentage with an increase in

Kollidon® SR concentration. This is an indication that the inclusion of Kollidon® SR led to a stronger matrix in the beads, and the beads were less brittle.

Swelling behaviour studies were conducted on the bead formulations by immersing a pre-weighed sample of the beads in a phosphate buffer solution (PBS) with a pH of 5.60 and pH 7.40 respectively as described in chapter 2, section 2.7.6. The inclusion of Kollidon® SR led to a decrease in swelling for especially the formulations that was cross linked for 60 minutes. Beads that contained Kollidon® SR swelled significantly more in PBS pH 5.60 than it did in a pH of 7.40.

Lastly, ketoprofen release from the beads and the granules in PBS pH 7.40 at 37 °C over a period of 6 hours were investigated. Dissolution testing on these formulations showed that the bead formulations succeeded in achieving controlled release. The beads achieved better controlled release than the granule formulations. This could be ascribed to the fact that the granule formulations did not form a matrix. Beads that were cross linked for a longer period was more efficient in achieving controlled release and especially the 1% w/v Kollidon® SR bead cross linked for 30 minutes and the 0.5% w/v Kollidon® SR bead cross linked for 60 minutes showed promising results.

5.2 Future prospects

Throughout this study a lot of obstacles had to be overcome to maximize drug loading and achieve efficient controlled release. Several factors were also identified that may prove meaningful if studied in future.

Beads

The beads did achieve controlled release to a certain extent. The controlled release, however, was insufficient and the following factors could contribute to achieve better controlled release.

- The cross linking time could be extended. With an increase in cross linking time the TPP-diffuse deeper into the formulation and could increase the mechanical strength of

the matrix as a result of better cross linking. Bead formulations that were cross linked for 60 minutes had lower AUC values (after 60 minutes) than the bead formulations that were cross linked for 30 minutes indicating better controlled release potential.

- Ketoprofen as drug of choice for this study may have affected the release profiles. Ketoprofen has a relative low pK_a value and has a high solubility in mediums with a high pH value. This also led to loss of drug to the external TPP phase during inotropic gelation. It is recommended that a drug be used that would not dissolve in the alkaline TPP-solution; the drug loading as well as controlled release might improve by this alteration.

- Another factor that may have an effect on the release characteristics of the beads is the viscosity of the polymer. Säkkinen (2003:24) suggested that the release rate of the drug through the polymer matrix is dependant on the gelled polymer matrix. An increase in viscosity of the polymer might improve controlled release.

- The addition of more than one polymer to the same formulation may also prove to be valuable in achieving controlled release. Each polymer has different physical and chemical characteristics and the addition of more than one polymer may increase the viscosity of the bead matrix.

Granules

With regard to granules several variable factors were identified that might have influenced the lack of controlled released achieved with these formulations. The factors include the following:

- Size of the granules. It was noticed that the granules were much smaller in size than the beads. The smaller particles meant the granules had a large surface area in contact with the dissolution medium compared to the bead formulations and the ketoprofen could easily dissolve in the dissolution medium. It is postulated that a bigger size granule may increase the controlled release ability of the granule.

- The viscosity of the polymer and the drug used in this study may have influenced the release characteristics of the granules as mentioned in the discussion of the future prospects for the beads.

The polymer used did not cause the granule to form a matrix, although it did cover the drug, it did not delay the release of the drug effectively. Therefore a new batch of granules was made with pectin as the polymer to investigate the influence of the type of polymer. Pectin is mainly used as a gelling agent, but can also act as thickener, water binder and stabilizer. Pectin forms thermoreversible gels at a low pH (Chaplin, 2007).

Method of preparation

Pectin was added at a 5% (w/w) concentration with chitosan and ketoprofen powders into a glass jar. The powders were then mixed in a Turbula mixer (Model T2C, Wily A. Bachofen Maschinefabrik, Basel, Switzerland) for 5 minutes. The powder was then wetted with a 2% v/v acetic acid solution while it was stirred in a Kenwood mixer (KM300, Kenwood Ltd. Britian). The wet mass was granulated through a 2 mm sieve. The granules were dried in an oven (Term-O-Mat oven, Labotec, South Africa) at 37 °C for 4 hours and then granulated through a fine sieve (Fritsch analysette, Germany). Only granules between 710 μm and 800 μm were used. The drug loading of the granules were determined and dissolution tests were preformed on the granules as described in chapter 2, section 2.7.3.

Results

The release of ketoprofen from the chitosan granule formulation containing 5% (w/w) pectin in PBS pH 7.4 is depicted in figure 5.1 and 5.2.

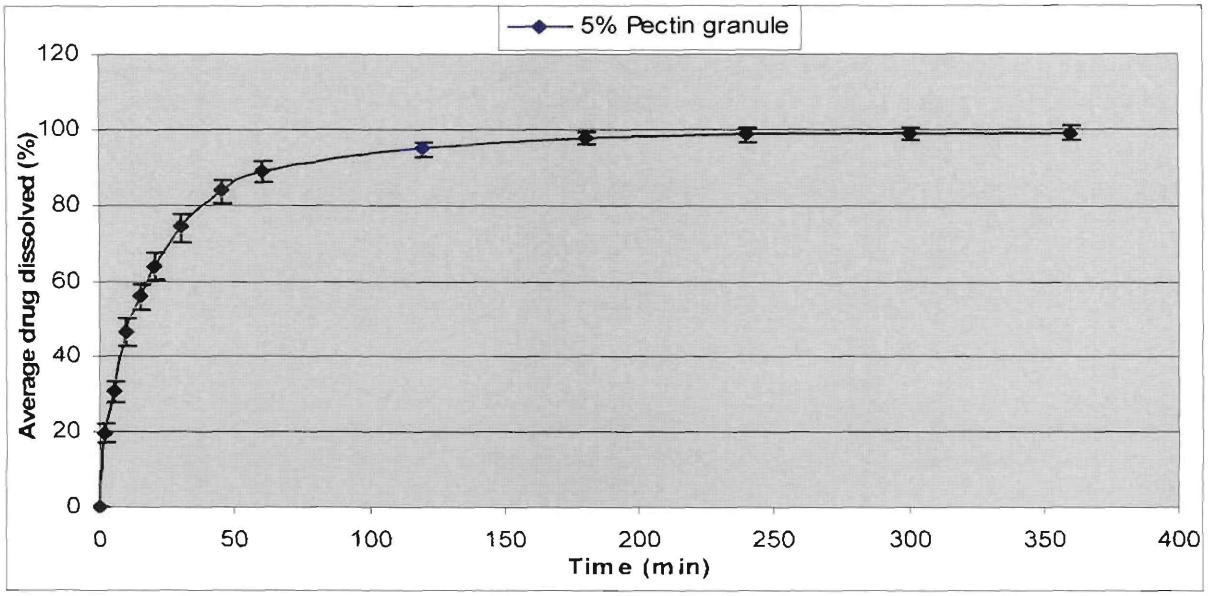


Figure 5.1: Ketoprofen release from a chitosan/pectin 5% (w/w) granule formulation in PBS pH 7.4 over 360 minutes.

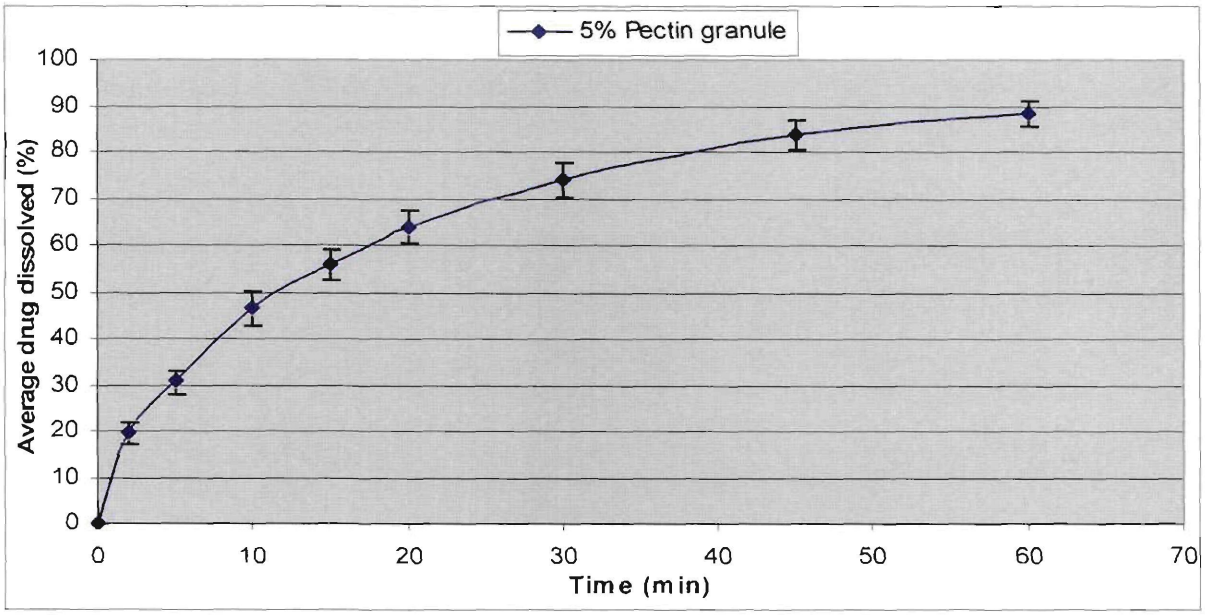


Figure 5.2: Ketoprofen release from chitosan/pectin a 5% (w/w) granule formulation in PBS pH 7.4 over the first 60 minutes of the dissolution experiment.

Discussion

The inclusion of pectin into the granule formulation made an obvious difference in the release modifying behaviour of the granule formulation. The release of the ketoprofen was significantly slower than the Kollidon® SR granule formulations and the pectin granules had a MDT value of 26.39 ± 1.09 minutes, (the Kollidon® SR granule formulations all had MDT values below 10). It is evident from figure 5.2 that the granule formulation had a burst effect during the first 5 minutes. The percentage ketoprofen released in the dissolution medium steadily increased over the first hour as seen in figure 5.2. The granules released 88.53 ± 2.79% of its ketoprofen content during the first 60 minutes, the remaining amount of ketoprofen in the granules was then slowly released in the dissolution medium and the curve only reached a plateau after 250 min as seen in figure 5.1. The pectin granules had an AUC value of 1350.56 ± 100.69 after 60 minutes of the dissolution experiment which is significantly lower than the AUC values of the Kollidon® SR granule formulations after 60 minutes of the dissolution experiment. It is therefore evident that the polymer had a significant influence on the release characteristics of the granules and further studies may prove rewarding.

ANNEXURE A

CERTIFICATES OF APPROVAL

CERTIFICATE OF ANALYSIS FOR KETOPROFEN

Boehringer Ingelheim
Bidachem

Certificate of analysis Nr° F229-04

Product : KETOPROFEN

Batch n°: 04040675

Tests	Results	Specifications
Characters:	conforms	A white or almost white crystalline powder, practically insoluble in water, freely soluble in acetone, in alcohol and in methylene chloride
Identification:		
IR Spectrum:	conforms	Conforms to reference spectrum
Clarity of solution (10% in acetone):	conforms	Not more than refer. suspen. I
Color of solution (10% in acetone):	conforms	Not more than reference solution Y6
Heavy metals:	conforms	Not more than 10 ppm
Loss on drying:	0,02 %	Not more than 0,5 %
Sulphated ash:	0,04 %	Not more than 0,1%
Potentiometric assay (0.1 N NaOH):	100,1 %	99,0 – 100,5 % (on dried substance)
Related substances (HPLC):		
Imp. A (3-Acetylbenzophenone):	n.d.	Not more than 0,2%
Imp C (2-(3carboxyphenyl) propionic acid:	n.d.	Not more than 0,2%
Max other single:	0,06 %	Not more than 0,2%
Total unknown impurities:	0,10 %	Not more than 0,4%
Residual solvents (GC-HS):		
Toluene:	815 ppm	Not more than 890 ppm
Benzene:	< 0,2 ppm	Not more than 2 ppm

Warren Chem Specialities
(PTY) LTD
P.O. BOX 29069
SANDRINGHAM
2131 R.S.A.
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REG. 2000/008419/07
34 BRUNTON CIRCLE
FOUNDERS HILL
LETHABONG
FAX (011) 452-2310

WC1.8743

This product corresponds to : Ph.Eur. latest Ed.

Manufacturing date: May 2004 Batch size: 1225,0 Kg
Retest date: May 2006 Internal Code: 153050

Release date: 20 May 2004

BIDACHEM s.p.a
Quality Control
Manager

Dr. T. De Quarti

Bidachem S.p.A. – S.S. n. 11 (Padana Superiore), 8 – 24040 Fornovo S. Giovanni (BG) – ITALY - Tel. 0363/3552221

XIAMEN JIANGYUAN IMPORT AND EXPORT COMPANY
4/F, NO.168 QIXING ROAD XIAMEN, FUJIAN CHINA
TEL: 86-592-5911378 , FAX: 86-592-5911318

9

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

厦门江源经济发展公司出口单证章

ABSA BANK LIMITED IB CENTRE CAPE TOWN
LETTER OF CREDIT NUMBER 827-01-0074500-G
"Drum" s No.: 1—67

WCI 7109

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2801
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South Africa

2006-05-15
GKA/M320
Dr.Leyendecker
+49 621 60-45308
Certificate No 1459

Certificate of Analysis according to DIN 55350-18-4.2.2

KOLLIDON SR POLYMER

Purchase Order/Customer Product#
PO058707

Material	51597764
Order	3003106318 000010
Delivery	3084230017 000010
Lot/No	63920836W0
Lot/Qty	0.300 KG
Total	0.300 KG
Transport	00000000000035335967

Test Parameter	Requirements	UoM	Results
Identification (IR)	must conform		conforms
pH-value (suspension 10 % in water)	Min.: 3.5 Max.: 5.5		4.7
Heavy metals	Max.: 20	mg/kg	<20
Loss on drying	Max.: 5.0	g/100g	3.2
Sulfated ash	Max.: 2.00	g/100g	1.40
Microbiological Quality (Ph.Eur., Cat.2 + 3A)	must conform		conforms
Total viable aerobic count (aerobic bacteria + fungi)	Max.: 100	CFU/g	<10
Enterobacteria and certain other gram-negative bacteria	Max.: 10	CFU/g	<1
Escherichia Coli	Max.: 0	CFU/g	0
Staphylococcus aureus	Max.: 0	CFU/g	0

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Certificate No 1459
Page 2 of 3

Certificate of Analysis according to DIN 55350-18-4.2.2

KOLLIDON SR POLYMER

Purchase Order/Customer Product#
PO058707

Material	51597764	
Order	3003106318	000010
Delivery	3084230017	000010
Lot/No	63920836W0	
Lot/Qty	0.300	KG
Total	0.300	KG
Transport	00000000000035335967	

Test Parameter	Requirements	UoM	Results
Pseudomonas aeruginosa	Max.: 0	CFU/g	0
Total acetates	Max.: 5000	mg/kg	3470
Vinyl acetate	Max.: 100	mg/kg	<10
content of polyvinylacetate	Min.: 75.0 Max.: 85.0	g/100g	78.2
content of povidone	Min.: 18.0 Max.: 21.0	g/100g	19.8

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Certificate of Analysis according to DIN 55350-18-4.2.2

KOLLIDON SR POLYMER

Purchase Order/Customer Product#
PO058707

Material	51597764	
Order	3003106318	000010
Delivery	3084230017	000010
Lot/No	63920836W0	
Lot/Qty	0.300	KG
Total	0.300	KG
Transport	00000000000035335967	

Total acetates corresponds to Residual solvents, Ph.Eur. class 3.

Manufacturer: BASF AG
Carl-Bosch-Str. 38
67056 Ludwigshafen
Germany

QC-Reference-No.	05C08572
Production date	11.2005
Release date	21.12.2005
Retest date	11.2007

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Quality Control
sig. Dr.Peter

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ANNEXURE B

EXPERIMENTAL DATA

Table B-1: Dissolution data for ketoprofen release from pure chitosan beads at pH 7.40 over a period of 360 minutes.

Time (min)	pH 7.4 – Beads 30 min cross linked		pH 7.4 – Beads 60 min cross linked	
	% Release	Standard deviation	% Release	Standard deviation
0	0	0	0	0
2	22.21328	1.65290754	23.6252	3.35060213
5	41.8198	5.10705974	41.61836	4.07397589
10	59.54576	6.11853002	58.44934	2.66695398
15	70.87837	6.20348186	69.77545	1.67242373
20	77.69403	6.00420064	78.04028	0.95241375
30	83.88619	6.39271524	86.48219	0.65361589
45	88.34454	6.0359458	92.44611	0.48647111
60	90.139	6.07791554	94.42999	0.17870729
120	90.61803	5.73921622	94.38545	0.2904851
180	91.05636	5.76327968	93.9924	0.41644431
240	91.39517	6.09326612	93.81658	0.25035168
300	90.77585	5.87899235	94.53507	0.16590327
360	91.30159	6.00094763	95.19227	0.35441552

Table B-2: Dissolution data for ketoprofen release from chitosan/Kollidon 0.25% bead at pH 7.40 over a period of 360 min.

Time (min)	pH 7.4 – Beads 30 min cross linked		pH 7.4 – Beads 60 min cross linked	
	% Release	Standard deviation	% Release	Standard deviation
0	0	0	0	0
2	22.1342	0.63583455	20.94993	1.87384456
5	36.71673	0.24338324	33.94829	2.61623376
10	52.34048	0.18254191	47.46904	2.54263311
15	63.80138	0.84208501	57.19778	1.9727332
20	71.33364	1.11965217	64.02329	2.00458129
30	79.99841	0.61896435	72.57569	1.34029364
45	87.3987	0.71139768	81.20753	1.17388498
60	90.99664	0.79881373	87.25718	0.79312709
120	95.68772	1.06276843	96.55364	0.52737091
180	96.65991	1.06919447	98.74844	0.87141829
240	98.69769	1.05715546	99.35917	0.95141337
300	98.72875	1.1592978	99.90003	1.12852961
360	99.36757	1.23378276	99.63787	1.00945284

Table B-3: Dissolution data for ketoprofen release from chitosan/Kollidon 0.5% bead at pH 7.40 over a period of 360 min.

Time (min)	pH 7.4 – Beads 30 min cross linked		pH 7.4 – Beads 60 min cross linked	
	% Release	Standard deviation	% Release	Standard deviation
0	0	0	0	0
2	22.26247	1.5942464	16.76959	0.35169173
5	34.79712	2.70056781	27.76956	1.2899383
10	50.26841	2.28590889	37.65789	1.9753321
15	61.66847	2.54801305	45.8292	1.36431538
20	68.4664	3.04185631	51.59801	2.02215561
30	77.09787	3.64033897	59.36392	2.03115346
45	84.36259	3.5982893	68.6817	2.61133356
60	89.35778	3.56973448	75.06632	3.03804236
120	94.55762	3.18700587	88.14467	2.04491075
180	96.9178	2.69390382	94.63073	1.00114468
240	98.4104	2.38326357	96.20985	0.39137573
300	99.18334	1.62777279	97.12705	0.39377375
360	99.6792	1.71982299	97.84281	0.39423726

Table B-4: Dissolution data for ketoprofen release from chitosan/Kollidon 1% bead at pH 7.40 over a period of 360 min.

Time (min)	pH 7.4 – Beads 30 min cross linked		pH 7.4 – Beads 60 min cross linked	
	% Release	Standard deviation	% Release	Standard deviation
0	0	0	0	0
2	16.52809	1.83440452	19.51283	2.98369672
5	27.51074	0.95691643	35.02983	2.63354671
10	41.72599	1.86938098	49.04599	2.30971743
15	51.79422	0.94216929	58.79352	2.23221332
20	59.28704	1.51487624	66.39983	1.7372266
30	69.39873	0.34603331	75.60836	1.67189351
45	78.85332	0.62068996	84.64753	1.50286748
60	85.26271	0.54701187	91.08935	1.1022929
120	93.52654	0.67789944	96.88532	0.82486262
180	96.64716	0.35821417	99.60239	0.1834746
240	98.04842	0.5398137	99.78616	0.12480089
300	98.93328	0.56325019	99.6437	0.39352098
360	99.58847	0.85772991	99.68155	0.55785048

Table B-5: Dissolution data for ketoprofen release from pure chitosan granule and chitosan/Kollidon 1% granule at ph 7.4 over a period of 6 hours.

Time (min)	Chitosan granule pH 7.4		Chitosan/Kollidon 1% pH 7.4	
	% Release	Standard deviation	% Release	Standard deviation
0	0	0	0	0
2	36.29652	6.52828937	38.9206	3.08575162
5	56.39384	3.67175593	58.86271	1.92462073
10	74.4671	3.46689917	70.04381	1.18307734
15	85.05053	2.27023798	84.7038	0.38693173
20	91.28935	1.79235322	89.54881	0.34093978
30	96.62816	0.99514274	94.55911	0.27565848
45	98.88122	0.34814352	96.70074	0.30877895
60	99.78874	0.25378433	99.02439	0.47275137
120	100.333	0.25376215	99.80135	0.44563106
180	100.6004	0.15529379	99.85871	0.5688008
240	100.7252	0.26108441	100.1603	0.57368402
300	100.7971	0.10872586	99.73814	0.56258922
360	100.8145	0.17206563	99.8087	0.43564818

Table B-6: Dissolution data for ketoprofen release from chitosan/Kollidon 5% granule and chitosan/Kollidon 10% granule at pH 7.4 over a period of 6 hours.

Time (min)	Chitosan/Kollidon 5% pH 7.4		Chitosan/Kollidon 10% pH 7.4	
	% Release	Standard deviation	% Release	Standard deviation
0	0	0	0	0
2	46.26603	6.39647092	36.57577	8.57715102
5	64.3037	5.64387294	54.88416	6.2403408
10	79.20361	3.45827802	72.06269	4.87553468
15	87.44129	2.13038943	81.99311	3.15324714
20	91.95368	1.17293527	87.89526	2.2259864
30	95.98495	0.85053723	93.80051	0.70751852
45	98.09963	0.37939846	97.235	0.72678859
60	98.80579	0.43766977	98.70651	0.95781107
120	99.36645	0.43550344	99.02016	0.90586211
180	99.54357	0.35551916	99.1335	1.12266402
240	99.42757	0.23662559	99.33561	0.99169076
300	99.72975	0.20364206	99.43345	1.06864361
360	99.69352	0.26195789	99.47465	1.08029001

Table B-7: Dissolution data for ketoprofen release from chitosan/Kollidon 5%-2-pyrrolidinone/ketoprofen-ethanol granule and chitosan/Kollidon 10%-2-pyrrolidinone granule at pH 7.4 over a period of 6 hours.

Time (min)	Chitosan/Kollidon 5%-2-pyrrolidinone/ketoprofen-ethanol pH 7.4		Chitosan/Kollidon 10%-2-pyrrolidinone pH 7.4	
	% Release	Standard deviation	% Release	Standard deviation
0	0	0	0	0
2	54.2353	11.633863	37.69554	4.01894072
5	73.71952	4.75847559	58.97498	3.81606967
10	86.02464	3.63096365	77.16032	2.71021791
15	90.31639	3.55238972	87.10327	1.86539197
20	92.72463	3.51131756	91.74348	1.2593831
30	93.88528	3.63122325	95.24948	1.0681647
45	94.2404	3.69624677	97.49731	1.43895579
60	94.63909	2.95558919	98.17202	1.39926329
120	94.88309	3.32051681	98.52352	1.40814324
180	95.47408	3.74979266	99.0292	1.46696829
240	95.6571	3.55250817	99.32004	1.34957475
300	95.83324	3.49696982	99.44401	1.25337992
360	96.05289	3.50050077	99.39971	1.16782349

Table B-8: Dissolution data for ketoprofen release from chitosan/Kollidon 10%-freeze dried granule.

Time (min)	Chitosan/Kollidon 10%-freeze dried pH 7.4	
	% Release	Standard deviation
0	0	0
2	38.68522	1.43639707
5	62.80585	3.3204323
10	79.05866	3.24994773
15	87.90307	2.16842159
20	92.68108	1.47034309
30	95.77908	0.50888916
45	96.70753	0.16786914
60	96.87271	0.08789721
120	97.17338	0.16407766
180	97.22962	0.34146771
240	97.46685	0.27911567
300	97.50731	0.27848398
360	97.68215	0.27868931

Bead formulations

The following abbreviations are used:

- Formula A = chitosan bead (CB)
- Formula B = 1% Kollidon® SR/chitosan bead (K1B)
- Formula C = 0.5% Kollidon® SR/chitosan bead (K0.5B)
- Formula D = 0.25% Kollidon® SR/chitosan bead (K0.25B)

Table B-8: p-values for drug loading (Tukey HSD test).

	Tukey HSD test; variable Drugloading (CBouwer1.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = .87669, df = 16.000								
Cell No.	Formule	Tyd	{1} 74.647	{2} 74.717	{3} 77.387	{4} 76.343	{5} 76.745	{6} 63.977	{7} 72.690	{8} 66.050
1	A	30		1.000000	0.039821	0.390374	0.178488	0.000175	0.239696	0.00017
2	A	60	1.000000		0.047330	0.438843	0.206942	0.000175	0.207661	0.00017
3	B	30	0.039821	0.047330		0.860113	0.987717	0 000175	0.000439	0.00017
4	B	60	0.390374	0.438843	0.860113		0.999319	0.000175	0.004029	0.00017
5	C	30	0.178488	0.206942	0.987717	0.999319		0.000175	0.001539	0.00017
6	C	60	0.000175	0.000175	0.000175	0.000175	0 000175		0.000175	0.18825
7	D	30	0.239696	0.207661	0.000439	0.004029	0.001539	0.000175		0.00017
8	D	60	0.000175	0.000175	0.000175	0.000175	0.000175	0.188258	0.000176	

Table B-9: p-values for friability (Tukey HSD test).

	Tukey HSD test; variable Friabilty (CBouwer1.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = .40004, df = 16.000								
Cell No.	Formule	Tyd	{1} 4.6900	{2} 6.9133	{3} 2.2667	{4} 2.7067	{5} 4.8633	{6} 3.6900	{7} 5.3733	{8} 5.496
1	A	30		0.009959	0.004740	0.024425	0.999965	0.547947	0.877163	0.7647
2	A	60	0.009959		0.000175	0.000178	0.019057	0 000394	0.119463	0.1789
3	B	30	0.004740	0.000175		0.986616	0.002543	0.175175	0.000513	0.0003
4	B	60	0.024425	0.000178	0.986616		0.012791	0.566995	0.001984	0.0013
5	C	30	0.999965	0 019057	0.002543	0 012791		0.363858	0.969892	0.9121
6	C	60	0.547947	0.000394	0.175175	0.566995	0.363858		0.072832	0.0468
7	D	30	0.877163	0.119463	0.000513	0.001984	0.969892	0.072832		0.9999
8	D	60	0.764749	0.178922	0.000388	0.001300	0.912195	0 046801	0.999997	

Table B-10: p-values for AUC 60 min (Tukey HSD test).

	Tukey HSD test; variable AUC60min (CBouwer1.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 5885.0, df = 16.000								
Cell No.	Formule	Tyd	{1} 1803.3	{2} 1795.0	{3} 1195.5	{4} 1391.4	{5} 1486.6	{6} 1040.7	{7} 1581.4	{8} 1353.7
1	A	30		1.000000	0.000175	0.000292	0.002419	0.000175	0.043183	0.000206
2	A	60	1.000000		0.000175	0.000324	0.003080	0.000175	0.055251	0.000216
3	B	30	0.000175	0.000175		0.092394	0.005155	0.274001	0.000430	0.252337
4	B	60	0.000292	0.000324	0.092394		0.786574	0.000941	0.109082	0.998361
5	C	30	0.002419	0.003080	0.005155	0.786574		0.000210	0.790100	0.441759
6	C	60	0.000175	0.000175	0.274001	0.000941	0.000210		0.000176	0.002691
7	D	30	0.043183	0.055251	0.000430	0.109082	0.790100	0.000176		0.036089
8	D	60	0.000206	0.000216	0.252337	0.998361	0.441759	0.002691	0.036089	

Table B-11: p-values for AUC 360 min (Tukey HSD test).

	Tukey HSD test; variable Auc360min (CBouwer1.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 7152E2, df = 16.000								
Cell No.	Formule	Tyd	{1} 31781.	{2} 32920.	{3} 32528.	{4} 33602.	{5} 33183.	{6} 31047.	{7} 33409.	{8} 33252.
1	A	30		0.716519	0.951900	0.212303	0.493981	0.955728	0.323613	0.437781
2	A	60	0.716519		0.998880	0.969892	0.999920	0.187828	0.995535	0.999620
3	B	30	0.951900	0.998880		0.768265	0.975844	0.429194	0.895390	0.958978
4	B	60	0.212303	0.969892	0.768265		0.998260	0.031897	0.999990	0.999457
5	C	30	0.493981	0.999920	0.975844	0.998260		0.098255	0.999970	1.000000
6	C	60	0.955728	0.187828	0.429194	0.031897	0.098255		0.054116	0.082088
7	D	30	0.323613	0.995535	0.895390	0.999990	0.999970	0.054116		0.999998
8	D	60	0.437781	0.999620	0.958978	0.999457	1.000000	0.082088	0.999998	

Table B-12: p-values for swelling after 10 min (Tukey HSD test).

	Tukey HSD test; variable min10 (CBouwer2.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00628, df = 32.000								
Cell No.	Formule	Tyd	{1} 2.0678	{2} 2.3652	{3} 1.8788	{4} 1.6585	{5} 2.3162	{6} 1.6498	{7} 2.3608	{8} 1.5615
1	A	30		0.000141	0.005337	0.000138	0.000259	0.000138	0.000143	0.000138
2	A	60	0.000141		0.000138	0.000138	0.958324	0.000138	1.000000	0.000138
3	B	30	0.005337	0.000138		0.000906	0.000138	0.000576	0.000138	0.000138
4	B	60	0.000138	0.000138	0.000906		0.000138	0.999999	0.000138	0.424114
5	C	30	0.000259	0.958324	0.000138	0.000138		0.000138	0.974680	0.000138
6	C	60	0.000138	0.000138	0.000576	0.999999	0.000138		0.000138	0.540995
7	D	30	0.000143	1.000000	0.000138	0.000138	0.974680	0.000138		0.000138
8	D	60	0.000138	0.000138	0.000138	0.424114	0.000138	0.540995	0.000138	

Table B-13: p-values for swelling after 10 min at two different pH values* (*L = pH 5.60, *H = pH 7.40) (Tukey HSD test).

	Tukey HSD test; variable min10 (CBouwer2.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00628, df = 32.000								
Cell No.	Formule	pH	{1} 2.0658	{2} 2.3672	{3} 1.7602	{4} 1.7772	{5} 1.8910	{6} 2.0750	{7} 1.8768	{8} 2.0455
1	A	H		0.000140	0.000140	0.000145	0.011979	0.999999	0.005337	0.999817
2	A	L	0.000140		0.000138	0.000138	0.000138	0.000143	0 000138	0.000138
3	B	H	0.000140	0.000138		0.999945	0.115469	0.000139	0.211503	0.000147
4	B	L	0.000145	0.000138	0.999945		0.236496	0.000141	0.390213	0.000168
5	C	H	0.011979	0.000138	0.115469	0.236496		0.007120	0.999984	0.036171
6	C	L	0.999999	0.000143	0.000139	0.000141	0.007120		0.003150	0.997892
7	D	H	0.005337	0.000138	0.211503	0.390213	0.999984	0.003150		0.016876
8	D	L	0.999817	0.000138	0.000147	0.000168	0 036171	0.997892	0.016876	

Table B-14: p-values for swelling after 60 min (Tukey HSD test).

	Tukey HSD test; variable min60 (CBouwer2.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00769, df = 32.000								
Cell No.	Formule	Tyd	{1} 2.1132	{2} 2.2818	{3} 1.9838	{4} 1.6610	{5} 2.3523	{6} 1.7312	{7} 2.3560	{8} 1.5160
1	A	30		0.040376	0.209994	0.000138	0.001147	0.000138	0.000957	0.000138
2	A	60	0.040376		0.000166	0.000138	0.854036	0.000138	0.819706	0.000138
3	B	30	0.209994	0.000166		0.000143	0.000138	0.000597	0.000138	0.000138
4	B	60	0.000138	0.000138	0.000143		0.000138	0.856968	0.000138	0.114599
5	C	30	0.001147	0.854036	0.000138	0 000138		0.000138	1.000000	0.000138
6	C	60	0.000138	0.000138	0.000597	0.856968	0.000138		0.000138	0.003913
7	D	30	0.000957	0.819706	0.000138	0.000138	1.000000	0.000138		0.000138
8	D	60	0.000138	0.000138	0.000138	0.114599	0.000138	0.003913	0.000138	

Table B-15: p-values for swelling after 60 min at two different pH values* (*L = pH 5.60, *H = pH 7.40) (Tukey HSD test).

	Tukey HSD test; variable min60 (CBouwer2.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00769, df = 32.000								
Cell No.	Formule	pH	{1} 2.0070	{2} 2.3880	{3} 1.7772	{4} 1.8677	{5} 1.9375	{6} 2.1460	{7} 1.8072	{8} 2.0648
1	A	H		0.000138	0.001833	0.143927	0.862736	0.145825	0.008668	0.942057
2	A	L	0.000138		0.000138	0.000138	0.000138	0.000996	0.000138	0.000143
3	B	H	0.001833	0.000138		0.632548	0.059169	0.000138	0.998776	0.000193
4	B	L	0.143927	0.000138	0.632548		0.859868	0.000236	0.927569	0.009943
5	C	H	0.862736	0.000138	0.059169	0.859868		0.005534	0.202509	0.225580
6	C	L	0.145825	0.000996	0.000138	0.000236	0 005534		0.000140	0.745041
7	D	H	0.008668	0.000138	0.998776	0.927569	0.202509	0.000140		0.000481
8	D	L	0.942057	0.000143	0.000193	0.009943	0.225580	0.745041	0.000481	

Table B-16: p-values for swelling after 360 min (Tukey HSD test).

	Tukey HSD test; variable min360 (CBouwer2.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00688, df = 32.000								
Cell No.	Formule	Tyd	{1} 2.1913	{2} 2.3947	{3} 2.3192	{4} 1.9033	{5} 2.4323	{6} 1.9682	{7} 2.4930	{8} 1.7737
1	A	30		0.003955	0.169268	0.000157	0.000544	0.001345	0.000145	0.00013E
2	A	60	0.003955		0.760240	0.000138	0.992740	0.000138	0.464616	0.00013E
3	B	30	0.169268	0.760240		0.000138	0.293115	0.000138	0.019499	0.00013E
4	B	60	0.000157	0.000138	0.000138		0.000138	0.870803	0.000138	0.15713S
5	C	30	0.000544	0.992740	0.293115	0.000138		0.000138	0.904343	0.000138
6	C	60	0.001345	0.000138	0.000138	0.870803	0.000138		0.000138	0.006427
7	D	30	0.000145	0.464616	0.019499	0.000138	0.904343	0.000138		0.000138
8	D	60	0.000138	0.000138	0.000138	0.157139	0.000138	0.006427	0.000138	

Table B-17: p-values for swelling after 360 min at two different pH values* (*L = pH 5.60, *H = pH 7.40) (Tukey HSD test).

	Tukey HSD test; variable min360 (CBouwer2.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00688, df = 32.000								
Cell No.	Formule	pH	{1} 2.0860	{2} 2.5000	{3} 2.0590	{4} 2.1635	{5} 2.0200	{6} 2.3805	{7} 1.9997	{8} 2.2670
1	A	H		0.000138	0.999130	0.736254	0.860374	0.000150	0.622887	0.013365
2	A	L	0.000138		0.000138	0.000138	0.000138	0.233660	0.000138	0.000807
3	B	H	0.999130	0.000138		0.388432	0.991062	0.000139	0.913823	0.003061
4	B	L	0.736254	0.000138	0.388432		0.086430	0.001873	0.032601	0.400373
5	C	H	0.860374	0.000138	0.991062	0.086430		0.000138	0.999865	0.000419
6	C	L	0.000150	0.233660	0.000139	0.001873	0.000138		0.000138	0.289766
7	D	H	0.622887	0.000138	0.913823	0.032601	0.999865	0.000138		0.000213
8	D	L	0.013365	0.000807	0.003061	0.400373	0.000419	0.289766	0.000213	

Granule formulations

The following abbreviations are used:

- Formula A = chitosan granule (CG)
- Formula B = 1% Kollidon/chitosan granule (K1G)
- Formula C = 5% Kollidon/chitosan granule (K5G)
- Formula D = 10% Kollidon/chitosan granule (K10G)
- Formula E = 10% Kollidon/chitosan granule freeze dried (K10Gfd)
- Formula F = 10% Kollidon dissolved in 2-Pyrrolidinone (10KPG)

- Formula G = 5% Kollidon/Pyrrolidinone/ketoprofen/etanol granule (5KPG)

Table B-18: p-values for drug loading of granule formulations (Kruskal-Wallis test).

	Multiple Comparisons p values (2-tailed); Drugloading (CBouwer3.sta) Independent (grouping) variable: Formule Kruskal-Wallis test: H (6, N= 21) =12.67532 p =.0485						
Depend.: Drugloading	A R:19.000	B R:9.0000	C R:11.333	D R:9.3333	E R:16.000	F R:3.0000	G R:9.3333
A		1.000000	1.000000	1.000000	1.000000	0.033340	1.000000
B	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000
C	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000
D	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000
E	1.000000	1.000000	1.000000	1.000000		0.216038	1.000000
F	0.033340	1.000000	1.000000	1.000000	0.216038		1.000000
G	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	

Table B-19: p-values of AUC 60 min of granule formulations (Kruskal-Wallis test).

	Multiple Comparisons p values (2-tailed); Auc60min (CBouwer3.sta) Independent (grouping) variable: Formule Kruskal-Wallis test: H (6, N= 21) =12.64069 p =.0491						
Depend.: Auc60min	A R:8.6667	B R:5.0000	C R:14.667	D R:4.3333	E R:14.667	F R:11.667	G R:18.000
A		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
B	1.000000		1.000000	1.000000	1.000000	1.000000	0.216038
C	1.000000	1.000000		0.869089	1.000000	1.000000	1.000000
D	1.000000	1.000000	0.869089		0.869089	1.000000	0.146666
E	1.000000	1.000000	1.000000	0.869089		1.000000	1.000000
F	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000
G	1.000000	0.216038	1.000000	0.146666	1.000000	1.000000	

Table B-20: p-values of AUC 360 min of granule formulations (Kruskal-Wallis test).

	Multiple Comparisons p values (2-tailed); Auc360min (CBouwer3.sta) Independent (grouping) variable: Formule Kruskal-Wallis test: H (6, N= 21) =14.96104 p =.0206						
Depend.: Auc360min	A R:19.667	B R:13.000	C R:14.667	D R:10.000	E R:4.0000	F R:11.667	G R:4.0000
A		1.000000	1.000000	1.000000	0.041698	1.000000	0.041698
B	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000
C	1.000000	1.000000		1.000000	0.740304	1.000000	0.740304
D	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000
E	0.041698	1.000000	0.740304	1.000000		1.000000	1.000000
F	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000
G	0.041698	1.000000	0.740304	1.000000	1.000000	1.000000	

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