

THE EFFECT OF PHARMACEUTICAL EXCIPIENTS ON RIFAMPICIN RELEASE FROM CHITOSAN BEADS

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'All religions, arts and sciences are branches of the same tree. All these aspirations are directed toward ennobling man's life lifting it from the sphere of mere physical existence and leading the individual towards freedom', *Albert Einstein*

I ONLY FELT READY FOR THIS COURSE
WHEN MY AMBITIONS OVERPOWERED MY
LACK OF MOTIVATION, WHEN MY HOPES
COULD SEE BEYOND THE NARROW END
AND WHEN ALL STARS WENT BEHIND THE
CLOUDS BUT ONE

*This study, this dissertation, the completion of my masters degree and the understanding of the scope of work I have completed, are fulfilled in loving memory of my late younger brother, **Lefa Mohlala** – 'memories of you make a day seem incomplete but the future still worth to living for'.*

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Introduction and Aim of the Study

The need for a healthy lifestyle and health awareness has increased dramatically over the past few years. With improving technology and some ongoing innovations, a better lifestyle becomes even more desirable despite the increasing cost thereof.

In pharmaceutical sciences the need to achieve better therapeutic outcomes, involving minimum cost and energy, is still a goal that lies ahead to be accomplished. Modern innovations into drug technology have to some extent managed to improve therapeutic outcomes, although the problem of cost-effectiveness remains to be tackled. This is because the technology that has been put into this effort has never been adequately explored to reduce the cost of improving therapeutic outcomes.

Research into controlled drug release systems aims at maintaining improved therapeutic outcomes, eliminate the high cost of drug use and reduce drug toxicities. Beads have become an interesting area of research as far as controlled release studies are concerned. However, the physical and chemical nature (physicochemistry) of the polymer plays a major role in determining its efficiency as a controlled release vehicle.

The aim of this study is to prepare and characterise rifampicin loaded chitosan beads and to incorporate several pharmaceutical excipients into the beads to improve the swelling behaviour and consequently rifampicin release from the beads. The study involves the evaluation of the beads based on their morphology, swelling behaviour, drug loading and drug release properties.

By incorporating rifampicin into the beads, with the motivation that *Mycobacterium* species is so notorious to monotherapy, the study aims at achieving pronounced drug

levels in both the gastric and intestinal environment and it involves the following as its main objectives:

- A literature review on polymeric drug delivery systems, with the emphasis on chitosan and beads and their advantages over conventional drug release systems.
- A literature study on the different methods used for the preparation and characterisation of beads.
- To prepare and characterise chitosan beads with a reliable and reproducible method and to investigate the effect of pharmaceutical excipients on the properties of the beads.
- To incorporate rifampicin into selected formulations and to conduct dissolution studies to evaluate rifampicin release from the beads.

The physicochemical properties of chitosan and its pharmaceutical and biotechnological uses are discussed in Chapter 1. Chapter 2 describes different methods that have been used to prepare and characterise beads. In Chapter 3 the preparation and characterisation of rifampicin loaded chitosan beads, containing several pharmaceutical excipients, are discussed while Chapter 4 describes the release of rifampicin from these beads.

Abstract

Controlled release systems aim at achieving a predictable and reproducible drug release over a desired time period. These systems allow reduced dosing frequency, constant drug levels in the blood, increased patient compliance and decreased adverse effects. In a recent study, Chitosan beads, containing *N*-trimethyl Chitosan chloride, have shown a potential in the delivery of rifampicin. However, because of inadequate amounts of rifampicin released over 24 hours, incorporation of other pharmaceutical excipients to increase the swelling behaviour of the beads to improve drug release, was considered in this study.

Chitosan beads were prepared through ionotropic gelation with tripolyphosphate (TPP) as a crosslinking agent. To increase the porosity of the Chitosan beads Explotab[®], Ac-Di-Sol[®] and vitamin C were added individually to Chitosan solutions at concentrations of 0.1, 0.25 and 0.5 % w/v before adding the mixture to the TPP solution. Swelling and morphology studies were used in the evaluation of the different formulations. The swelling and morphology results were then used to select a set of combination and concentrations of two excipients and then prepare and characterise beads containing two combinations. The combination formulations and formulations containing single excipients were then loaded with rifampicin. Pure chitosan beads exhibited a higher drug loading capacity (67.49 %) compared to the lowest loading capacity of 41.61 % exhibited by chitosan beads containing a combination of Explotab[®] and Ac-Di-Sol[®]. For all the other formulations the drug loading capacity ranged within 48 and 63 %.

These formulations were used for dissolution studies over a period of 6 hours at pH 5.60 and 7.40. The dissolution results showed that no chitosan has dissolved at both pH values. A significant amount of rifampicin was, however, released from the beads, especially at pH 7.40. Chitosan beads containing vitamin C also exhibited high rifampicin release (48.34 ± 1.00 %) at pH 5.60 compared to the other formulations and this makes vitamin C a potential excipient for enhanced drug release over a wide pH range (both acidic and alkaline). However, further studies are necessary to

optimise the preparation method to minimise drug loss during loading and to improve the drug loading capacity of the beads.

Key words: Chitosan; rifampicin; ionotropic gelation; tripolyphosphate (TPP); Explotab[®]; Ac-Di-Sol[®]; vitamin C

Uittreksel

Vrystellingsgeregleerde doseervorme word hoofsaaklik gebruik om voorspelbare en herhaalbare geneesmiddelvrystelling oor tyd te verseker. Die voordele van hierdie tipe doseervorme is onder andere konstate plasmavlakke van die geneesmiddel, verbeterde pasiënt samewerking en verminerde newe-effekte. In 'n vorige studie is bevind dat kitosaan krale, wat *N*-trimetiel kitosaan hidrochloried bevat het, groot potensiaal inhou vir die aflewering van rifampisien. Hierdie krale was egter nie in staat om genoegsame rifampisien vry te stel oor 'n periode van 34 uur nie. Die doel van hierdie studie was om, deur die insluiting van verskillende farmaseutiese hulpstowwe, die uitswellingsgedrag van kitosaan krale te verbeter om rifampisien vrystelling uit die krale te verhoog.

Kitosaan krale is berei deur ionotropiese jelering met triplifosfaat as kruisbindingsagent. Explotab[®], Ac-Di-Sol[®] en vitamien C is in konsentrasies van 0.1, 0.25 en 0.5 % m/v in die kitosaanoplossing gesuspendeer of opgelos voor die krale gevorm is. Die uitswellingsgedrag en struktuur van hierdie krale is ondersoek. Op grond van hierdie resultate is verskeie kitosaan krale berei wat 'n kombinasie van die farmaseutiese hulpstowwe bevat het. Rifampisien is in hierdie krale, asook krale wat 'n enkel hulpstof bevat het, geïnkorporeer. Suiwer kitosaan krale het 'n hoër rifampisieninhoud (67.49 %) gehad terwyl krale wat 'n kombinasie van Explotab[®] en Ac-Di-Sol[®] bevat het die laagste rifampisieninhoud (41.61 %) gehad het. Die rifampisieninhoud van die ander formules het gewissel tussen hierdie twee waardes.

Dissolusiestudies by 'n pH van 5.60 en 7.40 is op die krale uitgevoer oor 'n periode van 6 ure. Geen kitosaan het opgelos sedert die dissolusiestudies nie. Goeie rifampisien vrystelling is verkry, veral by 'n pH van 7.40. krale wat vitamien C as hulpstof bevat het, het die grootste rifampisienvrystelling (48.34 ± 1.00 %) by 'n pH van 5.60 vertoon. Uit die dissolusiedata is afgelei dat vitamien C groot potensiaal het vir insluiting as hulpstof in krale met die doel om geneesmiddelvrystelling te verhoog by beide suur en alkalise omgewings. Verdere studies is egter nodig om die

bereidingsmethode van de kralen te optimaliseren om geneesmiddelinsluiting te verhogen.

Sleutelwoorden: Kitosaan; rifampisine; ionotropische jellering; tripolifosfaat; Explotab[®]; Ac-Di-Sol[®]; vitamine C

Uittreksel

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Kitosaan krale is berei deur ionotropiese jelering met triplifosfaat as kruisbindingsagent. Explotab[®], Ac-Di-Sol[®] en vitamien C is in konsentrasies van 0.1, 0.25 en 0.5 % m/v in die kitosaanoplossing gesuspendeer of opgelos voor die krale gevorm is. Die uitswellingsgedrag en struktuur van hierdie krale is ondersoek. Op grond van hierdie resultate is verskeie kitosaan krale berei wat 'n kombinasie van die farmaseutiese hulpstowwe bevat het. Rifampisien is in hierdie krale, asook krale wat 'n enkel hulpstof bevat het, geïnkorporeer. Suiwer kitosaan krale het 'n hoër rifampisieninhoud (67.49 %) gehad terwyl krale wat 'n kombinasie van Explotab[®] en Ac-Di-Sol[®] bevat het die laagste rifampisieninhoud (41.61 %) gehad het. Die rifampisieninhoud van die ander formules het gewissel tussen hierdie twee waardes.

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bereidingsmethode van de kralen te optimaliseren om geneesmiddelinsluiting te verhogen.

Sleutelwoorden: Kitosaan; rifampisinen; ionotropische jellering; tripolifosfaat; Explotab[®]; Ac-Di-Sol[®]; vitamine C

Chapter 1

Chitosan For Enhanced Drug Delivery

1.1 Introduction

Polymers have played a major role in the development of controlled drug release systems. The first polymeric drug delivery systems only incorporated polymers that were commercially available and were approved by the U.S. Food and Drug Administration. The mechanisms by which drugs are released from these polymers and the process of manufacturing such controlled drug delivery devices have been well reviewed in the literature. Extensive research and major efforts are currently being made to improve both the polymers and the manufacturing processes, as well as to apply them to the controlled release of a wide variety of new pharmacological agents. Due to the continued development of controlled release technology, the need has arisen for materials with more specific drug delivery properties. These materials include new biodegradable polymers, polymers with both hydrophilic and hydrophobic characteristics and hydrogels that respond to temperature and pH changes (Dunn & Ottenbrite, 1991:xi).

Natural polymers such as cellulose and starch, as well as chitosan, which has gained considerable attention over the past few years, represent biodegradable and toxicological harmless raw materials of low cost. Especially cellulose and starch derivatives have been used as abundant excipients in various pharmaceutical formulations for many decades (Bernkop-Schnürch, 2000:2). The most important pharmaceutical application of these polymers is in controlled drug delivery. Because of their superior characteristics together with a very safe toxicity profile, these polymers serve as reliable drug carriers for controlled drug delivery (Bernkop-Schnürch, 2000:2).

Chapter 1: Chitosan For Enhanced Drug Delivery

The aim of controlled drug release (CDR) is to maintain a therapeutic plasma level of the drug with narrow fluctuations with a reduction in administration frequency. The advantages of controlled drug delivery systems over conventional dosage forms include the following (Kim, 2000:6):

- Improvement in patient compliance
- Decrease in total drug use
- Reduction in local or systemic side effects
- Minimisation of drug accumulation
- Reduction of potentiation or loss of drug activity during chronic treatment
- Improvement in treatment efficiency
- Improvement in speed of control of medical conditions
- Reduction in plasma drug fluctuation
- Improvement in bioavailability for some drugs
- Improvement in the ability to provide special effects e.g. morning relief of arthritis
- Reduction in cost

In addition to the above mentioned advantages, CDR also eliminates the necessity for multiple injections of some drugs that can be associated with the risks of possible toxic side reactions. CDR is often useful for drugs with short half-life, high systemic toxicity, frequent dosages and expensive drugs.

A number of polymers are employed for this purpose as well as for some other reasons such as their mucoadhesive and good absorption enhancing properties, which makes them useful tools for 'targeted drug delivery'. Targeted drug delivery describes a system in which an environmental factor stimulates drug release or activation. In controlled release the physical and chemical properties of the system are modified to realise the desired drug release kinetics. The two determining factors for this kinetics are biodegradation and the release method (diffusion, osmosis and/or erosion) (Pruzinsky, 1999: <http://www.rpi.edu/dept/materials/COURSES/NANO/bio.htm>).

As mentioned earlier chitosan is a polymer of particular interest and has received renewed attention in the last decade for potential application in drug delivery. This chapter describes the role of chitosan in drug delivery and its chemical and physicochemical properties.

1.2 Chemical Definition and Synthesis of Chitosan

In 1859 Rouget discovered that when chitin was boiled in a concentrated potassium hydroxide solution, a product was obtained that dissolved in dilute iodide and acids, unlike chitin that only stained brown. However, this chitin derivative was formally named 'chitosan' only in 1894 by Hoppe-Seyler. Chitosan possesses structural characteristics similar to glucosaminoglycans with a chemical formula of $(C_6H_{11}O_4N)_n$ (Paul & Sharma, 2000:5).

Chitosan is a linear polysaccharide, which is prepared by (partial) *N*-deacetylation of chitin, an abundant structural polysaccharide in crab and shrimp shells (Figure 1.1). Chitin is a high molecular weight linear polymer of *N*-acetyl-D-glucosamine (*N*-acetyl-2-amino-2-deoxy-D-glucopyranose) units linked by β -(1-4) bonds (Gupta & Ravi-kumar, 2001:639). Chitosan consists of (1-4)-linked 2-acetamido-2-deoxy- β -D-glucopyranose units (Figure 1.2) (Schipper *et al.*, 1999:336).

According to Khan *et al.* (2002:206) chitin with a degree of deacetylation of 75 % or above is generally known as chitosan. However, chitosan is not one specific compound (Romøren *et al.*, 2002:216); the term usually refers to a family of polymers that are characterised by a number of sugar units per molecule, which defines the molecular weight and the degree of deacetylation (Dodane & VIlivalan, 1998:246). The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin,

leaving behind an amino group (-NH_2). Chitosan's versatility depends mainly on these highly chemical reactive amino groups (Khan *et al.*, 2002:206).

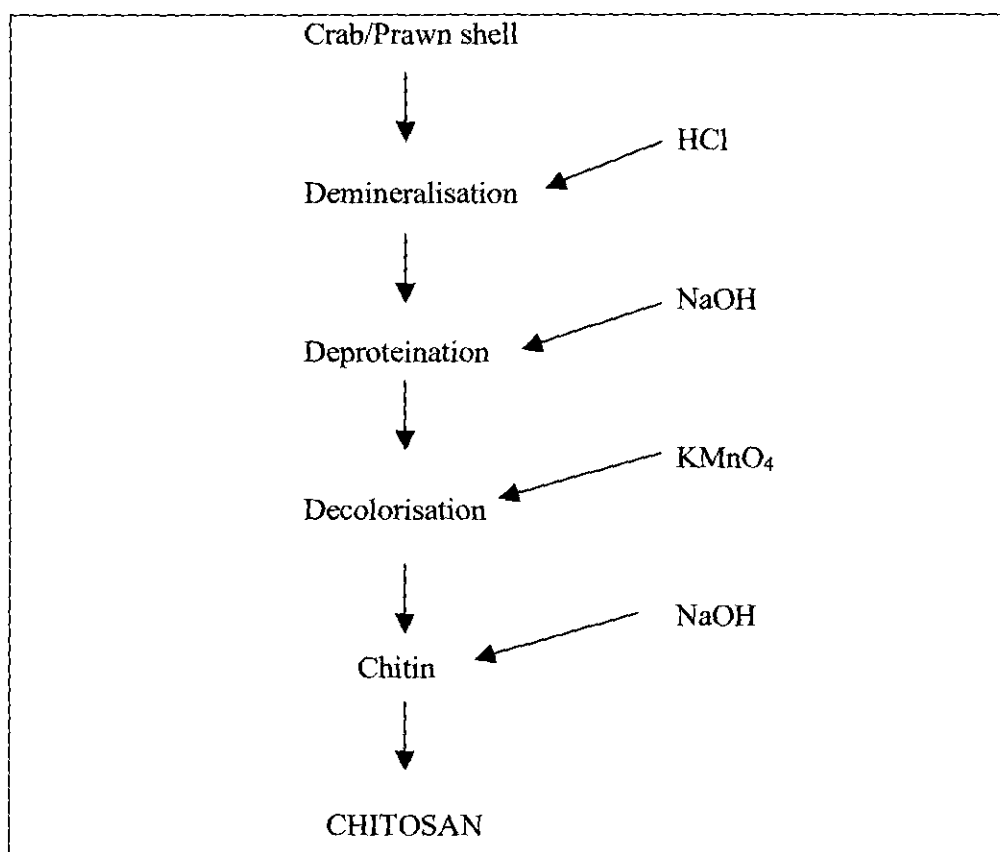


Figure 1.1: Chitosan production flow chart.

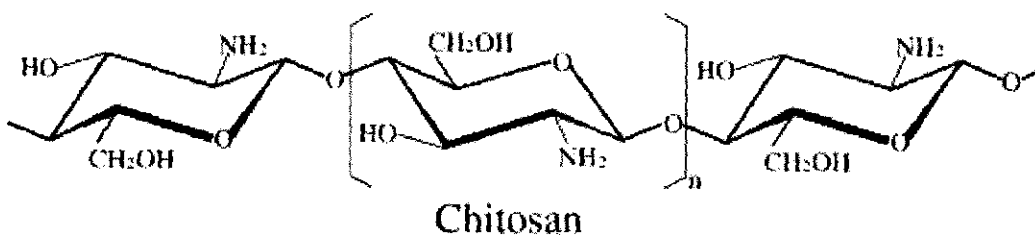


Figure 1.2: Chemical structure of chitosan.

Biomedical grade or purified chitosan is prepared by repeating the deacetylation process. Pharmaceutical grade chitosan is normally deacetylated between 90 and 95 % (Paul &

sharma, 2000:5). It has an apparent pK_a value of 6.5, which according to Liu & Yao (2002:2) renders it the property to aggregate at pH values above 6. It is therefore only soluble in acidic solutions (pH 1 - 6) where most of the amino groups are protonated (Liu & Yao, 2002:2).

1.3 Physicochemical Properties of Chitosan

Because of the increasing availability of commercial products containing chitosan as a component (powders, solutions, gels, films, beads) and due to the diversity of chitosan sources, each of which may have an effect on chitosan's properties, basic and applied research on chitosan will inevitably continue in several fields of applications. Of great importance are the molecular weight and the degree of deacetylation of chitosan, which play a pronounced role in various formulations intended for pharmaceutical and biotechnological applications. A number of studies have already been completed about the effects of chitosan's physicochemical properties on its performance in various applications *e.g.* the effect of molecular weight (MW) of chitosan on the thermal, mechanical and permeability properties of prepared membrane (Chen & Hwa, 1996:353), the effect of MW and degree of deacetylation (DDA) on *in vitro* degradation of chitosan by commercial enzyme preparation (Zhang & Neau, 2001:1653) and the effects of the MW and DDA of chitosan on lipase loaded chitosan beads characteristics (Alsarra *et al.*, 2002:3637).

1.3.1 Molecular weight (MW) and degree of deacetylation (DD)

Several studies have assessed the effects of the molecular weight of chitosan against its degree of deacetylation in the various fields where chitosan has been exploited (Puttipatkhachorn *et al.*, 2001:143; Chen *et al.*, 1996:353; Alsarra *et al.*, 2002:3637). One difficulty with the use of chitosan is that it is commercially available in a wide range of molecular weights and degrees of deacetylation (Alsarra *et al.*, 2002:3638). The DDA is one of the factors that are used to characterise chitosan. The term deacetylation refers,

in context, to the process by which chitosan is prepared from chitin. Functional properties of chitosan such as viscosity, antimicrobial activity immunoadjuvant activity, hypercholestoremic activity, mechanical properties, porosity of membranes, blood coagulation activity and wound healing activity depend on the molecular weight and degree of deacetylation of the chitonous product used (Chen *et al.*, 1997:287). Together with other parameters, the molecular weight of chitosan has been found to have a major effect on its functional properties.

The effects of the MW and DDA of chitosan on the characteristics of lipase-loaded chitosan beads were investigated by Alsarra *et al.* (2002:3638). The study revealed that chitosan with a high MW and DDA resulted in a high loading of the lipase. MW did not have a marked effect on the activity of the enzyme. Release studies revealed that enzyme release increased to a maximum when the bead was manufactured using a low MW and a moderate to high DDA chitosan sample. According to this study, chitosan with a high MW and DDA can thus improve loading and reduce the release of lipase in these beads. The choice of a chitosan sample with a suitable MW and DDA could affect the lipase entrapment efficiency and other desirable characteristics of the hydrogel beads (Alsarra *et al.*, 2002:3644). The DDA is the absolute measure of the amino group content of chitosan. It can be affected by manipulating the chemical process for the synthesis of chitosan. It is therefore essential to characterise chitosan by determining its degree of deacetylation prior to its utilisation at the developmental stage of drug delivery systems (Khan *et al.*, 2002:206).

Snyman *et al.* (2000:145) investigated the relationship between the absolute molecular weight and the degree of quartenisation of a chitosan derivative, *N*-trimethyl chitosan chloride (TMC). The study also established the correlation between the molecular weight and the intrinsic viscosities of the TMC polymers. A decrease in molecular weight, as determined by SEC/MALLS characterisation, with an increase in the degree of quartenisation of the TMC polymers, correlated well with the decrease in the intrinsic viscosities of the TMC polymers. In general, the intrinsic viscosity, as an indication of

the molecular weight, decrease with an increase in the degree of quaternisation of the TMC polymers (Snyman *et al.*, 2002:150).

A number of studies have been performed to evaluate the effect of varying chitosan DDA values on pharmaceutical aspects such as chitosan-drug interactions, drug release behaviour, as well as crystalline characteristics and thermal behaviour of drugs in chitosan systems (Liu *et al.*, 2002:1; Peniche *et al.*, 1998:6549; Liu *et al.*, 2004:243). These investigations are justifiable on the grounds that it is the density of the amino groups that accounts for the reactivity of chitosan in clear terms that have already been defined. For example, in a study conducted by Puttipatkhachorn *et al.* (2001:152) it was found that the DDA affected the solid-state solubility of salicylic acid in chitosan films. The report went further to indicate that as the DDA of chitosan increased the solid-state solubility of salicylic acid increased. Liu *et al.* (2002:2) reported that a high degree of deacetylation and a degree of polymerisation above 50 % is instrumental for chitosan's effect on the opening of the tight junctions of mucosal cells.

The effect of the DDA on the gelation of chitosan and glycerophosphate was investigated by Chenite *et al.* (2000:2157). It was found that chitosans with different degrees of deacetylation exhibited gelation at different temperatures. Apparently, an increase in the DD caused a decrease in gelation temperature. For example, for chitosan which is 70 % deacetylated, gelation occurs around 65 °C while for chitosan which is 95 % deacetylated the gelation temperature has been lowered to around 32 °C.

1.4 Pharmaceutical Applications of Chitosan

1.4.1 Biotechnological applications of chitosan

1.4.1.1 Absorption enhancement

Developments in the last decade demonstrated that chitosan and its derivatives are good candidates for controlled drug delivery. Research into the physicochemical properties of chitosan also described its mechanisms of absorption enhancement at various drug absorption sites in different routes of administration (Smith *et al.*, 2004:43; Kotze *et al.*, 1998:35; Tengamnuay *et al.*, 2000:53).

The ideal absorption enhancer should be non-toxic, effective over a wide pH range and act in a reversible way. Other characteristics like reliability towards site-specific drug release or controlled release may traditionally add to the potential utility of such an ideal absorption enhancing agent (Kotze *et al.*, 1999:343).

In the 1990's chitosan turned out to be a useful excipient in various pharmaceutical formulations. Modifications of certain structural features of chitosan make it feasible to carry out certain tasks in delivery systems focusing on specific pharmaceutical and technological challenges such as absorption enhancement (Bernkop-Schnürch, 2000:1). Because of its positive charge in acidic media, cationic chitosan can interact with the anionic components of the lipoproteins on the surface of epithelial cells. It is also known that the interior of the tight junction channel is hydrated and contains negatively charged sites which favour interaction with protonated chitosan (Kotze *et al.*, 1999:269).

Cationic chitosan can displace cations from electronegative sites on the cell membranes that require cationic co-ordination for dimensional stability. Changes in the concentrations of certain ions in the tight junction pore could result in alteration in tight

junction resistance leading to opening of the pore with increased paracellular permeability. The presence of negative sialic acid residues in mucus also contributes to the paracellular absorption of polypeptide drugs by increasing the mucoadhesivity of chitosan. Chitosan is able to interact with the opening mechanism of the tight junctions as observed by a change in cytoskeletal F-actin from a filamentous to a globular structure (Kotze *et al.*, 1999:270).

According to Liu and Yao (2002:3) only protonated chitosan i.e. in its uncoiled configuration can trigger the opening of the tight junctions, thereby facilitating the paracellular transport of hydrophilic compounds (Thanou *et al.*, 2001:99). This property implies that chitosan itself would be effective as an absorption enhancer only in a limited area of the intestinal tract where the pH values are close to or lower than its pK_a value (Liu & Yao, 2002:3).

1.4.1.2 Mucoadhesive agent

One of the objectives of formulating a dosage form is to ensure drug absorption at the targeted site of absorption over a desired period of time. This necessitates manipulation of the dosage form or the development of a drug carrier system that can effectively deliver the right amount of the drug over a desired period of time. The dosage form should be able to stay in contact with the surface of the absorption site for the time necessary to deliver the desired amount of drug.

By prolonging the residence time of drug carriers at the absorption site, sustained release and improved bioavailability of drugs can be achieved. The bioavailability of drugs has been improved by the use of mucoadhesive dosage forms (Dodane and Vilivalan, 1998:246).

One such example is chitosan, which is not only non-toxic, but also biocompatible. For these reasons it has found a number of applications in drug delivery (Thanou *et al.*, 2001:91). Chitosan has been shown to have good bioadhesive properties (He *et al.*,

1998:75; Lehr *et al.*, 1992:43). It possesses -OH and -NH₂ functional groups that can participate in hydrogen bonding while the polymer chain is flexible to a certain extent. These are both properties essential for mucoadhesion (Romøren *et al.*, 2002:216).

It is therefore acknowledged that chitosan possesses good mucoadhesive properties. This has been demonstrated *in vitro* by Lehr *et al.* (1992:47), who proved that chitosan has better mucoadhesive properties when compared with hydroxypropylcellulose and carboxymethylcellulose.

1.4.1.3 Gene delivery

The need to produce effective and safe drug therapy has resulted in several studies aimed at improving the bioavailability of new therapeutic agents with a minimum of side effects. This promotes current research to develop safe and effective means of gene delivery, the current focus on gene therapy in pharmaceuticals and medicine.

In the past several years, gene therapy has received significant attention for its potential in the replacement of dysfunctional genes and treatment of acquired diseases. Gene therapy refers to the transmission of DNA, encoding a therapeutic gene of interest in the targeted cells or organs with consequent expression of the transgene. The central problem of gene therapy lies in the development of a safe and effective gene transfection system (Liu & Yao, 2002:1).

Several studies implicated chitosan as a potential vehicle for gene delivery. The chemical nature of chitosan and its derivatives prompted thorough investigations into chitosan aided gene delivery. According to Liu & Yao (2002:1) viral and non-viral methods are some of the techniques that were recently developed for the introduction of DNA into cells. Viral vectors were reported by Liu & Yao (2002:1) as being effective in terms of transfection efficiency, but they exhibited fatal drawbacks such as immune responses and oncogenic effects when used *in vivo*. Non-viral vector delivery systems have been

increasingly produced as alternatives to viral vectors because of their safety, stability and ability to be produced in large quantities (Simon *et al.*, 1999:233).

Promising results were reported in the formation of complexes between chitosan and DNA (Park *et al.*, 2001:349; Sato *et al.*, 1996:725). Although chitosan increases transformation efficiency (Lee *et al.*, 1998:218; Li *et al.*, 2002:343), the addition of appropriate ligands to the DNA-chitosan complex seems to achieve more efficient gene delivery via receptor-mediated endocytosis (Park *et al.*, 2001:354; Lee *et al.*, 1998:218). Furthermore, incubation of cells with chitosan demonstrated a low cytotoxic activity (Lee *et al.*, 1998:218; Thanou *et al.*, 2000:20). These results suggest that chitosan has comparable efficiency without the associated toxicity of some of the other synthetic vectors and can therefore be an effective gene delivery system *in vivo* (Dodane *et al.*, 1998:249).

The accumulated information about the physicochemical and biological properties of chitosan has led to the recognition of this cationic polysaccharide as a promising and versatile non-viral vector for gene transfection. Chitosan-based gene delivery systems comprise a number of non-viral vectors that are currently extensively investigated (Liu & Yao, 2002:9). Some of these chitosan-derived vectors for gene delivery have been investigated are described below.

1.4.1.3.1 Deoxycholic acid modified-chitosan vectors

Deoxycholic acid is a main component of bile acid, which is biologically the most detergent-like molecule in the body. Since bile acid can assemble in water, the deoxycholic acid-modification of chitosan also self-dissociates to form micelles with a mean diameter of 160 nm. Hydrophobically modified chitosan by deoxycholic acid provides colloidally stable self-aggregates in aqueous media. These self-aggregate/DNA complexes are considered to be useful for the transfer of genes into mammalian cells *in vitro* and served as a good delivery system composed of biodegradable polymeric materials (Lee *et al.*, 1998:218). Complexes of plasmid DNA and this type of chemically

modified chitosan enhanced transfection efficiency as compared to pure DNA (Liu & Yao, 2002:5).

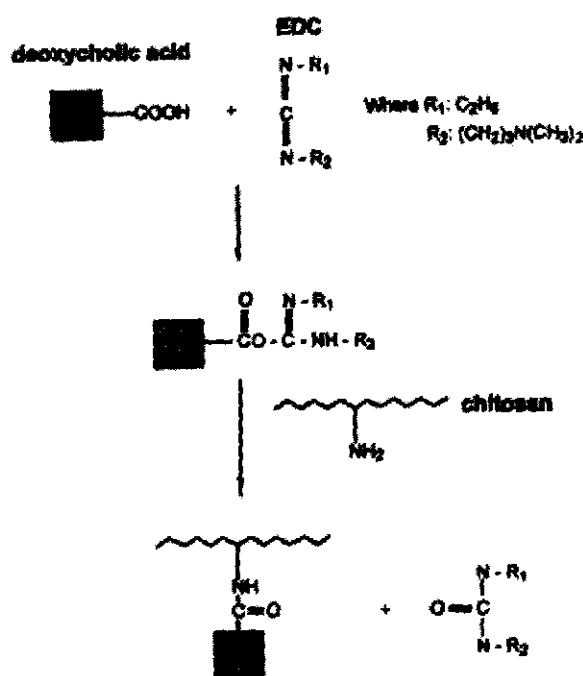


Figure 1.3: A scheme of the coupling mechanism between chitosan and deoxycholic acid using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) through amide linkage formation (Liu & Yao, 2002:5).

1.4.1.3.2 Dodecylated chitosan vectors

An *N*-dodecylated chitosan (CS-12) was synthesised from dodecyl bromide and chitosan. DNA/CS-12 complexes were prepared by assembling DNA to the modified chitosan vector forming a pectrolyte complex. Incorporation of dodecylated chitosan can enhance the thermal stability of DNA. Pure DNA in the absence of dodecylated chitosan is hydrolysed by DNase and has been broken into fragments (Liu & Yao, 2002:6).

1.4.1.3.3 Quaternised chitosan vectors

Modification of chitosan into quaternised forms eliminates the disadvantage of its insolubility at physiological pH values (Liu & Yao, 1998:6). Quaternised chitosan oligomers spontaneously formed complexes with plasmid DNA which is an indication of their stronger binding to DNA than unmodified chitosan (Thanou *et al.*, 2002:156). Quaternised chitosan oligomers with a degree of quaternisation of 40 % (TMO-40) and 50 % (TMO-50)-plasmid DNA complexes raised the transfection efficiency 2 – 4 times compared to control values (Liu *et al.*, 1998:7).

1.4.1.3.4 Transferrin-Knob protein conjugated chitosan vectors

Knob protein is a protein synthesised by the malaria parasite *Plasmodium falciparum* to induce protrusions on membranes of host erythrocytes, which are essential for the survival of the parasite in the host (Kilejian *et al.*, 1986:7938).

Transferrin receptors responsible for iron import to the cells are found on many mammalian cells. As a ligand, transferrin could efficiently transfer small molecular weight drugs, nonbioactive macromolecules and liposomes through a receptor-mediated endocytosis mechanism. Knob (C-terminal globular domain of the fiber protein) was conjugated to chitosan by disulfide linkages to enhance transfection efficiency of pRE-luciferase, which was used as a model plasmid. This knob conjugation to chitosan nanoparticles improved gene expression in HeLa cells by 130-fold (Liu *et al.*, 2002:8).

1.4.1.3.5 Galactosylated chitosan vectors

It has been known that animal cells have lectin-like receptors on their cell membranes which can bind to galactose/*N*-acetylgalactosamine. Since polygalactosamine has a primary amine on the C-2 position of galactose, the hydrochloride salt of the polygalactosamine can bind to polyanionic DNA. For these reasons, formation of DNA complexes with polygalactosamine would be an effective method to obtain cell-specific DNA complexes (Sato *et al.*, 1996:725).

Preparation of a galactosylated chitosan takes place when galactose groups are chemically bound to chitosan. When intended for liver-targeted delivery, this system could efficiently transfect Chang liver cells expressing asialoglycoprotein receptor (ASGR) which specifically recognises the galactose ligands on chitosan (Liu & Yao, 2002:8).

1.4.1.4 Controlled drug release

The ability to formulate chitosan into different dosage forms has made it an important polymer in drug delivery. By manipulating its chemical structure and exploiting its physicochemical properties, chitosan has been used in biotechnological and pharmaceutical applications for more than one purpose. However, its use in controlled drug delivery has gained considerable attention in recent years and is probably one of its most promising pharmaceutical applications. The desire to transport drugs to targeted sites in the body and to eliminate unwanted effects frequently encountered with conventional formulations, has lead to more focus on research in the use of biodegradable biopolymers for both rapid and sustained drug delivery. However, the biggest focus on the use of biopolymers is for targeted drug delivery (TDD) or site-specific drug delivery.

Sustained periodontal delivery of ipriflavone was achieved by Perugini *et al.* (2003:8) using a new chitosan/poly (D,L-lactide-co-glycolide (PLGA) film delivery system for this lipophilic drug. The *in vitro* release profile of the drug, paclitaxel, from chitosan-based films demonstrated release over 20 days (Perugini *et al.*, 2003:7). Chitosan has also been reported to be valuable in colonic drug delivery, for example, as enteric-coated chitosan capsules to enhance the uptake of insulin. Colon-specific drug delivery of 5-aminosalicylic acid for 2,4,6-trinitrobenzene sulfonic acid sodium salt (TNBS)-induced colitis in rats was studied by Tozaki *et al.* (2002:51) using chitosan capsules. The study revealed that after oral administration of chitosan capsules containing 5-aminosalicylic acid (5-ASA), the concentrations of 5-ASA in the large intestinal mucosa were higher compared to those in a carboxymethylcellulose suspension.

It is the mucoadhesive and absorption enhancing properties of chitosan and its derivatives that make them excellent excipients in various pharmaceutical formulations as well as in controlled and targeted drug delivery. It is from this background that chitosan finds various applications in drug delivery systems and for enhancement of drug delivery in various routes of administration.

1.4.2 Chitosan as a drug carrier

Both the physical and chemical properties of chitosan render it useful and compatible in several dosage forms. Some of these dosage forms and the role of chitosan in them are reviewed in the next sections.

1.4.2.1 Beads

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).

Beads are spherical gel pellets consisting of a biocompatible polymer cross-linked with cross-linking agents and loaded with a drug of choice (Bodmeier & Pramar, 1989:1475; Mi *et al.*, 2002:61) ranging in size between 0.8 mm and 1.5 mm (Shu & Zhu, 2000:53). Beads show ample potential for the formulation of controlled release dosage forms (CRDF) over conventional single unit dosage forms (Lubbe, 2002:vi). Chitosan, a polysaccharide with structural characteristics similar to glycosamino glycan, was studied for various biomedical applications including amongst others as an excipient in drug delivery systems such as (Paul & Sharma, 2000:6).

Chitosan is used in pharmaceutical preparations due to its availability, good biocompatibility and other desirable characteristics. It is inexpensive and indigestible, which makes it a promising vehicle for the development of drug delivery systems.

It was possible to prepare chitosan beads for the controlled delivery of salmon calcitonin (Aydin & Akbuga, 1996:101). A number of studies have been conducted to study chitosan beads as potential system for controlled drug delivery (Gupta & Ravi-Krumar, 2000:1115; Gupta *et al.*, 2001:639; Mi *et al.*, 2002:61). The pH-sensitivity of chitosan beads crosslinked with different crosslinking agents renders the beads their versatility for mucosal delivery of a wide range of pharmacological agents. Salty environments appeared to weaken the interaction of chitosan citrate cross-linked chitosan beads and thus resulting in accelerated drug release (Shu & Zhu, 2002:224).

Oven and freeze-dried chitosan beads containing rifampicin were prepared and characterised by Lubbe (2000:55). The beads exhibited significant drug loading capacity with oven dried beads being less porous than the freeze-dried beads. Although the beads exhibited very slow release profiles, they showed potential for sustained release of rifampicin. Drug release from the beads was however pH dependent with more drug being released at pH 7.40 compared to pH 6.40.

1.4.2.2 Hydrogels

There is evidence in literature documenting the delivery of hydrophobic drugs from hydrogels. This is despite the fact that an increase in hydrophobicity improves drug transport across the buccal mucosa (Hansen *et al.*, 1992:237) and also that a hydrogel platform may offer a means of sustaining drug delivery (Martin *et al.*, 2003:35). According to Martin *et al* (2003:35) the lack of hydrogel drug delivery devices for hydrophobic drugs could be due to inadequate attention paid to the incorporation of relatively hydrophobic drugs into hydrogels and only four such studies could be found in the literature namely 1) the loading of vephylline (a xanthine bronchodilator) in both poly (malic acid polyethylene glycol) gels and hydrophobised polyethylene glycol based

hydrogels (Belcheva *et al.*, 1995:43), 2) the incorporation of ibuprofen into poly (*N*-isopropylacrylamide) based gels (Lowe *et al.*, 1999:1031), 3) the incorporation of progesterone into poly (*N*-isopropylacrylamide) based gels (Yu & Grainger, 1995:117) and 4) the incorporation of cyclosporine in polyvinyl pyrrolidone-polyhydroxyethylmethacrylate hydrogels (Gallardo *et al.*, 2001:1).

A viscous solution of a photocrosslinkable chitosan solution has been reported to be easily crosslinked upon light (UV-) irradiation, resulting in an insoluble hydrogel. This chitosan hydrogel is a strong tissue-adhesive and, when compared with fibrin glue, is more effective in sealing air leakages from pinholes on isolated small intestine and aorta, as well as from incisions on isolated trachea (Ono *et al.*, 2001:848). Furthermore, this chitosan hydrogel has been found to induce wound contraction and healing. It has been shown that the application of chitosan hydrogels into open wounds induces significant wound contraction, thereby accelerating wound closure and the healing process (Ishihara *et al.*, 2002:833; Ishihara *et al.*, 2001:513). In addition, chitosan hydrogels showed the ability of controlled release of various growth factors thereby acting as novel carriers to induce neovascularisation *in vivo* (Ishihara *et al.*, 2003:248). A study conducted by Obara *et al.* (2003:3442) showed that fibroblast growth factor-2 (FGF-2) molecules incorporated into a chitosan hydrogel gradually release upon biodegradation of the hydrogel itself and that the FGF-2 incorporated molecules show a substantial effect to improve wound healing in mutant diabetic mice.

The antineoplastic agent paclitaxel, a hydrophobic molecule that is poorly soluble in water, has been incorporated into a thermosensitive chitosan-based hydrogel to obtain sustained release. This experiment showed that one intratumoral injection of the thermosensitive hydrogel containing paclitaxel was as effective as four intravenous injections of Taxol® (paclitaxel containing injection) in inhibiting the growth of EMT-6 cancer cells in mice, but in a less toxic manner. Histological analysis revealed that the proportion of necrotic area was similar for the chitosan/ β -glycerophosphate6/paclitaxel hydrogel and the Taxol®-treated tumors, a disparity between tumour-associated inflammatory cell populations may suggest different anti-tumor mechanisms (Ruel-

Gariépy *et al.*, 2003:53). Chitosan was also found to inactivate macrophages *in vivo* and suspended Meth-A tumor growth in Balb/c mice (Nishimura *et al.*, 1984:93).

1.4.2.3 Tablets

Studies on the behaviour of chitosan when subjected to various pH environments revealed its potential to be employed in tablet formulations. Chitosan has been studied mainly as a diluent or filler in tablet manufacturing. However, it has also been studied as an adhesive, a disintegrating agent and as an anti-adherent (Nigai *et al.*, 1984:21). An immediate release formulation was obtained when chitosan was used as a pharmaceutical excipient for directly compressed tablets (Miyazaki *et al.*, 1990:95). Bilayered tablets prepared by direct compression of chitosan/drug mixtures required no additional conventional compression excipients, due to the excellent suitability of chitosan in direct tableting processes and its binding and lubricating properties (Remuñán-Lopez *et al.*, 1998:147). A study by Inouye *et al* (1988:165), demonstrated sustained release of prednisolone from directly compressed chitosan tablets.

Giunchedi *et al.* (2002:235) formulated chlorhexidine buccal tablets by using drug-loaded chitosan microspheres. In this study the loading of chlorhexidine into chitosan microparticles lead to a remarkable improvement of its dissolution rate. The microbial killing of *C. albicans* was also increased with this formulation due to the antimicrobial effect of the polymer itself. The incorporation of tetracycline into tablets made from spray-dried chitosans was aimed at alleviating the unwanted gastric effects of tetracycline. This study concluded that tablets containing spray-dried chitosans were less friable and exhibited higher crushing strength (Rege *et al.* 2003:56).

1.4.2.4 Films

There is a tendency to replace plastic materials with films, which according to Doi *et al.* (1998:330), have become a serious problem because of pollution and the difficulty of the

disposal of plastics. According to Srinivasa *et al.* (2003:79) biopolymer films are generally prepared by using biological material such as polysaccharides and proteins and their derivatives which are naturally and abundantly available. Natural biopolymeric films have the advantage over synthetic biopolymers since they are totally biodegradable and are derived from natural raw materials. They can be used effectively as an alternative to synthetic plastics (Srinivasa *et al.*, 2003:79). Biopolymers also have desirable mechanical and barrier properties (Guilbert *et al.*, 1996:10).

In general, chitosan films are used in the separation of ethanol from water by evaporation (Lee, 1993:277), water purification (Muzzarelli *et al.*, 1989:293) and the controlled release of pharmaceutical compounds (Bovin & Bertorello, 1993:375). However, chitosan films have limited applications as packaging material. Conventional drug formulation for the mouth, such as toothpaste and mouthwashes have very low penetration of the drugs intended for periodontal drug delivery (Perugini *et al.*, 2002:1). Chitosan films were found to be a suitable dosage form to deliver drugs into the periodontal pocket. Moreover, the use of biodegradable films can increase patient compliance, as the inserted film does not need to be removed (Perugini *et al.*, 2003:2).

Chitosan-based films were also investigated by Amorim *et al.* (2003:35) as a support for lipase immobilisation. In this study, *C. cylindraceae* lipase was successfully immobilised on films of chitosan obtained from the mycelia of *S. racemosum* and from a crustacean source using glutaraldehyde as a bifunctional agent. The lipase activity dramatically dropped to about 45 % of the initial activity for the second use and remained stable until the fourth use in both preparations. Carneiro-da-Cuhna *et al.* (1999:403) also reported similar results for the residual activity of immobilised lipase in a chitosan film from a crustacean source, where the lipase activity was $12.0 \mu\text{mol s}^{-1} \text{m}^2$ after the fourth use, which represented 5 % of the initial activity ($245.0 \mu\text{mol s}^{-1} \text{m}^2$). From these studies it is clear that chitosan films prove to offer the advantage of combining chitosan's mucoadhesive properties with its prolonged release properties for several drug agents. Khan *et al.* (2000:307) was able to show that chitosan films prepared by using lactic acid

conformed to the general properties of films by exhibiting the following characteristics namely adequate softness, flexibility, pliability and bioadhesivity.

1.4.2.5 Microparticulate delivery systems (Microparticles or Microspheres)

Microparticulate drug delivery is one of the concepts that has been studied extensively and employed in drug delivery for various biomedical purposes. According to the laws of physics and chemistry, reduced particle size enhances chemical or physiological reactions. Microparticulate drug delivery systems have shown several advantages over other dosage forms, such as more uniform gastrointestinal transit, less variability and a smaller risk of dose dumping (Steenkamp, 2002:xi).

Microparticles have found a wide application in drug delivery and in controlled or targeted drug release systems. Today microparticle technology is an established technique that is used to deliver several types of drugs, including antigens, by injection or oral administration (e.g. steroids, proteins and antibiotics) (Steenkamp, 2002:1). Microspheres can be prepared with controlled particle sizes using different techniques and can be modified appropriately for the oral, nasal and parenteral delivery of drugs (Paul *et al.*, 2000:10).

Biocompatible polymers are predominantly favoured over small molecules to prepare microspheres (Steenkamp, 2002:1). Microspheres are ultrafine particulate formulations in the size ranging from 50 nm to 2 mm (Ravi-Kumar, 2000:17). Chitosan microspheres have been prepared and studied for various applications. According to Paul & Sharma (2000:10) most of the studies conducted on chitosan microspheres or granules involved oral delivery systems. A number of tests have already been done on chitosan microspheres to evaluate their feasibility as formulations for several therapeutic applications. These tests include the release of antibiotics, antihypertensive agents, cancer agents, etc (Paul & sharma, 2000:10).

Drug administration in any selected route of administration also necessitates consideration of the suitability of the kind of formulation used and the kind of drug delivery system employed. For example, according to Van der Lubben *et al.* (2001:687), oral vaccination suffers from the disadvantage of degradation of the vaccine in the gut and low uptake in the lymphoid tissue of the gastrointestinal tract (GIT). These problems can however be avoided by associating the vaccines with microparticles. Associating drugs or vaccines to microparticulate carrier systems may provide the following advantages:

- prolong release times,
- prevent degradation,
- target particular sites in the body and
- enable patients to take their medication less frequently, which might improve patient compliance (Van der Lubben *et al.*, 2001:687).

Several studies showed that chitosan can be used to prepare microparticles for different purposes. Lorenzo-Lamosa *et al.* (1998:109) had prepared chitosan microparticles with a prolonged release time for colonic drug delivery. Liu *et al.* (1997:65) used chitosan microparticles for the delivery of interleukin-2 in tumour immunotherapy. Remuñán-López *et al.* (1998:49) developed new chitosan-cellulose multicores microparticles for controlled drug delivery. In this study the results showed that the entrapment efficiency for sodium diclofenac by the microparticles was very high irrespective of the processing conditions. These microparticles were also stable at low pH and thereby making them suitable for oral delivery without any harmful crosslinking treatment.

It is from this background that chitosan microparticles now find wide applications in drug delivery systems. Enhancement of the systemic immune response against diphtheria after oral vaccination with chitosan-based microparticles has also been described by Van der Lubben *et al.* (2003:1406). This is a good example of the potential multipurpose application of chitosan-based microparticulate drug delivery systems.

1.5 Mucosal Administration Routes for Chitosan Drug Delivery Systems

The bioavailability of a drug and its therapeutic effectiveness are often influenced by the route selected for administration. For a medication to achieve its maximal efficacy, a drug should be able to be administered easily so that better patient compliance can be achieved; and it should be capable of being absorbed efficiently so that greater bioavailability can be accomplished (Chien *et al.*, 1989:1)

Most therapeutic peptide and protein drugs are poorly absorbed through biological membranes even upon formulation with penetration enhancers, possibly due to a combination of several factors including large molecular size (i.e., ≥ 1000 g/mol), ionisation, high surface charge, enzymatic and chemical instability and the low permeability of absorption barriers in the body. This raises questions regarding methods of delivery for drug compounds to their sites of action in the body. In some instances, drug dosimetry is increased by orders of magnitude to achieve the minimum systemic concentrations required for efficacy. In other cases the drug product is formulated with exotic absorption promoters with some toxicological liabilities to improve permeability across the absorption barrier. However, all these factors should be taken into consideration when a specific route of administration is selected in the formulation process of novel drug delivery systems.

Chitosan, a mucopolysaccharide of marine origin, has been claimed to act as both a bioadhesive and permeabiliser, making it a candidate compound for mucosal drug delivery (Senel *et al.*, 2000:2067).

1.5.1 The Oral Route

The oral route of drug administration is the most frequently used, simple, comfortable, convenient and physiological way of administering traditional drugs (Paul & Sharma,

2000:7). Some drugs are poorly absorbed across mucosal membranes due to their hydrophilic nature and molecular weight such as peptide drugs. Some of these compounds represent a class of valuable therapeutics that are still administered parenterally (Thanou *et al.*, 2001:92).

Chitosan has been thoroughly investigated as a potential oral delivery vehicle. An extensive review has been published by Paul & Sharma (2000:7). The different barriers encountered within the GI-tract, however, harm the efficacy of most oral formulations. In general, they can be divided into the absorption and the enzymatic barrier, which are mainly responsible for the low bioavailability of several orally administered drugs. Because of its permeation enhancing effect, enzyme inhibitory capabilities and mucoadhesive properties, chitosan and its derivatives are able to reduce both barriers, which make these polymers important excipients for peroral drug delivery (Bernkop-Schnürch, 2000:2).

1.5.2 The Buccal Route

The transmucosal routes of drug delivery (i.e. the mucosal linings of the nasal, rectal, vaginal and oral cavity) offer distinct advantages over peroral administration for systemic drug delivery. Within the oral mucosal cavity, drug delivery is classified into three categories (i.e. buccal, sublingual and local drug delivery). The buccal route offers an attractive route of administration for systemic drug delivery (Shojaei, 1998:15).

According to Martin *et al.* (2003:35), the buccal route has been advocated as a possible route of administration of drugs that undergo extensive hepatic first pass metabolism or which are susceptible to degradation in the gastrointestinal tract. This route is well vascularised with venous blood draining the buccal mucosa, reaching the heart directly via the jugular vein. Various advantages have been elucidated between the buccal and the sublingual route. Limitations to the use of the buccal route also saw extensive technological research in enhancing drug absorption through this route despite the reflected limitations. It is evident that the buccal route is considerably less permeable

than the sublingual area, and is generally not able to provide the rapid absorption and good bioavailabilities seen with sublingual administration (Shojaei, 1998:18). Although drug fluxes via this route are inferior to those obtained via the sublingual mucosa, due to a permeability barrier (Harris & Robinson, 1992:1), the relative immobility, when compared to the sublingual route, of the buccal musculature makes this route ideally suited for mucoadhesive sustained release dosage forms (Shojaei *et al.*, 1998:15; Harris & Robinson, 1992:1). Furthermore, the sublingual route is not suited for transmucosal delivery systems. This is because this route lacks an expanse of smooth muscle or immobile mucosa and is constantly washed by a considerable amount of saliva making it difficult for device placement (Shojaei, 1998:19).

In general drug fluxes across the buccal mucosa are poor and typical peak plasma levels for peptide drugs of between 0.0004 % (Bhatt & Johnson, 1997:272) and 0.05 % (Hoogstraate *et al.*, 1996:84) of the administered dose have been obtained even in the presence of penetration enhancers. Peak levels of 0.1 % of the administered dose have been obtained for morphine (Stanley *et al.*, 1997:163). The lack of dosage form retention of the buccal route and its suitability for sustained delivery applications prompted the employment of bioadhesive polymers, including chitosan, in buccal drug delivery systems (Shojaei, 1998:22). Chitosan has also been reported to enhance the permeability of the buccal mucosa (Senel *et al.*, 2000:2070).

The mucoadhesive and absorption enhancing mechanisms discussed elsewhere (1.4.1.1 and 1.4.1.2) are a basic principle of chitosan's suitability for transmucosal drug delivery. Together with this, the biocompatibility of chitosan may make it a drug carrier of choice for sustained drug delivery via the buccal route.

1.5.3 The Nasal Route

As an alternative route therapy, nasal delivery seems beneficial for the administration of several classes of drugs such as protein and peptide drugs because of faster absorption, faster onset of action and lack of enzymatic activity. Compared with oral or subcutaneous

administration, nasal administration can enhance bioavailability and improve safety and efficacy. This painless, user-friendly route of administration also eliminates first-pass metabolism and has fewer side effects. Chitosan effectively enhances the absorption of hydrophilic drugs (such as proteins and peptide drugs) across nasal epithelia (Paul & Sharma, 2000:9). For example, nasal administration of insulin with chitosan has led to reduced glucose levels in rats (Illum *et al.*, 1994:1186).

1.6 Conclusion

Several studies have demonstrated to a great extent that chitosan is a good candidate for controlled drug delivery. This polymer offers added effects besides most of the purposes for which it is intended. The mucoadhesive properties of chitosan lead to wide applicability in many dosage formulations. The chemical structure of chitosan also added to its suitability for drug delivery. Its ability to open tight junctions and to adhere to mucosal membranes makes it a suitable candidate for both sustained and targeted drug delivery as a drug carrier as well as an absorption enhancer. Improved delivery of several drugs is possible by using chitosan and its derivatives in novel drug delivery systems.

Chapter 2

Beads For Controlled Drug Delivery: Preparation and Characterisation

2.1 Introduction

The development of a new drug involves more than the synthesis of the substance that has a particular effect on the body. It must also be considered how to deliver the drug to the appropriate part of the body and once there, how to make it available for absorption and distribution. This is not a trivial issue; in some cases the development of an appropriate delivery system can be as complex as the development of the drug itself (Thrash T., 1999: <http://www.denison.edu/chem/DCS/journal>).

According to Langer (1993:537) most controlled release systems fall into one of three categories namely release by diffusion, solvent activated release or release by polymeric degradation. Although a number of controlled release systems have been developed in recent years, there is still a big need to optimise drug activity by increasing its bioavailability at the absorption site (Thrash T., 1999: <http://www.denison.edu/chem/DCS/journal>).

The application of polymers in controlled drug release systems have become important in the pharmaceutical industry. As discussed in Chapter 1 polymers have several advantages rendering them suitable for controlled drug delivery. Especially beads have become important as a microparticulate drug delivery system. The advantages of beads in drug delivery have been reviewed in Chapter 1. In this chapter the different methods of preparation and characterisation of beads for drug delivery are described..

2.2 Preparation of Beads

Bead formation can be accomplished employing various technological methods, which may employ different materials. Some of the following methods, which have been used to prepare beads, have also been reviewed by Lubbe *et al.* (2002:3-11).

2.2.1 Ionotropic gelation method

The ionotropic gelation method used for the preparation of beads is one of the most trusted methods that is widely used and has been described by Bodmeier & Pramar (1989:1478). This method is based on the principle of interaction between two opposite charges. This can be depicted by the interaction between polycationic chitosan and compounds like tripolyphosphate (TPP), sodium sulfate and sodium citrate. A solution of the polymer can be prepared with a drug loaded into it. The solution or dispersion, of the polymer and the drug, are dropped through a disposable syringe into a gently agitated TPP solution. The bead forms on contact of the solution or dispersion with the TPP solution, entrapping the drug within a three-dimensional network of ionically linked polymer (Lubbe *et al.*, 2002: 3-5).

2.2.2 Emulsification ionotropic gelation method

This method involves ionotropic gelation technology and therefore several crosslinking agents have been employed in the process. According to Poncelet *et al.* (1999a:171) the emulsification-internal ionotropic gelation is an alternative method to encapsulation and extrusion technologies when a large bead size distribution has to be avoided. The method allows encapsulation of a bioactive substance in small beads. Chan *et al.* (2002:259) explored the emulsification-internal ionotropic gelation method to prepare microspheres. The method may provide an alternative method to produce discrete microspheres with the use of less calcium salts (Chan *et al.*, 2002:260).

2.2.3 Extrusion spheronisation technique

The extrusion spheronisation technique is gaining popularity and increasing acceptance in the pharmaceutical industry. The number of excipients available for successful spheronisation by this technique are, however, limited (Goskonda & Uadrashta, 1993:916). Based on literature evidence, chitosan is a potentially useful excipient and has shown increasing value in this technology.

The method of extrusion spheronisation has also gained attention as a pelletisation technique, largely because it is a simple and fast process. It has processing advantages with high dose drugs especially when compared to other pelletisation techniques such as layering, spray congealing, cryopelletisation and encapsulation (Neau *et al.*, 2000:130). The main processing steps are:

- Dry powder blending
- Wet mixing
- Extrusion of the wet mass
- Spheronisation of the cylindrical extrudate.

In a study conducted by Krogars *et al.* (2000:187) pellets containing 30 % ibuprofen were prepared by blending the dry substance in a high shear mixer for 1 minute. EudragitTM S 100 (methacrylic acid, methylmethacrylate) in ethanol (60 %) was used as a binder solution. The mass was extruded after granulation. The resulting pellets were dried on trays as a monolayer at room temperature (21 ± 2 °C). It is also possible to produce sustained release beads without coating by using the extrusion spheronisation technique (Goskonda & Uadrashta, 1994:89).

2.2.4 Melt solidification technique

The melt solidification technique was used by Maheshwari *et al.* (2003:57) and Pradkar *et al.*, (2003:34) for the preparation of ibuprofen-cetylalcohol beads and for the preparation of ibuprofen beads, respectively. The drug or drug complex was melted and stirred on a water bath maintained at 80 °C to form a uniform molten mass. The melt was then poured into 1000 ml deionised water. The system was then stirred continuously using a constant speed stirrer with a propeller blade to obtain beads. The beads formed in this process were separated by filtration and dried at room temperature.

2.2.5 Electric dispersion of polymer solutions

Another method that uses electrostatic potential to prepare beads has been described by Poncelet *et al.* (1999:2018). This method has been widely exploited in industry and has been used for the production of microcapsules on the same principle that microbeads are prepared.

This method involves the dropwise addition of a polymer solution into a solidifying bath. By applying an electrostatic potential between the droplet formation device and the collecting solution, it is possible to obtain smaller droplets which are desirable for many applications. Droplet formation may be divided into two phases. Under a certain critical value for the electrical potential (U_c) liquid exits the nozzle as droplets. The surface tension decreases with increasing potential resulting in a reduction of droplet diameter to approximately 200 μm . At a higher electric potential, the liquid exits the nozzle as a jet stream which subsequently breaks into droplets, which are smaller than 200 μm . In this case, droplet size is mainly determined by the jet instability (theory of Rayleigh).

2.3 Characterisation of Beads

2.3.1 IR spectra analysis

Infrared (IR) spectra analysis is performed on beads on a number of occasions to monitor any structural changes of the beads. The presence of functional groups and any other chemical interactions can be analyzed with IR spectra.

Gupta & Ravi-Kumar (2001:640) used IR spectra analysis to compare the spectra of pure chitosan beads to that of chitosan-poly ethyleneglycol (CHI-PEG) beads and that of crosslinked chitosan beads. IR analysis was performed on cross-linking reinforced chitosan-alginate beads to monitor the reaction for the preparation of the beads. Gupta & Ravi-Kumar (2001:641) used a combination of IR and UV spectra to monitor structural changes of CHI-PEG beads, pure chitosan beads and crosslinked chitosan beads after they were subjected to swelling studies. Swelling experiments were performed on the beads and at predetermined time intervals the swelling solution was filtered and UV spectra of the filtrate were then recorded. The beads were then dried at 35 to 45 °C and IR spectra were then recorded to note any structural changes by comparing the IR spectra of the initial dry beads (Mi *et al.*, 2002:63).

Lin *et al.* (1999:6308) used a thermal micro attenuated total reflection/Fourier transform infrared/differential scanning calorimetry (ATR/FT-IR/DSC) microscopic system to determine the inter- and/or intra-molecular weight of poly (*N*-isopropylacrylamide) (PNIPAAM) molecules and water in PNIPAAM microgel beads prepared by different drying methods after the beads were water-equilibrated for 24 hours.

By comparing newly recorded IR spectra to standard ones, chemical interactions within beads can be detected and explained as either normal or attributable to any experimental errors, which can then be investigated.

2.3.2 Morphology: Scanning electron microscopy (SEM)

The shape and surface morphology of beads can be examined by using a scanning electron microscope (SEM). According to Gupta & Ravi-Kumar (2001:641) SEM studies are conducted by mounting the samples on metal stubs and then vacuum coated with gold. Coated samples are then examined using a scanning electron microscope. By using scanning electron microscopy studies, Lubbe *et al.* (2002) was able to examine the surface topography of chitosan beads as well as the pore structure of the beads. With cross-sectional views the pore nature of the bead matrix can be exactly described.

2.3.3 Solubility

The solubility of beads depends on the degree of crosslinking (Gupta & Ravi-Kumar, 2001:641). A number of techniques have been used to investigate the solubility of beads in various media of varying pH values. One method followed by Gupta *et al.* (2001:641) involves the weighing of a sample of the beads. The sample is then immersed in a 2 % aqueous acetic acid solution for 24 hours and the percentage solubility (% *S*) is then determined by the following equation:

$$S (\%) = (W_0 - W_t) / W_0 \times 100 \quad (2-1)$$

where W_0 is the initial weight of the beads and W_t is the weight of vacuum-dried beads after immersion for 24 hours in acidic solution.

2.3.4 Swelling and degradation

Mi *et al.* (2002:63) used swelling studies to determine the water sorption capacity of chitosan-alginate beads. The beads were swelled in media of pH 1 – 7 at room temperature. A known weight (200 mg) of the chitosan-alginate beads were placed in the media for the required period of time. The swollen beads were collected and the net

weight of the beads was determined by first blotting the beads with filter paper to remove adsorbed water on the surface and then weighing immediately on an electronic balance. The percentage swelling of the beads in the media were then calculated from the formula:

$$E_{sw} = [(W_e - W_0)/W_0] \times 100 \quad (2-2)$$

where E_{sw} is the percentage swelling of chitosan-alginate beads at equilibrium. W_e denotes the weight of the beads at equilibrium swelling and W_0 is the initial weight of the chitosan-alginate beads. Each swelling experiment was repeated three times and the average value was taken as the percentage swelling value.

Gupta & 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-polyethyleneglycol for the controlled release of drugs, Gupta & Ravi-Kumar (2001:639) studied the swelling behaviour of the beads at different pH values. The degree of swelling for each sample at a time t was calculated by using the following relationship:

$$\text{Degree of swelling (E}_{sw}) = (W_t - W_0)/W_0 \quad (2-3)$$

where W_t and W_0 are the weights of the beads at time t and in a dry state, respectively.

The crosslinked chitosan beads were expected to undergo degradation by hydrolysis of the amino/imine bonds; hence, hydrolytic degradation of the beads was studied under physiological conditions. Chitosan beads were placed in 100 mL solutions at pH 2.0 and pH 7.40 at 37 °C under stirred conditions and the hydrolytic degradation of the beads was observed microscopically at different time intervals. The swelling process of the beads at pH values lower than 6 involves protonation of the amino/imine groups in the beads with mechanical relaxation of the coiled polymeric chains. The process of swelling is expected to be completed in two stages. In the first stage the amino/imine groups at the bead surface are protonated leading to dissociation of the hydrogen bonds between the amino/imine groups and other groups. The protonation facilitates solvent penetration

from the sample surface forming a sharp boundary or moving front separating the insolvent polymer region with that of the swollen portion of the beads. In the second stage, protons and counterions diffuse into the bead to protonate the inner amino/imine groups, dissociating the hydrogen bonds. This process of protonation continues until the whole structure of the bead collapses and is solvated (Gupta & Ravi-Kumar, 2001:641).

2.3.5 Mechanical/Crushing strength

This technique, called the ‘mercury load cell method’ uses either 50 or 100 ml glass hypodermic syringes. The tip of the syringe and the top of the plunger were removed as a modification to the method. The barrel was used as a hollow support and guide tube with close-fitting tolerances to the plunger. The hollow plunger with one open end served as a load cell to which mercury could be added. A window was cut into the barrel to facilitate placement on the base platen. The plunger acted as the movable platen and was set directly on the bead positioned on the lower platen. As the rate of loading may affect crushing load, mercury was introduced from a reservoir into the upper chamber at the rate of 10 g/sec until a single bead crushed. The total weight of the plunger and the mercury required to fracture a bead was the crushing load. A minimum of 10 beads were tested and the average load in grams was taken as the crushing strength (Maheshwari *et al.*, 2003:57; Pradkar *et al.*, 2003:35).

2.3.6 Friability

Friability tests are usually performed on most solid pharmaceutical dosage forms to assess their resistance to crack or develop chips. Friability is determined as the percentage weight loss after subjecting the samples to mechanical stress using a friabilator set at a predetermined number of revolutions per minute (rpm) (Alsarra *et al.*, 2002:3639).

Alsarra *et al.* (2002:3639) characterised chitosan beads by subjecting them to a series of tests. In the same study, friability tests were performed in triplicate. The beads were passed through a standard sieve series. The beads retained on sieve number 16 (aperture 1.19 mm) were weighed and placed in the plastic chamber of the friabilator with 25 3mm glass beads. The beads were subjected to 100 rotations at a constant speed of 25 rpm. The fragments were separated by resieving the beads and the beads were weighed again. The friability was determined as the percent in weight of the beads.

2.3.7 Drug loading capacity

Only a certain amount of an active compound can be incorporated into beads. For beads to be of pharmacotherapeutic advantage as drug carriers, they must be able to incorporate adequate amounts of the drug for release and absorption within the therapeutic range of the drug.

Drug loading capacity tests, also called entrapment studies (Alsarra *et al.*, 2002:639) or drug incorporation studies (Mi *et al.*, 2002:61), are mostly conducted by employing a UV spectrophotometer. A method employed by Gupta & Ravi-Kumar (2001) involved keeping a weighed sample of the drug loaded beads in 100 mL solution of acetic acid (2 %) at 30 °C for 48 hours. After centrifugation, the drug in the supernatant and washings of the beads was assayed by recording absorbance with a UV spectrophotometer. The drug loading capacity (DLC) of the beads and the drug encapsulation efficiency (EE) were calculated from the following equations:

$$\text{LC} = \frac{\text{Total amount of drug} - \text{Free amount of drug}}{\text{Weight of the Beads}} \quad (2-4)$$

$$\text{EE} = \frac{\text{Total amount of drug} - \text{Free amount of drug}}{\text{Total amount of drug}} \quad (2-5)$$

Alsarra *et al.* (2002:3641) used another method for entrapment studies on lipase loaded chitosan beads. After the enzyme loaded beads were prepared, 20 mL of the tripolyphosphate (TPP) solution and two 3 mL washings of the beads were analysed by using the bicinchoninic acid (BCA) protein assay described by Smith *et al.* (1985:76). Briefly, a known volume of each sample was drawn and diluted with an appropriate volume of Tris-HCl buffer. A combination of reagent A (sodium carbonate, sodium bicarbonate and sodium tartrate in 0.2 N sodium hydroxide), reagent B (BCA solution) and reagent C (4 % cupric sulfate pentahydrate) was added to the sample and the mixture was incubated for 1 hour at 60 °C. Decanted solutions of the placebo beads were used as a blank. Different concentrations (0.01 – 0.04 mg/ml) of *C. Rugosa* lipase were used as standards. The enzyme lost in these solutions was quantified by measuring the absorbance at 562 nm using a UV-Vis spectrophotometer. The percentage entrapment efficiency was obtained by calculating the percentage of the lipase entrapped, based on the initial mass of lipase present in each chitosan solution.

2.3.8 Dissolution and drug release

Dissolution or drug release studies are normally conducted to evaluate the ability of a drug vehicle to release the right amount of drug over an acceptable period of time. Most orally administered drugs are targeted for release in the stomach and in the duodenum. Therefore, specially designed dosage forms should be able to release the active ingredient in a short space of time before excretion through normal processes, for example defecation, occurs. Dissolution or drug release studies are normally performed by placing the final dosage form in media of varying pH values under stirred or unstirred conditions and then collecting aliquots of predetermined volume to measure (spectrophotometrically) the amount of the drug released into the media (Maheshwari *et al.*, 2003:59).

Gupta & ravi-kumar (2001:642) conducted release experiments on beads in a glass apparatus at 37 °C under unstirred conditions in acidic (pH 2.0) and basic (pH 7.40) solutions. Beads (0.1 g) containing a known amount of the drug were added to the release

medium (100 mL). At predecided intervals, aliquots of 1 mL were withdrawn, filtered, and assayed by recording the absorbance spectrophotometrically.

Alsarra *et al.* (2002:3637) determined the release of lipase enzyme from beads in 0.05 M Tris-HCl buffer (pH 7.20) over a 36 hour period to evaluate the loss of the enzyme from the beads after the manufacturing process was complete. Release studies were conducted in Tris-HCl buffer because this buffer was also used during the manufacturing process.

2.4 Conclusion

Currently beads receive a lot of attention in controlled drug delivery research. The advantages of beads in drug delivery have been discussed in Chapter 1. With improving technology, the manufacturing processes of beads have been simplified to a great extent rendering them suitable for various pharmaceutical applications. Several techniques are used for the preparation of beads, which include the emulsification ionotropic gelation method, the ionotropic gelation method, extrusion spheronisation technique, melt solidification technique and electric dispersion of polymer solutions. These methods can be modified to give a desired type of bead formulation for a specific pharmaceutical purpose. Ionotropic gelation proves to be a very reliable and simple method for the preparation of beads and this method is frequently used in the preparation of drug loaded beads. Beads can be characterised by IR analysis, morphology studies, solubility, swelling and degradation, mechanical or crushing strength measurements, friability, drug loading capacity and drug release profiles. The morphology of the beads, their swelling behaviour, drug loading and drug release characteristics are important characteristics determining the quality and efficacy of beads and are therefore an integral part in the characterisation process of beads.

Chapter 3

Formulation and Characterisation of Chitosan Beads Containing Pharmaceutical Excipients

3.1 Introduction

The properties of beads that make them a convenient dosage form for optimal drug delivery have been discussed Chapter 1 and 2. This chapter describes the formulation and characterisation of rifampicin loaded chitosan beads and rifampicin loaded chitosan beads containing different pharmaceutical excipients. In a previous study, it was shown that less than 45 % rifampicin was released over a period of 24 hours from chitosan beads (Lubbe *et al.*, 2002:71). From this background, it was hypothesised that the inclusion of pharmaceutical excipients into chitosan beads might improve the swelling behaviour of the beads and consequently improve the rifampicin release over a shorter period of time. Rifampicin, a first line agent in the treatment of tuberculosis (TB), was used as the drug of choice because of the widespread occurrence of TB and the recent increase in multi-drug resistant TB strains due to inadequate rifampicin absorption.

3.2 Study Design

The ionotropic gelation method of Bodmeier and Prammar (1989:1478) was used in this study to prepare both pure chitosan beads and chitosan beads containing a single pharmaceutical excipient. Tripolyphosphate (TPP) was used as a crosslinking agent. The pharmaceutical excipients and their concentrations used in this study are given in Table

3.1. The prepared formulations were characterised on the basis of their morphology and swelling behaviour and is described in section 3.3.

Based on the results obtained with chitosan beads containing a single pharmaceutical excipient, beads containing selected combinations of two pharmaceutical excipients were also prepared and characterised and this is described in Section 3.4. Based on all the results obtained, chitosan beads containing either single pharmaceutical excipients (chitosan/SPE beads) or multiple pharmaceutical excipients (chitosan/MPE beads) were then prepared with the inclusion of rifampicin as described in Section 3.5. The rifampicin loaded chitosan/SPE and chitosan/MPE beads were characterised on the basis of their morphology, drug loading capacity and swelling behaviour. Dissolution studies on rifampicin loaded beads are described in Chapter 4.

Table 3.1: Pharmaceutical excipients incorporated into chitosan beads.

Pharmaceutical Excipient	Concentration (% w/v)		
	0.1	0.25	0.5
Ac-Di-Sol® (ADS)	X	X	X
Explotab® (EXPL)	X	X	X
Vitamin C (Vit C)	X	X	X

3.3 Preparation and Characterisation of Chitosan/Single Pharmaceutical Excipient (Chitosan/SPE) Beads

3.3.1 Materials

The following materials were used in the preparation of the different beads: Chitosan (91.4 % deacetylated) [Annexure A, C.1], Glacial acetic acid 99.8 % (UnivAR, South

Africa), Tripolyphosphate (Sigma, South Africa), Ac-Di-Sol[®] (FMC, Ireland), Explotab[®] (Mendell, England) and Vitamin C (Merck, South Africa). The chemicals used in all procedures were of analytical grade and were used as received.

3.3.2 Method

The ionotopic gelation method of Bodmeier and Prammar (1989: 1478) with TPP was used to prepare the beads. Beads were prepared from a single concentration of bubble-free chitosan solution that was added dropwise through a peristaltic pump into a 5 % w/v tripolyphosphate (TPP) solution in deionised water, using the setup shown in Figure 3.1. The chitosan concentration was maintained at 3 % w/v throughout all the experiments. The chitosan solution was prepared by dissolving chitosan powder in a 2 % w/v acetic acid solution in deionised water. The solution was stirred until the chitosan was completely dissolved and was left over night over night for air bubbles to clear. The excipients were dissolved or suspended in the polymer solution in the specified concentrations (Table 3.1). The beads were allowed to stir for 30 minutes in the TPP solution to allow crosslinking after which the beads were removed and washed twice with 100 ml deionised water to remove the excess TPP. Beads were then taken for freeze-drying overnight in a Freezemobile 6 freeze-dryer (Virtis Gardiner, USA). Freeze-dried beads were characterised as described in Section 3.3.3.

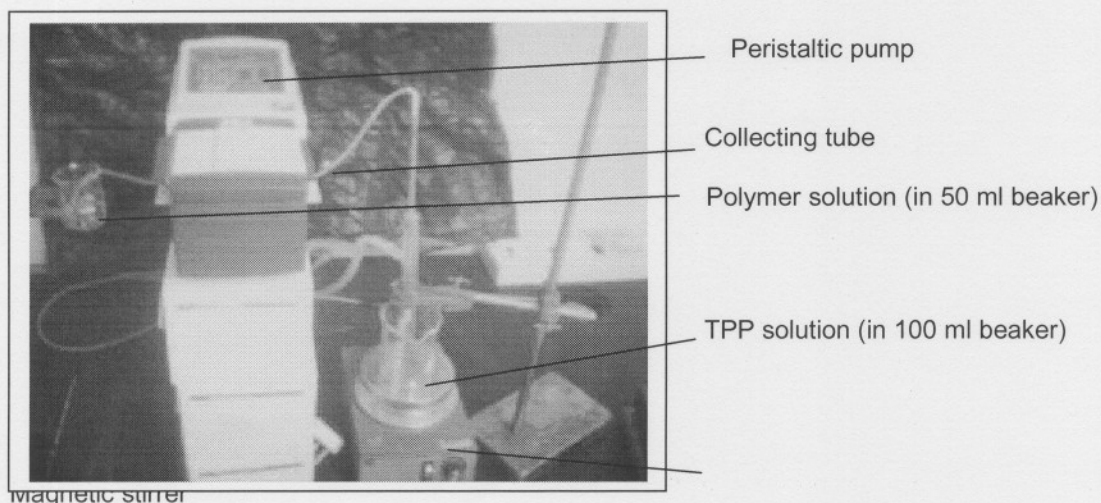


Figure 3.1: Setup for the preparation of beads using a Watson-Marlow 205S peristaltic pump (Watson-Marlow Ltd., England).

3.3.3 Characterisation of chitosan/SPE beads

3.3.3.1 Morphology

Morphology studies were carried out using a scanning electron microscope (SEM). Full and cross-cut beads were mounted on aluminium sample mounts and coated under vacuum with a 20 nm layer of gold-palladium alloy for better imaging. A Phillips ESEM Quanta 200 scanning electron microscope (Phillips, The Netherlands) was used.

3.3.3.2 Swelling behaviour

Swelling behaviour studies were conducted in triplicate over 6 hours in a phosphate buffer solution (PBS) at pH 5.60 and 7.40 at 37 °C. Samples of beads (100 mg) were weighed into test tubes and 5 ml PBS was introduced into each test tube. The test tubes

were then immersed and left in a water bath (Stuart Scientific, UK) set at 37 °C. The weight of the beads after removing the media and excess PBS droplets around the beads was recorded as W_t , i.e. the weight of the beads after time t . The weight of the beads was determined after 10, 60 and 360 minutes (6 hours). The swelling behaviour was then expressed as the **degree of swelling** (E_{sw}) by

$$E_{sw} = (W_t - W_0)/W_0 \quad (3-1)$$

where W_t is the weight of the beads after time t and W_0 is the initial weight.

3.3.4 Results and discussion

3.3.4.1 Morphology

The SEM micrographs are depicted in Figures 3.2 to 3.5. A general increase in pore size of the beads was observed upon addition of the pharmaceutical excipients compared to pure chitosan beads (Figure 3.2). An increase in the Vit C concentration produced beads with a smoother outer surface and a slight decrease in pore size (Figure 3.4a-c). Chitosan beads containing 0.1 % w/v ADS appeared solid and spherical with little porosity (Figure 3.5a). However, a pronounced increase in pore size was observed with an increase in the ADS concentration to 0.25 and 0.5 % w/v, while the beads became less spherical (Figure 3.5b-c). There were no major differences in the pore size of the EXPL formulations (Figure 3.3a-c) although the beads appeared more spherical compared to the Vit C and ADS formulations. For all the formulations, it was noticed that the outer layer became more distinguishable with an increase in the pore size.

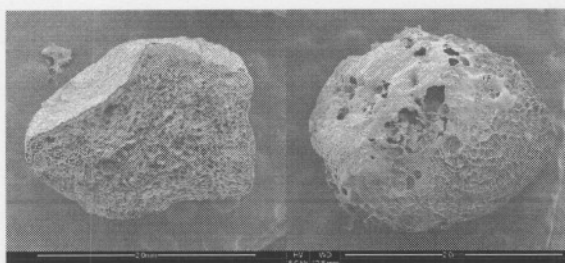
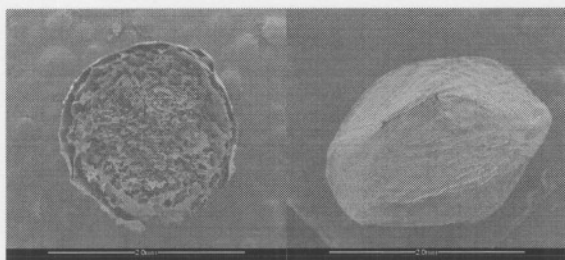
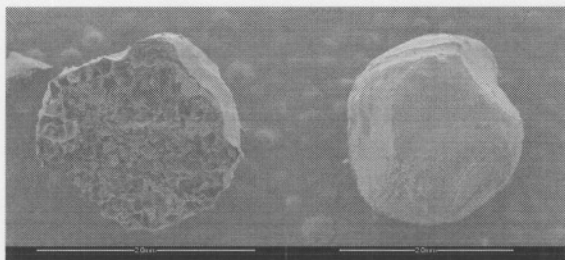


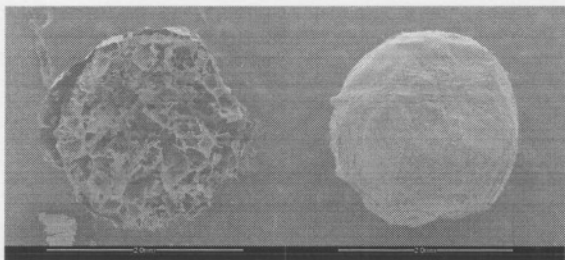
Figure 3.2: Cross-section and full view of a pure chitosan (3 % w/v) bead.



(a)



(b)



(c)

Figure 3.3: Cross-section and full view of a (a) 0.1 % w/v, (b) 0.25 % w/v and (c) 0.5 % w/v Explotab[®] loaded chitosan (3 % w/v) bead.

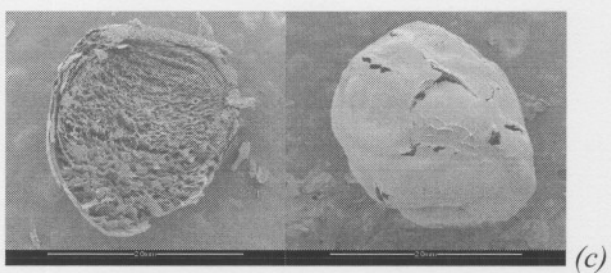
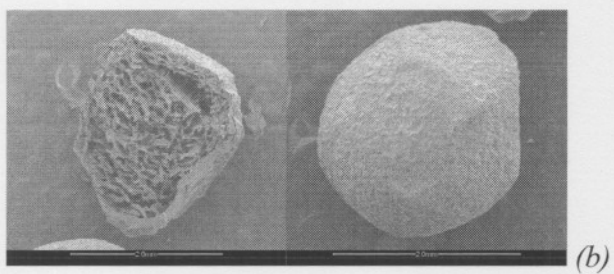
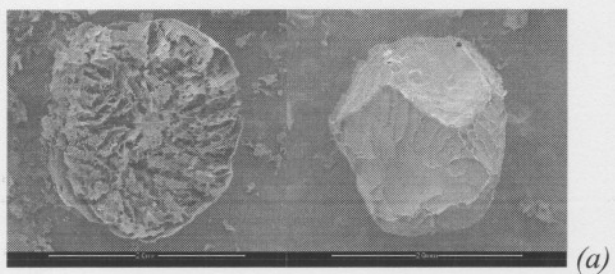


Figure 3.4: Cross-section and full view of a (a) 0.1 % w/v, (b) 0.25 % w/v and (c) 0.5 % w/v Vitamin C loaded chitosan (3 % w/v) bead.

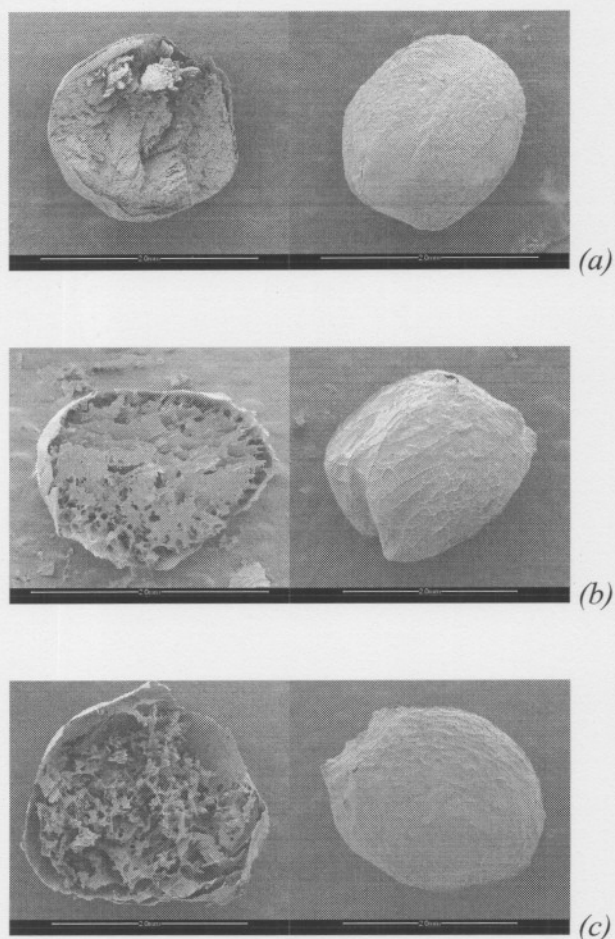


Figure 3.5: Cross-section and full view of a (a) 0.1 % w/v, (b) 0.25 % w/v and (c) 0.5 % w/v Ac-Di-Sol[®] loaded chitosan (3 % w/v) bead.

3.3.4.2 Swelling behaviour

Table 3.2 and figures 3.6 to 3.8 depict the swelling behaviour of the different bead formulations at pH 5.60 and 7.40 over a period of 360 minutes. It is clear that the addition of pharmaceutical excipients led to an increase in the swelling behaviour of all the bead formulations compared to the pure chitosan beads. The bar graphs depict a general increase in swelling as the pH is changed from 5.60 to 7.40 for both the Vit C and EXPL formulations. A rapid increase in swelling is seen with Vit C loaded beads after the first 10 minutes at both pH values. No significant difference was observed in the swelling of

the ADS formulations at both pH values compared to the pure chitosan formulation except at a concentration of 0.5 % w/v where significant swelling was observed. In the Explotab[®] formulations the 0.25 % w/v concentration exhibited the best swelling behaviour at both pH values. In general, for all the formulations more pronounced swelling was observed during the first 10 minutes after which time the swelling rate decreased, although all formulations showed further swelling up to 360 minutes.

Table 3.2: Degree of swelling (Esw) of pure chitosan and chitosan/SPE beads at pH 5.60 and 7.40.

Bead Formulation	Degree of Swelling (Esw)					
	10 min		60 min		360 min	
	pH 5.60	pH 7.40	pH 5.60	pH 7.40	pH 5.60	pH 7.40
Chitosan 3%	0.77 ± 0.21	1.17 ± 0.15	0.80 ± 0.17	1.20 ± 0.35	1.80 ± 0.20	2.47 ± 0.11
Chit/ADS 0.1 %	1.50 ± 0.30	1.73 ± 0.11	1.53 ± 0.30	1.90 ± 0.65	2.17 ± 0.15	2.10 ± 0.46
Chit/ADS 0.25 %	0.63 ± 0.23	0.77 ± 0.30	0.90 ± 0.61	1.10 ± 0.10	2.17 ± 0.58	2.30 ± 0.70
Chit/ADS 0.5 %	1.93 ± 0.06	2.23 ± 0.11	2.10 ± 0.20	2.37 ± 0.11	3.33 ± 0.40	3.30 ± 0.53
Chit/EXPL 0.1 %	0.77 ± 0.06	1.33 ± 0.29	1.03 ± 0.15	1.53 ± 0.15	1.60 ± 0.60	3.10 ± 0.53
Chit/EXPL 0.25 %	1.33 ± 0.15	1.23 ± 0.25	1.43 ± 0.20	1.83 ± 0.47	1.83 ± 0.47	3.67 ± 0.42
Chit/EXPL 0.5 %	0.60 ± 0.10	1.27 ± 0.25	0.73 ± 0.25	1.37 ± 0.11	1.87 ± 0.40	3.87 ± 0.25
Chit/Vit C 0.1 %	0.87 ± 0.11	1.53 ± 0.30	0.93 ± 0.15	2.20 ± 0.46	2.97 ± 0.32	2.90 ± 0.52
Chit/Vit C 0.25 %	1.47 ± 0.11	1.67 ± 0.32	1.47 ± 0.11	1.87 ± 0.75	1.93 ± 0.25	3.20 ± 0.61
Chit/Vit C 0.5 %	1.27 ± 0.25	1.60 ± 0.43	1.70 ± 0.53	2.00 ± 0.26	2.77 ± 0.63	3.70 ± 0.30

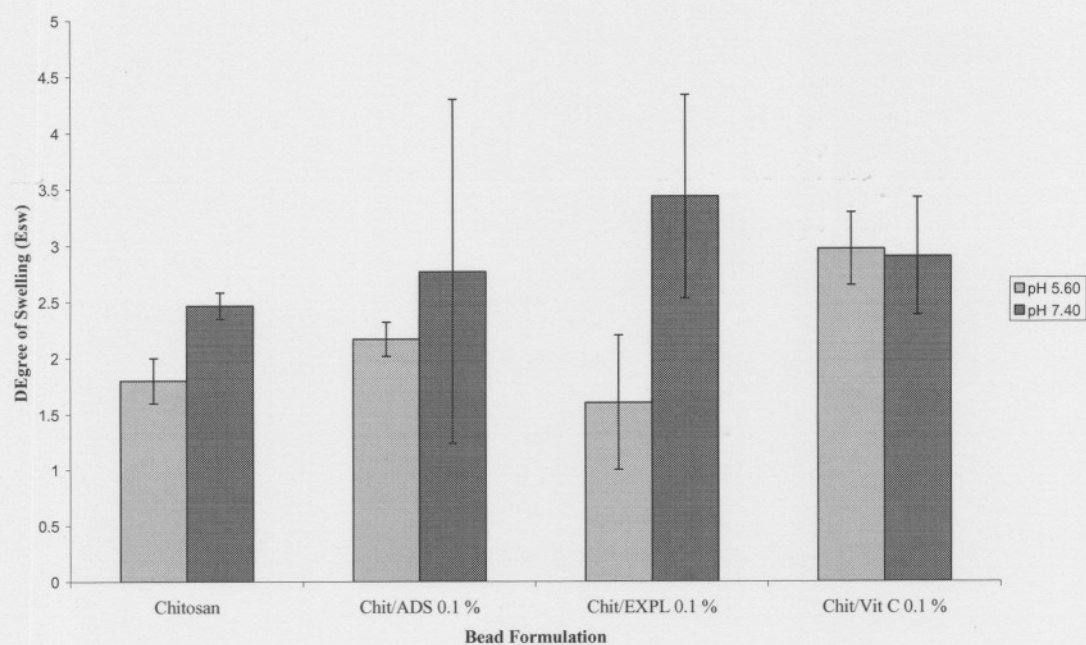


Figure 3.6: The effect of pharmaceutical excipients (0.1 % w/v) on the swelling behaviour of chitosan/SPE beads at pH 5.60 and 7.40 after 6 hours.

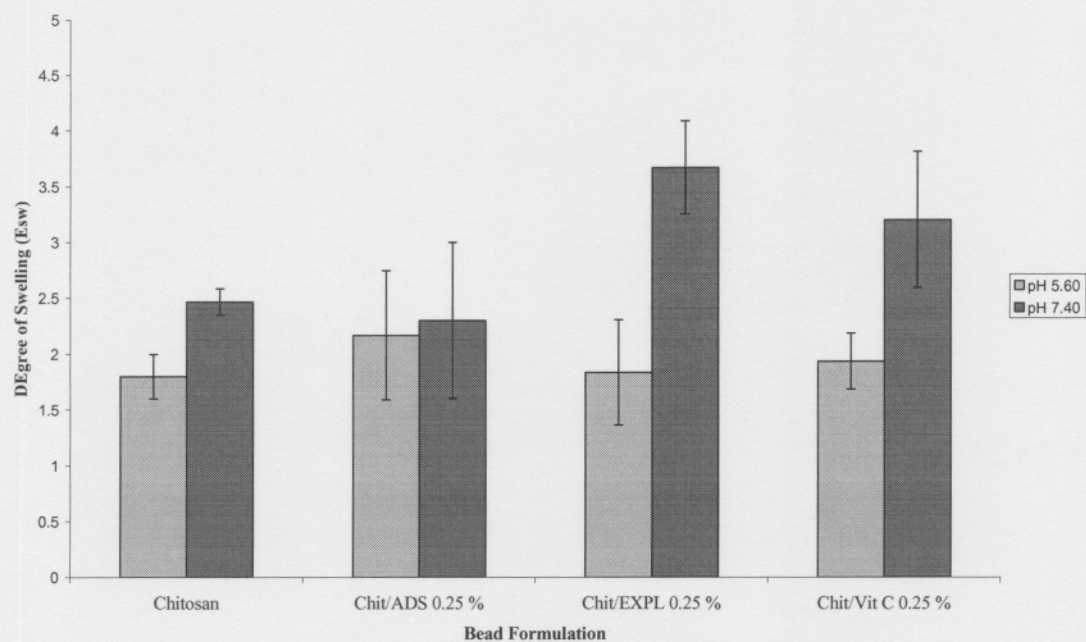


Figure 3.7: The effect of pharmaceutical excipients (0.25 % w/v) on the swelling behaviour of chitosan/SPE beads at pH 5.60 and 7.40 after 6 hours.

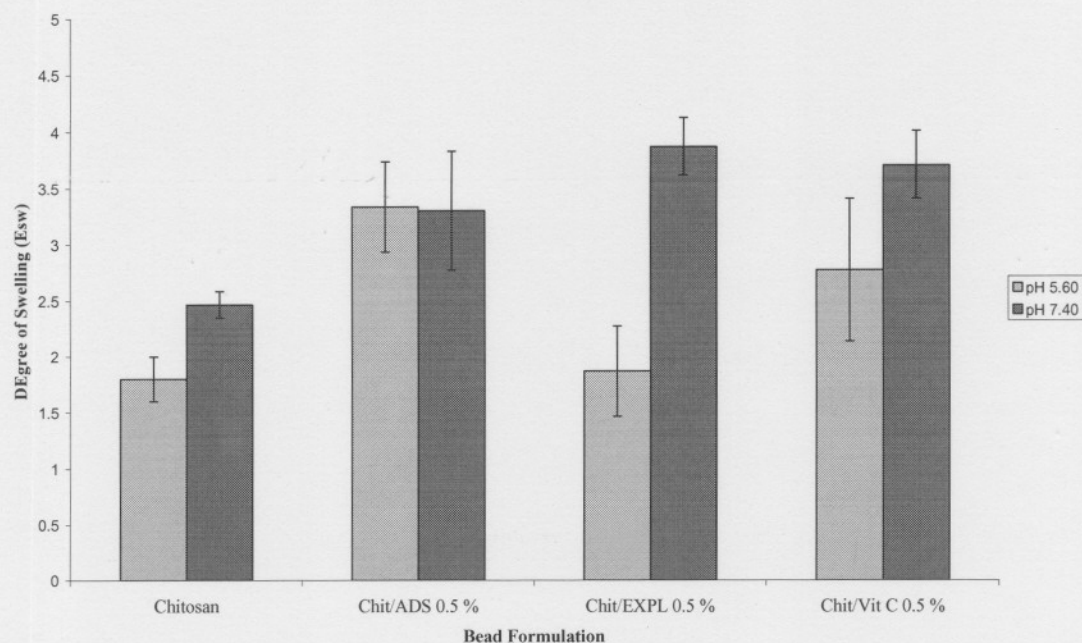


Figure 3.8: The effect of pharmaceutical excipients (0.5 % w/v) on the swelling behaviour of chitosan/SPE beads at pH 5.60 and 7.40 after 6 hours.

3.3.5 Summary

Pure chitosan beads and chitosan/SPE beads were prepared successfully using the ionotropic gelation procedure. The prepared formulations were characterised for their morphology and swelling behaviour. The results indicate a significant influence of the pharmaceutical excipients on the morphology and swelling behaviour of the beads. In general, addition of the pharmaceutical excipients produced beads with increased pore sizes and improved swelling behaviour. Based on these results, a single concentration of each excipient was selected for the preparation of chitosan/multiple pharmaceutical excipients (chitosan/MPE) beads, which are discussed in the next section. Although it was very difficult to select a single concentration of each excipient on the basis of the morphology and swelling behaviour results it was decided to continue the studies with a 0.25 % w/v concentration of Vit C and EXPL. Based on the better porosity of the 0.5 % w/v ADS bead (Figure 3.5c) this concentration of this excipient was selected for further studies.

3.4 Preparation and Characterisation of Chitosan/Multiple Pharmaceutical Excipients (Chitosan/MPE) beads

3.4.1 Materials

The same materials were used to prepare the chitosan/MPE beads as described in Section 3.3.1.

3.4.2 Method

The same methodology as described in Section 3.3.2, was used to prepare the chitosan/MPE beads. The following combinations of pharmaceutical excipients were selected and incorporated into chitosan (3 % w/v) beads:

- Explotab[®] 0.25 % w/v + Vitamin C 0.25 % w/v (Chit/EXPL/Vit C)
- Ac-Di-Sol[®] 0.5 % w/v + Vitamin C 0.25 % w/v (Chit/ADS/Vit C)
- Ac-Di-Sol[®] 0.5 % w/v + Explotab[®] 0.25 % w/v (Chit/ADS/EXPL)

3.4.3 Characterisation of chitosan/MPE beads

3.4.3.1 Morphology

Morphology studies on chitosan/MPE beads were conducted as described in section 3.3.3.1.

3.4.3.2 Swelling behavior

Swelling behavior studies on chitosan/MPE beads were conducted as described in section 3.3.3.2.

3.4.4 Results and discussion

3.4.4.1 Morphology

Figures 3.9 to 3.11 depict the morphology of the different chitosan/MPE beads. The addition of a combination of excipients produced beads with a higher porosity compared to the chitosan/SPE beads. The effect of vitamin C 0.25 % w/v when combined with another excipient seem similar as the effect obtained with the chitosan/SPE beads (Vit C). The outer surface of these formulations appear smoother as compared to the combination of ADS and EXPL which, however, showed a more increased pore size (Figure 3.11) compared to beads containing these two excipients alone.

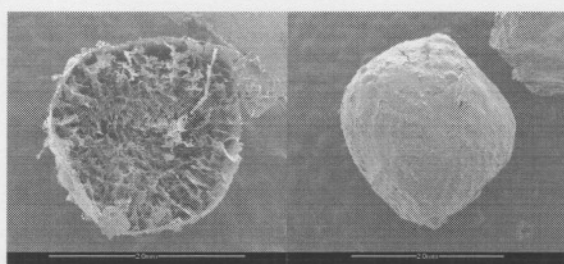


Figure 3.9: Cross-section and full view of a Chit/ADS/Vit C bead.

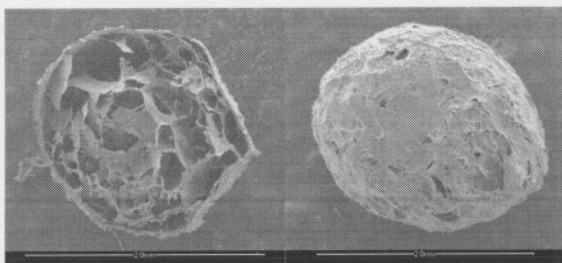


Figure 3.10: Cross-section and full view of a Chit/ADS/EXPL bead.

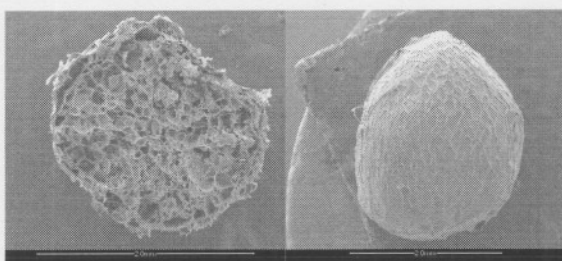


Figure 3.11: Cross-section and full view of a Chit/EXPL/Vit C bead.

3.4.4.2 Swelling behaviour

The swelling behaviour results of the chitosan/MPE beads are given in Table 3.3 and are depicted graphically in Figure 3.12. The effect of pH on the swelling behaviour of the chitosan/MPE beads is similar to that obtained for the chitosan/SPE beads. More pronounced swelling was observed at pH 7.40 compared to pH 5.60. The rate of swelling of the beads was high during the first 10 minutes and thereafter more constant up to 6 hours although increased swelling was still evident. It was also observed that the swelling values of the chitosan/MPE beads were much lower compared to those of the chitosan/SPE beads (Table 3.2 and Table 3.3) and no major differences between the chitosan/MPE beads and pure chitosan beads were evident. These results seem to be in conflict given the higher porosity of the beads compared to pure chitosan beads but may

be explained by the nature of the outer surface of the beads which is less permeable than that of the pure chitosan beads (Figure 3.2).

Table 3.3: Degree of swelling (Esw) of pure chitosan beads and chitosan/MPE beads at pH 5.60 and 7.40.

Bead Formulation	Degree of Swelling (Esw)					
	10 min		60 min		360 min	
	pH 5.6	pH 7.4	pH 5.6	pH 7.4	pH 5.6	pH 7.4
Chitosan 3%	0.77 ± 0.21	1.17 ± 0.15	0.80 ± 0.17	1.20 ± 0.35	1.80 ± 0.20	2.47 ± 0.12
Chit/EXPL/Vit C	1.03 ± 0.21	1.00 ± 0.06	1.03 ± 0.06	1.67 ± 0.28	1.22 ± 0.11	1.88 ± 0.26
Chit/ADS/Vit C	1.30 ± 0.10	1.55 ± 0.31	1.23 ± 0.06	1.80 ± 0.28	2.08 ± 0.13	2.24 ± 0.05
Chit/ADS/EXPL	0.83 ± 0.12	0.84 ± 0.21	1.37 ± 0.15	2.13 ± 0.21	1.79 ± 0.02	2.47 ± 0.14

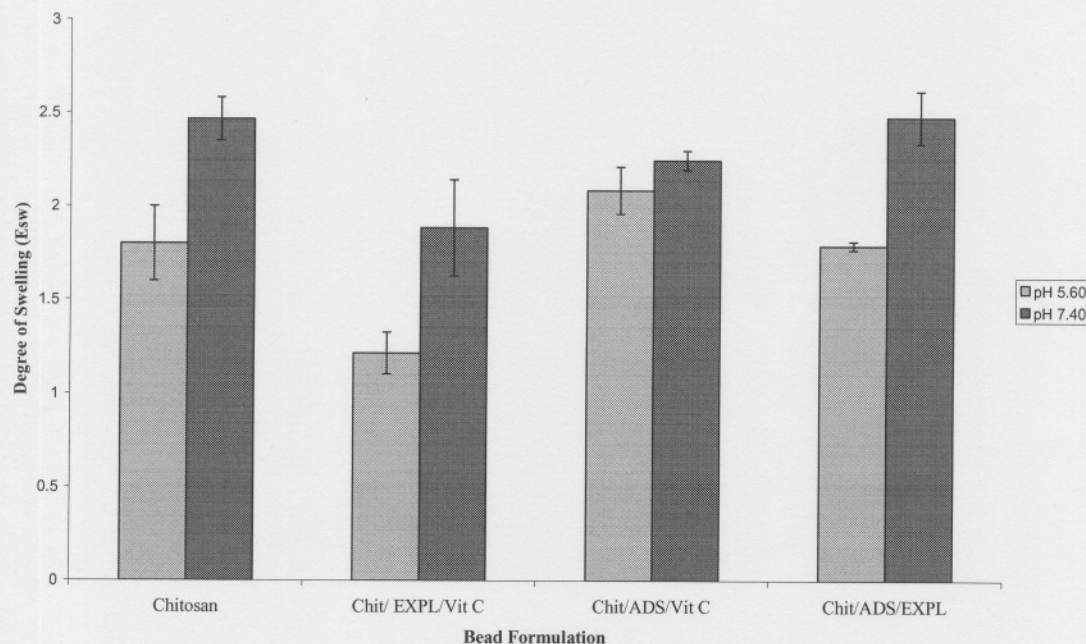


Figure 3.12: The effect of combined pharmaceutical excipients on the swelling behavior of chitosan/MPE beads at pH 5.60 and 7.40 after 6 hours.

3.4.5 Summary

The inclusion of a combination of excipients led to a higher porosity of the beads compared to the chitosan/SPE beads although a decrease in the swelling behavior was observed for the chitosan/MPE beads. This could possibly be explained by the nature of the outer surfaces of the beads and needs to be investigated further. However, chitosan/MPE beads might still prove useful in drug delivery and the preparation and the evaluation of rifampicin loaded chitosan/SPE and chitosan/MPE beads are described in the next section.

3.5 Preparation of Rifampicin Loaded Chitosan/SPE and Chitosan/MPE beads

3.5.1 Materials

The same materials used for the preparation of drug free beads were used for the preparation of rifampicin loaded beads. Rifampicin was a gift from Rolab (Pty) Ltd. (Spartan, South Africa). Methanol (100 %) (SMM Instruments, South Africa) was used as a solvent for rifampicin. Sodium hydroxide pellets and monobasic potassium phosphate were obtained from Merck, South Africa. Plastibrand[®] disposable cuvettes (Merck, South Africa) were used for the spectrophotometric analysis of rifampicin.

3.5.2 Method

Rifampicin loaded beads were prepared by the same method used for the preparation of drug free beads (Sections 3.3. and 3.4.2). Table 3.4 gives a summary of the prepared chitosan beads and the excipients used in the preparation of the beads. Rifampicin was added to the polymer solution to obtain a chitosan/rifampicin dispersion of 2 % w/v rifampicin prior to bead formation. The water washings and the TPP solution were used

for spectrophotometric detection of the amount of drug lost from the beads during bead preparation. This was done to determine the drug loading capacity of the beads (Section 3.5.3.2).

Table 3.4: Rifampicin loaded chitosan/SPE and chitosan/MPE beads

Chitosan/SPE		Chitosan/MPE			
Excipient	(% w/v)	Excipient	(% w/v)	Excipient	(% w/v)
ADS	0.5	EXPL	0.25	Vit C	0.25
EXPL	0.25	ADS	0.5	Vit C	0.25
Vit C	0.25	ADS	0.5	EXPL	0.25

3.5.3 Characterisation of beads

3.5.3.1 Morphology

Morphology studies were done with a scanning electron microscope (SEM) as described in Section 3.3.3.1.

3.5.3.2 Drug loading

The amount of rifampicin incorporated in the beads was determined by spectrophotometric analysis. An amount of 0.1 g rifampicin was dissolved in 100 ml methanol and 3 ml of this solution was diluted to 100 ml with phosphate buffer solution (PBS) at pH 7.40. From this stock solution standard concentrations (22.0, 20.0, 18.0, 16.0, 14.0, 12.0, 10.0, 8.0, 6.0, 4.0 and 2.0 µg/ml) were prepared. The UV absorbance of these standard concentrations was determined at 475 nm with a Helios α Unicam

spectrophotometer (Unicam, Cambridge, UK) and plotted as a graph of absorbance versus concentration. A typical example of this standard curve is shown in figure 3.13.

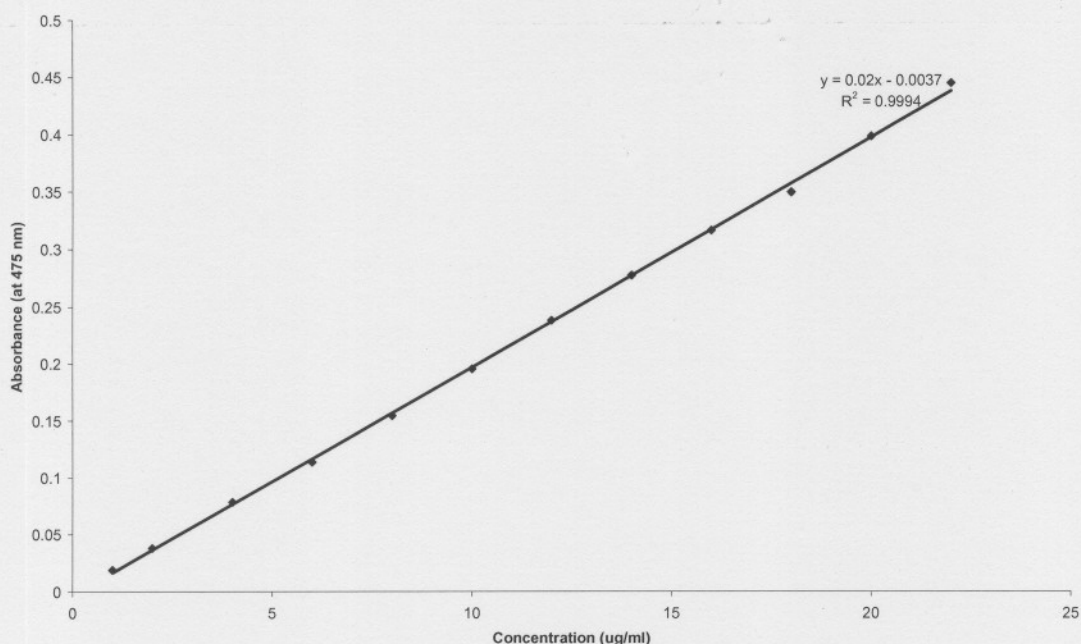


Figure 3.13: Rifampicin standard curve used for drug loading and dissolution studies.

A new standard curve was constructed on everyday during the preparation of new batches of beads and until completion of the dissolution studies.

Drug loading capacity was determined during the preparation of the beads and was calculated as the difference in the amount of drug loaded into the polymer solution and the amount of drug determined spectrophotometrically from the TPP solutions and the water washings. The drug loading capacity was expressed as **% Drug Loading** and was calculated according to the following equation:

$$\% \text{ Drug Loading} = \frac{(M_0 - M_s)}{M_0} \times 100 \% \quad (3-2)$$

where M_0 is the original amount of drug in the prepared polymer solution and M_s is the amount of the drug determined in the TPP solution and the water washings.

3.5.3.3 Swelling behaviour

Swelling behaviour studies were performed in triplicate with the same method described in Section 3.3.3.2.

3.5.4 Results and discussion

3.5.4.1 Morphology

The different micrographs obtained with the SEM are depicted in Figures 3.14 – 3.26.

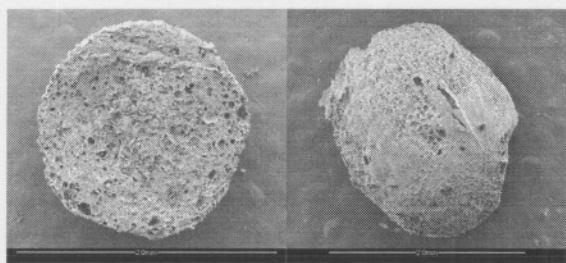


Figure 3.14: Cross-section and full view of a rifampicin (2 % w/v) loaded chitosan (3 % w/v) bead.

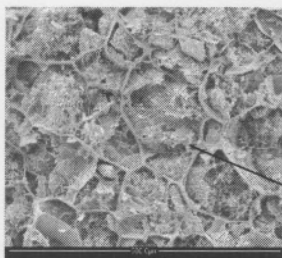


Figure 3.15: Microscopic view of drug particles inside a cross-cut chitosan (3 % w/v) bead.

Drug particles

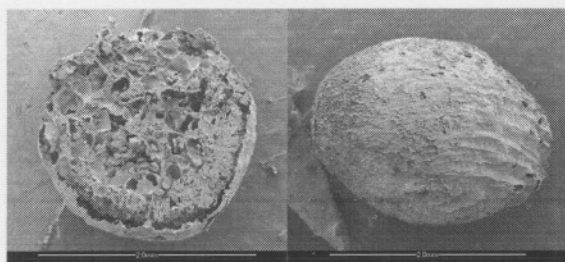
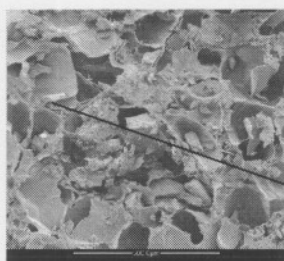


Figure 3.16: Cross-section and full view of a rifampicin (2 % w/v) loaded Chit/EXPL bead.



Drug particles

Figure 3.17: Microscopic view of drug particles inside a cross-cut Chit/EXPL bead.

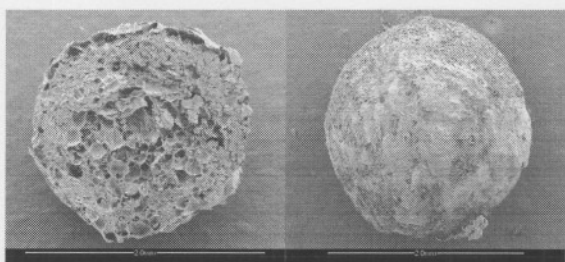
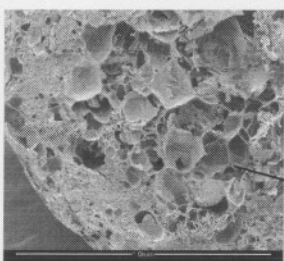


Figure 3.18: Cross-section and full view of a rifampicin (2 % w/v) loaded Chit/ADS bead.



Drug particles

Figure 3.19: Microscopic view of drug particles inside a cross-cut Chit/ADS bead.

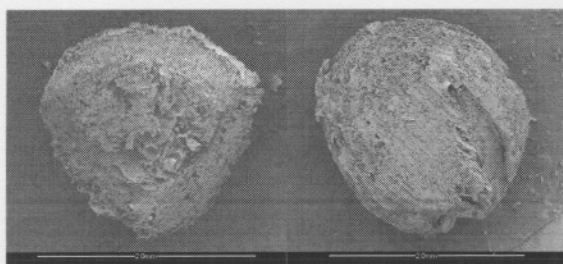
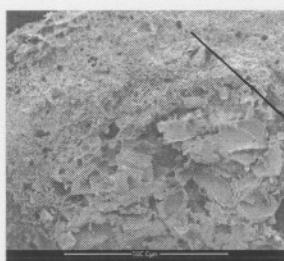


Figure 3.20: Cross-section and full view of a rifampicin (2 % w/v) loaded Chit/Vit C bead.



Drug particles

Figure 3.21: Microscopic view of drug particles inside a Chit/Vit C bead.

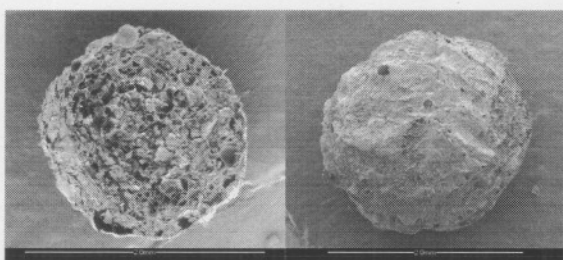
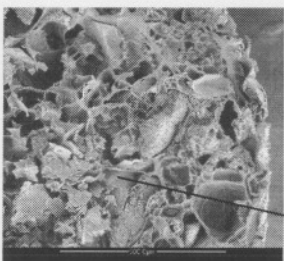


Figure 3.22: Cross-section and full view of a rifampicin (2 % w/v) loaded Chit/ADS/EXPL bead.



Drug particles

Figure 3.23: Microscopic view of drug particles inside a cross-cut Chit/ADS/EXPL bead.

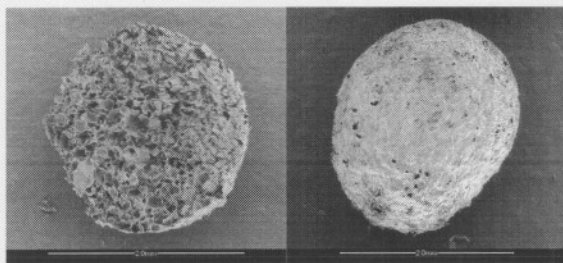


Figure 3.24: Cross-section and full view of a rifampicin loaded Chit/ADS/Vit C bead.

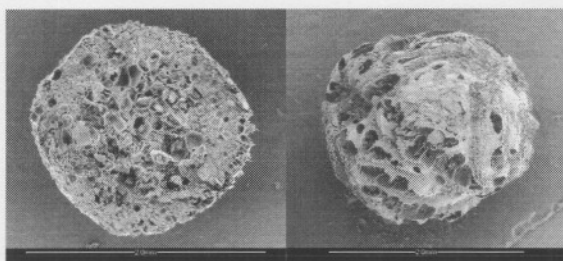
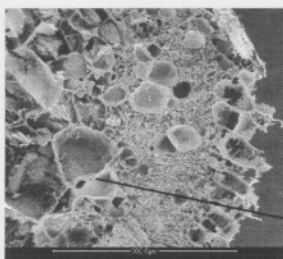


Figure 3.25: Cross-section and full view of a rifampicin loaded Chit/EXPL/Vit C bead.



Drug particles

Figure 3.26: Microscopic view of drug particles inside a cross-cut Chit/EXPL/Vit C bead.

The SEM micrographs of rifampicin loaded beads show altered morphology due to the incorporation of rifampicin. It was expected that drug particles would fill the pores of the beads depending on the drug loading capacity of the beads. Few empty pores can be seen on cross-cut samples with drug particles inside most pores. Most rifampicin loaded beads appear to have rough outer surfaces with small edges due to the presence of drug particles on the outer surface and this is typically depicted in Figure 3.25.

3.5.4.2 Drug Loading

Table 3.5 gives the % *Drug Loading* values for the different rifampicin loaded beads. Pure chitosan beads exhibited a higher drug loading capacity while the inclusion of pharmaceutical excipients resulted in a lower drug loading capacity. This lower drug loading capacity values can probably be explained by a loss of rifampicin to the TPP solution during crosslinking when a pharmaceutical excipient or combinations thereof are present.

Table 3.5: Drug loading capacity values of the bead formulations.

Bead Formulation	% Drug Loading
Pure chitosan	67.49
Chit/ADS	48.74
Chit/EXPL	62.74
Chit/Vit C	48.49
Chit/EXPL/Vit C	56.66
Chit/ADS/Vit C	60.99
Chit/ADS/EXPL	41.61

3.5.4.3 Swelling behaviour

The results obtained in the swelling behaviour studies are given in Table 3.6 and are depicted in Figure 3.27. The effect of pH on the swelling behaviour of the rifampicin loaded beads is similar to that obtained with the drug-free beads. However, rifampicin loaded beads exhibited in general lower swelling values when compared to drug-free

beads. No logical explanation for this could be found and this phenomenon needs further investigation.

Table 3.6: Degree of Swelling (Esw) of rifampicin loaded beads at pH 5.60 and 7.40.

Rifampicin loaded Formulations	Degree of Swelling (Esw)					
	10 min		60 min		360 min	
	pH 5.60	pH 7.40	pH 5.60	pH 7.40	pH 5.60	pH 7.40
Chitosan 3 %	0.77 ± 0.06	0.94 ± 0.10	0.93 ± 0.15	1.83 ± 0.07	1.20 ± 0.10	2.10 ± 0.10
Chit/ADS	1.53 ± 0.06	0.89 ± 0.10	1.73 ± 0.06	1.73 ± 0.06	1.80 ± 0.00	2.27 ± 0.12
Chit/EXPL	0.87 ± 0.06	0.88 ± 0.15	1.50 ± 0.20	1.53 ± 0.07	1.93 ± 0.15	1.97 ± 0.12
Chit/Vit C	0.57 ± 0.06	1.00 ± 0.17	1.20 ± 0.10	1.47 ± 0.57	1.20 ± 0.10	1.70 ± 0.00
Chit/EXPL/Vit C	1.03 ± 0.06	1.00 ± 0.06	1.03 ± 0.06	1.67 ± 0.28	1.13 ± 0.15	1.77 ± 0.25
Chit/ADS/Vit C	1.23 ± 0.20	1.55 ± 0.31	1.30 ± 0.10	1.8 ± 0.28	1.90 ± 0.40	1.97 ± 0.15
Chit/ADS/EXPL	0.83 ± 0.12	0.84 ± 0.21	1.37 ± 0.15	2.13 ± 0.21	1.60 ± 0.20	2.13 ± 0.21

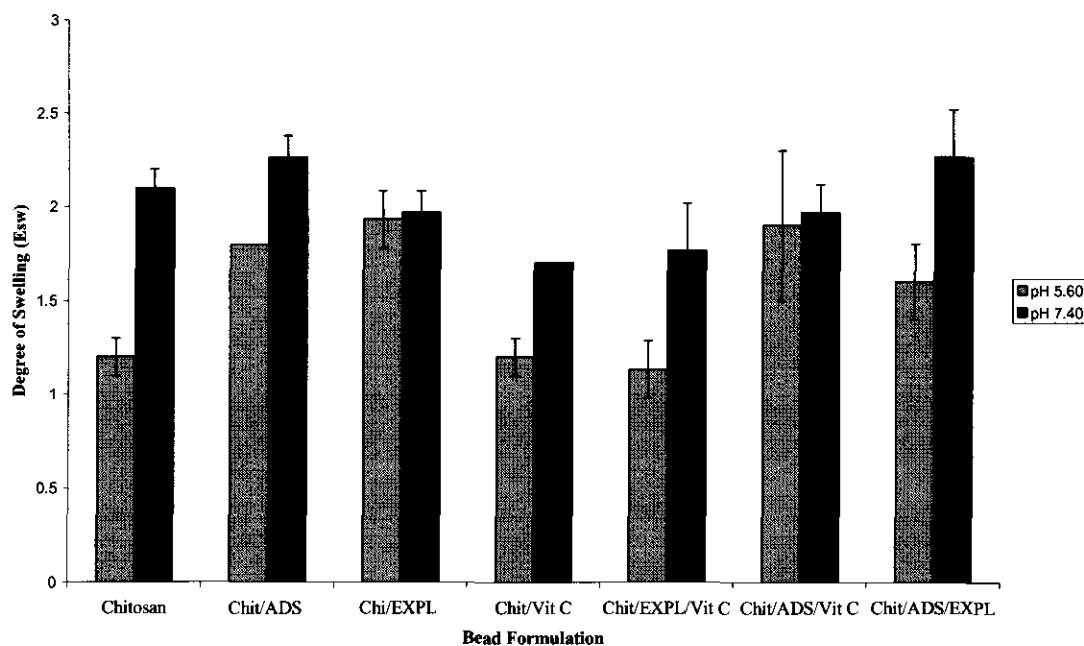


Figure 3.27: Degree of swelling (Esw) of rifampicin loaded beads after 6 hours at pH 5.60 and 7.40.

3.5.5 Summary

Rifampicin loaded beads were prepared successfully using the ionotropic gelation method. It was observed during the study that inclusion of rifampicin into the chitosan solution resulted in a more viscous suspension, although no needle size alteration was necessary. The rifampicin loaded beads were characterised on the basis of their morphology, swelling behaviour and drug loading capacity. The SEM micrographs showed that rifampicin has an effect on the morphology of the beads. The outer surface of the beads seem to have changed due to the presence of drug particles on the outer surface of the beads. The swelling behaviour values of rifampicin loaded beads appeared lower compared to drug free beads and this might necessitate further investigation.

3.6 Conclusion

The effect of Ac-Di-Sol[®], Explotab[®] and Vitamin C on the morphology, swelling behaviour and drug loading capacity of chitosan beads was studied. In general, the results of this study show that addition of Vitamin C, Explotab[®] and Ac-Di-Sol[®] all increase the porosity and swelling behaviour of chitosan beads. Increased pore size is of importance as it might improve drug loading capacity of the beads. Fast swelling chitosan beads may show great potential as targeted controlled release delivery systems for various drugs. The presence of drug particle on the outer surface of the beads might results in a burst release during the first few minutes and this is important for rapid onset of action for drugs with low bioavailability. Dissolution studies on the prepared chitosan beads are described and discussed in Chapter 4.

Chapter 4

Rifampicin Release From Chitosan/SPE and Chitosan/MPE Beads

4.1 Introduction

The preparation and characterisation of rifampicin loaded chitosan/SPE and chitosan/MPE beads were described in Chapter 3. This chapter describes the rifampicin release from these formulations. Drug loading studies have been described as part of the characterisation of the beads in Chapter 3 and these results have been used during the dissolution studies to determine the amount of rifampicin released from the beads. Dissolution studies were conducted at pH 5.60 and 7.40 over a period of 6 hours (360 minutes). Both the released rifampicin and chitosan (for any dissolved traces) were determined spectrophotometrically.

4.2 Experimental Design and Methodology

4.2.1 Calculations

Seven formulations were used for dissolution studies (Table 4.1). From each formulation a 0.1 g sample was used for dissolution studies. The amount of rifampicin in each sample differed from formulation to formulation, depending on the drug loading capacity of each formulation (Table 3.5). The amounts of rifampicin in 0.1 g of each formulation were calculated from the drug loading capacity and mass yield values and are shown in Table 4.1.

Table 4.1: Amount of rifampicin (in μg) per 0.1 g of dissolution sample as calculated from the drug loading capacity (DLC) and the Mass Yield values.

Bead Formulation	DLC (%)	DLC (μg)	Mass Yield (g)	Rifampicin in (μg)
Chitosan 3 %	67.49	404950	1.13	35836
Chit/ADS	48.74	292440	1.51	19367
Chit/EXPL	62.74	376440	1.21	31110
Chit/Vit C	48.49	290940	1.34	21712
Chit/EXPL/Vit C	56.66	339950	1.24	27415
Chit/ADS/Vit C	60.99	365950	1.36	26908
Chit/ADS/EXPL	41.61	249660	1.31	19058

4.2.2 Dissolution Studies

Dissolution studies on all formulations were performed in phosphate buffered solution (PBS) at pH 5.60 and 7.40 at 37 °C. The PBS was prepared according to the Merck's Tables for the Laboratory (Merck Tables: 59, 60). A 1/15 M solution of potassium dihydrogen orthophosphate (Merck, South Africa) was prepared by dissolving 9.073 g of the compound in 1000 ml of deionised water, while a 1/15 M of disodium hydrogen orthophosphate (Merck, South Africa) solution was prepared by dissolving 11.87 g of the compound in 1000 ml of deionised water. To make PBS of pH 5.60, 95.5 ml of potassium dihydrogen orthophosphate solution was made up to 100 ml with the solution of disodium orthophosphate. Similarly, 19.7 ml of potassium dihydrogen orthophosphate solution was needed to prepare PBS of pH 7.40 when made up to 100 ml with disodium

hydrogen orthophosphate solution. The above formula was used to prepare 5 liter quantities of dissolution media.

The dissolution method used to determine the drug release from the beads consists of *apparatus 1* in the USP (2002:1243). The basket stirring element was used. An Erweka DT6R (Erweka® Apparatebau GmbH, Germany) dissolution apparatus was used. A sample of 0.1 g of the beads was placed in the basket. The basket was lowered into 500 ml dissolution medium and rotation was started after the basket was submerged into the medium. The rotation was kept constant at 50 revolutions per minute. The dissolution studies were conducted in four-fold.

4.2.3 Rifampicin analysis

Three standard curves of rifampicin were constructed everyday during drug loading and dissolution studies. It was convenient to use the same standard curve for both the drug loading and dissolution studies since the amount of rifampicin released was calculated from drug loading capacity values. The preparation of rifampicin stock and standard solutions for spectrophotometric analysis has already been described in chapter 3.

Samples of 5 ml dissolution media were taken at 1 – 10, 20, 30 and 60 minutes after which samples were taken at 2, 3, 4, 5, and 6 hours. Of the PBS solution 5 ml was added to the dissolution bath after each sampling interval. The concentration of the released rifampicin was calculated from the UV absorbance of rifampicin at 475 nm (British Pharmacopoeia, 2001:1347). The following equation was used to correct for dilution of the dissolution media after each sample extraction and addition of PBS:

$$Y_n^* = Y_n + \frac{V_s}{V_m} \cdot \sum^{n-1} Y^* \quad (4-1)$$

where Y_n^* is the corrected absorbency of the n^{th} sample, Y_n is the measured absorbency of the n^{th} sample, V_s is the sample volume, V_m is the dissolution medium volume and $\sum^{n-1} Y^*$ is the sum of all the corrected absorbencies prior to the n^{th} sample.

4.2.4 Chitosan analysis

Materials

The following materials were used during the construction of a chitosan standard curve: Chitosan (91.4 % deacetylated) [Annexure A, C.1]. Cibacron brilliant red 3B-A (Aldrich, USA). Glycine sodium chloride and sodium hydrochloride (Merck, South Africa). Glacial acetic acid (99.5 %) and hydrochloric acid (SAARchem, South Africa).

Method

The method followed the colorimetric method for determination of chitosan as described by Muzzarelli (1998:255). Chitosan powder (0.5 g) was suspended in 50 ml demineralised water. After stirring for 30 minutes, glacial acetic acid (99.5 %, 0.5 ml) was added to dissolve the chitosan powder. The solution was made up to 100 ml with demineralised water and filtered through a glassfibre prefilter to remove any trace impurities. The resulting solution was further diluted 10-fold to 0.5 g/liter.

A glycine hydrochloride buffer (0.1 M) was prepared by dissolving glycine (1.87 g) and sodium chloride (1.46 g) and made up to 250 ml with demineralised water. Aliquots (81 ml) of this solution were made up to 100 ml with 0.1 M HCl. The final pH was 3.20. A solution of cibacron red dye was prepared by dissolving 150 mg of the powder in 100 ml demineralised water. Aliquots of the dye solution (5 ml) were made up to 100 ml with the prepared glycine hydrochloride buffer.

For the construction of a chitosan standard curve 15, 30, 45, 60, 80, 150, 200 and 250 μ l of chitosan stock solution were pipetted into disposable cuvettes and made up with the buffer to 300 μ l. Aliquots of the dye were added to each cuvette to 3 ml. The absorbancy values were measured at 572 nm with a Helios α Unicam spectrophotometer (Unicam, Cambridge, UK). A new standard curve was constructed for each day of analysis. A typical standard curve for chitosan is depicted in Figure 4.1.

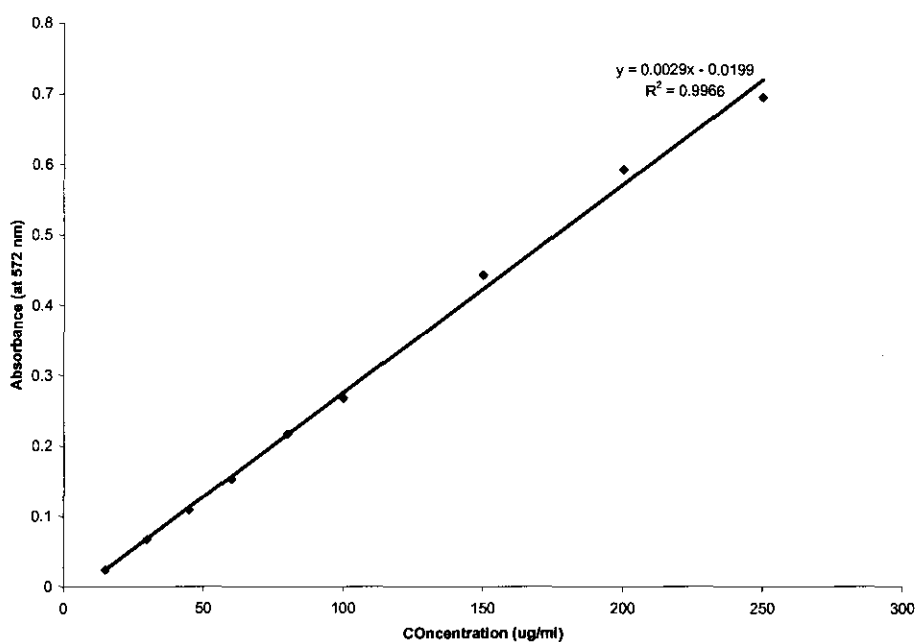


Figure 4.1: A typical standard curve used for Chitosan analysis.

From the 5 ml sample volumes taken during dissolution studies 300 μ l was transferred into disposable cuvettes and cibacron red dye was added to 3 ml. The UV absorbance of these solutions was determined at 572 nm. However, no chitosan was released from all the formulations and therefore the next section only describes results on rifampicin release.

4.3 Results and Discussion

4.3.1 Chitosan beads

Figure 4.2 and Table 4.2 depict the rifampicin release from pure chitosan beads at pH 5.60 and 7.40 over a period of 6 hours. Due to the presence of drug particles on the surface of the beads, a burst effect was observed in the first 10 minutes. The rate of rifampicin release seemed to be higher in the first 15 minutes after which the rate declined although drug release was still observed up to 6 hours. Drug release was higher at pH 7.40 with just over 29,56 $\pm$ 0.70 % of the drug released compared to pH 5.60 where 19.71 $\pm$ 0.75 % of the drug was released after 6 hours.

Table 4.2: Amount of rifampicin released from pure chitosan beads at pH 5.60 and 7.40 after 6 hours.

Time (min)	pH 5.60		pH 7.40	
	% Release	Standard Deviation	% Release	Standard Deviation
0	0	0	0	0
1	2.05	0.12	2.54	0.46
2	2.75	0.09	4.28	0.51
3	3.34	0.11	5.04	0.051
4	4.03	0.09	5.33	0.31
5	4.60	0.19	6.50	0.51
6	4.90	0.04	7.12	0.41
7	5.12	0.06	7.46	0.07
8	5.44	0.12	7.87	0.11
9	5.90	0.12	8.35	0.23
10	6.14	0.15	8.97	0.42
20	7.40	0.21	10.87	0.48
30	8.80	0.23	12.43	0.89
60	10.87	0.42	16.30	0.21
120	13.19	0.44	19.59	1.23
180	15.17	0.40	22.84	1.47
240	17.03	0.56	25.78	1.78
300	18.20	0.50	27.85	2.00
360	19.71	0.75	29.56	0.70

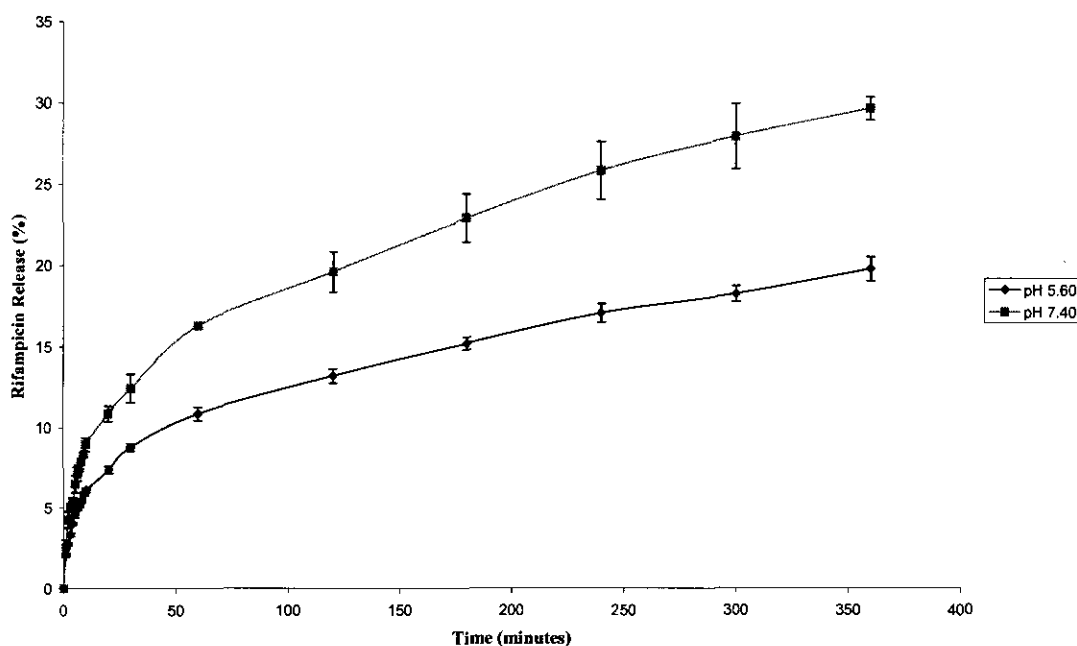


Figure 4.2: Rifampicin release from pure chitosan beads at pH 5.60 and 7.40.

4.3.2 Chitosan/SPE beads

Tables 4.3 to 4.5 and Figures 4.3 to 4.7 depict the rifampicin release from the Chit/ADS, Chit/EXPL and Chit/Vit C beads respectively at pH 5.60 and 7.40 over a period of 6 hours. A burst release effect was observed in the first 10 minutes due to the presence of drug particles on the outer surface of the beads. With the Chit/ADS formulation the rate of release was much higher at pH 7.40 compared to pH 5.60 while the drug release seemed to decline after 20 minutes at pH 5.60. Rifampicin release was higher at pH 7.40 with 51.50 ± 3.82 % of the drug being released compared to pH 5.60 where 23.62 ± 1.11 % of the drug was released after 6 hours. With the Chit/EXPL beads 39.63 ± 1.98 % of the rifampicin was released at pH 7.40 while 22.87 ± 1.31 % of the drug was released from this formulation at pH 5.60 after 6 hours. In general, better rifampicin release was observed with the Chit/Vit C formulations at pH 5.60 (48.34 ± 1.00 %) and pH 7.40 (55.83 ± 2.47 %) compared to the other two formulations at the same pH values. Although the Chit/Vit C beads exhibited small pore sizes the better rifampicin release

obtained with the incorporation of vitamin C are attributable to the acidic microenvironment created by the vitamin C thus resulting in more pronounced swelling.

Table 4.3: Amount of rifampicin released from Chit/ADS beads at pH 5.60 and 7.40 after 6 hours.

Time (min)	pH 5.60		pH 7.40	
	% Release	Standard Deviation	% Release	Standard Deviation
0	0	0	0	0
1	2.41	0.18	3.35	0.27
2	3.30	0.07	5.11	0.29
3	3.93	0.08	6.82	0.26
4	4.72	0.08	7.60	0.13
5	5.04	0.16	7.96	0.15
6	5.45	0.13	8.75	0.12
7	5.84	0.27	9.44	0.12
8	6.22	0.29	10.11	0.24
9	6.55	0.33	10.55	0.31
10	7.00	0.33	10.67	0.24
20	7.91	0.18	13.15	0.31
30	9.44	0.12	15.61	0.38
60	11.75	0.17	20.93	0.64
120	14.65	0.44	27.95	1.57
180	17.45	0.68	36.96	2.19
240	19.93	0.73	42.88	3.77
300	21.82	1.08	47.46	3.04
360	23.62	1.11	51.50	3.82

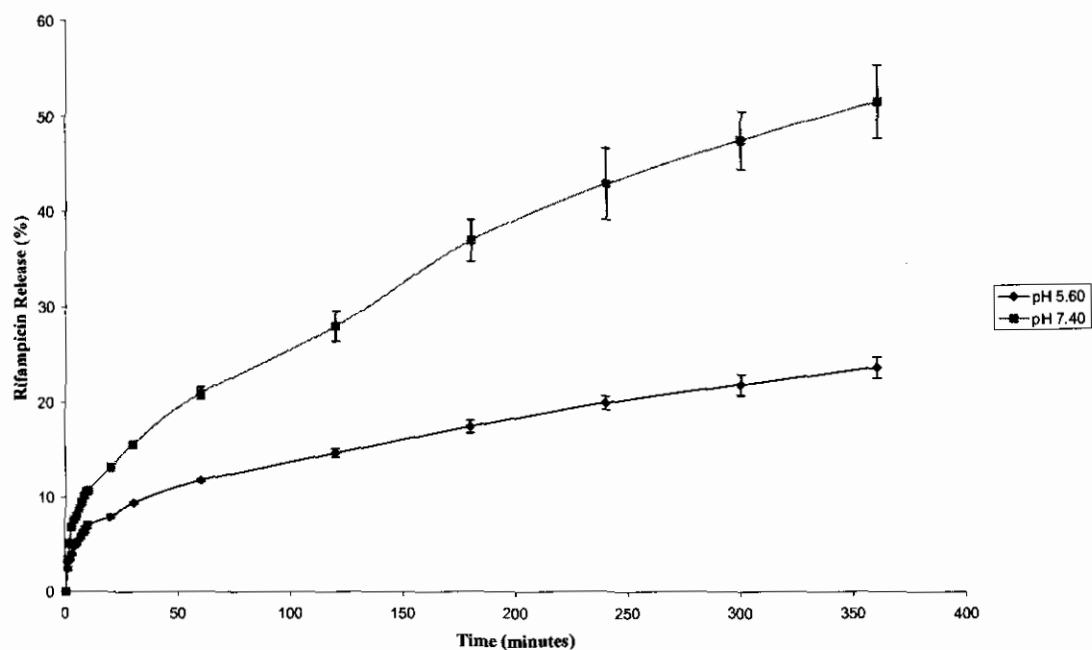


Figure 4.3: Rifampicin release from Chit/ADS beads at pH 5.60 and 7.40.

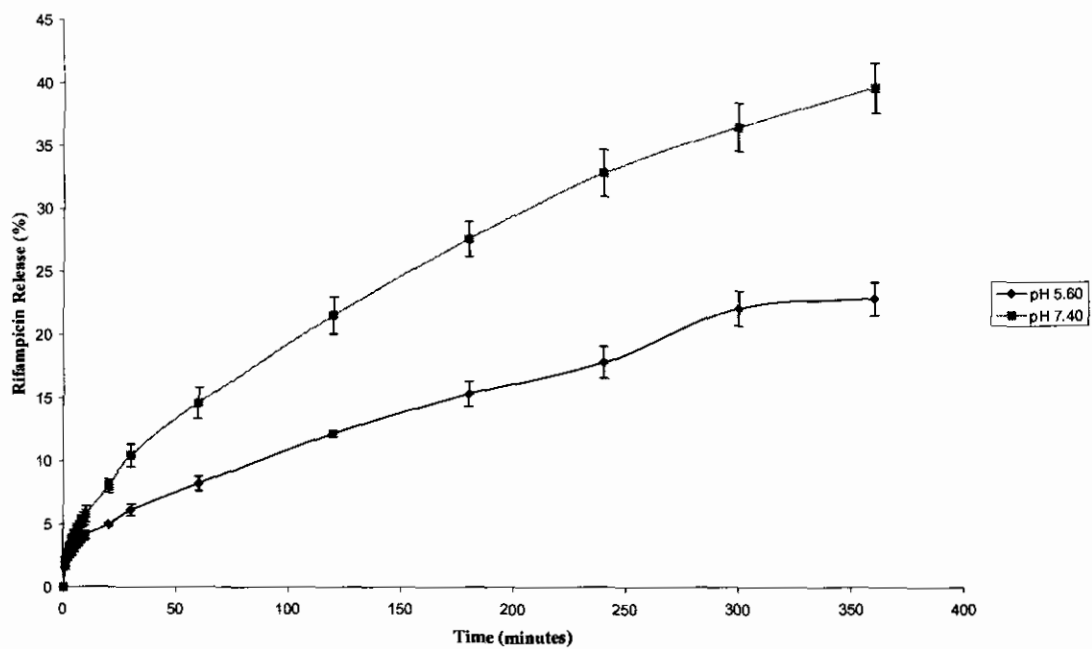


Figure 4.4: Rifampicin release from Chit/EXPL beads at pH 5.60 and 7.40.

Table 4.4: Amount of rifampicin released from Chit/EXPL beads at pH 5.60 and 7.40 after 6 hours.

Time (min)	pH 5.60		pH 7.40	
	% Release	Standard Deviation	% Release	Standard Deviation
0	0	0	0	0
1	1.52	0.14	2.11	0.10
2	2.19	0.10	2.76	0.30
3	2.51	0.17	3.30	0.20
4	2.72	0.20	3.86	0.29
5	3.00	0.21	4.14	0.44
6	3.29	0.25	4.54	0.46
7	3.53	0.29	4.77	0.46
8	3.79	0.31	5.24	0.45
9	3.97	0.30	5.41	0.51
10	4.13	0.34	5.82	0.64
20	4.98	0.11	8.02	0.52
30	6.17	0.45	10.50	0.91
60	8.22	0.57	14.59	1.23
120	12.18	0.23	21.53	1.46
180	15.34	1.00	27.60	1.38
240	17.84	1.25	32.82	1.87
300	22.10	1.39	36.51	1.93
360	22.87	1.31	39.63	1.98

Table 4.5: Amount of rifampicin released from Chit/Vit C beads at pH 5.60 and 7.40 after 6 hours.

Time (min)	pH 5.60		pH 7.40	
	% Release	Standard Deviation	% Release	Standard Deviation
0	0	0	0	0
1	1.87	0.20	5.61	0.41
2	4.61	0.26	7.74	0.57
3	6.19	0.38	9.42	0.54
4	8.09	0.23	11.55	0.43
5	9.12	0.42	12.28	0.44
6	10.03	0.30	13.15	0.48
7	10.95	0.31	14.27	0.61
8	12.20	0.26	15.04	0.71
9	12.78	0.26	15.58	0.55
10	13.66	0.31	16.33	0.68
20	15.78	0.22	20.00	0.66
30	17.48	0.55	23.50	1.00
60	21.27	0.74	29.86	1.61
120	27.89	1.44	37.48	1.56
180	36.14	1.01	42.94	1.61
240	40.98	1.04	48.74	1.96
300	46.00	1.04	52.73	2.04
360	48.34	1.00	55.83	2.47

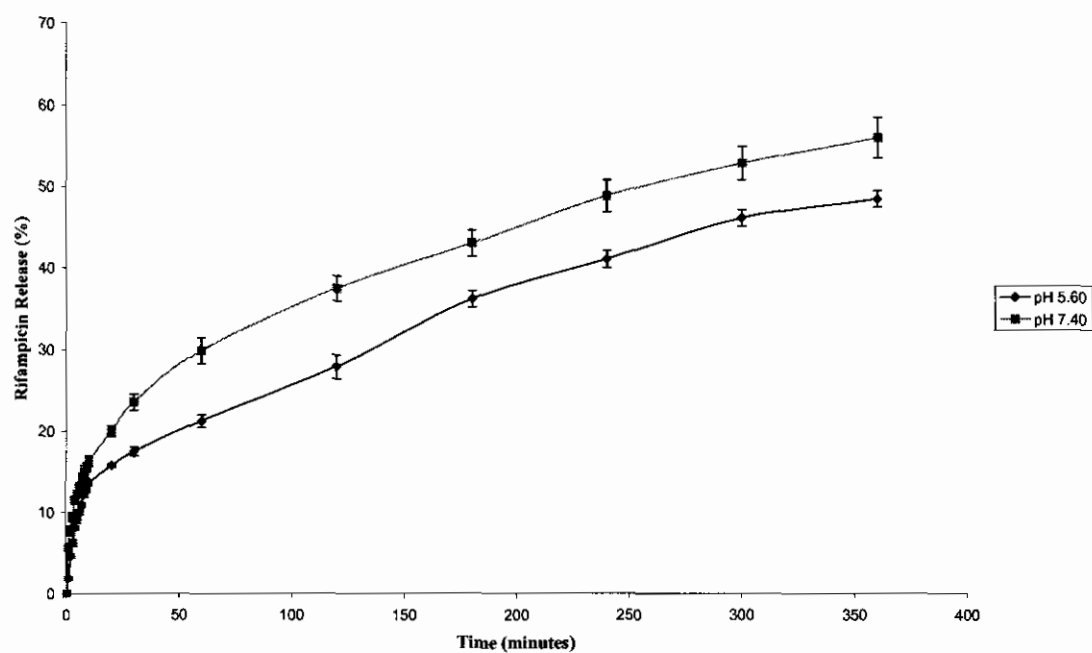


Figure 4.5: Rifampicin release from Chit/Vit C beads at pH 5.60 and 7.40.

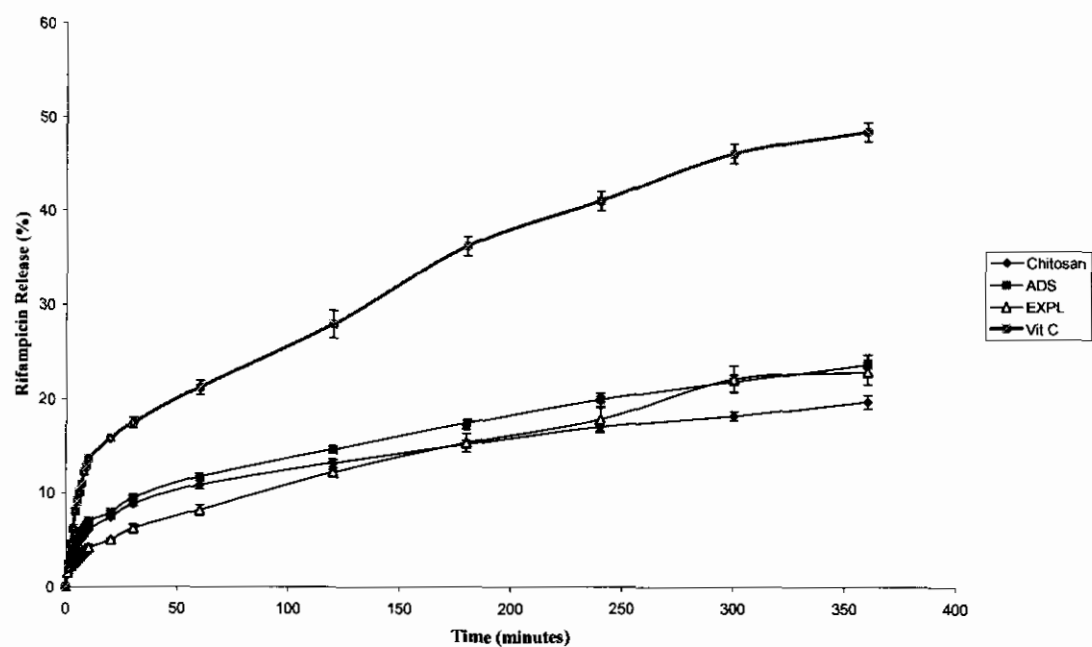


Figure 4.6: Rifampicin release from chitosan/SPE beads at pH 5.60 after 6 hours.

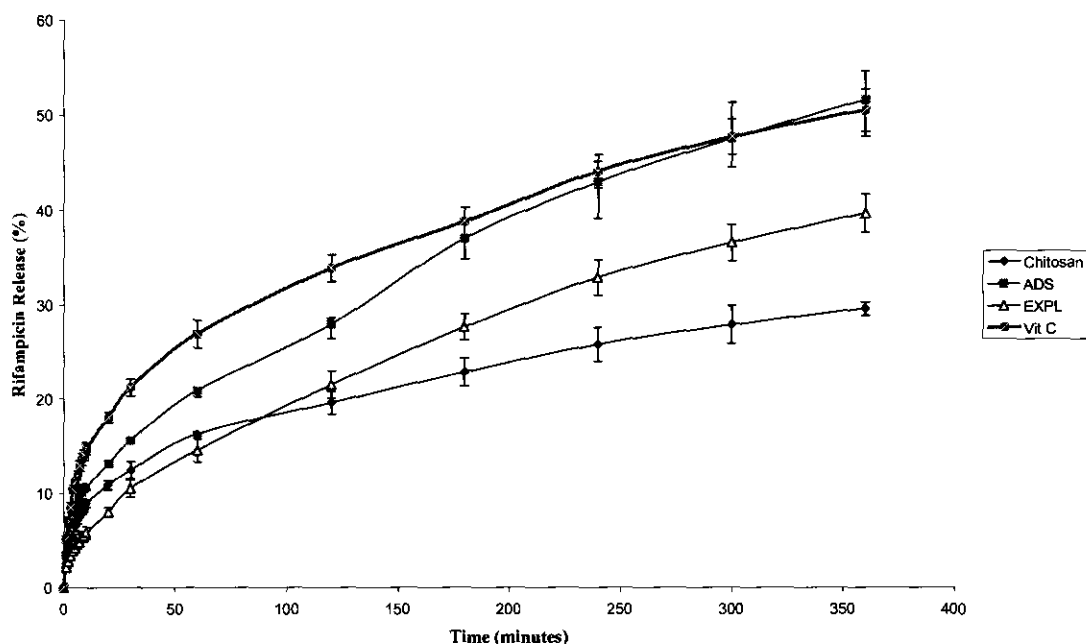


Figure 4.7: Rifampicin release from chitosan/SPE beads at pH 7.40 after 6 hours.

4.3.3 Chitosan/MPE beads

Tables 4.6 to 4.8 and Figures 4.8 to 4.12 depict the rifampicin release from the different chitosan/MPE beads. The effect of combinations of the pharmaceutical excipients on rifampicin release from the beads is not easily comparable to that of single excipients. The effect of pH, however, remained the same on all the formulations. Better rifampicin release was observed at pH 7.40 compared to pH 5.60. With the observed high porosity and better swelling of Chit/ADS/EXPL beads (Figure 3.10) better drug release than obtained was expected. A 61.69 ± 1.41 % of rifampicin was released from the Chit/ADS/EXPL formulation at pH 7.40 whereas only 32.70 ± 1.82 % of the drug was released from the same formulation at pH 5.60. A burst release effect was also observed with all the formulations at both pH values due to the presence of drug particles on the outer surfaces of the beads.

Table 4.6: Amount of rifampicin released from Chit/EXPL/Vit C beads at pH 5.60 and 7.40 after 6 hours.

Time (min)	pH 5.60		pH 7.40	
	% Release	Standard Deviation	% Release	Standard Deviation
0	0	0	0	0
1	1.43	0.13	3.55	0.44
2	2.17	0.17	5.03	0.39
3	3.80	0.17	6.40	0.21
4	4.57	0.24	7.44	0.27
5	5.71	0.23	8.46	0.13
6	6.54	0.20	9.17	0.25
7	7.58	0.24	9.64	0.28
8	8.06	0.33	10.24	0.25
9	8.76	0.33	10.77	0.25
10	9.50	0.32	11.29	0.37
20	10.55	0.49	13.95	0.40
30	12.12	0.49	16.49	0.67
60	14.63	0.67	20.97	0.49
120	16.49	0.87	27.61	0.36
180	17.97	0.63	33.30	0.48
240	20.06	0.67	37.51	0.47
300	21.26	0.62	40.71	0.63
360	23.55	0.78	43.64	0.55

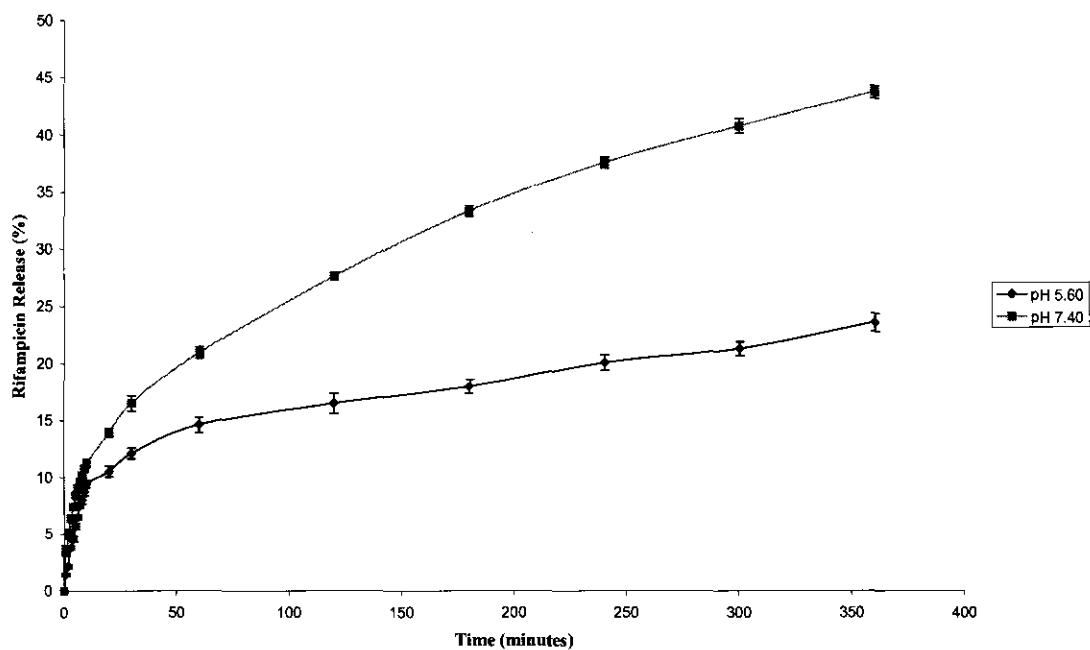


Figure 4.8: Rifampicin release from rifampicin loaded Chit/EXPL/Vit C beads at pH 5.60 and 7.40.

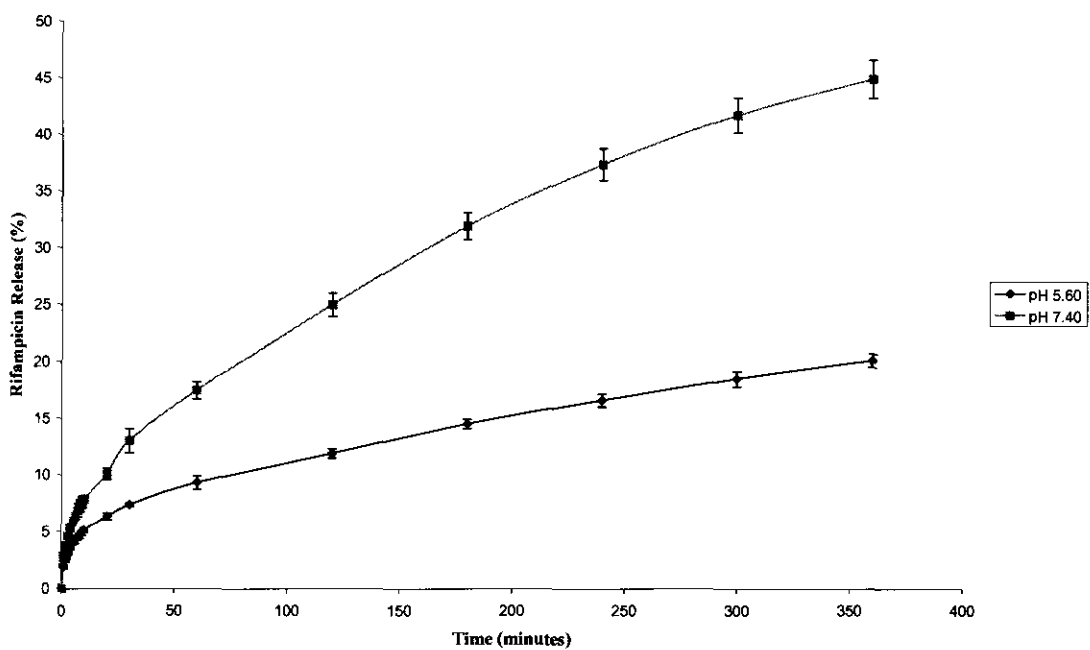


Figure 4.9: Rifampicin release from Chit/ADS/Vit C beads at pH 5.60 and 7.40.

Table 4.7: Amount of rifampicin released from Chit/ADS/Vit C beads at pH 5.60 and 7.40 after 6 hours

Time (min)	pH 5.60		pH 7.40	
	% Release	Standard Deviation	% Release	Standard Deviation
0	0	0	0	0
1	1.92	0.17	2.74	0.37
2	2.56	0.19	3.68	0.40
3	3.13	0.14	4.63	0.16
4	3.61	0.14	5.35	0.16
5	4.03	0.13	5.89	0.30
6	4.23	0.24	6.37	0.29
7	4.51	0.19	6.89	0.57
8	4.65	0.23	7.28	0.51
9	4.91	0.20	7.62	0.51
10	5.17	0.19	7.92	0.33
20	6.33	0.31	10.12	0.51
30	7.34	0.15	13.03	1.07
60	9.29	0.58	17.47	0.75
120	11.90	0.42	24.96	1.05
180	14.52	0.42	31.89	1.18
240	16.60	0.56	37.30	1.41
300	18.51	0.66	41.65	1.55
360	20.10	0.59	44.82	1.70

Table 4.8: Amount of rifampicin released from Chit/ADS/EXPL beads at pH 5.60 and 7.40 after 6 hours.

Time (min)	pH 5.60		pH 7.40	
	% Release	Standard Deviation	% Release	Standard Deviation
0	0	0	0	0
1	2.68	0.51	4.52	0.42
2	4.14	0.75	6.90	1.04
3	5.05	0.41	8.32	0.88
4	5.63	0.53	9.99	0.92
5	6.25	0.52	10.42	1.06
6	6.78	0.48	11.30	1.05
7	7.21	0.63	12.12	0.92
8	7.71	0.50	12.88	0.85
9	7.95	0.56	13.57	0.75
10	8.28	0.43	14.07	0.75
20	9.94	0.53	17.73	0.50
30	11.96	0.28	21.80	0.76
60	14.62	0.82	28.74	1.02
120	18.63	1.09	38.84	1.07
180	23.10	1.28	47.23	1.23
240	26.84	1.16	53.18	1.41
300	29.68	1.34	58.04	1.66
360	32.70	1.82	61.69	1.41

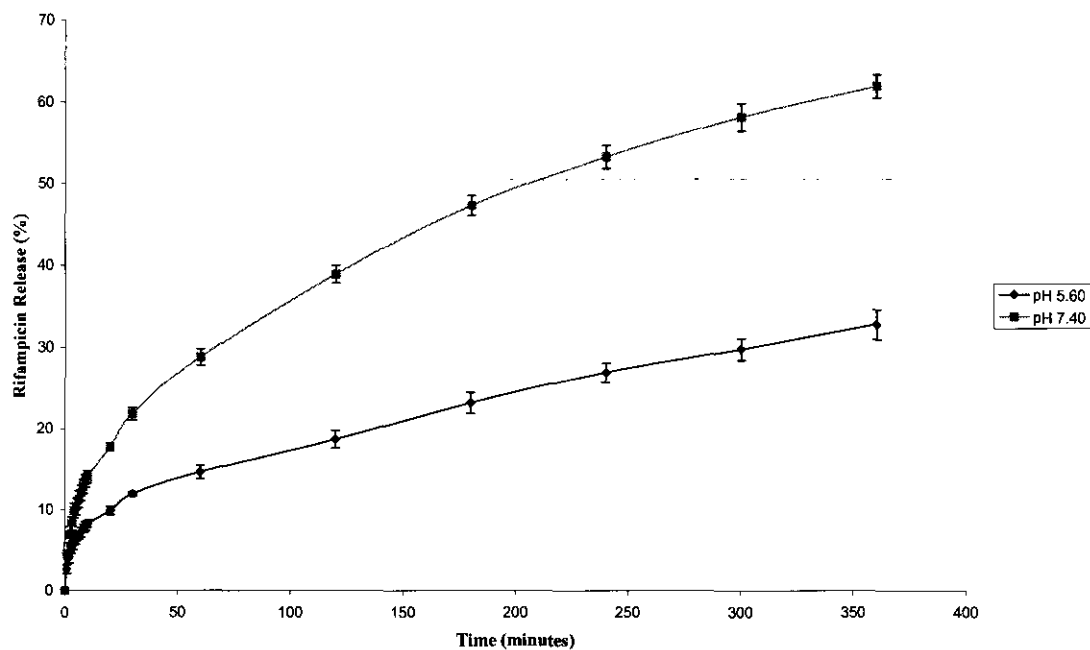


Figure 4.10: Rifampicin release from Chit/ ADS/ EXPL beads at pH 5.60 and 7.40.

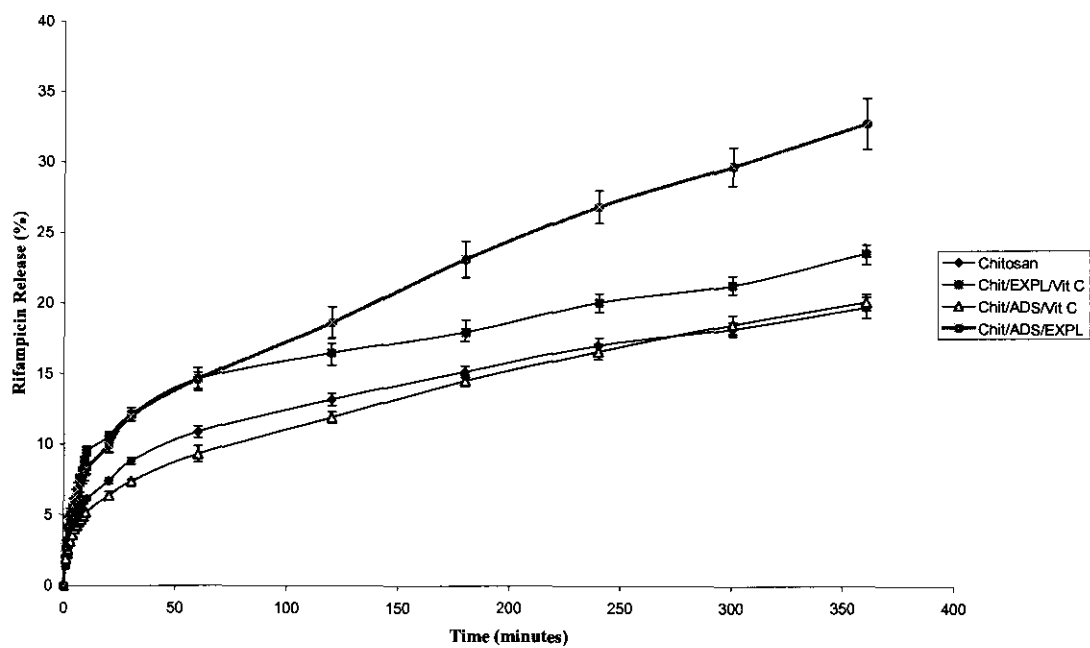


Figure 4.11: Rifampicin release from chitosan/MPE beads at pH 5.60 after 6 hours.

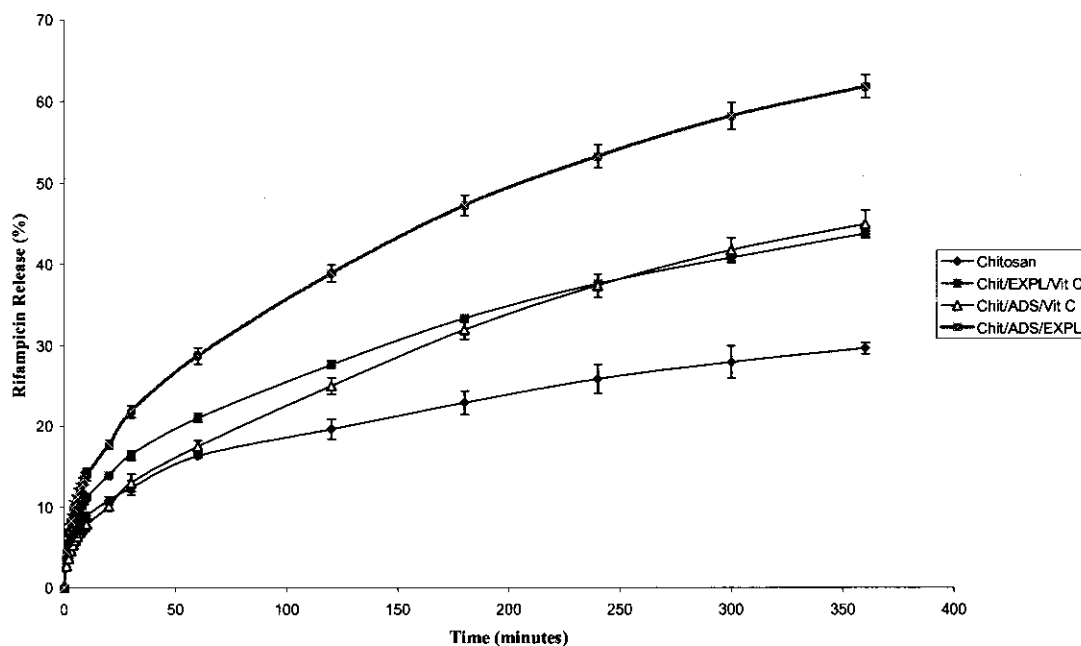


Figure 4.12: Rifampicin release from chitosan/MPE beads at pH 7.40 after 6 hours.

4.3.4 Comparison of dissolution data

A comparison of the dissolution results is given in Figure 4.13. The results compare in the following order: **pH 7.40 > pH 5.60 and Chit/Expl/ADS > Chit/Vit C > Chit/ADS > Chit/ADS/Vit C > Chit/Expl/Vit C > Chit/Expl > pure Chitosan** beads. It can be seen from these results that the excipients had little or no effect on the release of rifampicin at a pH of 5.60 except for vitamin C (0.25 % w/v) (Figure 4.6). However, at a pH of 7.40 it is clear that the addition of pharmaceutical excipients had a major effect on rifampicin release (Figures 4.7 and 4.2). It is also evident that vitamin C and Ac-Di-Sol[®] had a more pronounced effect compared to Explotab[®] at pH 7.40 (Figure 4.7). The best release was obtained with a combination of Ac-Di-Sol[®] and Explotab[®] at pH 7.40. This was comparable with vitamin C alone. It can therefore be concluded that vitamin C alone is a good choice for incorporation into beads when drug release is intended over a wide pH range (both acidic and basic) and that Ac-Di-Sol[®] and vitamin C or a combination of

Explotab[®] and Ac-Di-Sol[®] can be used when drug release is intended in neutral and slightly basic environments.

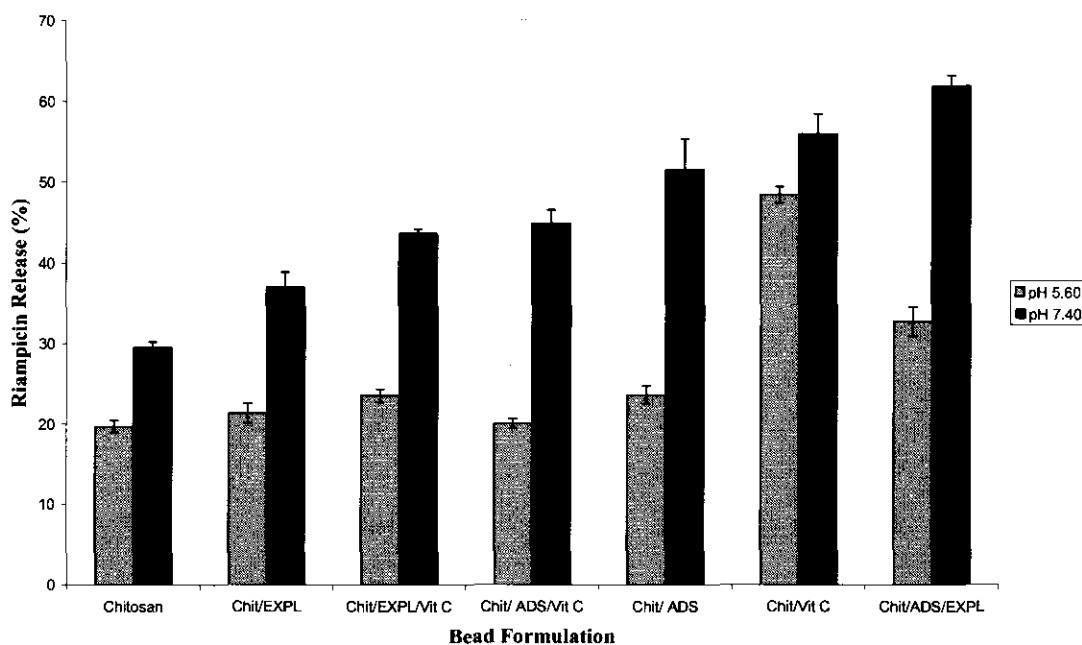


Figure 4.13: Rifampicin release from rifampicin loaded beads at pH 5.60 and 7.40 after a period of 6 hours.

4.4 Conclusion

Rifampicin release studies on all the selected formulations of beads were conducted with success with the described method. In general, it was found that the inclusion of Ac-Di-Sol[®], Explotab[®] and vitamin C greatly enhance rifampicin release from the beads. A significant amount of rifampicin was released from the beads, especially at pH 7.40. At a pH of 5.60 only vitamin C was able to enhance rifampicin release compared to pure chitosan beads. A burst release effect was observed with all the formulations at both pH values due to the presence of rifampicin particles on the surface of the beads which was a result of the preparation process. The effect of a combination of pharmaceutical excipients on rifampicin release from the beads was not easily comparable to the effect of

single excipients. It can therefore be speculated that other properties of chitosan that have not been investigated in this study, can also influence the rate and extent of rifampicin release from the beads at both pH values. For example, according to Alsarra *et al.* (2002:3637), both the molecular weight and degree of deacetylation of chitosan were found to have an effect on the release of the enzyme, lipase, from chitosan beads. The lower release of rifampicin at pH 5.60 can probably be explained by a good structural integrity of the beads at this environment while the structural integrity of the beads is more compromised at a pH of 7.40, leading to beads that easily collapse in this environment after swelling occurred with consequent higher rifampicin release. However, on the other hand the better rifampicin release at pH 7.40 can also be explained by its better solubility at an alkaline pH than at an acidic pH (Steenkamp, 2002:78).

Chapter 5

Summary and Future Prospects

5.1 Summary

Chitosan is a polycationic, biodegradable, non-pharmacological and non-toxic polymer, which has found wide application both in biotechnology and pharmaceuticals. Many experimental drug delivery systems that are chitosan based have been designed, developed and optimised for desired drug release profiles. The desire to develop chitosan-based microparticulate drug delivery systems led to the formulation of microparticles and nanoparticles. In all these systems, chitosan has been found to perform more than one role. Apart from being also exploited as a non-viral gene transfectant, chitosan provides both mucoadhesive and absorption enhancing advantages in the delivery of hydrophilic drugs.

This study demonstrated that the abilities of chitosan to provide several advantages in drug delivery were not limited to micro- and nano-particles alone. Beads are also part of microparticulate drug delivery systems and it has been demonstrated in many studies that beads are a versatile dosage system for controlled drug delivery. A number of techniques can be used to prepare beads but the ionotropic gelation method is the most reproducible method and was used in this study for the preparation of chitosan beads. This method also provides easy entrapment of the drug inside the beads. Enforced by crosslinking, bead formation takes place with ease due to the cationic nature of chitosan which allows drug entrapment within a three-dimensional network of ionically linked polymer. In this way the drug is locked within the polymeric matrix of the bead.

Rifampicin, a first line drug in the treatment of tuberculosis, was chosen as the study drug for incorporation into chitosan beads. The conventional treatment regimen for tuberculosis has led to incompliance in most patients and resulted in many deaths over decades. Incorporating rifampicin into chitosan beads can improve its absorption and bioavailability thereby eliminating the need for multi-drug treatment in the post-acute phase of tuberculosis. In a previous study the incorporation of rifampicin into chitosan beads, containing *N*-trimethyl chitosan chloride (TMC), has only resulted in a minimal release of the drug over 24 hours. In this study the ability of pharmaceutical excipients such as Ac-Di-Sol®, Explotab® and Vitamin C was investigated to enhance the swelling behaviour of the beads to increase the rate and extent of rifampicin release over 6 hours at pH 5.60 and 7.40. The excipients were suspended or dissolved in the polymer solution prior to drug loading.

For preparation of the rifampicin loaded chitosan beads, a rifampicin/chitosan suspension containing several pharmaceutical excipients was dripped into a tripolyphosphate (TPP) solution resulting in precipitation of the polymer solution into spherical gel pellets. The beads were washed twice with deionised water to remove the excess TPP and were then freeze-dried. The water washings and the TPP solution were used for spectrophotometric analysis to calculate the amount of rifampicin lost during bead preparation and to determine the drug loading capacity of the various beads formulations. A scanning electron microscope (SEM) was used to investigate the effect of the excipients, either single or in combination, on the structure of the beads with the emphasis on their porosity. Swelling behaviour studies were also conducted by immersing a pre-weighed sample of the beads in phosphate buffer solution (PBS) of pH 5.60 and 7.40 at 37 °C. The weight increase, after removing the beads from the PBS and blotting any excess droplets, was used to calculate the degree of swelling (Esw).

Rifampicin release from the beads in PBS was studied over a period of 6 hours at pH 5.60 and 7.40 at 37 °C. It was found that less drug was released at pH 5.60 compared to pH 7.40. The dissolution studies proved that Vitamin C, Ac-Di-Sol® and Explotab® increased the swelling of the beads thereby leading to much improved drug release compared to

pure chitosan beads. With the addition of these excipients, rifampicin release was significantly improved over a period of 6 hours as opposed to a previous study where minimal rifampicin was released after 24 hours.

Although no chitosan was released from the beads for paracellular absorption enhancement, inclusion of these excipients improved rifampicin release to significant amounts and this may necessitate future attention on the optimisation of the method used for bead preparation to minimise drug loss to further improve drug loading.

5.2 Future Prospects

A number of variables were identified which may have had an influence on the characteristics of the prepared beads, thus emphasising the need to optimise certain techniques in this study. Many factors documented in literature have been evaluated for any effects on the characteristics of beads and the results provided proof around many of the hypotheses concerning beads.

Firstly, the concentration of the polymer solution itself will have an influence on the structure and integrity of the beads and consequently on drug loading and drug release. The viscosity of the polymer solution might influence drug loading because more viscous polymer solutions might lead to better drug suspension. Rifampicin does not dissolve in the polymer solution and therefore an amount of the drug might sediment easily to the bottom of a less viscous solution. The influence of viscosity on drug loading and drug release is attributable to the molecular weight and the degree of deacetylation of chitosan. In order to maintain an optimal bead size for improved drug release, the viscosity of the polymer solution has to be such that it can easily flow through a gauge 22 or 21 needle without blocking the needle.

The quality of the beads in terms of shape has a bearing on the extent of crosslinking or the entire preparation technique. The preparation technique with regard to stirring times

will have an influence on the extent of crosslinking and therefore on the integrity and shape of the bead. Crosslinking increases shape or structural integrity as well as the ability of the beads to maintain roundness. Beads with edges on the surface might show a character of fragility and less structural integrity. Although prolonged stirring times might improve crosslinking and mechanical strength of the beads, more drug can be lost during this time. It is therefore important to study the effect of stirring times on drug loading, drug release, the friability and mechanical strength of the beads to optimise the preparation process.

Another factor that may influence rifampicin release from the beads is the chemical interaction of chitosan with the dissolution media. More attention can be focused in future on the chemical interactions of the excipients on the behavior of chitosan in the dissolution media by studying the *ionic strength effect*. Possible interactions of the excipients with chitosan can be assessed by infrared (IR) analysis of pure chitosan beads and beads containing the excipients prior to drug loading and release studies.

It is therefore advisable to repeat the same study with more emphasis to control bead size. The selected excipients can also be combined with *N*-trimethyl chitosan chloride (TMC) for possible TMC release to act as absorption enhancer. The concentration of the excipients, particularly vitamin C, can also be increased for even better swelling and also to study the pH sensitivity of the beads relative to the concentration of the excipients. The degree of deacetylation, which defines the amino content of chitosan, can be studied as another factor that influences the pH sensitivity of chitosan beads.

Annexure A

**Certificates of
Approval**

CERTIFICATE OF ANALYSIS FOR RIFAMPICIN

GRUPPO LEPETIT S.p.A.
STABILIMENTO DI BRINDISI

ANALYTICAL REPORT

Analytical Register No 4950

No 10352 / BR

Product Rifampicin USP 23 - BP 1998

Batch 1892

Quantity Kg 2000

DETERMINATION	RESULT
IDENTIFICATION (infrared identity test)	POSITIVE
DESCRIPTION	RED-BROWN CRYSTALLINE POWDER
PH	5.1
CRISTALLINITY	COMPLIES
SULPHATED ASH	0.02 %
LOSS ON DRYING	0.21 %
HEAVY METALS (Pb)	LESS THAN 20 ppm
BULK DENSITY (tapped)	0.797 g/ml
ASSAY (calculated on the dried substance):	
- SPECTROPHOTOMETRIC	99.8 %
- HPLC	99.4 %
RELATED SUBSTANCES by HPLC:	
- 3-Formyl Rifamycin SV	<0.05 %
- Quinone	0.09 %
- Any single other	NMT 1.0 %
- Total (except Quinone)	0.36 %

Confirmation: BR 02 / 280000147

QUALITY CONTROL MANAGER

Manufacturing: 11 / 99

Expiration: 11 / 2003

Dr. M. Di Gerardo

Date: 04 / 05 / 2000

FUJIAN SHIPBUILDING INDUSTRY GROUP CORP.

XIAMEN IMPORT AND EXPORT CO., LTD

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E-MAIL: XIAMEN@XIAMEN.COM

CERTIFICATE OF ANALYSIS FOR CHITOSAN

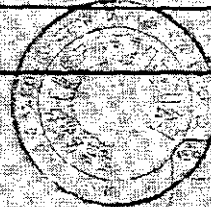
Report No. 000001

WCI 4219

Batch No. 000201	Quantity 50kg	Report Date: Feb 03, 2000
ITEMS TESTED	INSPECTION RESULTS	
Appearance	Off-white	
Mbisture	5.80%	
Ash	9.33%	
Decomposition	61.48%	
Viscosity (0.5%)	200s	
Mesh	80mesh	

L/C No. IC04760069800102

Drum's No. 69-102



2000-02-29

Annexure B
Dissolution Data

Annexure B: Dissolution Data

Data B.1 Dissolution data for rifampicin release from pure chitosan (3 % w/v) beads at pH 5.60 and 7.40 over a period of 6 hours.

Time (min)	pH 5.60				pH 7.40			
	Absorbency Values	Corrected Absorbency	% Release	Standard Deviation	Absorbency Values	Corrected Absorbency	% Release	Standard Deviation
0	0	0	0	0	0	0	0	0
1	0.02575	0.02575	2.054482	0.119141	0.03275	0.03275	2.542814	0.457015
2	0.0355	0.035655	2.745437	0.090657	0.0575	0.057697	4.283124	0.513411
3	0.04375	0.044118	3.33583	0.105691	0.068	0.068542	5.03969	0.051883
4	0.0535	0.05413	4.034319	0.091367	0.07175	0.0727	5.329759	0.313815
5	0.06125	0.062201	4.597366	0.185567	0.088	0.08938	6.493419	0.513045
6	0.06525	0.066569	4.90205	0.038031	0.0965	0.098408	7.123228	0.413545
7	0.068	0.06971	5.121206	0.059406	0.10075	0.103237	7.460107	0.066388
8	0.07225	0.074368	5.446156	0.116306	0.106	0.109092	7.868527	0.113125
9	0.07825	0.080802	5.894968	0.121909	0.11225	0.115978	8.348906	0.231188
10	0.08125	0.084271	6.137006	0.146716	0.1205	0.124901	8.971424	0.423407
20	0.09875	0.102259	7.391845	0.211366	0.147	0.152124	10.87055	0.478912
30	0.11825	0.122351	8.793532	0.23439	0.1685	0.174506	12.43195	0.885104
60	0.14725	0.152061	10.86612	0.418235	0.223	0.230017	16.30449	0.21017
120	0.1795	0.185194	13.17757	0.440358	0.26875	0.277105	19.58943	1.230889
180	0.207	0.213771	15.17115	0.40167	0.31375	0.323718	22.8412	1.472518
240	0.2325	0.240513	17.03671	0.560864	0.354	0.36585	25.78044	1.781911
300	0.24775	0.257158	18.1979	0.497325	0.3815	0.395474	27.84706	2.003019
360	0.268	0.278895	19.71427	0.745653	0.40375	0.420013	29.55894	0.703226

Annexure B: Dissolution Data

Data B.2 Dissolution data for rifampicin release from Chitosan/Ac-Di-Sol® 0.5 % w/v beads at pH 5.60 and 7.40 over a period of 6 hours.

Time (min)	pH 5.60				pH 7.40			
	Absorbency values	Corrected Absorbency	% Release	Standard Deviation	Absorbency values	Corrected Absorbency	% Release	Standard Deviation
0	0	0	0	0	0	0	0	0
1	0.015	0.015	2.413914	0.182556	0.02225	0.02225	3.34979	0.266118
2	0.02175	0.02184	3.296864	0.065577	0.03575	0.03584	5.109688	0.287773
3	0.0265	0.026721	3.92687	0.07521	0.04875	0.049098	6.815499	0.262863
4	0.0325	0.03288	4.721913	0.075657	0.0545	0.055141	7.595503	0.13051
5	0.03475	0.035325	5.037529	0.164431	0.057	0.057968	7.96043	0.151074
6	0.03775	0.038533	5.451702	0.125367	0.06275	0.06406	8.746824	0.123699
7	0.0405	0.04151	5.835927	0.271758	0.06775	0.069436	9.440856	0.122006
8	0.04325	0.044503	6.222283	0.290692	0.0725	0.074593	10.10649	0.267675
9	0.0455	0.047012	6.546225	0.330418	0.0755	0.078028	10.5499	0.307463
10	0.04875	0.050535	7.000996	0.329716	0.076	0.078981	10.67292	0.237254
20	0.0555	0.057578	7.910086	0.176362	0.09475	0.098187	13.15215	0.308715
30	0.067	0.069411	9.437565	0.115878	0.11325	0.117255	15.61363	0.375863
60	0.0845	0.087313	11.74847	0.166148	0.15375	0.158435	20.92934	0.643373
120	0.1065	0.10982	14.65381	0.441403	0.20725	0.212857	27.95454	1.573322
180	0.1275	0.131459	17.44711	0.680532	0.27575	0.282601	36.95747	2.18706
240	0.146	0.150724	19.93396	0.730901	0.32	0.328505	42.88312	3.768844
300	0.15975	0.16535	21.82198	1.07524	0.3535	0.363925	47.45535	3.042434
360	0.17275	0.179308	23.62383	1.111603	0.38275	0.395296	51.50492	3.815614

Annexure B: Dissolution Data

Data B.3 Dissolution data for rifampicin release from pure Chitosan/Explotab® 0.25 %
w/v beads at pH 5.60 and 7.40 over a period of 6 hours

Time (min)	pH 5.60				pH 7.40			
	Absorbency values	Corrected Absorbency	% Release	Standard Deviation	Absorbency values	Corrected Absorbency	% Release	Standard Deviation
0	0	0	0	0	0	0	0	0
1	0.01525	0.01525	1.522786	0.137238	0.0225	0.0225	2.105382	0.103742
2	0.0235	0.023592	2.193093	0.104428	0.0305	0.030635	2.759095	0.297289
3	0.02725	0.027483	2.505766	0.167008	0.037	0.037318	3.296128	0.198806
4	0.02975	0.030146	2.7198	0.20321	0.04375	0.04429	3.856385	0.291494
5	0.033	0.033575	2.995308	0.210909	0.047	0.047803	4.138643	0.438794
6	0.0365	0.037273	3.292472	0.254219	0.05175	0.052835	4.543005	0.462994
7	0.03925	0.040242	3.531055	0.294615	0.05425	0.055645	4.768851	0.455857
8	0.04225	0.043477	3.791054	0.310742	0.05975	0.061471	5.236977	0.448692
9	0.04425	0.045731	3.972141	0.297562	0.0615	0.063579	5.406412	0.511943
10	0.046	0.047746	4.134102	0.338554	0.06625	0.068698	5.817765	0.635893
20	0.05625	0.058272	4.979952	0.112318	0.09325	0.096096	8.019376	0.516829
30	0.07075	0.07311	6.172265	0.450154	0.1235	0.126905	10.49517	0.911233
60	0.09575	0.098534	8.215329	0.566446	0.17375	0.177896	14.59271	1.23028
120	0.1445	0.147859	12.17895	0.229269	0.259	0.264189	21.52701	1.456663
180	0.183	0.187226	15.34241	0.999764	0.333	0.339743	27.59838	1.384793
240	0.213	0.218324	17.84138	1.249358	0.396	0.404741	32.8215	1.870912
300	0.26475	0.271352	22.10261	1.387285	0.4395	0.450617	36.50801	1.926798
360	0.27275	0.28094	22.87313	1.309867	0.47575	0.489504	39.63289	1.978603

Annexure B: Dissolution Data

Data B.4 Dissolution data for rifampicin release from pure Chitosan/Vitamin C 0.25 %
w/v beads at pH 5.60 and 7.40 over a period of 6 hours

Time (min)	pH 5.60				pH 7.40			
	Absorbency values	Corrected Absorbency	% Release	Standard Deviation	Absorbency Values	Corrected Absorbency	% Release	Standard Deviation
0	0	0	0	0	0	0	0	0
1	0.0125	0.0125	1.865333	0.199435	0.045	0.045	5.607514	0.409801
2	0.03625	0.036325	4.608639	0.255266	0.06325	0.06352	7.739981	0.574303
3	0.04975	0.050043	6.188127	0.378891	0.0775	0.07815	9.42448	0.544382
4	0.066	0.066591	8.093588	0.230982	0.0955	0.096615	11.55061	0.42834
5	0.0745	0.075487	9.117909	0.424116	0.10125	0.102938	12.27867	0.438705
6	0.082	0.083434	10.03296	0.298554	0.10825	0.110545	13.15463	0.479921
7	0.0895	0.091426	10.95319	0.306138	0.11725	0.120195	14.26571	0.605559
8	0.09975	0.102213	12.19525	0.259629	0.12325	0.126898	15.03758	0.714762
9	0.10425	0.107312	12.78231	0.261313	0.12725	0.131638	15.5833	0.551534
10	0.11125	0.114937	13.66034	0.30997	0.133	0.138151	16.33329	0.683566
20	0.129	0.133355	15.78101	0.218708	0.164	0.169949	19.99464	0.660087
30	0.143	0.148129	17.48214	0.545353	0.1935	0.200433	23.50469	0.995344
60	0.175	0.180987	21.26555	0.74276	0.2475	0.255594	29.85615	1.613085
120	0.2315	0.238537	27.89208	1.442576	0.31225	0.321829	37.48272	1.562819
180	0.30175	0.310176	36.14089	1.012781	0.35775	0.369203	42.93749	1.609949
240	0.342	0.352236	40.9839	1.038189	0.406	0.419599	48.74035	1.955294
300	0.3835	0.395788	45.99865	1.039439	0.43825	0.454285	52.73423	2.039578
360	0.4015	0.416089	48.33619	0.999331	0.4625	0.481165	55.82925	2.470637

Annexure B: Dissolution Data

Data B.5 Dissolution data for rifampicin release from Chitosan/ Explotab® 0.25 % w/v / Vitamin C 0.25 % w/v beads at pH 5.60 and 7.40 over a period of 6 hours.

Time (min)	pH 5.60				pH 7.40			
	Absorbency Values	Corrected Absorbency	% Release	Standard Deviation	Absorbency Values	Corrected Absorbency	% Release	Standard Deviation
0	0	0	0	0	0	0	0	0
1	0.012	0.012	1.431681	0.128962	0.03525	0.03525	3.551846	0.436538
2	0.02	0.020072	2.167766	0.167189	0.05125	0.051462	5.030171	0.392115
3	0.03775	0.037942	3.797329	0.173972	0.066	0.066519	6.403262	0.214718
4	0.046	0.046419	4.5703	0.236664	0.077	0.077915	7.442462	0.271725
5	0.05825	0.058945	5.712544	0.230991	0.08775	0.089127	8.464883	0.13107
6	0.067	0.068044	6.542327	0.20172	0.095	0.096904	9.174021	0.250701
7	0.078	0.079446	7.582074	0.240418	0.0995	0.101974	9.636354	0.278815
8	0.08275	0.084664	8.057903	0.334193	0.1055	0.108571	10.23793	0.245956
9	0.09	0.092411	8.764305	0.333215	0.11075	0.114454	10.7744	0.247128
10	0.0975	0.100451	9.497472	0.32208	0.11575	0.120118	11.29095	0.372602
20	0.1085	0.112036	10.55391	0.486046	0.14425	0.149313	13.95319	0.398695
30	0.125	0.129187	12.1179	0.485686	0.17125	0.177178	16.49424	0.669305
60	0.15175	0.156687	14.62563	0.668561	0.21925	0.226206	20.96506	0.48656
120	0.17125	0.177097	16.48686	0.871009	0.29075	0.299021	27.60509	0.359153
180	0.1865	0.193375	17.9712	0.628189	0.3515	0.361516	33.30396	0.475977
240	0.20825	0.216244	20.05662	0.668107	0.3955	0.407625	37.50863	0.469522
300	0.22025	0.229493	21.26484	0.616426	0.42825	0.442748	40.71149	0.634648
360	0.244	0.254565	23.55111	0.778046	0.45775	0.474817	43.63591	0.546901

Annexure B: Dissolution Data

Data B.6 Dissolution data for rifampicin release from Chitosan/ Ac-Di-Sol® 0.5 % w/v /
Vitamin C 0.25 % w/v beads at pH 5.60 and 7.40 over a period of 6 hours

Time (min)	pH 5.60				pH 7.40			
	Absorbency Values	Corrected Absorbency	% Release	Standard Deviation	Absorbency Values	Corrected Absorbency	% Release	Standard Deviation
0	0	0	0	0	0	0	0	0
1	0.012	0.012	1.431681	0.128962	0.03525	0.03525	3.551846	0.436538
2	0.02	0.020072	2.167766	0.167189	0.05125	0.051462	5.030171	0.392115
3	0.03775	0.037942	3.797329	0.173972	0.066	0.066519	6.403262	0.214718
4	0.046	0.046419	4.5703	0.236664	0.077	0.077915	7.442462	0.271725
5	0.05825	0.058945	5.712544	0.230991	0.08775	0.089127	8.464883	0.13107
6	0.067	0.068044	6.542327	0.20172	0.095	0.096904	9.174021	0.250701
7	0.078	0.079446	7.582074	0.240418	0.0995	0.101974	9.636354	0.278815
8	0.08275	0.084664	8.057903	0.334193	0.1055	0.108571	10.23793	0.245956
9	0.09	0.092411	8.764305	0.333215	0.11075	0.114454	10.7744	0.247128
10	0.0975	0.100451	9.497472	0.32208	0.11575	0.120118	11.29095	0.372602
20	0.1085	0.112036	10.55391	0.486046	0.14425	0.149313	13.95319	0.398695
30	0.125	0.129187	12.1179	0.485686	0.17125	0.177178	16.49424	0.669305
60	0.15175	0.156687	14.62563	0.668561	0.21925	0.226206	20.96506	0.48656
120	0.17125	0.177097	16.48686	0.871009	0.29075	0.299021	27.60509	0.359153
180	0.1865	0.193375	17.9712	0.628189	0.3515	0.361516	33.30396	0.475977
240	0.20825	0.216244	20.05662	0.668107	0.3955	0.407625	37.50863	0.469522
300	0.22025	0.229493	21.26484	0.616426	0.42825	0.442748	40.71149	0.634648
360	0.244	0.254565	23.55111	0.778046	0.45775	0.474817	43.63591	0.546901

Annexure B: Dissolution Data

Data B.7 Dissolution data for rifampicin release from Chitosan/ Ac-Di-Sol® 0.5 % w/v /
Explotab® 0.25 % w/v beads at pH 5.60 and 7.40 over a period of 6 hours.

Time (min)	pH 5.60				pH 7.40			
	Absorbency Values	Corrected Absorbency	% Release	Standard Deviation	Absorbency Values	Corrected Absorbency	% Release	Standard Deviation
0	0	0	0	0	0	0	0	0
1	0.01675	0.01675	2.682598	0.506639	0.03075	0.03075	4.519096	0.419976
2	0.02775	0.027851	4.138744	0.747988	0.04875	0.048935	6.90451	1.04265
3	0.0345	0.034767	5.04604	0.41478	0.05925	0.059727	8.320253	0.880169
4	0.03875	0.039224	5.630702	0.526823	0.071	0.071833	9.908233	0.915393
5	0.04325	0.043957	6.251504	0.519159	0.0745	0.075759	10.42324	1.059075
6	0.047	0.047966	6.777463	0.482213	0.08075	0.082456	11.30174	1.048386
7	0.05	0.051248	7.207991	0.633522	0.0865	0.08869	12.11957	0.915639
8	0.0535	0.055048	7.706469	0.50491	0.09175	0.094459	12.87634	0.845045
9	0.055	0.056869	7.945345	0.559969	0.0965	0.09976	13.57165	0.754155
10	0.05725	0.059449	8.283785	0.43107	0.09975	0.103589	14.07393	0.748472
20	0.0695	0.072043	9.93578	0.533151	0.127	0.131437	17.72706	0.502481
30	0.0845	0.08746	11.95816	0.280449	0.15725	0.162449	21.79516	0.755393
60	0.10425	0.107717	14.61544	0.820701	0.20925	0.215393	28.7402	1.016091
120	0.13425	0.138342	18.63284	1.086725	0.285	0.292398	38.84166	1.070803
180	0.1675	0.172398	23.10019	1.279607	0.34725	0.356358	47.23183	1.234311
240	0.195	0.200903	26.83943	1.161798	0.3905	0.401692	53.17861	1.414333
300	0.2155	0.222573	29.68207	1.343808	0.42525	0.438785	58.04441	1.661988
360	0.23725	0.245616	32.70481	1.819209	0.4505	0.466586	61.69137	1.409495

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