THE EFFECT OF PHARMACEUTICAL EXCIPIENTS ON THE RELEASE OF INDOMETHACIN FROM CHITOSAN BEADS

Riana Havinga

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Promotor: Prof A.F. Marais

Co-Promotor: Prof A.F. Kotzé

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"I am among those who think that science has great beauty. A scientist in his laboratory is not only a technician: he is also a child placed before natural phenomena which impress him like a fairy tale."

♦Marie Curie♦

FOREWORD

Firstly I would like to thank my **Creator God** for the wonderful opportunity He gave me to continue my studies. He taught me patience and determination, skills a researcher need in abundance, for which I will always be grateful. Without His grace and love I would not have been able to complete my studies.

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Table of Contents

Abstract

Chitosan has proven through the years as a versatile biomaterial to be used in pharmaceutical applications. Its mucoadhesive properties as well as its ability to manipulate the tight junctions in epithelium membranes have qualified it as an effective drug carrier in controlled drug delivery systems. Microparticles or beads as they are forward called in this study have advantages over conventional drug dosage forms because of a large surface to volume ratio and have the ability to target a specific site for drug release. Indomethacin is an anti-inflammatory drug that causes gastrointestinal side effects in conventional immediate-release dosage forms. The goal is to manipulate the drug delivery vehicle to target the intestines/colon as the site for drug delivery and to minimize this side effect. Thus chitosan beads have been chosen as a drug delivery system for indomethacin in this study.

Chitosan beads have been prepared through the ionotropic gelation method using tripolyphophate (TPP) as a cross-linking agent. To prepare the most effective bead to encapsulate indomethacin different formulation and system variables (pH of the TPP solution, the concentration of the TPP solution as well as the indomethacin concentration) have been evaluated according to the following parameters: morphology, drug loading capacity and swelling capability. The ideal pH of the TPP solution was determined at 8.7 and the most effective TPP and indomethacin concentration were 5% w/v and 4% w/v respectively. The chitosan concentration was kept at 3% w/v throughout the study. These concentrations were used to examine the effect of pharmaceutical excipients on the indomethacin release from chitosan beads.

The effect of the different excipients namely, Explotab® (0.25% w/v), Ac-Di-Sol® (0.5% w/v) and Vitamin C (025% w/v), on the morphology, drug loading capacity, swelling capability as well as the drug release of indomethacin chitosan beads (ICB's) were also studied. The excipients were used in the individually above mentioned concentrations and in combination with each other in the same concentrations. These formulations were used in dissolution studies over a period of 6 hours in PBS pH 7.4 solutions. The indomethacin release rate increased when an excipient was added to the formulation and it dramatically increased when the excipients were added in their various combinations, compared to the formulation that did not contain excipients.

Keywords: Chitosan; controlled drug delivery; indomethacin; inotropic gelation; tripolyphosphate(TPP); Explotab®; Ac-Di-Sol®; Vitamin C.

Uittreksel

Die afgelope paar jaar het navorsing getoon dat kitosaan 'n veelsydige biomateriaal is wat nuttig in farmaseutiese toepassings gebruik kan word. Kitosaan kwalifiseer as 'n effektiewe geneesmiddelabsorbsiebevorderaar in vrystellingsreguleerde doseervorme as gevolg van sy mukoklewende eienskappe asook sy vermoë om die digsluitende hegtingskomplekse ("tight junctions") in die epiteel membrane te verbreed. Mikropartikels of krale soos dit in die studie bekend staan, het die voordeel bo konvensionele doseervorme a.g.v die groot oppervlak tot volume verhouding asook krale se vermoë om 'n spesifieke area vir absorbsie te teiken. Indometasien is 'n anti-inflammatoriese middel wat verantwoordelik is vir gatrointestinale newe-effekte. Dit sal uiters voordelig wees om die kolon as area van absorbsie te teiken om sodoende newe-effekte te verminder. Kitosaankrale word in die studie as vervoermiddel vir indometasien gebruik.

Kitosaankrale is deur middel van die inotropiese jelerings metode voorberei waartydens tripolifosfaat (TPP) as kruisbindingsagent gebruik is. Verskillende vervaardigings en formulerings veranderlikes (pH van die die TPP oplossing, konsentrasie van die TPP oplossing asook die indometasienkonsentrasie) is geëvalueer na aanleiding van die volgende eienskappe van die krale: morfologie, geneesmiddelkapasiteit en swellingskapasiteit, om sodoende die mees effektiewe geneesmiddeldraersisteem vir indometasien te verkry. Die ideale pH vir die TPP oplossing is by 8.7 verkry en die mees effektiewe TPP- en indometasienkonsentrasie was 5% w/v en 4% w/v onderskeidelik. Die kitosaankonsentrasie was 3% w/v gedurende die hele studie. Laasgenoemde konsentrasies was gebruik om die effek van farmaseutiese hulpstowwe op die vrystelling van indometasien uit die kitosaankrale te ondersoek.

Die effek van die hulpstowwe, Explotab® (0.25% w/v), Ac-Di-Sol® (0.5% w/v) en Vitamien C (0.25% w/v), is na aanleiding van die morfologie, swel- en geneesmiddelkapasiteit en geneesmiddelvrystelling van indometasien uit kitosaankrale ondersoek. Die hulpstowwe is gebruik in die bogenoemde konsentrasies en in kombinasie met mekaar in dieselfde konsentrasies. Hierdie formules is gebruik om dissolusiestudies mee uit te voer oor 'n periode van 6 ure in PBS pH 7.4 oplossing. Die indometasienvrystellingstempo het verhoog wanneer 'n hulpstof bygevoeg is en die tempo het nog meer verhoog wanneer die hulpstowwe in kombinasie met mekaar gebruik is in vergelyking met die standaardformule.

Keywords: Kitosaan; vrystellingsreguleerde doseervorme; indometasien; inotropiese jelering; tripolifosfaat (TPP); Explotab®; Ac-Di-Sol®; Vitamien C

Introduction and Aim of Study

Drug delivery systems (DDS) that can precisely control the release rates or target drugs to a specific body site have had an enormous impact on the healthcare system. The last two decades in the pharmaceutical industry have witnessed an avant-garde interaction among the fields of polymer and material science, resulting in the development of novel drug delivery systems.

Research into controlled drug delivery systems aim at eliminating the high cost of drug use; reduce drug toxicities and maintaining improved therapeutic outcomes. Chitosan beads have been proven as an ideal controlled drug delivery system. The purpose of pharmaceutical excipients in the conventional dosage form has already been established. The aim of this study is to determine its effects on indomethacin loaded chitosan beads.

The oral route is still the route of choice when administrating drugs because of the excellent patient compliance and easy administration. Indomethacin, an anti-inflammatory drug, however causes gastrointestinal irritation. The aim is to develop a system that will carry the drug to the intestines where drug release will occur, thus eliminating indomethacin' main side effect and actually targets diseases such as ulcerative colitis in the colon.

This study aims to achieve pronounced drug levels in the intestinal environment and it involved the following as its main objectives:

- To conduct study on chitosan beads as a polymeric drug delivery system with the emphasis on their advantages over conventional drug delivery systems.

- To prepare and characterize indomethacin chitosan beads with a reliable and reproducible method and to investigate various formulation and system variables on the properties of the beads.
- To evaluate the effect of pharmaceutical excipients (Ac-Di-Sol®, Explotab® and Vitamin C) on the properties of the indomethacin chitosan beads.
- To conduct dissolution studies on the selected formulations and to evaluate the effect of pharmaceutical excipients on the release rate of indomethacin from the chitosan beads.

The physio-chemical properties of chitosan and the motivation for chitosan beads as an effective drug delivery vehicle are discussed in chapter 1. Chapter 2 describes the preparation of the indomethacin chitosan beads and characterization tests that are done on the beads. In chapter 3 the various formulation variables' effect on the indomethacin chitosan beads are investigated as well as the effect of pharmaceutical excipients on the beads. Chapter 4 describes the effect of the pharmaceutical excipients on the release rate of indomethacin from chitosan beads.

Chapter 1

Chitosan beads for the enhanced drug delivery of indomethacin

1.1 Introduction

The use of microsphere-based therapy allows drug release to be carefully tailored to the specific treatment site through the choice and formulation of various drug–polymer combinations. The total dose of medication and the kinetics of release are the variables, which can be manipulated to achieve the desired result. Using innovative microencapsulation technologies, and by varying the copolymer ratio, molecular weight of the polymer, etc., microspheres can be developed into an optimal drug delivery system which will provide the desired release profile. Microsphere-based systems may increase the life span of active constituents and control the release of bioactive agents (Sinha *et al.*, 2004:3).

Being small in size, microspheres have large surface to volume ratios and can be used for controlled release of insoluble drugs. Extensive research is being carried out to exploit chitosan as a drug carrier to attain the desirable drug release profile. Chitosan microspheres are used to provide controlled release of many drugs and to improve the bioavailability of degradable substances such as protein or enhance the uptake of hydrophilic substances across the epithelial layers. These microspheres are being investigated both for parenteral and oral drug delivery (Queen *et al.*, 2000:95-100).

Chitosan has also been used as a potential carrier for prolonged delivery of drugs, macromolecules and targeted drug delivery. Magnetic chitosan microspheres used in targeted drug delivery are expected to be retained at the target site capillaries under the influence of an external magnetic field (Gallo *et al.*, 1988:300). Also, strong interaction between cationic microspheres and anionic glycosaminoglycan receptors can retain the microspheres in the capillary region (Gallo *et al.*, 1988:300; Hassan *et al.*, 1992:390).

1.2 Microspheres of chitosan

A 'microcapsule' is defined as a spherical particle with size varying from 50 nm to 2 mm, containing a core substance. Microspheres are, in a strict sense, empty spherical particles. However, the terms microcapsules and microspheres are often used synonymously. In addition, some related terms are used as well. For

example, 'microbeads' and 'beads' are used alternatively. Spheres and spherical particles are also used for a large size and rigid morphology. Recently, Yao *et al.* (1995:155), highlighted the preparation and properties of microcapsules and microspheres related to chitosan. Due to the attractive properties and wider applications of chitosan-based microcapsules and microspheres, a survey of the applications in controlled drug release formulations is appropriate. Moreover, microcapsule and microsphere forms have an edge over other forms in handling and administration.

1.2.1 The chemical definition of chitosan

Chitosan is a polysaccharide, similar in structure to cellulose. Both are made by linear β-(1→4)-linked monosaccharides [see figure 1.1 (a)]. However, an important difference to cellulose is that chitosan is a co-polymer composed of 2-amino-2-deoxy-β-$_D$-glucan combined with glycosidic linkages. The primary amine groups render special properties that make chitosan very useful in pharmaceutical applications. Compared to many other natural polymers, chitosan has a positive charge and is mucoadhesive (Berscht *et al.*, 1994:593). Therefore, it is used extensively in drug delivery applications. Chitosan is obtained from the deacetylation of chitin, a naturally occurring and abundantly available (in marine crustaceans) biocompatible polysaccharide. However, applications of chitin are limited compared to chitosan because chitin is structurally similar to cellulose, but chemically inert. The acetamide groups of chitin can be converted into amino groups to give chitosan, through the treatment of chitin with concentrated alkali solution. Chitin and chitosan represent long-chain polymers having molecular mass up to several million Daltons. Chitosan is relatively reactive and can be produced in various forms such as powder, paste, film, fiber, etc (Sunil *et al.*, 2004:6). Commercially available chitosan has an average molecular weight ranging between 3800 and 20,000 Daltons and is 66% to 95% deacetylated.

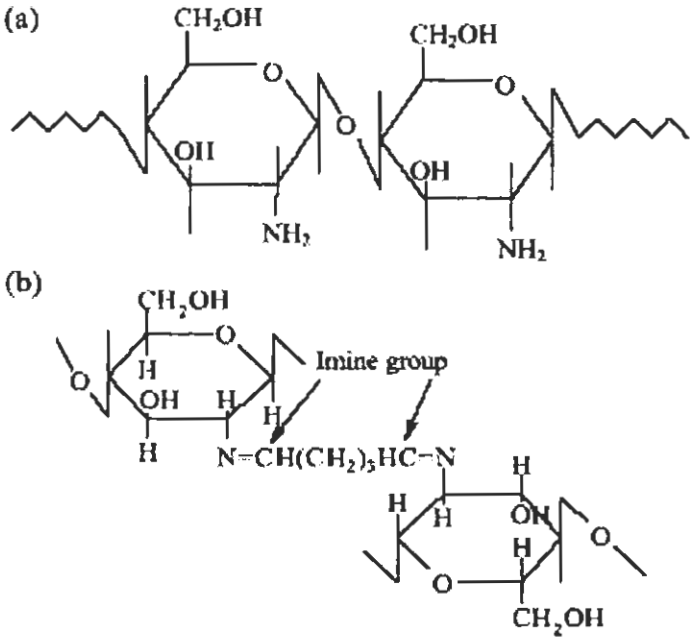


Figure 1.1 : (a) Structure of chitosan. (b) Structure of cross-linked chitosan (Sunil *et al.*, 2004:7).

1.2.2 The physiochemical properties of chitosan

Chitosan, being a cationic polysaccharide in acidic pH environments, contains free amino groups and hence, is insoluble in water. In an acidic pH environment the amino groups can undergo protonation thus, making it soluble in water. The solubility of chitosan depends upon the distribution of free amino and N-acetyl groups (Sannan *et al.*, 1976:3589-3600). Usually 1–3% aqueous acetic acid solutions are used to solubilize chitosan. Chitosan is biocompatible with living tissues since it does not cause allergic reactions and rejection. It breaks down slowly to harmless products (amino sugars), which are completely absorbed by the human body (Nicol, 1991:46-48). Chitosan degrades under the action of ferments, it is nontoxic and easily removable from the organism without causing concurrent side reactions. It possesses antimicrobial property and absorbs toxic metals like mercury, cadmium, lead, etc. In addition, it has good adhesion, coagulation ability, and immunostimulating activity (Sunil *et al.*, 2004:6).

Chitosan has been shown to possess mucoadhesive properties (Lehr *et al.*, 1992:43; Needleman *et al.*, 1995:617; Rillosi *et al.*, 1995:669; He *et al.*, 1998:75; Shimoda *et al.*, 2001:567; Kockisch *et al.*, 2003:1614) due to molecular attractive forces formed by electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces. These properties may be attributed to:

(a) strong hydrogen bonding groups like OH^-, $COOH^-$ (Schipper *et al.*, 1997:923);

(b) strong charges (Dodane *et al.*, 1999:21);

(c) high molecular weight (Schipper *et al.*, 1996:1686; Kotze *et al.*, 1998:35);

(d) sufficient chain flexibility (He *et al.*, 1998:75); and

(e) surface energy properties favoring spreading into mucus (Lueβen *et al.*, 1994:329).

If the degree of deacetylation and molecular weight of chitosan can be controlled, then it would be a material of choice for developing micro/nanoparticles. Chitosan has many advantages, particularly for developing micro/nanoparticles. These include: its ability to control the release of active agents, it avoids the use of hazardous organic solvents while fabricating particles since it is soluble in aqueous acidic solution, it is a linear polyamine containing a number of free amine groups which are readily available for crosslinking, its cationic nature allows for ionic crosslinking with multivalent anions, it has mucoadhesive character, which increases residual time at the site of absorption (Sunil *et al.*, 2004:6).

Chitin and chitosan have very low toxicity; LD_{50} of chitosan in laboratory mice is 16 g/kg body weight, which is close to sugar or salt. Chitosan is proven to be safe in rats up to 10% in the diet (Arai *et al.*, 1968:89-94). Various sterilization methods such as ionizing radiation, heat, steam and chemical methods can be suitably adopted for sterilization of chitosan in clinical applications (Chandy *et al.*, 1990:1-24).

In view of the above-mentioned properties, chitosan is extensively used in developing drug delivery systems. Particularly, chitosan has been used in the preparation of mucoadhesive formulations, improving the dissolution rate of poorly soluble drugs, drug targeting and enhancement of peptide absorption (Sunil *et al.*, 2004:7).

1.2.3 Mechanisms of action of chitosan

Chitosan is being studied extensively as an enhancer for transmucosal drug delivery *in vitro* and *in vivo*. However, there is still much to be accomplished in understanding its mechanisms of action on mucosal epithelium.

The mechanism of action of chitosan was suggested to be a combination of mucoadhesion and an effect on tight-junction (TJ) regulation (Artursson *et al.*,1994:253-267). Using a human colon carcinoma cell line (Caco-2) as an *in vitro* model of intestinal epithelium, cell permeability was shown to increase following

treatment with chitosans of various salt forms and molecular weights (Artursson *et al.*, 1994:253-267; Borchard *et al.*, 1996:131-138; Dodane *et al.*, 1999:21-32). Epithelial permeability can be assessed by measuring transepithelial electrical resistance (TER), which is inversely proportional to the permeability of the epithelial layer to organic ions. Measurements of TER revealed that chitosan's effects were concentration-dependent and reversible (Figure 1.2).

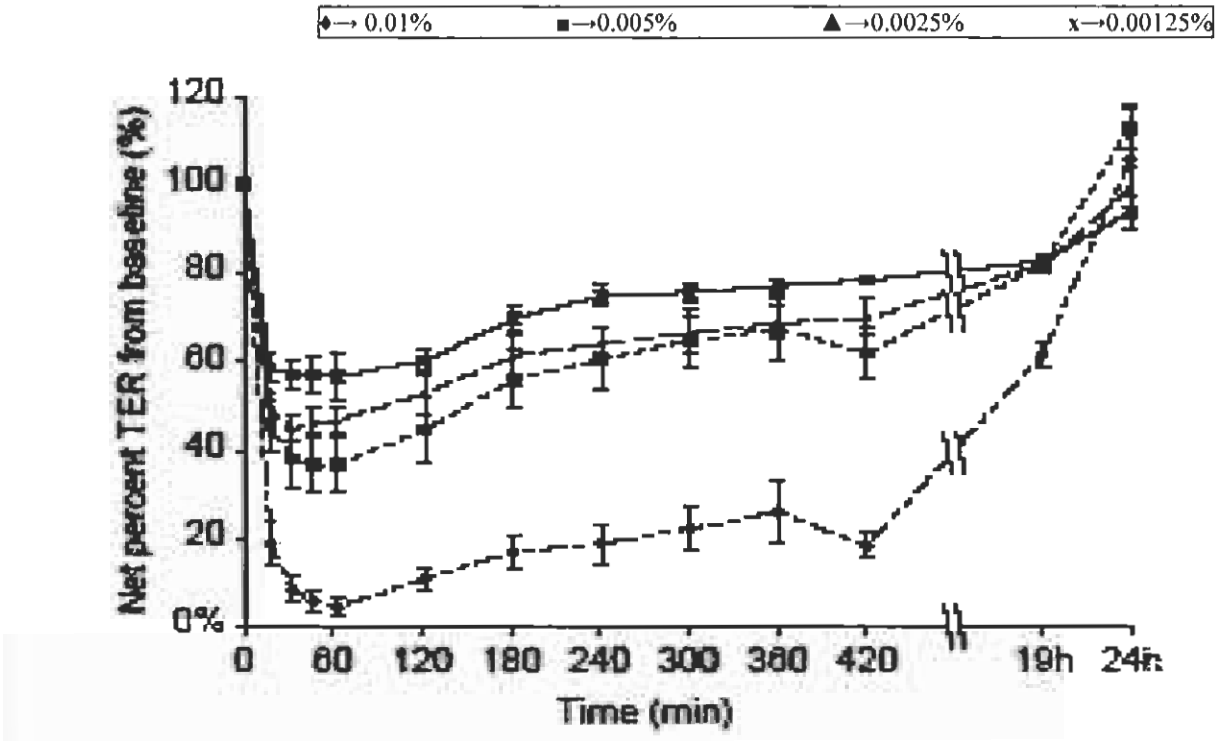


Figure 1.2. : Time course and reversal of chitosan effects on transepithelial electrical resistance (TER) in Caco-2 cells. Apical side of Caco-2 cells were incubated with various concentrations of chitosan (%w/v) for 1h and TER was measured. Cells were then washed twice with PBS, incubated with Caco-2 culture medium and TER was assessed over 24h. TER baseline was 1060 $^{+}$ 27 $\Omega.cm^2$. n > 10.(Dodane *et al.*, 1999:21-32).

However, additional studies showed no significant difference in the resistance values obtained between 0.1 and 0.5% w/v, suggesting a threshold effect of chitosan above 0.1% (Dodane *et al.*, 1999:21-32). This observation is in accordance with the data obtained by several groups with chitosan glutamate (Illum *et al.*, 1994:1186-1189; Artursson *et al.*, 1994:253-267; Leuβen *et al.*, 1994:15-23). The apparent permeability coefficient of mannitol, a marker of the paracellular pathway, reached a plateau at polymer concentrations of 0.25 and 0.5% w/v (Artursson *et al.*, 1994:253-267). A comparable effect was obtained by Lueβen *et al.*, 1994:15-23, where 0.4 and 1% w/v chitosan elicited similar values for the transport rate of DGAVP peptide (9-desglycinamide, 8-larginine vasopressin). Illum *et al.*, 1994:1186-1189, had also reported that

administration of 0.2 to 1% w/v chitosan, in combination with insulin, produced a similar action on the reduction of blood glucose levels in rats. As shown by an increase in paracellular flux of mannitol, chitosan seems to enhance Caco-2 cell permeability by affecting TJ (Artursson *et al.*, 1994:253-267). These results were corroborated by a structural study performed in cell lines of various types and origins (Dodane *et al.*, 1999:21-32). Tight junctional changes were monitored by using antibodies against occludin, a transmembrane protein of the TJ, Furuse *et al.* (1993:1777), and ZO-1, a TJ-associated protein (Stevenson *et al.*, 1986:755-766). Occludin and ZO-1 staining revealed a decrease in fluorescent intensity, accompanied by a ZO-1 cytoplasmic localization in chitosan-treated monolayers compared with control cells (Dodane *et al.*, 1999:21-32). Furthermore, chitosan induced a redistribution of F-actin, a protein of the cytoskeleton stained by bodipy phalloidin. Because actin has been shown to be important in regulating paracellular flow across cultured intestinal epithelia, Meza *et al.* (1980:746-754), the described effects of chitosan on epithelial barrier function might result, at least partially, from alterations of the cytoskeleton. Reversible effects of chitosan on Caco-2 cell structure were observed, indicating that chitosan acts temporarily on the cellular barrier (Dodane *et al.*, 1999:21-32).

Transmission electron micrographs of cells exposed to 0.1% chitosan for 30 minutes resulted in the appearance of large intracellular vacuoles and swollen endoplasmic reticulum cisternae (Dodane *et al.*, 1999:21-32). However, the cells displayed a continuous apical membrane, normal microvilli, intact organelles and TJ as observed in control cells. The absence of apparent changes in the junctional morphology accompanied by increased paracellular permeability has been previously reported (Gonzalez-Mariscal *et al.*, 1991:193-202). This observation reinforces the existence of additional factors in TJ modulation, such as: the number and length of strands in the junctions; the existence of channels; the biochemical state of the junctional components; and the regulation of the junctional complex by the cytoskeleton or secondary messenger systems.

In conclusion, these studies demonstrate that chitosan appears to increase cell permeability by affecting paracellular and intracellular pathways. Chitosan causes relatively mild and reversible effects on epithelial function and morphology, which makes it a promising absorption-enhancing compound for the mucosal delivery of drugs.

1.3 Methods of preparation of chitosan microparticles

Different methods have been used to prepare chitosan particulate systems. Selection of any of the methods depends upon factors such as particle size requirement, thermal and chemical stability of the active agent, reproducibility of the release kinetic profiles, stability of the final product and residual toxicity associated with the final product.

1.3.1 The ionic gelation method

The use of complexation between oppositely charged macromolecules to prepare chitosan microspheres has attracted much attention because the process is very simple and mild (Polk *et al.*, 1994:178-185; Liu *et al.*, 1997:65). In addition, reversible physical cross-linking by electrostatic interaction, instead of chemical cross-linking, has been applied to avoid the possible toxicity of reagents and other undesirable effects. Tripolyphosphate (TPP) is a polyanion, which can interact with the cationic chitosan by electrostatic forces (Kawashima *et al.*, 1985:2469). Bodmeier *et al.* (1989:1475) reported the preparation of TPP–chitosan complex by dropping chitosan droplets into TPP solution, many researchers have explored its potential pharmaceutical usage. In the ionic gelation method, chitosan is dissolved in aqueous acidic solution to obtain the cation of chitosan. This solution is then added dropwise under constant stirring to polyanionic TPP solution. Due to the complexation between oppositely charged species, chitosan undergoes ionic gelation and precipitates to form spherical particles. However, TPP-chitosan microparticles formed have poor mechanical strength thus, limiting their usage in drug delivery.

Ko *et al.* (2002:165) prepared chitosan microparticles with TPP by the ionic cross-linking method. Particle sizes of TPP-chitosan microparticles varied from 500 to 710 μm with drug encapsulation efficiencies more than 90%. Morphologies of TPP-chitosan microparticles have been examined by SEM (scanning electron microscopy). As the pH of the TPP solution decreased and molecular weight of chitosan increased, microparticles acquired better spherical shapes having smooth surfaces. Release of felodipine as a model drug was affected by the preparation method. Chitosan microparticles prepared at lower pH or higher concentration of TPP solution resulted in a slower release of felodipine. With a decreasing molecular weight and concentration of chitosan solution, the drug release increased. The drug release from TPP-chitosan microparticles decreased when the cross-linking time was increased.

The ionic gelation method will be the method used in this study to produce chitosan beads.

1.3.2 Emulsion cross-linking

This method utilizes the reactive functional amino group of chitosan to cross-link with aldehyde groups of the cross-linking agent. In this method, a water-in-oil (w/o) emulsion is prepared by emulsifying the chitosan aqueous solution in the oil phase. Aqueous droplets are stabilized using a suitable surfactant. The stable emulsion is cross-linked by using an appropriate cross-linking agent such as glutaraldehyde to harden the droplets. Microspheres are filtered and washed repeatedly with n-hexane followed by alcohol and then dried (Akbuga *et al.*, 1994:217).

1.3.3 Coacervation/precipitation

This method utilizes the physicochemical property of chitosan since it is insoluble in alkaline pH medium, but precipitates/coacervates when it comes in contact with alkaline solution. Particles are produced by blowing chitosan solution into an alkali solution like sodium hydroxide, NaOH-methanol or ethanediamine using a compressed air nozzle to form coacervate droplets. Separation and purification of particles was done by filtration/centrifugation followed by successive washing with hot and cold water (Nishimura *et al.*, 1986:1359).

1.3.4 Spray-drying

Spray-drying is a well-known technique to produce powders, granules or agglomerates from the mixture of drug and excipient solutions as well as suspensions. The method is based on drying of atomized droplets in a stream of hot air. In this method, chitosan is first dissolved in aqueous acetic acid solution, drug is then dissolved or dispersed in the solution and then, a suitable cross-linking agent is added. This solution or dispersion is then atomized in a stream of hot air. Atomization leads to the formation of small droplets, from which solvent evaporates instantaneously leading to the formation of free flowing particles (He *et al.*, 1999:53-65).

1.3.5 Emulsion-droplet coalescence method

The novel emulsion-droplet coalescence method was developed by Tokumitsu *et al.* (1999:1830) which utilizes the principles of both emulsion cross-linking and precipitation. However, in this method, instead of cross-linking the stable droplets, precipitation is induced by allowing coalescence of chitosan droplets with

NaOH droplets. First, a stable emulsion containing aqueous solution of chitosan along with drug is produced in liquid paraffin oil and then, another stable emulsion containing chitosan aqueous solution of NaOH is produced in the same manner. When both emulsions are mixed under high-speed stirring, droplets of each emulsion would collide at random and coalesce, thereby precipitating chitosan droplets to give small size particles.

1.4 Drug loading into microparticles of chitosan

Drug loading in micro/nanoparticulate systems can be done by two methods, i.e., during the preparation of particles (incorporation) and after the formation of particles (incubation). In these systems, drug is physically embedded into the matrix or adsorbed onto the surface. Various methods of loading have been developed to improve the efficiency of loading, which largely depends upon the method of preparation as well as physicochemical properties of the drug. Maximum drug loading can be achieved by incorporating the drug during the formation of particles, but it may get affected by process parameters such as method of preparation, presence of additives, etc. Both water-soluble and water-insoluble drugs can be loaded into chitosan-based particulate systems. Water-soluble drugs are mixed with the chitosan solution to form a homogeneous mixture, and then, particles can be produced by any of the methods discussed before. For instance, cisplatin was loaded during the formation of particles with encapsulation efficiency as high as 99% (Akbuga *et al.*, 1999:697). The initial concentration of cisplatin and volume of glutaraldehyde had no effect on the encapsulation efficiency. Drug encapsulation increased as the concentration of chitosan increased. Water-insoluble drugs and drugs that can precipitate in acidic pH solutions can be loaded after the formation of particles by soaking the preformed particles with a saturated solution of drug.

Drug loading capacity tests are mostly conducted by using a spectrophotometer. Gupta *et al.* (2001:639-649) employed a method that 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 was calculated from the following equation:

$$\text{DLC} = \frac{\text{Total amount of drug} - \text{Free amount of drug}}{\text{Weight of the beads}} \qquad (1\text{-}1)$$

1.4.1 Parameters affecting entrapment efficiency of the drugs in chitosan microspheres

Many factors affect the entrapment efficiency of the drugs in the chitosan microspheres, e.g. nature of the drug, chitosan concentration, drug polymer ratio, stirring speed, etc. Generally a low concentration of chitosan shows low encapsulation efficiency (Orienti *et al.*, 1996:463). However, at higher concentrations, chitosan forms highly viscous solutions, which are difficult to process.

A number of reports have shown that entrapment efficiency increases with an increase in chitosan concentration. This may be explained on the basis that an increase in the viscosity of the chitosan solution with an increase in the chitosan concentration prevents drug crystals from leaving the droplet. A study carried out by Nishioka *et al.* (1990:2871) also revealed that the cisplatin content increased with increasing chitosan concentration. Further Nishioka *et al.* (1990:2871-2873) also proved that the incorporation of chitin in the carrier matrix produced a more pronounced increase in drug content.

Genta *et al.* (1998:779) obtained satisfactory ketoprofen contents in all batches of chitosan microspheres with a theoretical polymer/drug ratio of 1:2 w/w. Microspheres made with a mixture of high molecular weight/low molecular weight chitosan (1:2 w/w) showed good drug content and encapsulation efficiency and these were independent of polymer/drug ratio.

Pavanetto *et al.* (1996:679) revealed that the acetic acid concentration in the polymeric solution influenced the ketoprofen content of the microspheres. Maximum drug encapsulation efficiency was obtained for the lowest theoretical drug chitosan ratio.

Singla *et al.* (2001:171) reported that when nifedipine was dispersed in the chitosan solution with stirring during preparation of mirospheres, the entrapment efficiency increased. Further, Dhawan *et al.* (2003:243) reported that with increase in loading, the entrapment efficiency decreased. Scanning electron microscopy indicated that the roughness on the surface of the microspheres increased with increase in loading.

1.5 Drug release from chitosan microparticles

Drug release from chitosan-based particulate systems depends upon the extent of cross–linking, morphology, size and density of the particulate system, physicochemical properties of the drug as well as the presence of adjuvants. *In vitro* release also depends upon pH, polarity and presence of enzymes in the dissolution media. The release of drug from chitosan particulate systems involves three different mechanisms: (a) release from

the surface of particles, (b) diffusion through the swollen rubbery matrix and (c) release due to polymer erosion. These mechanisms are shown schematically in Figure 1.3.

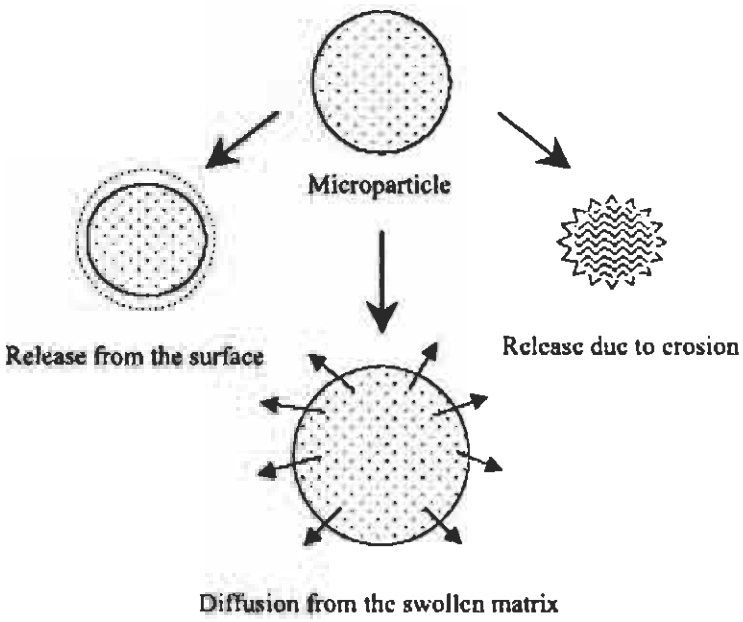


Figure 1.3: Mechanism of drug release from particulate systems (Sunil *et al.*, 2004:17).

In the majority of cases, drug release follows more than one type of mechanism. In case of release from the surface, adsorbed drug instantaneously dissolves when it comes in contact with the release medium. Drug entrapped in the surface layer of particles also follows this mechanism. This type of drug release leads to burst effect. He *et al.* (1999:53-65) observed that cemetidine-loaded chitosan microspheres have shown a burst effect in the early stages of dissolution. Most of the drug was released within a few minutes when particles were prepared by the spray drying technique. Increasing the cross-linking density can prevent the burst release. This effect can also be avoided by washing microparticles with a proper solvent, but it may lead to low encapsulation efficiency. Drug release by diffusion involves three steps. First, water penetrates into the particulate system, which causes swelling of the matrix; secondly, the conversion of glassy polymer into rubbery matrix takes place, while the third step is the diffusion of drug from the swollen rubbery matrix. The release is slow initially and accelerates later on. This type of release is more prominent in case of hydrogels (Sunil *et al.*, 2004).

1.5.1 Parameters affecting the release characteristics of drugs from chitosan microspheres

Many parameters determine the drug release behavior from chitosan microspheres. These include concentration and molecular weight of the chitosan, the type and concentration of crosslinking agent, variables like stirring speed, type of oil, additives, crosslinking process used, drug chitosan ratio, etc.

1.5.1.1 Effect of molecular weight of chitosan

Drug release studies from chitosan microspheres have generally shown that the release of the drug decreases with an increase in molecular weight of chitosan. Shiraishi *et al.* (1993:217) investigated the effect of molecular weight of chitosan hydrolysate on the release and absorption rate of indomethacin from gel beads. The release rate of indomethacin was found to decrease with increasing molecular weight of chitosan.

1.5.1.2 Effect of concentration of chitosan

Nishioka *et al.* (1990:2871) reported that the rate of cisplatin release reduced with the increasing concentration of chitosan. Aiedeh *et al.* (1997:567) observed that the method of chitosan interfacial crosslinkage by ascorbyl palmitate in water/oil dispersion was suitable to produce biodegradable system for insulin. The microcapsules obtained had release kinetics approaching zero order and a release rate, which could be increased by decreasing the chitosan content in the prepared solution.

1.5.1.3 Effect of drug content in the microspheres

A number of reports studying the effect of drug release have shown that the release of the drug from the microspheres increases with increase in drug content in the microspheres Bayomi *et al.* (1998:187). However, contrary results have also been reported. Akbuga *et al.* (1994:217) reported that furosemide release from chitosan microspheres followed the Higuchi matrix model. As the amount of furosamide incorporated increased, furosemide release was also increased. While Bodmeier *et al.* (1989:1475) reported that the release of sulfadiazine (a water insoluble drug) decreased with increase in drug content in the microspheres.

1.5.1.4 Physical state of the drug in the microspheres

The physical state of a drug is also an important parameter while investigating the drug release kinetics from a dosage form. The physical state of the drug may vary from molecular dispersion to well defined crystalline structures. He *et al.* (1999:53-65) observed that cimetidine and famotidine were molecularly dispersed inside

the microspheres, in the form of a solid solution. Therefore, the drug release was fast accompanied by a burst effect.

1.5.1.5 Effect of density of crosslinking

The crosslinking density has a remarkable effect on the release of drugs from the microspheres. Jameela *et al.* (1998:17-24) revealed that highly crosslinked microspheres released only 35% of the progesterone in 40 days compared to 70% release from microspheres crosslinked lightly.

1.5.1.6 Effect of additives

Lim *et al.* (1998:319) prepared chitosan microspheres by emulsification–coacervation technique using pentasodium tripolyphosphate as a counterion. This led to a high degree of aggregation. The aggregation was markedly reduced by the incorporation of magnesium stearate in the dispersed phase. However, this addition did not affect the drug release. Additionally, with increasing magnesium stearate content, larger sized microspheres were produced.

1.5.2 Drug release kinetics of tripolyphosphate chitosan microparticles

Swelling and drug release of tripolyphosphate/chitosan beads were usually insensitive to media pH. Chitosan beads, crosslinked by a combination of tripolyphosphate and citrate (or sulfate) together, not only had a spherical shape, but also improved pH-responsive drug release properties. These results indicated that ionically cross-linked chitosan beads might be useful in stomach specific drug delivery. Ko *et al.* (2002:165-174) prepared felodipine loaded chitosan microparticles with tripolyphosphate (TPP) by ionic crosslinking. On examining the morphologies of TPP–chitosan microparticles with scanning electron microscopy, it was observed as the pH of TPP solution decreased and molecular weight of chitosan increased, microparticles had a more spherical shape and smooth surface. Chitosan microparticles prepared with a lower pH or higher concentration of TPP solution resulted in slower felodipine release from microparticles. With decreasing molecular weight and concentration of chitosan solution, release of the drug was increased. The release of drug from TPP–chitosan microparticles decreased when crosslinking time increased.

1.6 Factors influencing the structure of chitosan beads (Ionic gelation method)

Molecular weight is one of the major factors to influence the phase-inversion. The beads prepared from high molecular weight of chitosan are covered with a dense layer on its surface. The chitosan solution (1.5 wt%)

degraded by lysozyme (1000 U/ml) can be concentrated (1.5-8.0 wt%) to prepare chitosan bead consisting of interconnected pores throughout the entire bead. Chemically cross-linking should be another important factor to influence the microstructure of chitosan beads. However, the factor of cross-linking is controlled (all beads are cross-linked at the same condition) in this study to examine the effect of phase-inversion on the variation of structures of chitosan bead. Chitosan beads prepared by such a process are very brittle. The pore size and effective porosity of the bead can be varied by altering synthesis conditions, such as initial polymer concentration, and the pH value and concentration of TPP solution. The key factors affecting the micro structural characteristics of the beads are discussed below (Fwu-Long Mi *et al.*, 2002:761).

1.6.1 Effect of pH value of the TPP solution

The solidification of chitosan beads in acidic TPP solution was attributed to electrostatic attraction like ionic cross-linking. The phase inversion of chitosan droplets in basic TPP solution was dependent on the competition between OH^- induced deprotonation with particulates and TPP ions induced ionic cross-linking. The deprotonation may induce chitosan solubility due to the transformation of more hydrophilic $-NH_3^+$ to hydrophobic $-NH_2$, and then induces phase separation. Whereas, the TPP ions are electrostatically attracted to chitosan, which results in the solid-liquid demixing. The competed liquid-liquid and solid-liquid phase separations promote the formation of an interconnected porous structure with particulates surrounding the pores. The ionic cross-linking of linear polymer chain by TPP ions locks the three dimensional network, which could help to repair phase separated structures. From SEM micrograph of chitosan gel beads, it is evident that the chitosan bead prepared in acidic TPP solution solution is macroporous, while the beads prepared in basic TPP solution solution is non-porous (Fwu-Long Mi *et al.*, 2002:761).

1.6.2 Effect of polymer concentration

The initial polymer concentration plays a crucial role in the development of gel microstructure, since it governs the relative amount of polymer-dilute and polymer-rich phases produced upon phase separation, which can effect both the liquid-liquid or solid-liquid demixing. An increase in the intial polymer concentration induces a significant decrease in the effective porosity of the microporous chitosan beads. The reduction in effective porosity of macroporous gel beads accompanied by an increase in the initial polymer concentration can be readily explained according to the varation of polymer-rich and polymer-lean phase. Reducing the initial polymer concentration consequently leads to a greater percentage of the dilute phase. Since the polymer-dilute phase leads to pores and the concentrated phase forms continuous structures, the

effective porosity of macroporous gel beads prepared by the phase-inversion technique is expected to decrease with an increase in the initial polymer concentration (Fwu-Long Mi *et al.*, 2002:761).

1.6.3 Effect of TPP concentration

Only minor changes in the porosity is observed in macroporous beads upon changing the TPP concentration. But as TPP concentration is lower than 3 wt%, porosity of chitosan beads significantly decreases. If porous structure is dominated only by liquid-liquid demixing, the pore size and porosity should not be affected by the TPP concentration. However, the early stage of phase inversion of chitosan beads were dominated by neutralization induced liquid-liquid demixing, but the final stage of phase-inversion of these chitosan beads were affected by the TPP ions induced solid-liquid demixing. In the liquid-liquid demixing process, nucleation of the liquid micelles composed of acetic acid and OH^- commenced when the droplet solution entered the binodal phase envelop. These micelles are in equilibrium at their interface with the surrounding polymer-rich gel phase. Radical growth of the micelles continues until the polymer-rich phase fused and solidified.The supersaturated polymer-rich gel eventually crystallizes into solid matrix in contact with the TPP ions. It is during this stage that the bicontinuous network is actually formed (Fwu-Long Mi *et al.*, 2002:762).

1.7 Pharmaceutical applications of chitosan microparticulate systems

Chitosan-based particulate systems are attracting pharmaceutical and biomedical applications as potential drug delivery devices. Some important applications are discussed below.

1.7.1 Colon targeted drug delivery

Chitosan is a promising polymer for colon drug delivery since it can be biodegraded by the colonic bacterial flora, Zhang *et al.* (2002:197), and it has mucoadhesive characteristics. The pH-sensitive multicore microparticulate system containing chitosan microcores entrapped into enteric acrylic microspheres was reported (Lorenzo-Lamosa *et al.*, 1998:109-118).

1.7.2 Mucosal delivery

Currently, mucosal surfaces such as nasal, peroral and pulmonary are receiving a great deal of attention as alternative routes of systemic administration. Chitosan has mucoadhesive properties and therefore, it seems particularly useful to formulate the bioadhesive dosage forms for mucosal administration (ocular, nasal,

buccal, gastro-enteric and vaginal-uterine therapy) (Genta *et al.*, 1998:1-88). Nasal mucosa has high permeability and easy access of drug to the absorption site. The particulate delivery to peroral mucosa is easily taken up by the Peyer's patches of the gut associated lymphoid tissue. Chitosan has been found to enhance the drug absorption through mucosae without damaging the biological system. Here, the mechanism of action of chitosan was suggested to be a combination of bioadhesion and a transient widening of the tight junctions between epithelial cells (Artursson *et al.*, 1994:1358-1361). Genta *et al.* (1998:1-88) studied the influence of glutaraldehyde on drug release and mucoadhesive properties of chitosan microspheres. A new *in vitro* technique was developed based on electron microscopy to study the effect of polymer cross-link density on the mucoadhesive properties of chitosan microspheres modulating the rate of theophylline release. The ability of insulin loaded chitosan nanoparticles to enhance the nasal absorption of insulin was investigated in a conscious rabbit model. Chitosan nanoparticles enhanced the nasal absorption of insulin to a greater extent than the aqueous solution of chitosan (Fernandez-Urrusuno *et al.*, 1999:1576).

1.7.3 Cancer therapy

Gadopentetic acid-loaded chitosan nanoparticles have been prepared for gadolinium neutron-capture therapy (Tokumitsu *et al.*, 1999:1830-1835). Their releasing properties and ability for longterm retention of gadopentetic acid in the tumor indicated that these nanoparticles are useful as intratumoral injectable devices for gadolinium neutroncapture therapy. The accumulation of gadolinium loaded as gadopentetic acid (Gd-DTPA) in chitosan nanoparticles designed for gadolinium neutron-capture therapy (Gd-NCT) for cancer have been evaluated *in vitro* in cultured cells (Shikata *et al.*, 2002:57).

Jameela *et al.* (1996:685) have prepared glutaraldehyde cross-linked chitosan microspheres containing mitoxantrone. The antitumor activity was evaluated against Ehrlich ascites carcinoma in mice by intraperitoneal injections. The tumor inhibitory effect was followed by monitoring the survival time and change in the body weight of the animal for 60 days. Mean survival time of animals which received free mitoxantrone was 2.1 days and this was increased to 50 days when mitoxantrone was given via microspheres.

1.7.4 Gene delivery

Gene therapy is a challenging task in the treatment of genetic disorders. In case of gene delivery, the plasmid DNA has to be introduced into the target cells, which should get transcribed and the genetic information should ultimately be translated into the corresponding protein. To achieve this goal, a number of hurdles is to be overcome by the gene delivery system. Transfection is affected by: (a) targeting the delivery system to target cell, (b) transport through the cell membrane, (c) uptake and degradation in the endolysosomes and (d)

intracellular trafficking of plasmid DNA to the nucleus. Chitosan could interact ionically with the negatively charged DNA and forms polyelectrolyte complexes. In these complexes, DNA becomes better protected against nuclease degradation leading to better transfection efficiency (Sunil *et al.*, 2004:20-21).

1.7.5 Topical delivery

Due to good bioadhesive property and ability to sustain the release of the active constituents, chitosan has been used in topical delivery systems. Bioadhesive chitosan microspheres for topical sustained release of cetyl pyridinium chloride have been evaluated (Conti *et al.*, 1998:822).

Improved microbiological activity was shown by these microparticulate systems. Conti *et al.* (2000:101) prepared microparticles composed of chitosan and designed as powders for topical wound-healing properties. Blank and ampicillin-loaded microspheres were prepared by spray-drying technique. *In vivo* evaluation in albino rats showed that both drug-loaded and blank microspheres have shown good wound healing properties.

1.7.6 Ocular delivery

De Campos *et al.* (2001:159-168) investigated the potential of chitosan nanoparticles as a new vehicle to improve the delivery of drugs to ocular mucosa. Cyclosporin A (CyA) was chosen as a model drug. A modified ionic gelation technique was used to produce CyA-loaded chitosan nanoparticles. These nanoparticles with a mean size of 293 nm, a zeta potential of +37 mV, high CyA association efficiency and loading of 73% and 9%, respectively were obtained. The *in vitro* release studies, performed under sink conditions, revealed the fast release during the first hour followed by a more gradual drug release during the 24-h period. The *in vivo* experiments showed that after topical instillation of CyA-loaded chitosan nanoparticles to rabbits, therapeutic concentrations were achieved in the external ocular tissues (i.e., cornea and conjunctiva) within 48 h while maintaining negligible or undetectable CyA levels in the inner ocular structures (i.e., iris/ ciliary body and aqueous humour), blood and plasma. These levels were significantly higher than those obtained following the instillation of chitosan solution containing CyA and an aqueous CyA suspension. The study indicated that chitosan nanoparticles could be used as a vehicle to enhance the therapeutic index of the clinically challenging drugs with potential application at the extraocular level.

1.7.7 Chitosan as a coating material

Chitosan has good film forming properties and hence, it is used as a coating material in drug delivery applications. Chitosan-coated microparticles have many advantages such as improvement of drug payloads,

bioadhesive property and prolonged drug release properties over the uncoated particles. Chitosan-coated microspheres composed of poly(lactic acid)– poly(caprolactone) blends have been prepared (Chandy *et al.*, 2002:275). These microspheres showed good potential for the targeted delivery of antiprolifcrative agents to treat restenosis. Shu *et al.* (2000:51) have prepared the alginate beads coated with chitosan by three different methods. The release of brilliant blue was not only affected by chitosan density on the particle surface, but also on the preparation method and other factors. Chiou *et al.* (2001:613-625) have used different molecular weight chitosans for coating the microspheres. The initial burst release was observed in the first hour with 50% release of lidocaine. But, 19.2% release occurred at 25th hour for the un-coated particles and 14.6% at the 90th hour for the chitosan-coated microspheres.

1.8 Indomethacin in chitosan beads as a controlled drug delivery system

Over the past 20 years, interaction among the fields of polymer and material science and the pharmaceutical industry has resulted in the development of what are known as drug delivery systems (DDS's), or controlled drug release. The advantages of using polymer-based devices over traditional dosage forms include:

- The ability to optimize the therapeutic effects of a drug by controlling its release on the body,
- Lower and more efficient doses,
- Less frequent dosing,
- Better patient compliance,
- Flexibility in physical state, shape, size, and surface,
- The ability to stabilize drugs and protect against hydrolytic or enzymatic degradation, and
- The ability to mask unpleasant taste or odor.

Controlled drug release is useful for drugs with short half-life, high systemic toxicity, frequent dosages, possible toxic side reactions and expensive drugs (Mathiowitz, 1999:9).

Systemic drug delivery via absorption into the bloodstream through the gastrointestinal (GI) epithelium can be limited by drug degradation during the first pass through the liver; however, the GI mucosal offers several advantages as an administration site over other mucus membranes (Mathiowitz, 1999:10). These advantages include the following:

- The oral administration route is familiar, convenient, and an accepted means of dosing most people, and
- The GI epithelium offers a large surface area for absorption and a close connection with a vast blood supply,

Owing to the fact that intimate contact between delivery device and the absorbing cell layer will improve both effectiveness and efficiency of the product, many researchers have recently focused on the developing bioadhesive drug delivery systems (BDDS's). The term *bioadhesion/mucoadhesion* refers to either adhesion between two biological materials or adhesion between some biological material and an artificial substrate (Mathiowitz, 1999:10). Chitosan has mucoadhesive properties and is thus the ideal material for BDDS. The development of efficient orally delivered BDDS's could enable the following important effects:

- Enhanced bioavailability and effectiveness of drug due to targeted drug delivery to a specific region in the GI tract,
- Maximized absorption rate due to intimate contact with the absorbing membrane and decreased diffusion barriers,
- Improved drug protection by polymer encapsulation and direct contact with absorbing cell layers, and
- Longer gut transit time resulting in extended periods for absorption.

The goal of incorporating indomethacin into chitosan beads is to produce a controlled drug delivery system as well as a bioadhesive drug delivery system as to capitalize on all the above mentioned factors. The main objective however for indomethacin chitosan beads is to target the colon as the main absorption site for indomethacin, thus decreasing the gastrointestinal side effects that indomethacin normally induces.

Diclofenac sodium (DFS) is a potent anti-inflammatory drug with pronounced analgesic and antipyretic properties. Due to DFS's short half life (1-2 hours) and its gastrointestinal adverse effects such as bleeding, ulcerations or perforations of the intestinal walls, it would be preferable to administer DFS in a controlled drug release device to lengthen the therapeutic effect and to decrease the side effects (Gupta *et al.*, 2000:1116). Drug release studies conducted on DFS loaded chitosan beads displayed a higher drug bioavailability from chitosan beads in a medium with an acidic pH, than in a medium with a basic pH (Gupta *et al.*, 2000:1116).

Indomethacin is also an anti-inflammatory drug where the most frequent adverse effects are gastrointestinal disturbances such as gastro intestinal bleeding, ulceration and perforation. To minimize this adverse effect is would be advisable to design a controlled drug delivery that targets the colon specifically. Drug targeting to the colon for systemic delivery is also very useful in the treatment of various diseases such as ulcerative colitis, Chron's disease and colon carcinomas. Because indomethacin is insoluble in media with a low pH, it makes for an ideal drug to target the colon and because it has anti-inflammatory properties it will also be useful in treating the above mentioned diseases.

1.9 Conclusion

Chitosan microspheres prove to be an ideal dosage form to deliver a wide variety of drugs, including indomethacin. Chitosan's mucoadhesive properties as well as its ability to increase cell permeability by affecting paracellular and intracellular pathways, enhances the absorption of mucosal delivery of drugs. Chitosan is also responsible for the controlled release of drugs and its pharmaceutical application seems limitless.

The preparation of chitosan beads are quite simple, but many factors should be taken into account when formulating a chitosan bead that will deliver optimal drug loading as well as optimal drug release. The above mentioned studies have proved that by manipulating the formulation of the chitosan bead by taking the physiochemical properties of the specific drug into account an optimal drug delivery system can be formulated. To achieve this goal one must have a good understanding of the factors that influence drug loading, drug release as well as the structure of the beads.

In this study, the factors that will achieve an optimal drug delivery system, in the form of chitosan beads, for indomethacin, will be explored. The effect of adding different pharmaceutical excipients, in various combinations and concentrations will also be studied and used to achieve the optimal drug release of indomethacin.

Chapter 2

Methods used for the preparation and characterization of chitosan-indomethacin beads

This chapter deals with the materials, methods and apparatus employed during this study.

2.1 Materials

Table 2.1 contains the materials used in this study. All materials used in this study were of analytical grade.

Table 2.1: Materials used during the preparation and characterization of indomethacin chitosan beads (ICB's).

Materials	Supplier
*Chitosan (91,4% deacetylated)	Xiamen, South Africa
*Indomethacin	Kirsch Pharma, South Africa
Glacial acetic acid	Labchem, South Africa
Tripolyphoshate	Sigma, South Africa
Ac-Di-Sol®	FMC, Ireland
Explotab®	Mendell, England
Vitamin C	Merck, South Africa
32% Hydrochloric acid	Labchem, South Africa
Sodium hydroxide	Merck, South Africa
Potassium dihydrogen orthophosphate	Merck, South Africa
Disodium hydrogen orthophosphate	Merck South Africa
Ethanol 99.9%	Ilovo, South Africa
Citric acid	Merck, South Africa

* See Annexure A and B for the certificates of analysis.

2.1.1 Motivation for the use of indomethacin as active ingredient

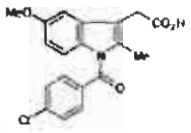
Indomethacin is a non-steroid anti-inflammatory drug (NSAID) used for its antipyretic and analgesic properties. It is not a simple analgesic and because of its untoward effects, it should not be used unnecessary (Flower *et al.*, 1985:695). It has been used effectively in the treatment of rheumatoid arthritis for more than a decade. The high incidence and severity of side-effects, the most common being gastro-intestinal ulceration, headache and dizziness, which are dose-related and associated with long-term administration, have limited its use (Eis *et al.*, 1998:120). This has led to the search for a new drug delivery system which can overcome the side effects by controlling the drug release (Joseph *et al.*, 1995:161-168). One of the physio-chemical properties of indomethacin is its insoluble character in acidic medium.

2.1.1.1 Physiochemical properties of indomethacin

The physiochemical properties of indomethacin play an important role in the incorporation of the drug into chitosan beads. Thus it should be studied intensively. Most of the properties indicated in Table 2.2 were taken into account when the indomethacin-chitosan beads where formulated.

Slide 2.1: Scanning electron microscope photo of indomethacin.

Table 2.2: Physiochemical properties of indomethacin (O'Brien *et al.*, 1984:211-238).

Structure :	 Indomethacin : [1-(4-Chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid $C_{19}H_{16}ClNO_4$ = 357.8
Description :	Pale yellow to yellow-tan crystalline powder that is odorless or almost odorless. Indomethacin exhibits polymorphism (see slide 2.1).
Melting point :	158° - 162°
Dissociation constants :	A pKa of 4.5 for the carboxyl group of indomethacin was calculated from aqueous solubility data.
Solubility :	Indomethacin is practically insoluble in water; soluble 1 in 50 of alcohol, 1 in 30 of chloroform, and 1 in 45 of ether.
Stability :	Indomethacin should be protected from light because exposure to light induces an increase in color and slight degradation. Indomethacin is unstable in alkaline solution and undergoes alkaline hydrolysis to p-chlorobenzoate and 2-methyl-5-methoxy-indole-3-acetate.
Ultraviolet Absorbance :	Indomethacin was first characterized by Shen *et al.* (1963:488) as having ultraviolet absorbance maxima at 319 and 230 nm with an inflection at 260 nm in ethanol. The USP lists a UV absorbance maximum at 318 nm in methanolic 0.1N hydrochloric acid.

2.1.1.2 Pharmacokinetics of indomethacin

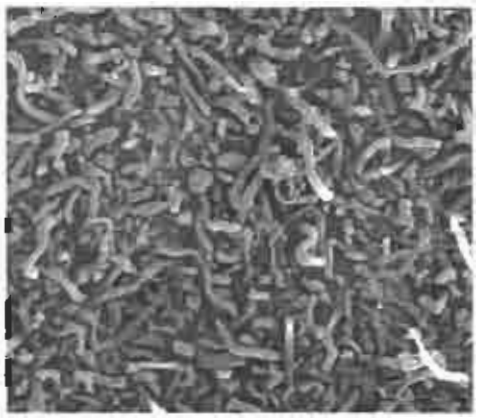
Indomethacin is readily absorbed from the gastrointestinal tract in adults; peak plasma concentrations are reached about two hours after an oral dose. Indomethacin is about 99% bound to plasma proteins. It is distributed into synovial fluid, the central nervous system, placenta and breast milk. The terminal plasma half-life has been reported to range from 2.6 to 11.2 hours in adults. Indomethacin is metabolized in the liver to its glucoronide conjugate and to desmethylindomethacin, desbenzoylindomethacin, desmethyl-desbenzoylindomethacin and to their glucoronides. Some indomethacin undergoes N-deacetylation. Indomethacin and its conjugates undergo enterohepatic circulation. Excretion of indomethacin metabolites is predominantly in the urine with lesser amounts appearing in the feaces (Reynolds, 1989:22).

2.1.2 Motivation for the inclusion of certain pharmaceutical excipients

In this study the effect of pharmaceutical excipients on the dissolution rates of indomethacin were investigated. The excipients: Explotab®, Ac-Di-Sol® and Vitamin C were used. These excipients have different properties that will have an influence on the behavior of the beads during the dissolution studies.

2.1.2.1 Ac-Di-Sol® (Croscarmelose sodium)

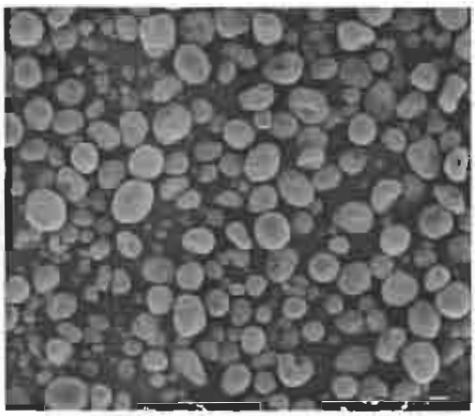
Ac-Di-Sol® is an odorless white colored powder used in solid oral dosage formulations as a disintegrant (see slide 2.2). It is insoluble in water and rapidly swells to 4-8 times its original volume when in contact with water (Rowe *et al.*, 2003:160-161).



Slide 2.2: Scanning Electron Microscope (SEM) photo of Ac-Di-Sol®.

2.1.2.2 Explotab® (Sodium starch glycolate)

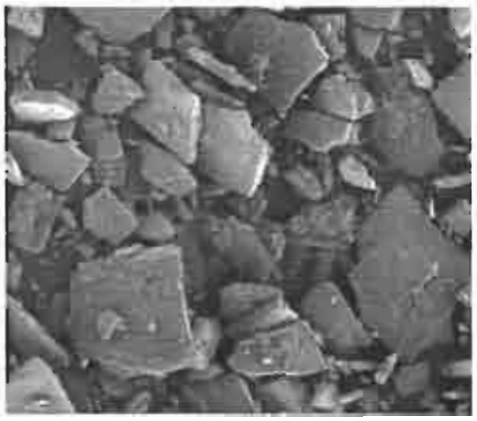
Explotab® is a white to off-white, odorless, tasteless, free-flowing powder. It consists of oval or spherical granules, 30-100 μm in diameter with some less-spherical granules ranging from 10-35 μm in diameter (see slide 2.3). Explotab® is used in solid oral dosage forms as a disintegrant. Although the effectiveness of many disintegrants is affected by the presence of hydrophobic excipients, the disintegrant efficiency of sodium starch glycolate is unimpaired. It has also been investigated for use as a suspending vehicle. At a concentration of 2% w/v it disperses in cold water and settles in the form of a highly hydrated layer. It swells up to 300 times its volume in water (Rowe *et al.*, 2003:501-503).



Slide 2.3: SEM photo of Explotab®.

2.1.2.3 Vitamin C

Vitamin C is a white to light yellow colored, non hygroscopic, odorless, crystalline powder or colorless crystals with a sharp acidic taste (see slide 2.4). Color gradually darkens upon exposure to light. It is used as an antioxidant in aqueous pharmaceutical formulations. It has 1 in 3.5 solubility in water (Rowe *et al.*, 2003:21-23). Vitamin C was added as an excipient in the beads to improve the release rate of the indomethacin from the chitosan beads. Vitamin C makes the micro-environment more acidic and this allows for the chitosan to expand/swell more, thus improving the indomethacin release rate from the chitosan beads. It also causes an increase in the porosity of the chitosan matrix due to its uneven structure.



Slide 2.4: SEM photo of Vitamin C.

2.2 Methods used for the preparation of beads

The following section describes the method used for the preparation of the "standard" indomethacin chitosan beads (ICB's) as well as the preparation of the ICB's containing various formulation excipients.

2.2.1 Preparation of "standard" ICB's

The chitosan-drug solution was prepared by adding the indomethacin to 1 ml acetic acid followed by the addition of deionised water up to 50 ml. Chitosan 3% w/v was then added and the system was stirred for 60 minutes to produce a homogenous solution. The indomethacin chitosan solution was then sonicated until bubble-free. A TPP solution was prepared by dissolving the TPP in 50 ml of deionised water. The pH was noted with a pH meter. The chitosan-indomethacin solution was added drop wise (50 rpm) to the TPP solution using a peristaltic pump (Watson-Marlow, England) which resulted in the formation of the beads (see Figure 2.1). The solution containing the beads was stirred for 30 minutes to allow for optimum crosslinking. The beads were then washed twice with 50 ml aliquots of deionised water and subsequently dried in a freeze drier (Virtis, USA) for 24 hours at -59 °C and 100 mTorr.

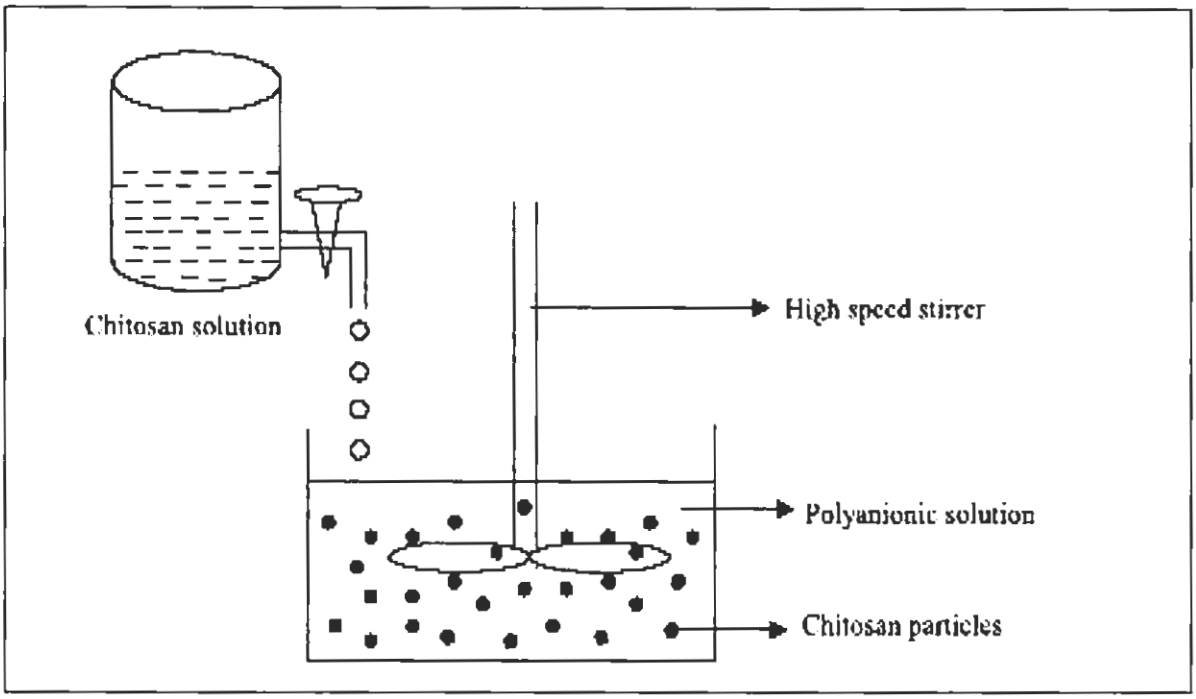


Figure 2.1: Schematic representation of preparation of chitosan particulate systems by ionic gelation method (Sunil *et al.*, 2004:13).

The process and formulation variables that were employed during the preparation of the beads are given in Table 2.3.

Table 2.3: Shows the process and formulation variables that were employed during preparation of the beads.

Variables	Levels
*pH of TPP solution	2, 3, 4, 5, 6, 7, 8, 9
TPP concentration	2, 3, 4, 5, 6 (%w/v)
Indomethacin concentration	1, 2, 3, 4, 5, 6, 7 (%w/v)

* The pH of the 5% w/v TPP solution (8.7) was lowered or increased to the required level by adding sufficient amounts of 0.1 M HCl or 0.1 M NaOH. The chitosan content was kept constant at 3% w/v.

2.2.2 Preparation of ICB's containing various formulation excipients

In order to investigate the effects of the formulation excipients on the characteristics of the beads and drug release from the beads two disintegrants (Ac-Di-Sol® and Explotab®) and ascorbic acid (alone and in combination with each other) were incorporated in the ICB's. Table 2.4 shows the various systems which

was added to / incorporated into the ICB's. The excipients were included in the initial chitosan-indomethacin solution and then added to the TPP solution as described in section 2.2.1.

Table 2.4: Excipient systems incorporated into ICB's.

System	Concentration (%w/v)
Explotab®	0.25
Ac-Di-Sol®	0.5
Vitamin C	0.25
Explotab®/Ac-Di-Sol®	0.25/0.5
Ac-Di-Sol®/ Vitamin C	0.5/0.25
Vitamin C/Explotab®	0.25 /0.25

2.3 Methods used for bead characterization

The characterization of a dosage form is of the utmost importance to ensure a safe, effective and viable product. Certain tests are performed on the beads to determine its mechanical strength, its swelling and dissolution behavior, drug loading capacity and solubility. In this study the morphology of the beads, which were determined by using scanning electron microscopy, was an important method to differentiate between the different behavior patterns of the beads in various experiments. Swelling and dissolution studies were also performed to determine the behavior of the beads.

2.3.1 Morphology: Scanning electron microscopy

The purpose of scanning electron microscopy (SEM) study was to obtain topographical characteristics of the beads. The sample was deposited on an aluminum hold and sputtered with gold palladium alloy to minimize the surface charging. A Philips XL30DX4i scanning microscope was used. Lubbe *et al.* (2002:48) was able to examine the exact pore nature of the bead matrix by cutting the sample in half with a razor blade and using a scanning electron microscope.

2.3.2 Drug loading capacity

During the preparation of the beads only a certain amount of drug was incorporated into the bead. For the bead to be considered as a viable dosage form adequate amounts of the drug should be incorporated and the

amount of drug that is lost should be minimized. It is important to determine the amount of drug that is loaded into the bead as to determine the amount of product needed per prescribed dose.

Indomethacin however is insoluble in water and also in a TPP solution. The method described in section 1.4 could not be used because the indomethacin is suspended in the TPP solution and thus can not be read on the spectrophotometer. Another method was adopted to determine the drug loading capacity of the indomethacin loaded beads. An amount of the dried beads (100 mg) were weighed and then crushed to produce a fine powder. The powder was then dissolved in 20 ml of ethanol and made up to 100 ml of PBS pH 7.2 solution. From this solution 2 ml were taken and made up to 50 ml of PBS pH 7.2 solution. The PBS 7.2 solution was prepared using a 7.15% w/v disodium hydrogen orthophosphate solution and a 2.1% w/v citric acid solution. These solutions were then added in the ratio 87:13 (disodium hydrogen orthophosphate:citric acid). This solution was then read on the spectrophotometer (Unicam, Cambridge, UK) at 318 nm wavelength and the drug loading was calculated by using a standard curve (see section 2.3.5 for the construction of a standard curve).

2.3.3 Swelling and degradation behavior

The swelling property of the beads was studied by measurement of percentage water uptake as a function of time. A known amount of beads (100 mg) was weighed and placed in 5 ml of a PBS solution, pH 5.6 and pH 7.4 respectively, at a temperature of 37 °C. Swollen beads were then collected at time intervals of 10, 60 and 360 minutes. The swollen beads were placed on a filter paper and the additional water on the surface of the sample was removed by using a vacuum filtration unit fitted with a Sartorius membrane filter. During this process care was taken while handling the swollen beads to avoid any weight loss due to breaking or erosion of the beads. The sample was then immediately weighed on an electronic balance. The percentage swelling of the sample in the media were calculated using the following formula:

$$\mathbf{Esw = [(We - Wo)/Wo] \times 100} \qquad (2\text{-}1)$$

Where:

Esw is the percentage swelling of the beads. Wo is the initial weight of the sample and We are the weight of the beads at equilibrium swelling.

The swelling media that were used were PBS pH 7.4 and PBS pH 5.6 solutions. 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 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 solution was prepared by dissolving 11.87 g of the compound in 1000 ml of deionised water. To make PBS of pH 5.6, 95.5 ml of the 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 7.4 when made up to 100 ml with disodium hydrogen orthophosphate.

According to Gupta *et al.* (2001:641) the process of swelling of chitosan beads 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 insoluble polymer region with that of the swollen portion of the beads. In the second stage, protons and counter ions diffuse into the bead to protonate the inner amino/imine groups, dissociating the hydrogen bonds. The process of protonation continues until the whole structure of the bead collapses and is solvated.

2.3.4 Dissolution studies

Dissolution studies are usually conducted to determine the ability of the dosage form to release the right amount of drug over an acceptable period of time. Chitosan beads containing indomethacin are an orally administrated drug that targets the release of indomethacin in the duodenum. Thus the indomethacin chitosan beads should be able to release indomethacin in a minimum amount of time before excretion through normal processes.

In this study the release of indomethacin that is encapsulated in chitosan beads containing different excipients was determined. Thus the effect of the different excipients was studied. Apparatus 1 of the USP was use to determine the drug release from the beads. The Erweka DT6R dissolution apparatus (Erweka® Apparatebau GmbH, Germany), with the basket stirring element, was used. A sample amount of 0.05 g beads were weighed (Precisa 240A, Precisa balances, Zurich, Switzerland) and placed in a basket. The basket was then lowered into 900 ml PBS (pH 7.4), dissolution medium and rotation was started after the basket was submerged into the medium. The rotations were kept constant at 50 revolutions per minute. Samples of 10 ml were withdrawn through a 3 µm membrane filters (Millipore, Bedford, Marlborough, England) to eliminate any suspended particles. Samples were withdrawn at time intervals 2, 5, 10, 15, 20, 30, 45, 60, 120, 180, 240,

300, 360 minutes. After each withdrawal dissolution medium was replenished with fresh preheated dissolution medium.

All the experiments were done in triplicate and assayed spectrophotometrically, using a Unicam® spectrophotometer (Unicam, Cambridge, UK) at 318 nm wavelength. The following equation was used to correct for dissolution media after each sample withdrawal and addition of pure medium:

$$Y^*_n = Y_n + \frac{V_s}{V_m} \bullet \Sigma^{n-1} Y^* \qquad (2\text{-}2)$$

Where:

Y^*_n is the corrected absorbancy of the n^{th} sample; Y_n is the measured absorbancy of the n^{th} sample; V_s is the sample volume; V_m is the dissolution medium volume and $\Sigma^{n-1} Y^*$ is the sum of the corrected absorbencies prior to the n^{th} sample.

2.3.5 Dissolution parameters: AUC_n and DR_i

The area under the dissolution profile up to 360 minutes (AUC) would be an indication of the extent of drug dissolution at the end of the dissolution test and the initial slope of the dissolution curve between t_0 and t_{15} was suggested to be an estimate for the initial dissolution rate of indomethacin (DR_i).

The DR_i (% drug dissolved.minute^{-1}) of each ICB formulation was determined from the slope of the dissolution curve between t_0 and t_{15}, while the AUC (% drug dissolved. minute^{-1}) of the drug between t_0 and t_{360} was determined using the trapezoidal rule (see equation 2.3). The dissolution curve is divided into intervals (the dissolution time points) and the arc between the intervals is connected linearly. Thus, a number of trapeziums are formed. The AUC is then calculated as the sum of the areas of each trapezium.

$$\mathbf{AUC} = 0.5 * \sum_{t=n}^{t=0} (t_n - t_{n-1}) * (c_n + c_{n-1}) \qquad (2\text{-}3)$$

Where:

t_n-t_{n-1} is the time difference between two consecutive sampling times and c_n and c_{n-1} is the drug concentration (% drug dissolved) in samples at sampling times corresponding to t_n and t_{n-1}.

In order to compare the different formulations in terms of their respective AUC's, each individual AUC was related to the AUC of the standard ICB's. The calculated value, termed the normalized AUC, AUC_n, is a dimensionless parameter and was calculated as follows:

$$AUC_n = \frac{AUC\ test\ formulaion}{AUC\ standard\ formulation} \qquad (2\text{-}4)$$

2.3.6 Construction of a standard curve

Before each drug loading capacity test and dissolution study a standard curve was constructed. Two stock solutions were prepared by respectively, dissolving 100 mg (A) and 30 mg (B) of indomethacin in 10 ml ethanol. These solutions were sonicated to ensure complete solubility of the drug. Both solutions were then made up to volume (A to 250 ml and B to 100 ml) with PBS solution. Standard solutions were then prepared from each stock solution by diluting exact volumes with PBS solution to give solutions with concentrations ranging from 4 – 40 µg/ml (from A) and 6 – 54 µg/ml (from B). The UV absorptions of the standard solutions were measured against a blank (PBS solution) at 318 nm and a standard curve was constructed (see Figure 2.2).

The preparation of the PBS solutions (pH 7.2 for the drug loading tests and pH 7.4 for the dissolution tests) was described in section 2.3.2 and 2.3.3 respectively.

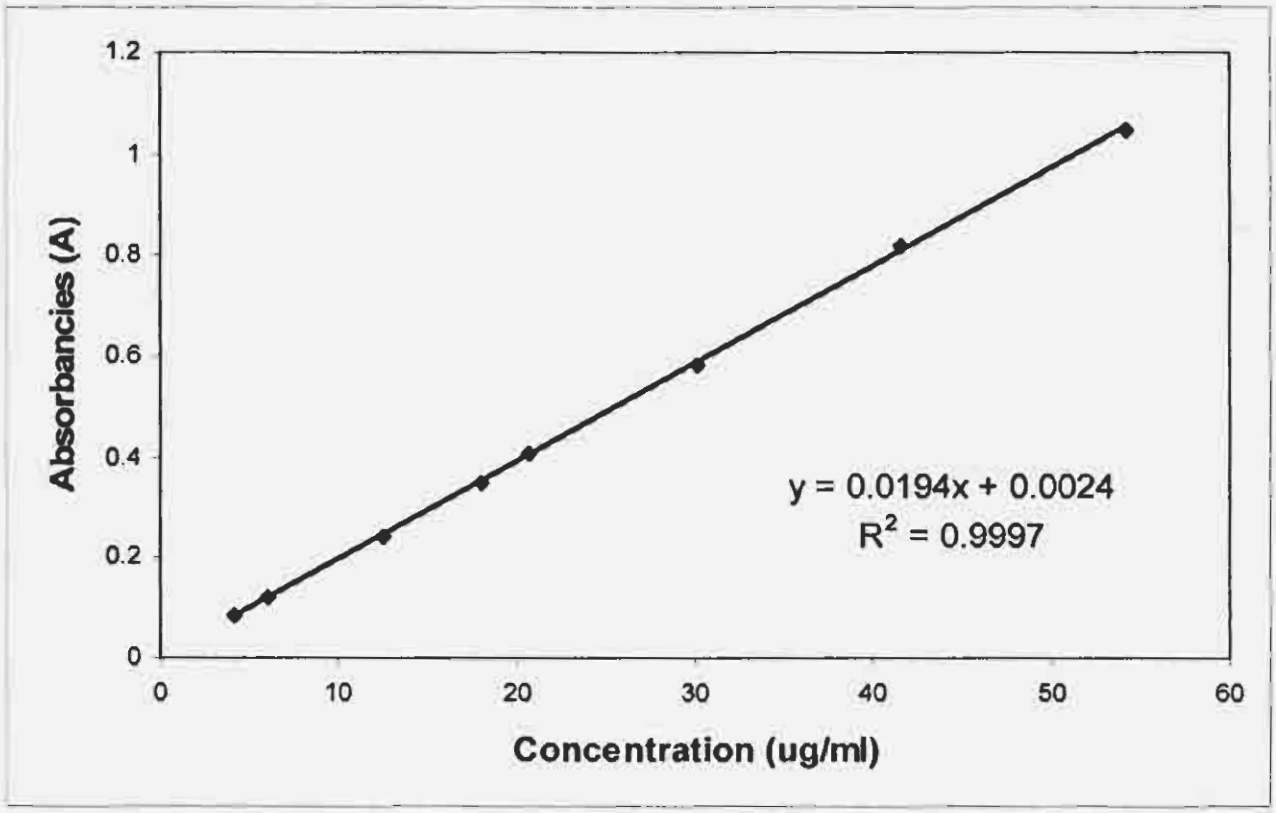


Figure 2.2: Example of a standard curve plotted for the determination of drug loading and dissolution of the ICB's.

2.3.7 Calculations

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

Chapter 3

The effect of process and formulation variables on the physical characteristics of indomethacin-chitosan beads

3.1 Introduction

It has been well documented that process and formulation variables have significant effects on the characteristics and properties of dosage forms. These variables not only affect the physical properties of the dosage form, but also influence the drug release profiles from the dosage form. Optimization of formulations normally entails the evaluation of the effect of the various variables in the presence of one another.

This chapter deals with the characterization of indomethacin-chitosan beads (ICB's) prepared by the ionic gelation method as described in section 1.3.1 (general method) and 2.2.1 (method used in this study). The effect of various process variables (the TPP concentration, the pH of the TPP solution, the indomethacin concentration) and formulation variables (type of disintegrant, combinations of disintegrants and ascorbic acid) on the morphology, drug loading and swelling behavior of the beads were examined.

3.2 Effect of pH of the TPP solution on the ICB's

Indomethacin is soluble in an alkaline media thus it was assumed that the drug would dissolve when the indomethacin-chitosan solution was added to the TPP solution (pH of 8.7). It was then argued that the drug loading would increase if the TPP solution was more acidic because indomethacin is insoluble in acidic media. ICB's (3% w/v chitosan and 3% w/v indomethacin, arbitrarily chosen) were prepared according to the method described in section 2.2.1 using a 5% w/v solution of TPP in which the pH (8.7) was altered by adding 0.1 M HCl (to lower the pH) or 0.1 M NaOH (to increase the pH). The determination of the optimum drug percentage in the beads will be discussed in section 3.4. The various pH levels of the TPP solution that were used in preparation of the beads were pH = 2, 3, 4, 5, 6, 7, 8, 9.

3.2.1 Morphology

The morphology of the ICB's prepared from the TPP solution at different pH levels were studied using a scanning electron microscope [SEM] (method described in section 2.3.1). The results are presented as SEM-slides (3.1 – 3.16) showing a cross section of the beads (uneven numbered slides) and an in-depth view of the chitosan matrix of the beads (evenly numbered slides).

Slide 3.1: Cross-section view of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 2.**

Slide 3.2: View of the chitosan matrix of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 2.**

Slide 3.3: Cross-section view of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 3.**

Slide 3.4: View of the chitosan matrix of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 3.**

Slide 3.5: Cross-section view of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 4.**

Slide 3.6: View of the chitosan matrix of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 4.**

Slide 3.7: Cross-section view of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 5.**

Slide 3.8: View of the chitosan matrix of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 5.**

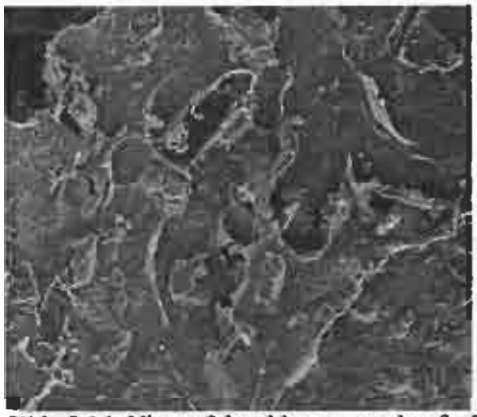
Slide 3.9: Cross-section view of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 6.**

Slide 3.10: View of the chitosan matrix of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 6.**

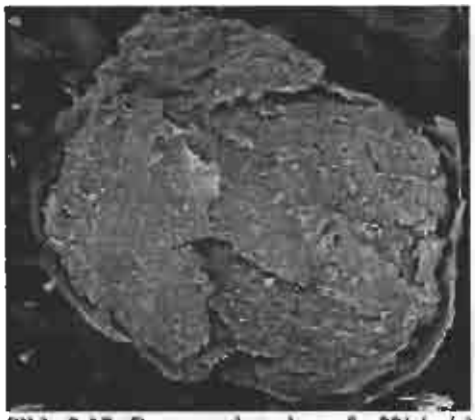
Slide 3.11: Cross-section view of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 7.**

Slide 3.12: View of the chitosan matrix of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 7.**

Slide 3.13: Cross-section view of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 8.**



Slide 3.14: View of the chitosan matrix of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 8.**



Slide 3.15: Cross-section view of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 9.**

Slide 3.16: View of the chitosan matrix of a 3% (w/v) chitosan bead with 3% (w/v) indomethacin at **pH 9.**

The effect of the pH level of the TPP solution on indomethacin loaded chitosan beads can clearly be observed in the morphology of the SEM photos. As the pH increased the chitosan coated the indomethacin particles more effectively as seen on the slides of the chitosan matrix. At lower pH levels most of the

indomethacin particles were exposed and not coated with chitosan. This phenomenon will probably play a role on the release of the drug in dissolution studies. The more the drug particles were coated with chitosan the more effective the controlled drug delivery will be. Thus indomethacin will be released in a more controlled way when the indomethacin particles are coated sufficiently with the chitosan matrix.

In the cross-section view of the beads, it appeared that the surface membrane became thicker with an increase in the pH. The surface membrane plays an important role in preserving the structural integrity of the bead. The chitosan matrix is also more porous in an acidic TPP solution than in a basic TPP solution. This could be explained as a result of a reduction between crosslinking TPP and the chitosan with a decrease in the pH (Fwu-Long Mi *et al.*, 2002:761). The chitosan matrix should however not be so macroporous that the structural integrity of the bead is compromised.

3.2.2 Drug loading

The actual drug loading in the beads were determined using the method described in section 2.3.2 and the results are presented in Figure 3.1. Eight samples were prepared as previously described.

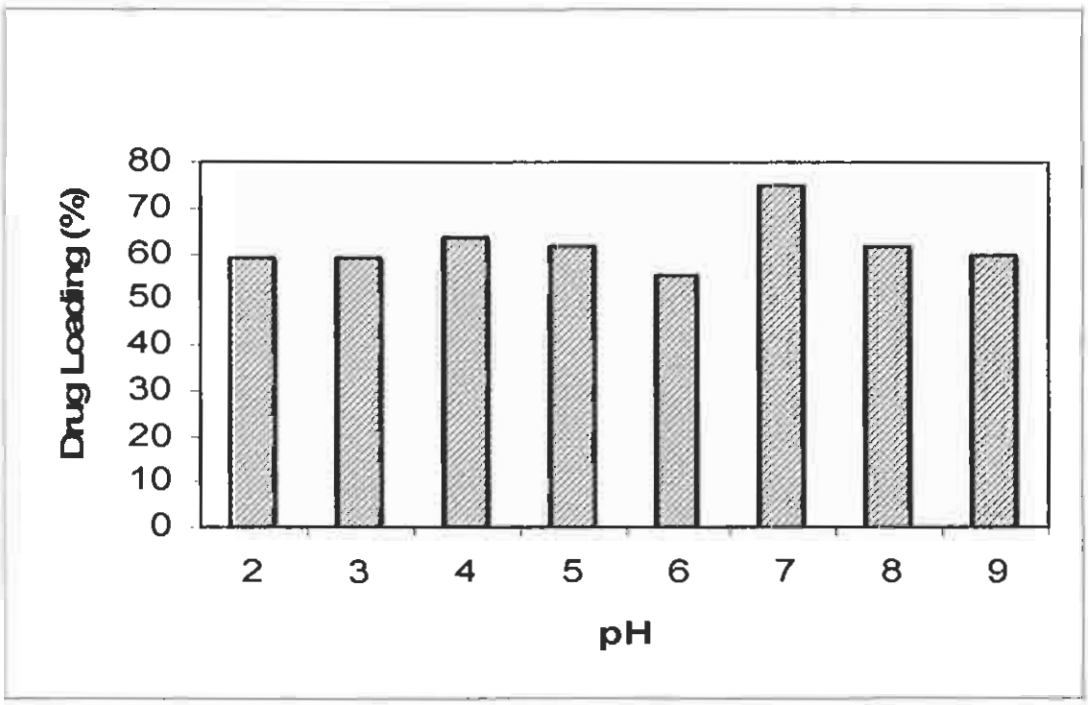


Figure 3.1: Drug loading of 3% (w/v) chitosan beads containing 3% (w/v) indomethacin where the variant was the pH of the TPP solution.

The pH of the TPP solution did not show significant changes in the drug loading. Although the TPP solution with a pH 7 resulted in the best drug loading, the difference in drug loading across the pH range is so insignificant that it could not be regarded as the peak drug loading at that particular pH. Thus the hypothesis that the pH of the cross-linking solution would affect the drug loading capability of the beads was proved wrong.

3.2.3 Swelling

The swelling was only determined on three of the eight samples. The formulations used were those prepared in TPP solutions with pH 2, 5 and 8 respectively. The degree of swelling was determined as described in section 2.3.3. The results are presented in Figures 3.2 and 3.3.

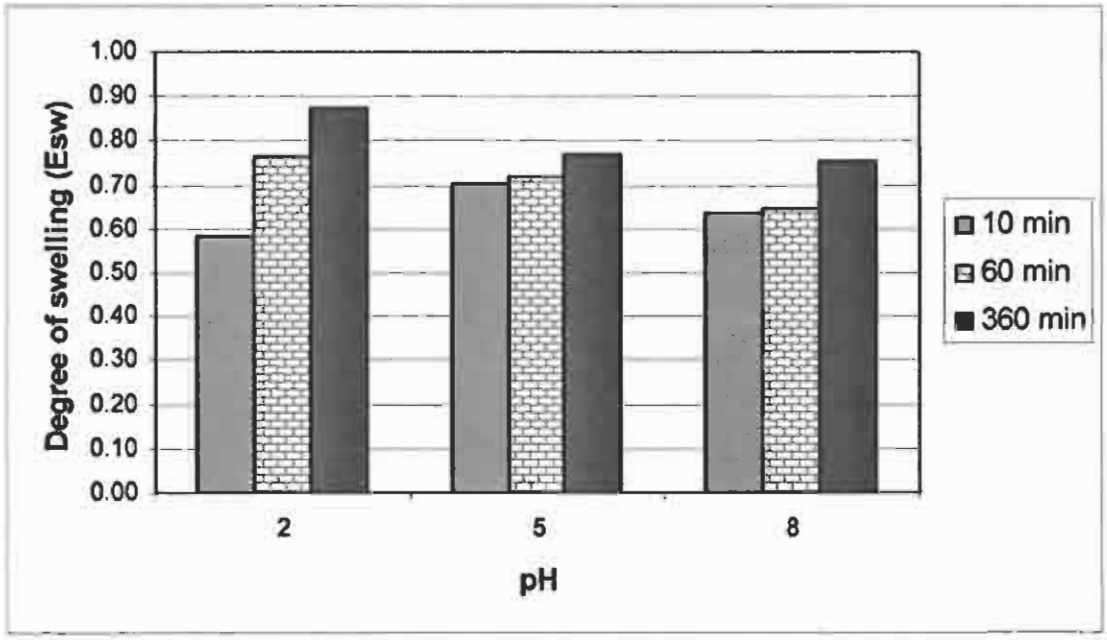


Figure 3.2 : The degree of swelling (Esw) of indomethacin loaded chitosan beads at 10, 60 and 360 minute intervals in a PBS pH 7.4 solution where the variant was the pH of the TPP solution.

The degree of swelling was higher in PBS pH 5.6 than in PBS pH 7.4. The difference between the swelling at 60 and 360 minutes is minimal. This indicated that the beads almost reach maximum swelling after one hour. There was no definite difference in the swelling capability between the different pH levels.

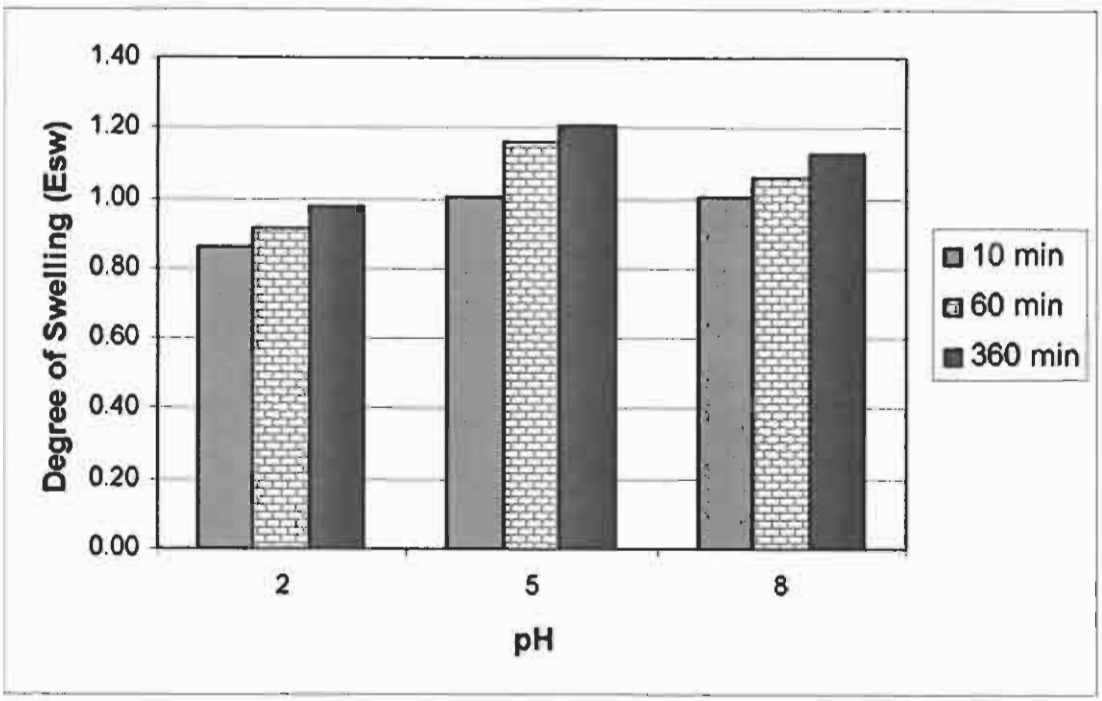


Figure 3.3 : The degree of swelling (Esw) of indomethacin loaded chitosan beads at 10, 60 and 360 minute intervals in a PBS pH 5.6 solution where the variant was the pH of the TPP solution.

3.2.4 Conclusion

TPP-chitosan microparticles were prepared by the ionic interaction between a positively charged amino group of chitosan and a negatively charged counterion of TPP. The ionization degree of TPP is dependent on the pH value of solution. In a standard TPP solution (pH 8.7), TPP is dissociated into OH^- and TPP ions ($HP_3O_{10}^{4-}$ and $P_3O_{10}^{5-}$). However at low pH, only $P_3O_{10}^{5-}$ anions exists. Moreover, chitosan is a weak polybase, and as the pH of the solution decreases, the ionization of amine group of chitosan increases. Therefore, TPP-chitosan microparticles prepared in the original TPP solution are dominated by deprotonation and slightly ionic-crosslinking, but chitosan microparticles prepared in acidic TPP solution are completely ionic-crosslinking dominated (Mi *et al.*, 1999a:1868, b:1551; Shu and Zhu 2000:51, 2001:237; Lee *et al.*, 2001:1879).

The most significant effect of the pH of the TPP solution was on the morphology of the beads. It was thus decided to retain the structural integrity of the bead which was achieved at a basic TPP solution. The study was further conducted with the natural pH of a 5% (w/v) TPP solution which is at 8.7.

3.3 Effect of indomethacin concentration on ICB's

To achieve an efficient drug delivery system, the system should be able to retain a maximum amount of the drug. The effect of the indomethacin concentration was tested in this study to determine the maximum drug loading capacity of the beads. The maximum dosage of indomethacin is 200 mg a day. To produce a viable product a minimum amount of beads should be taken to achieve an accurate and effective dosage. The beads in this experiment were produced by using the ionic gelation method as described in section 2.2.1. The TPP solution was kept at 5% (w/v) at pH 8.7 and the chitosan solution 3% (w/v), with the indomethacin concentration varying from 1 - 7% w/v.

3.3.1 Morphology

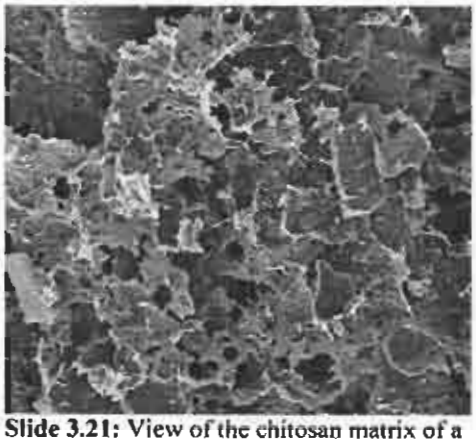
SEM was used to determine the morphology of the beads. Slide 3.17 – 3.23 present a view of the chitosan matrix at the different indomethacin concentrations.

Slide 3.17: View of the chitosan matrix of a 3% (w/v) chitosan, **1% (w/v) indomethacin** bead at pH 8.7.

Slide 3.18: View of the chitosan matrix of a 3% (w/v) chitosan, **2% (w/v) indomethacin** bead at pH 8.7.

Slide 3.19: View of the chitosan matrix of a 3% (w/v) chitosan, **3% (w/v) indomethacin** bead at pH 8.7.

Slide 3.20: View of the chitosan matrix of a 3% (w/v) chitosan, **4% (w/v) indomethacin** bead at pH 8.7.



Slide 3.21: View of the chitosan matrix of a 3% (w/v) chitosan, **5% (w/v) indomethacin** bead at pH 8.7.

Slide 3.22: View of the chitosan matrix of a 3% (w/v) chitosan, **6% (w/v) indomethacin** bead at pH 8.7.

Slide 3.23: View of the chitosan matrix of a 3% (w/v) chitosan, **7% (w/v) indomethacin** bead at pH 8.7.

The only significant difference on the morphology that could be observed from these slides was the marked increase of indomethacin particles in the chitosan matrix as the indomethacin concentration increased. The even structure of the chitosan matrix also became rougher with an increase in indomethacin concentration because of the uneven structure of the indomethacin particles that prevented the TPP to evenly cross-link with the chitosan particles. An uneven structure caused the structural integrity of the bead to decrease.

3.3.2 Drug loading

The drug loading of the beads was determined as described in section 2.3.2. To achieve a viable product, a minimum amount of beads should be able to give an accurate dosage. In Table 3.2 the amount of indomethacin that can be expected in 100 mg beads containing 3% (w/v) chitosan is shown in the last column. This means that 100 mg beads containing 3% (w/v) chitosan and 4% (w/v) indomethacin will provide a dosage of approximately 39.39 mg indomethacin. This is not a precise amount but an estimate that

can be expected. Currently indomethacin are available in both 25 mg and 50 mg capsules. The results are graphically presented in Figure 3.4.

Table 3.2: Variation in the concentration of indomethacin shows a variation in the amount of indomethacin expected in 100 mg beads containing 3% (w/v) chitosan as determined by the drug loading percentage.

Concentration Indomethacin (w/v)	Theoretic amount of indomethacin in 100mg beads (mg)	Drug loading Percentage (%)	Experimental amount of indomethacin in 100 mg beads (mg)
1%	25	57.16	14.29
2%	40	58	23.20
3%	50	64.18	32.09
4%	57.14	68.94	39.39
5%	62.5	70.85	44.28
6%	66.67	66.1	44.07
7%	70	71.28	49.90

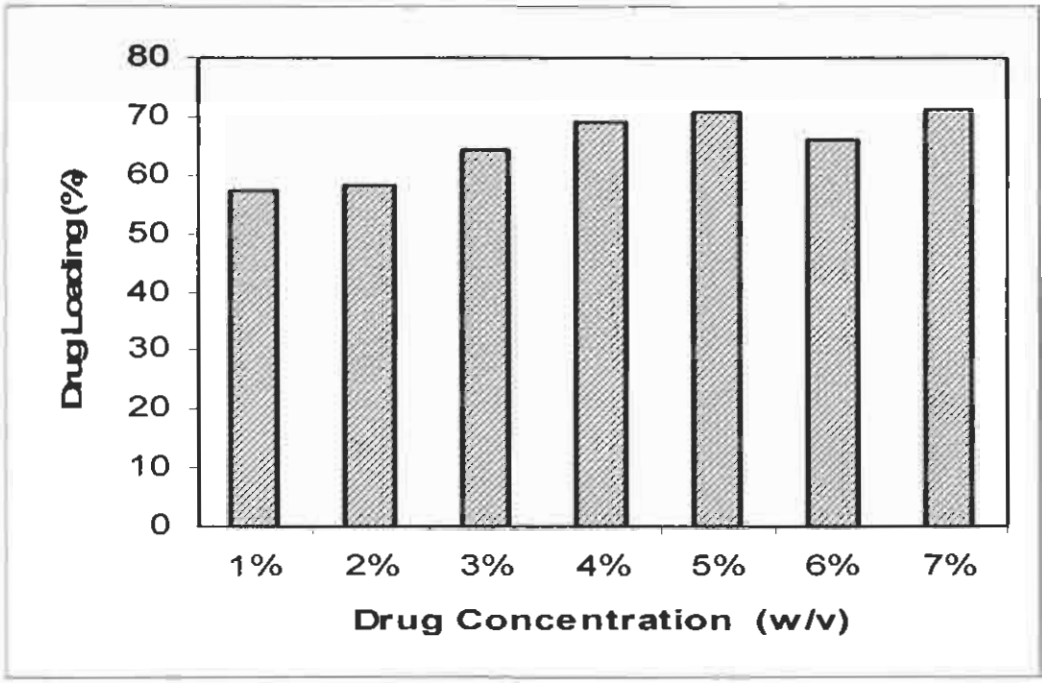


Figure 3.4: Drug loading of 3% (w/v) chitosan beads cross-linked with 5% (w/v) TPP where the variant was the concentration of the indomethacin % (w/v).

The drug loading percentage increased with an increase in the indomethacin concentration. It is important to consider the dosage that is to be achieved and the amount of beads that should be taken to achieve the

required dosage. Although the 7% (w/v) indomethacin provided an estimate of 10 mg more drug than the 4% (w/v) indomethacin per 100 mg beads, the percentage of the lost drug in the 7% (w/v) indomethacin does not justify that minor difference. Thus the 4% (w/v) indomethacin will be a better choice as it produces a practical dosage per 100 mg beads even if it does not prove to give the best drug loading percentage. The drug loss during the manufacturing process is also less than that of the 7% (w/v) indomethacin.

3.3.3 Swelling

The degree of swelling was determined as described in section 2.3.3. The results are presented in Figures 3.5 and 3.6.

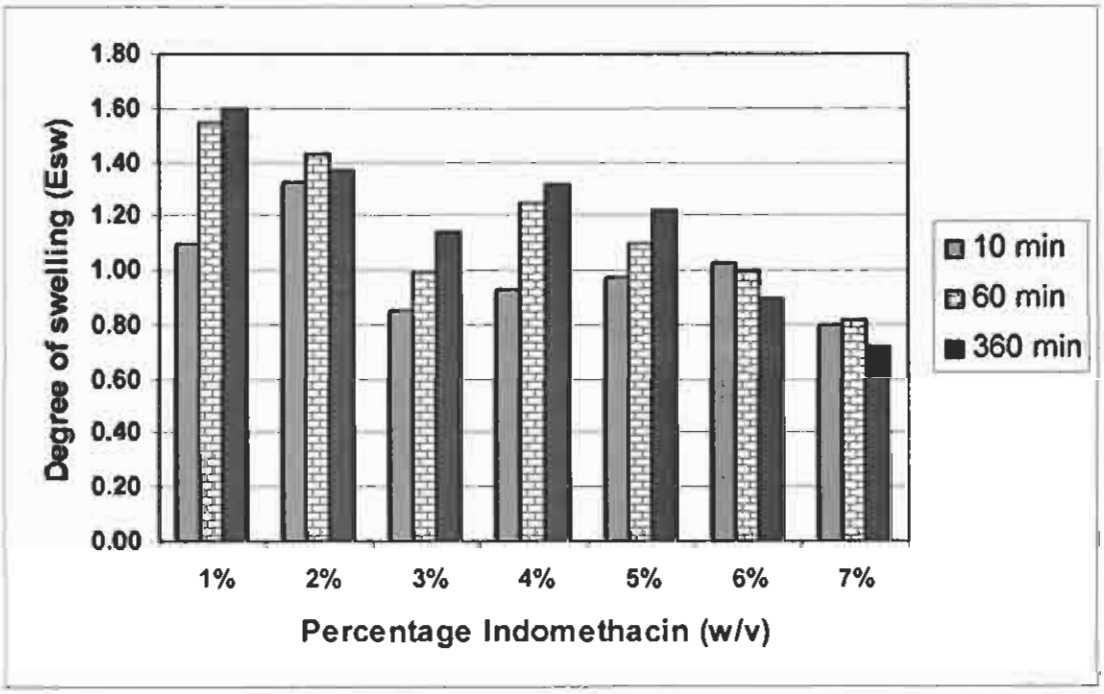


Figure 3.5 : The degree of swelling (Esw) of indomethacin loaded chitosan beads at 10, 60 and 360 minute intervals in a PBS pH 7.4 solution where the variant was the indomethacin concentration (w/v).

The degree of swelling gradually decreased as the indomethacin concentration increased. This may be due to the fact that the amount of indomethacin particles that increased as the indomethacin concentration increased and allowed less room for the PBS solution to enter the chitosan matrix. Because the structural integrity of the bead is compromised with an increase in indomethacin concentration the beads had a tendency to disintegrate after a certain amount of time. This is also the reason why the degree of swelling was less after 360 minutes than after 60 minutes in some of the cases. There was not a marked difference in the degree of swelling between the PBS pH 7.4 and PBS pH 5.6.

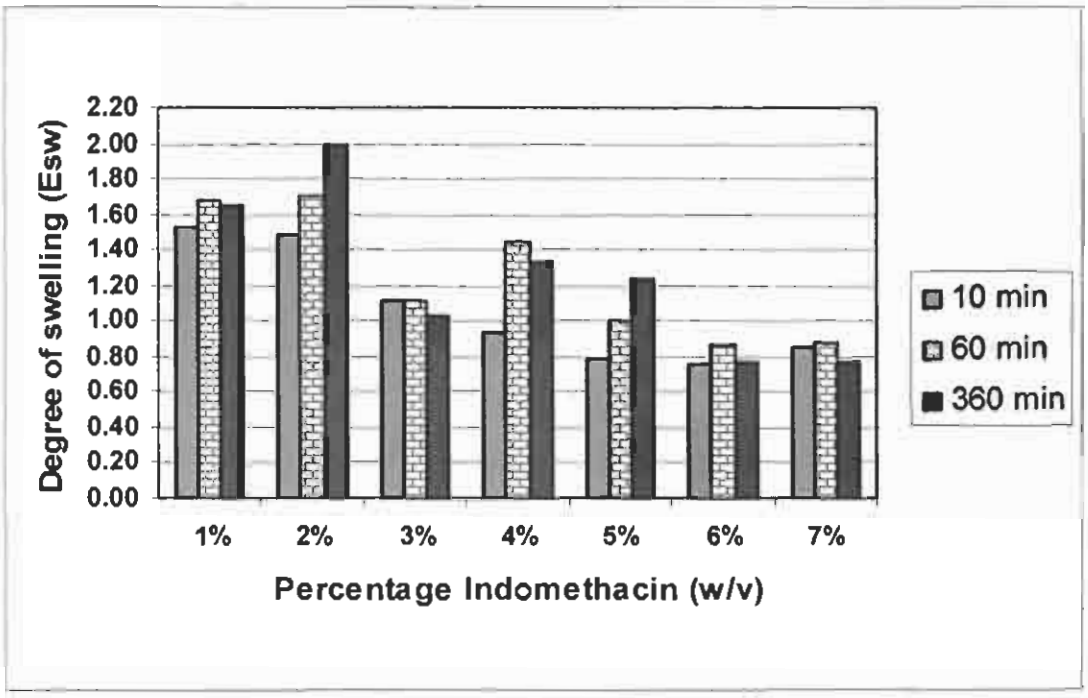


Figure 3.6 : The degree of swelling (Esw) of indomethacin loaded chitosan beads at 10, 60 and 360 minutes intervals in a PBS pH 5.6 solution where the variant was the indomethacin concentration (w/v).

3.3.4 Conclusion

The concentration indomethacin is an important parameter to consider when formulating a bead that will effectively encapsulate a sufficient amount of the drug. After reviewing the results it was decided that 4% (v/w) indomethacin concentration would be the concentration of choice for this study. It showed satisfying drug loading and competent swelling without compromising the structural integrity of the microspheres.

3.4 Effect of TPP concentration on ICB's

It was argued that the TPP concentration would definitely affect the characteristics of the beads, since the TPP cross-links with the chitosan to form a polymer matrix, with the magnitude of the cross-linking dependant on the TPP concentration. In order to determine its effect, TPP solutions of varying strength (3, 4, 5, 6% w/v) were prepared and used in the preparation of the ICB's (containing, 4% w/v indomethacin and 3% w/v chitosan) according to the ionic gelation method described in section 2.2.1. The beads were characterized in terms of morphology, drug loading and swelling behavior. [The pH of a 5% w/v solution was approximately 8.7].

3.4.1 Morphology

The morphology of the beads was determined with a scanning electron microscope as described in section 2.3.1. Only the 3% and 6% (w/v) TPP concentrations were used, in order to demonstrate more effectively the effect that TPP concentration has on the morphology of the beads. A view of the surface of the bead and a view of the chitosan matrix portrayed the most evident differences (Slide 3.24 – 3.27).

Slide 3.24: View of the surface of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **3% (w/v) TPP.**

Slide 3.25: View of the chitosan matrix of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **3% (w/v) TPP.**

Slide 3.26: View of the surface of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **6% (w/v) TPP.**

Slide 3.27: View of the chitosan matrix of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **6% (w/v) TPP.**

The surface of the beads (Slide 3.24 and 3.26) indicates how an increase in the TPP concentration increases the thickness of the polymer covering the bead. The 6% (w/v) TPP gave the impression that the bead is covered in plastic cling wrap. This might probably decrease the drug release of the beads. The 6% (w/v) TPP concentration's chitosan matrix was less porous than the 3% (w/v) TPP concentration's as can be seen on slides 3.25 and 3.27. Thus the higher the TPP concentration the less porous the polymer matrix appeared. The

3% (w/v) TPP's structure could be considered too porous, and the surface of the bead was not fully intact and this might decrease the structural integrity of the beads.

3.4.2 Drug loading

The drug loading was determined as described in section 2.3.2 and these results are presented in Figure 3.7.

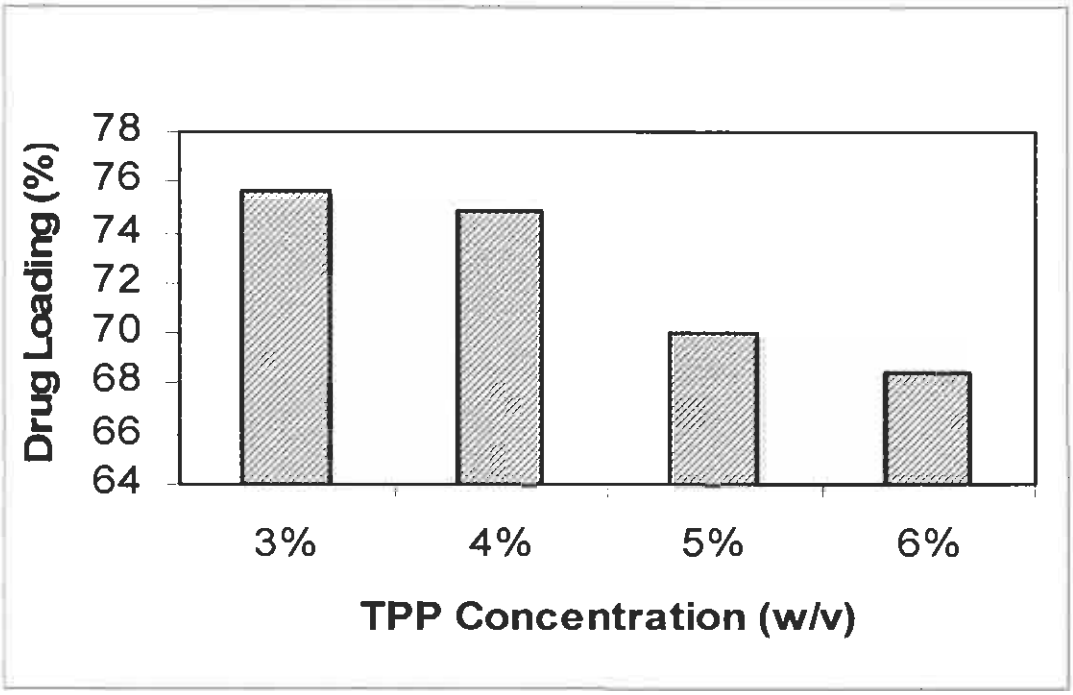


Figure 3.7: Drug loading of 3% (w/v) chitosan, 4% (w/v) indomethacin beads where the variant was the concentration of the TPP (w/v).

An increase in the TPP concentration causes a decrease in the drug loading. This might be because an increase in TPP concentration caused a decrease in the porosity of the chitosan matrix. Thus there was less room for indomethacin particles to bind with the chitosan and more of the drug was lost during the manufacturing process.

3.4.3 Swelling

The degree of swelling was determined as described in section 2.3.3. From these results it is evident that the degree of swelling is also dependant on the concentration of the TPP solution. The results are presented in Figures 3.8 and 3.9.

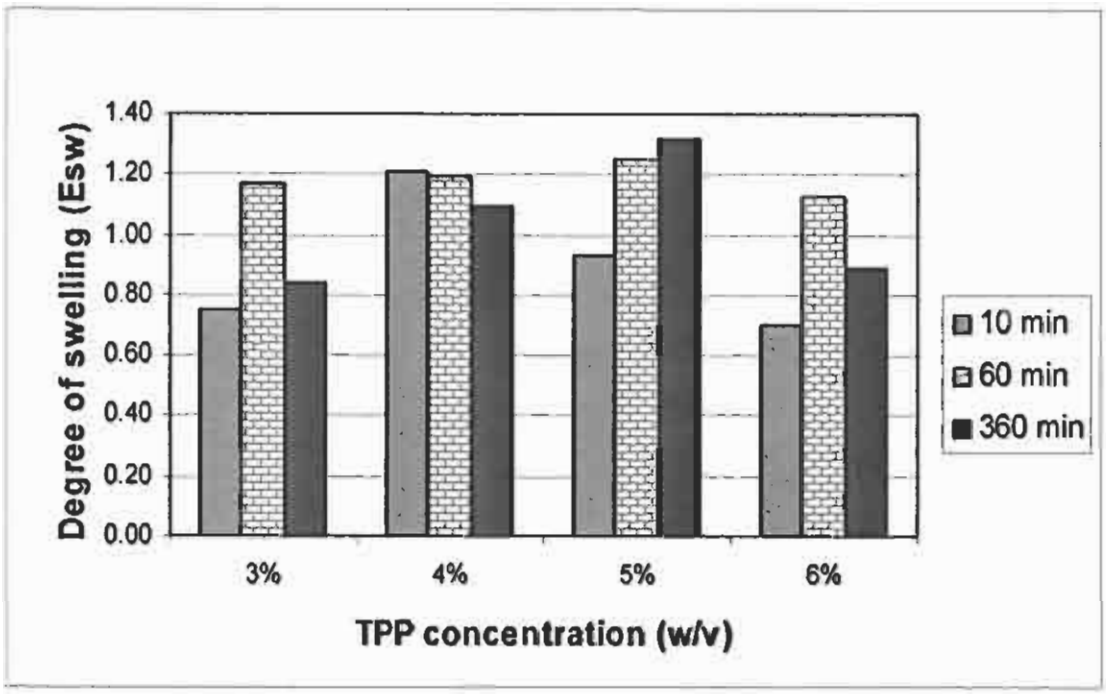


Figure 3.8 : The degree of swelling (Esw) of indomethacin loaded chitosan beads at 10, 60 and 360 minute intervals in a PBS pH 7.4 solution where the variant was the TPP concentration (w/v).

The swelling tests showed that the 5% (w/v) TPP concentration resulted in the optimum swelling ability (see Figure 3.8 and 3.9). In most of the tests the beads reached its optimum swelling after an hour. The degree of swelling is dependant on the porous structure of the beads. If the beads are too porous the three dimensional structure of the chitosan-polymer matrix will collapse during swelling and the bead will disintegrate. If the bead is not porous as was the case with 6% (w/v) TPP, the polymer forms an enclosed and tight matrix for a solution to penetrate and will thus give minimal swelling. The decrease in swelling at 360 minutes indicated that the beads disintegrated and lost their swelling ability. The beads displayed better swelling by a minor margin in the PBS pH 5.6 solution than in the PBS pH 7.4 solution. This indicated that the swelling capability of the beads were slightly dependant on the pH of the PBS solution.

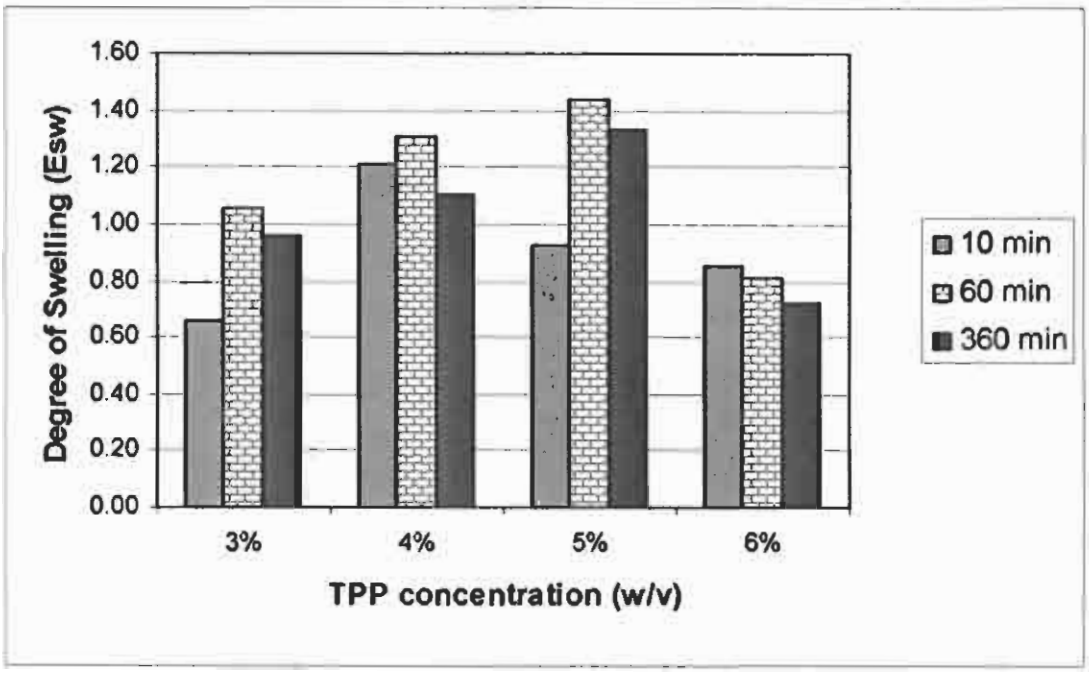


Figure 3.9 : The degree of swelling (Esw) of indomethacin loaded chitosan beads at 10, 60 and 360 minute intervals in a PBS pH 5.6 solution where the variant was the TPP concentration (w/v).

3.4.4 Conclusion

The drug loading, swelling and morphology of the beads is dependant on the concentration of the TPP. In light of the results discussed the TPP 5% (w/v) was chosen to continue the study with as it gave the best swelling results in both the PBS 5.6 and 7.4 solutions. Although it did not display the best drug loading, it displayed the best swelling results. The optimum concentration according to the morphology test indicated between 3% and 6% TPP (w/v), with 6% too high and 3% too low.

In general, the release profile of drug from TPP chitosan microparticles decreased with the increased crosslinking agent concentration. It also depends on the density of TPP-chitosan matrix. Remunan-Lopez *et al.* (1997:215-225) reported that the diffusion of drug from chitosan films decreased as the concentration of the TPP solution increased. In addition, they showed that the swelling and permeability characteristics of chitosan films were dependent on pH and concentration of the crosslinking agent.

3.5 Effect of single pharmaceutical excipients (SPE) on ICB's

The effect of the pH and concentration of the TPP solution and the effect of the concentration of the indomethacin have been studied. From these studies a 3% (w/v) chitosan, 4% (w/v) indomethacin bead cross-linked with a 5% TPP solution with a pH of 8.7 was chosen as the most ideal bead to encapsulate the drug indomethacin. The following step is to determine the effects of pharmaceutical excipients on the morphology, drug loading and swelling of the beads. The effect of pharmaceutical excipients on the drug release of the beads will also be studied, which will be further explored and discussed in chapter 4. This study will be based on a previous masters degree study that was conducted by Mahlala. (2004). In this study the optimal concentrations of the pharmaceutical excipients in chitosan beads, Explotab®, Ac-Di-Sol® and Vitamin C were determined. The optimal concentrations were as follows: 0.25% (w/v) Explotab®, 0.25% (w/v) Vitamin C and 0.5% (w/v) Ac-Di-Sol®. The beads were prepared using the ionic gelation method as described in section 2.2.2. The pharmaceutical excipients were added in their various concentrations to the chitosan/indomethacin solution. The viscosity of this solution increased dramatically with the inclusion of the excipients.

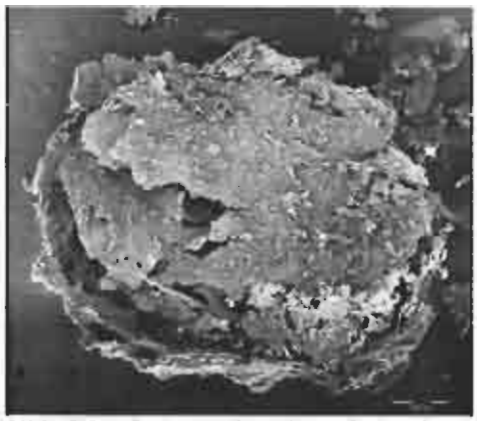
3.5.1 Morphology

The surface of the bead as well as a cross-section view of the bead containing a single pharmaceutical excipient was studied by means of a scanning electron microscope as described in section 2.3.1.

Slide 3.28: *View of the* **surface of a bead** containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **0.25 % (w/v) Explotab®.**

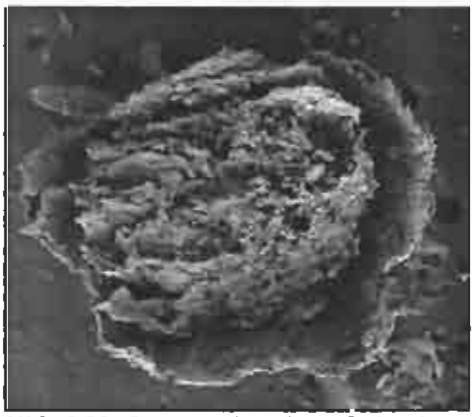
Slide 3.29: Cross-section view of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **0.25 % (w/v) Explotab®.**

Slide 3.30: View of the surface of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **0.25 % (w/v) Vitamin C.**



Slide 3.31: Cross-section view of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **0.25 % (w/v) Vitamin C.**

Slide 3.32: View of the surface of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **0.5 % (w/v) Ac-Di-Sol®.**



Slide 3.33: Cross-section view of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **0.5 % (w/v) Ac-Di-Sol®.**

Because morphology of all the excipients differs (see section 2.1.2), it is logical that the morphology of their beads will differ. Both Explotab® and Ac-Di-Sol® produced spherical beads with a thick membrane and a porous chitosan-polymer matrix. Ac-Di-Sol® beads however had a rough surface. The Vitamin C bead had a thinner membrane but was more porous than the other two. The particles of the excipients could be seen on the surface of the beads. This would certainly play an important role in the burst effect during the dissolution studies.

3.5.2 Drug loading

The drug loading was determined with the method described in section 2.3.2. The results are presented in Figure 3.10.

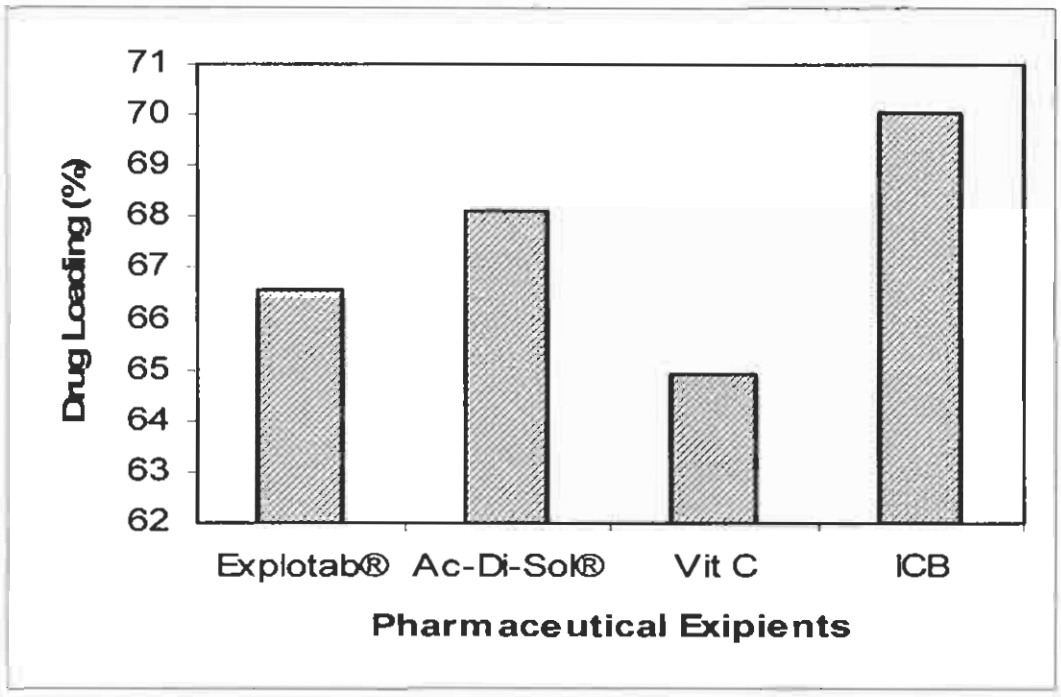


Figure 3.10: Drug loading of 3% (w/v) chitosan, 4% (w/v) indomethacin beads cross-linked with 5% (w/v) TPP where the variant was the single pharmaceutical excipient.

The inclusion of the excipients decreased the beads' drug loading ability compared to the indomethacin chitosan beads (ICB's) without any excipients. This could be explained that the inclusion of the excipients decreased the chance of the chitosan to effectively cross-link with the TPP. Ac-Di-Sol® produced the best drug loading of the three excipients.

3.5.3 Swelling

The degree of swelling was determined with the method described in section 2.3.3 at three intervals 10, 60 and 360 minutes. Swelling was also conducted in two solutions, PBS pH 7.4 and PBS pH 5.6 at 37 °C and the results are graphically presented in Figures 3.11 and 3.12.

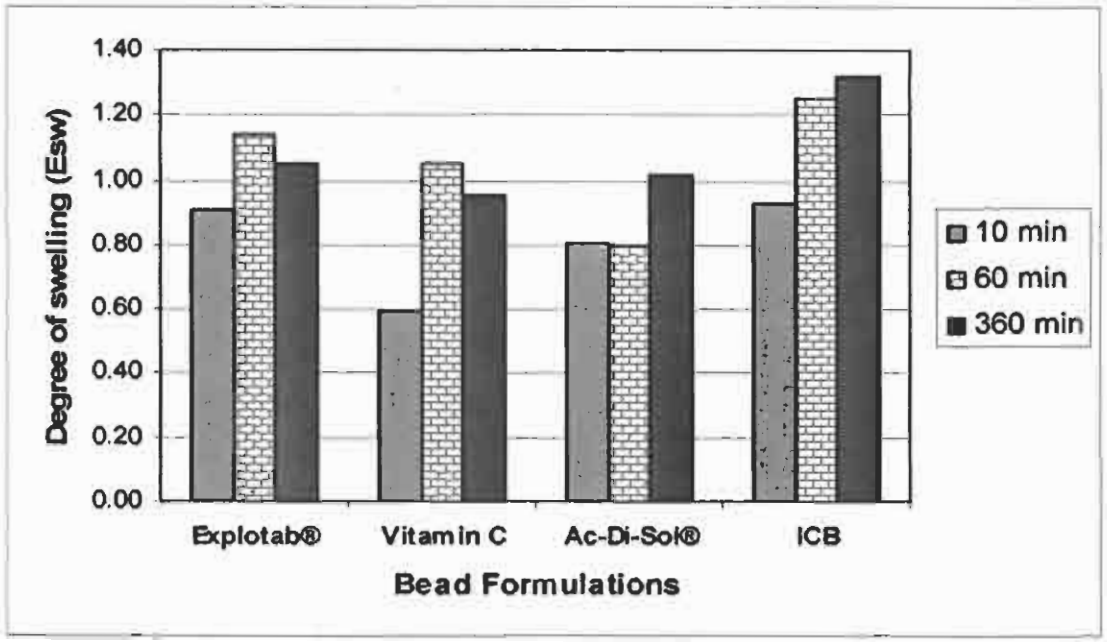


Figure 3.11 : The degree of swelling (Esw) of indomethacin loaded chitosan beads at 10, 60 and 360 minute intervals in a PBS pH 7.4 solution where the variant was the single pharmaceutical excipient.

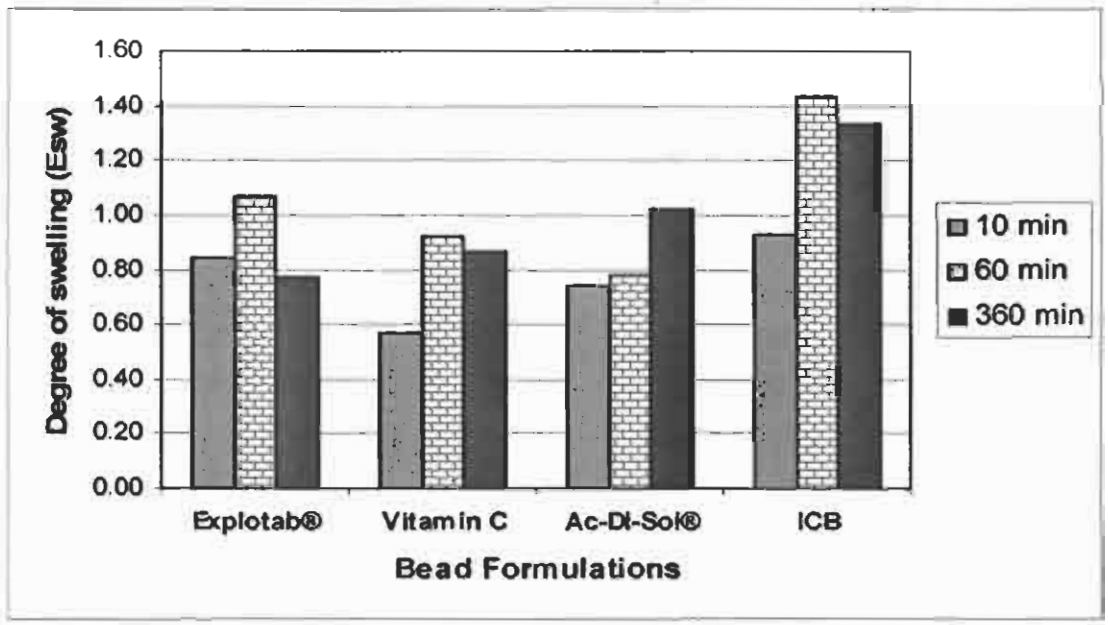


Figure 3.12 : The degree of swelling (Esw) of indomethacin loaded chitosan beads at 10, 60 and 360 minute intervals in a PBS pH 5.6 solution where the variant was the single pharmaceutical excipient.

The pattern of the degree of swelling in both PBS solutions was almost identical for all three excipients. Ac-Di-Sol[®]'s beads presented with the best structural integrity because they still managed to undergo swelling

after one hour and did not start to disintegrate. The swelling capability of the beads did decrease when the excipients were included.

3.5.4 Conclusion

The inclusion of the excipients did not make an significant difference in the drug loading and swelling capability of the beads. In fact the swelling and drug loading capabilities of the beads decreased with the inclusion of the excipients. The morphology of the beads changed on a minor scale because of the unique shape and size particles of the excipient being included. Ac-Di-Sol® presented as the best single pharmaceutical excipient to be used in ICB's because it demonstrated the best drug loading, swelling and morphology compared to the beads containing Explotab® and vitamin C. In chapter 4 the effect of the excipients on the indomethacin release from the chitosan beads will be tested.

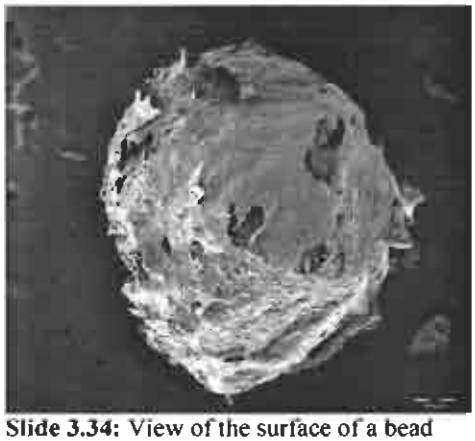
3.6 Effect of multiple pharmaceutical excipients (MPE) on ICB's

This section was approached in exactly the same way as the previous one except that the excipients were combined. The same concentrations were used. The ionic gelation method that was described in section 2.2.2 was used to prepare the beads and the excipient combinations were added to the chitosan-indomethacin solution. The three systems used were as follows:

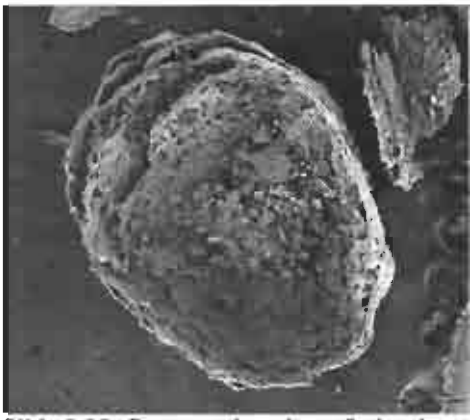
- 0.25% (w/v) Explotab® and 0.25% (w/v) Vitamin C
- 0.25% (w/v) Explotab® and 0.5% (w/v) Ac-Di-Sol®
- 0.25% (w/v) Vitamin C and 0.5% (w/v) Ac-Di-Sol®

3.6.1 Morphology

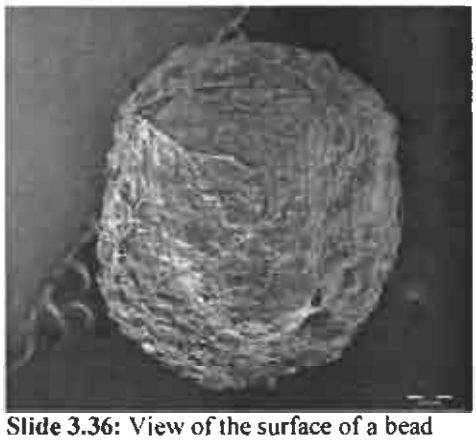
The morphology of the beads was studied using a scanning electronic microscope (SEM) as described in section 2.3.1 A view of the surface of the beads as well as a cross section view of the beads was used to study the beads (see slides 3.34 – 3.39).



Slide 3.34: View of the surface of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **0.25% (w/v) Explotab® and 0.25% (w/v) Vitamin C.**



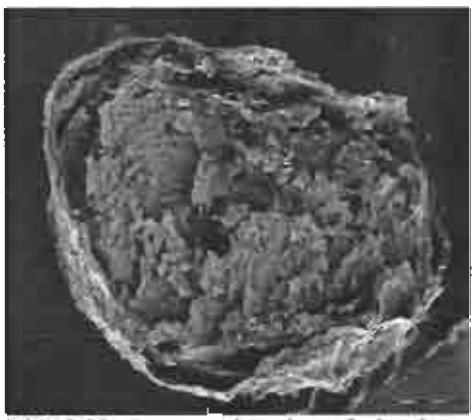
Slide 3.35: Cross-section view of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **0.25 % (w/v) Explotab® and 0.25% (w/v) Vitamin C.**



Slide 3.36: View of the surface of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **0.25% (w/v) Explotab® and 0.5% (w/v) Ac-Di-Sol®.**

Slide 3.37: Cross-section view of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **0.25 % (w/v) Explotab® and 0.5% (w/v) Ac-Di-Sol®.**

Slide 3.38: View of the surface of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **0.25% (w/v) Vitamin C and 0.5% (w/v) Ac-Di-Sol®.**



Slide 3.39: Cross-section view of a bead containing 3% (w/v) chitosan, 4%(w/v) indomethacin and **0.25 % (w/v) Vitamin C and 0.5% (w/v) Ac-Di-Sol®.**

The Ac-Di-Sol® combinations presented with the best morphology. Thick, intact membranes, see slide 3.36 and slide 3.38, ensured the integrity of the bead and the porous chitosan matrix ensured good swelling and

drug loading capabilities. It seemed that the vitamin C combinations presented with the best porosity of the chitosan matrix although the surface of the Explotab®/vitamin C bead severely compromised the structural integrity of the bead. Vitamin C has big uneven particles that might cause the surface membrane failing to cover the bead effectively.

3.6.2 Drug loading

Drug loading was studied as described in section 2.3.2. These results are presented in figure 3.13.

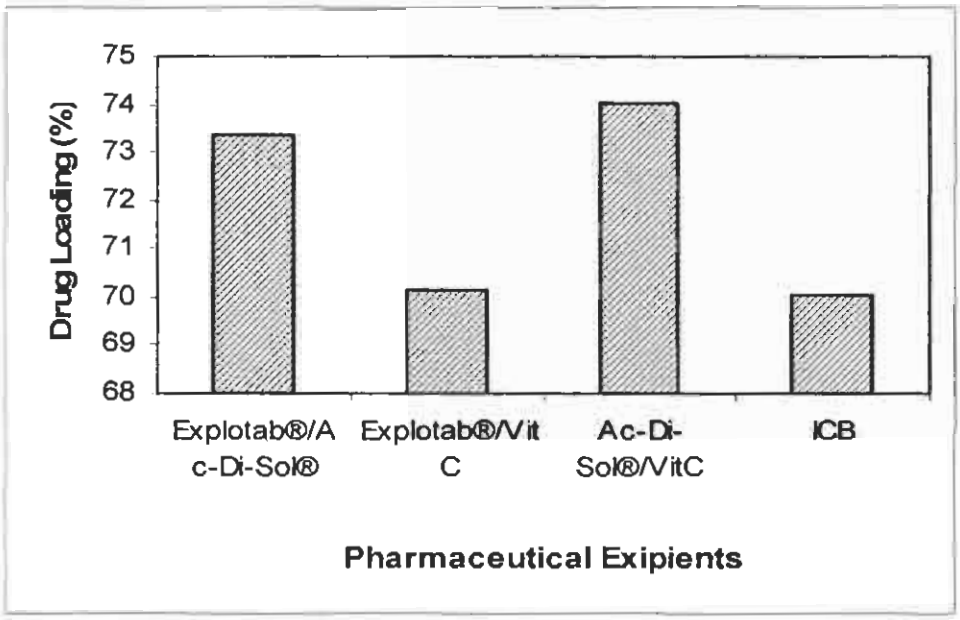


Figure 3.13: Drug loading of 3% (w/v) chitosan, 4% (w/v) indomethacin beads cross-linked with 5% (w/v) TPP where the variant was the single pharmaceutical excipients.

The Ac-Di-Sol® combinations presented with the best drug loading percentages. These results were already predicted when the morphology of the beads was studied. The multiple pharmaceutical excipients however presented with better drug loading than the single pharmaceutical excipients as well as the ICB's that did not contain excipients. This could be explained that when the various excipients were combined their difference in particle structure caused an increase in the porosity of the chitosan matrix that allowed for more indomethacin particles to be encapsulated into the bead.

3.6.3 Swelling

Swelling tests were performed as described in section 2.3.3 and the results are presented in Figures 3.14 and 3.15.

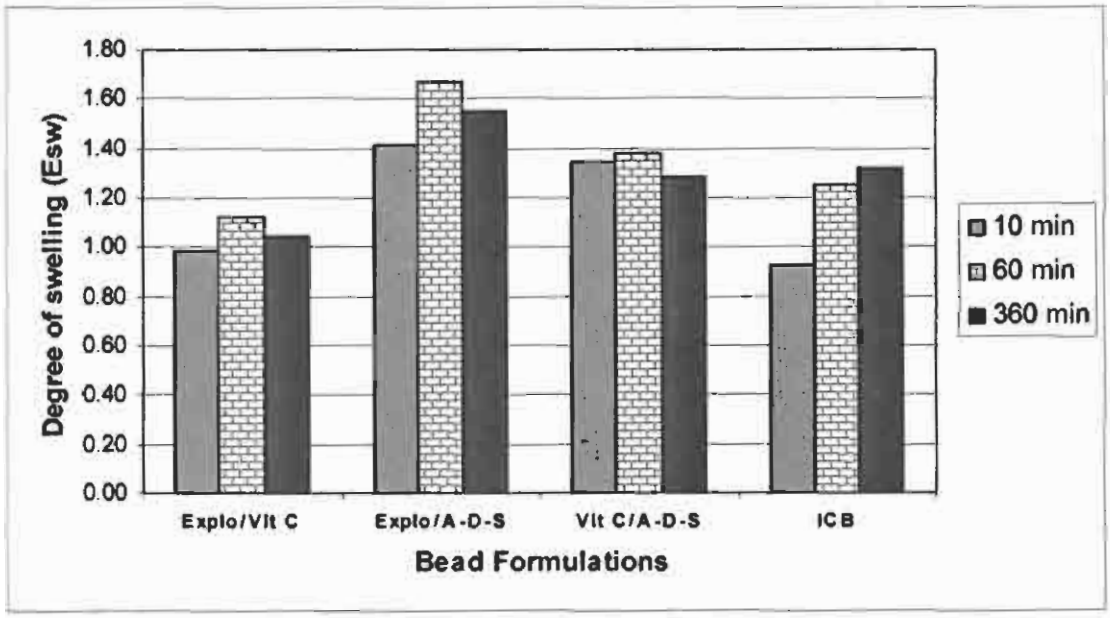


Figure 3.14 : The degree of swelling (Esw) of indomethacin loaded chitosan beads at 10, 60 and 360 minute intervals in a PBS pH 7.4 solution where the variant was the multiple pharmaceutical excipients.

The degree of swelling in both the PBS pH 7.4 and PBS pH 5.6 solutions are almost identical. The Ac-Di-Sol® combinations presented with the best swelling capabilities, with the Explotab®/Ac-Di-Sol® combination having a slight advantage over the vitamin C/Ac-Di-Sol® combination. The swelling capability increased dramatically of the beads containing combination excipients compared to the single excipient beads (see Figures 3.11 and Figures 3.12) as well as the ICB's without excipients.

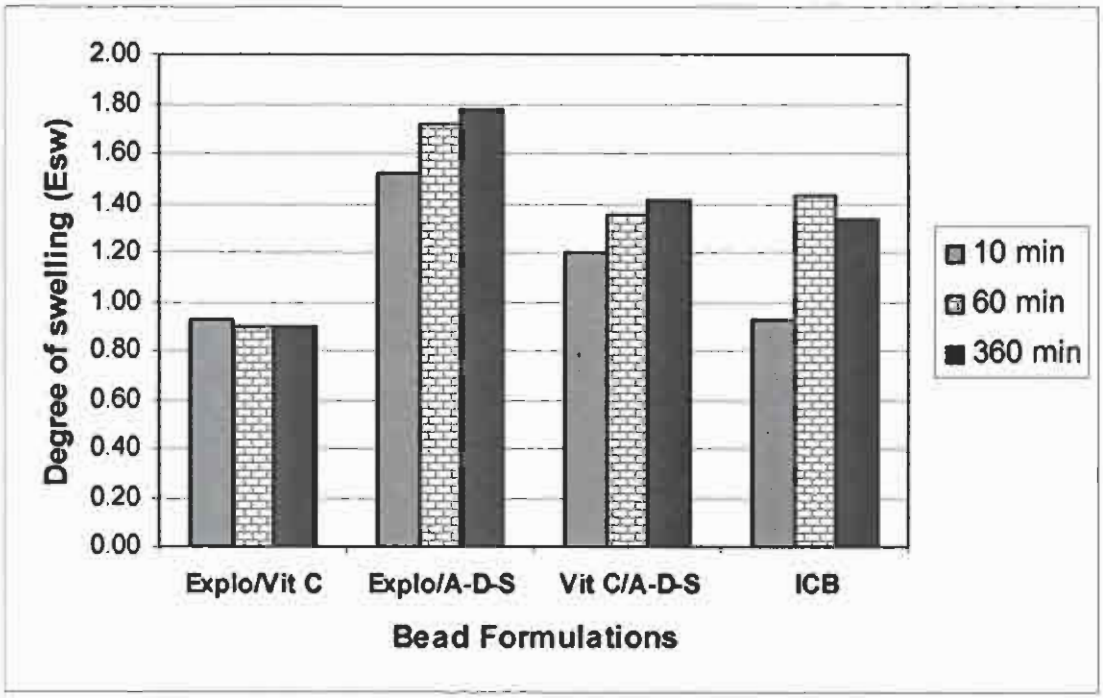


Figure 3.15 : The degree of swelling (Esw) of indomethacin loaded chitosan beads at 10, 60 and 360 minute intervals in a PBS pH 5.6 solution where the variant was the multiple pharmaceutical excipients.

3.6.4 Conclusion

The inclusion of multiple pharmaceutical excipients (MPE) markedly changed the properties of the ICB's. Both the capacity of drug loading and the degree of swelling of the beads increased following the incorporation of the different combinations of excipients used compared to the standard beads and those containing the single excipients (SPE's). This could be the result of the changes brought about in the structure of the beads. Since the drug loading capacity and swelling behavior of the beads were dependent on the porous structure of the beads, the presence of these excipient combinations enhanced the porosity of the beads, allowing better drug entrance and solvent penetration.

The beads containing Ac-Di-Sol®, in combination with either Explotab® or ascorbic acid, gave better drug loading and swelling, whilst these beads also showed a more intact bead membrane than the formulations containing the other excipient, either alone or in combination. SEM photos clearly indicate that in the formulations containing ascorbic acid, the bead surface was not fully intact. This resulted in a decrease in structural integrity, which in turn led to weak beads that disintegrate easily.

3.7 Summary

This chapter dealt with the preparation if indomethacin-chitosan beads and the effect of process variables (pH of the TPP solution, concentration of the drug and concentration of the TPP solution) and formulation variables (single and multiples excipients) on the characteristics, especially the morphology, swelling behavior and drug loading, of these beads.

The results indicated that the formulation that encapsulated the drug most effectively consisted of 3% w/v chitosan, 4% w/v indomethacin prepared in a 5% w/v TPP solution at pH 8.7. Furthermore, studies showed that the inclusion of combinations of pharmaceutical excipients enhanced the properties of the beads compared to the inclusion of these excipients alone. The results of the various tests used to characterize the properties of the beads suggested that the swelling behavior of the beads was to a large extent dependant on the porosity of the chitosan matrix, which in turn depended on the process and formulation variables, especially the pharmaceutical excipient added to the formulation.

The following chapter deals with the drug release profiles of indomethacin from the various formulations.

Chapter 4

Indomethacin release from ICB's

4.1 Introduction

Many pharmaceutical systems are essentially consists of a polymeric carrier hosting the active agent (drug) inside its three-dimensional network. Especially in the case of oral administration, they are often prepared as particulate systems since these forms present remarkable advantages over the single unit devices. The easier dispersion inside the stomach reflects into an appreciable reduction of the local drug concentration which is usually responsible for gastric irritation. Moreover, they are very versatile and this is another reason for their wide use (Tapia *et al.*, 1993:211).

Depending on the particulate therapeutic target, polymeric carriers can host highly or sparingly soluble drugs and, furthermore, various polymers can be used as carriers (Gander *et al.*, 1990:63-71; Sriamornsak *et al.*, 1998:207). More recently, they have shown to be profitable employed also as pulsed drug release-systems by employing pH and / or temperature sensitive polymers (Cho *et al.*, 1999:803; Kikuchi *et al.*, 1997:21-29). Moreover drug loading into cross-linked polymers can represent a profitable tool to increase the drug dissolution rate in aqueous media and, thus, the bioavailability of slightly water soluble drugs (Carli *et al.*, 1986:115-124). Indeed, the drug can be molecularly dispersed inside the polymeric network by means of solvent swelling or co grinding techniques (Grassi *et al.*, 1998:260), so that an amorphous drug state is eventually attained. Such a drug state is metastable, since the dry polymeric network hinders the recombination of the drug molecules in the more thermodynamically stable crystalline state. The hindering action is mainly due to both the polymer–drug interactions (indeed, drug crystals can form on condition that the dry network meshes be sufficiently wide).

The positive key factor of these release systems is that the amorphous drug solubility in hydrophilic fluids (water or physiological medium) is usually much higher than that of the crystalline form, so that the drug dissolution rate is considerably increased. Even if the amorphous drug tends to return to the more stable crystalline state (recrystallisation), nevertheless, the average solubility and bioavailability of the drug are neatly increased, and this explains the use of the term 'activated drug' (Grassi *et al.*, 2000:97-100). Figure 4.1 demonstrates the release of a drug, hosted in a polymeric network, when it comes into contact with the release environment.

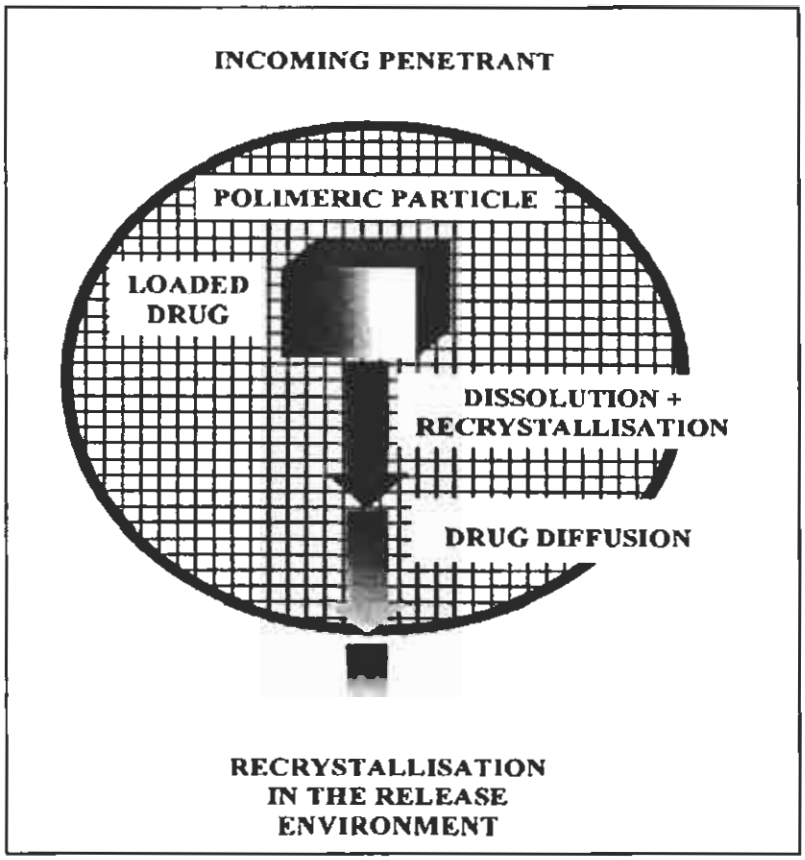


Figure 4.1: Phenomenological situation to be modeled. As soon as the penetrant molecules swell the polymeric network, the drug dissolution, coupled with a possible recrystallisation, can start. Then, the dissolved drug molecules diffuse through the polymeric meshes to reach the release environment where a new recrystallisation can take place (Grassi *et al.*, 2000:97).

In this chapter the effect of single and multiple pharmaceutical excipients on the dissolution rate of indomethacin beads will be studied. The formulas used were tested in term of swelling, drug loading and morphology as described in chapter 2 and 3.

4.2 Colon-specific drug delivery

The necessity and advantages of colon-specific drug delivery systems have been well recognized and documented. In addition to providing more effective therapy of colon related diseases such as irritable bowel syndrome, inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis, colon specific delivery has the potential to address important unmet therapeutic needs including oral delivery of macromolecular drugs. It has been reported that at least 1 million Americans are believed to have IBD with 15 000 – 30 000 new cases diagnosed annually (DiPirio & Bowden, 1997:733-749). Therefore, it appears that

targeted drug delivery with an appropriate release pattern could be crucial in providing effective therapy for this chronic disease.

The colon is also viewed as the preferred absorption site for oral administration of protein and peptide drugs, because of the relatively low proteolytic enzyme activities in the colon. It has been demonstrated that insulin, calcitonin and vasopressin can be absorbed in that region (Saffran *et al.*, 1986:1081; Antonin *et al.*, 1992:627).

However, the poor intrinsic permeability across colon luminal epithelium of peptide and protein drugs resulted in very low bioavailability following colon-specific delivery (less than 1%). To facilitate the transport of peptide drugs across the intestinal epithelium, the approach of applying absorption enhancers has been proposed (Fix, 1996:149). Studies indicated that absorption enhancers performed more effectively in the colon than in the upper gastrointestinal (GI) tract (Mrsny, 1992:15-34; Leone-Bay *et al.*, 1998:1163).

Because of the distal location of colon in the GI tract, a colon-specific drug delivery system should prevent drug release in the stomach and small intestine, and affect an abrupt onset of drug release upon entry into the colon. This necessitates a triggering element in the system that can respond to physiological changes in the colon. Overall, the physiological changes along the GI tract can be generally characterized as a continuum, with decreases in enzymatic activity, motility, and fluid content and an increase in pH. These gradual changes in physiological parameters are not suitable for triggering elements to effect a sudden and dramatic change in the performance of a delivery system in order to obtain colon-specific delivery. However, the presence of specific bacterial populations in the colon and an apparent transient, small reversal in the otherwise increasing pH gradient are the exceptions that have been extensively explored as triggering components for initiating colon-specific drug release (Yang *et al.*, 2001:1-3).

In general, four primary approaches have been proposed for targeted colon delivery, namely, prodrugs, pH- and time-dependent systems, and microflora-activated systems (Yang *et al.*, 2001:1-3).

4.3 Indomethacin release from chitosan beads

The standard formula containing: chitosan 3% (w/v), indomethacin 4% (w/v), TPP 5% (w/v), was used as the control group as to study the effect of excipients on the release of indomethacin chitosan beads. The release of indomethacin from chitosan beads will be compared with the release of the single and multiple

pharmaceutical excipient indomethacin beads. The indomethacin release was only studied in PBS pH 7.4 solution because of indomethacin's incapability to dissolve in an acidic medium. The indomethacin was not released from the chitosan beads in a PBS pH 5.6 solution.

4.3.1 Results

The release of indomethacin from chitosan beads was determined as described in section 2.3.4 and 2.3.5 and the results are presented Figure 4.2 and annexure C1.

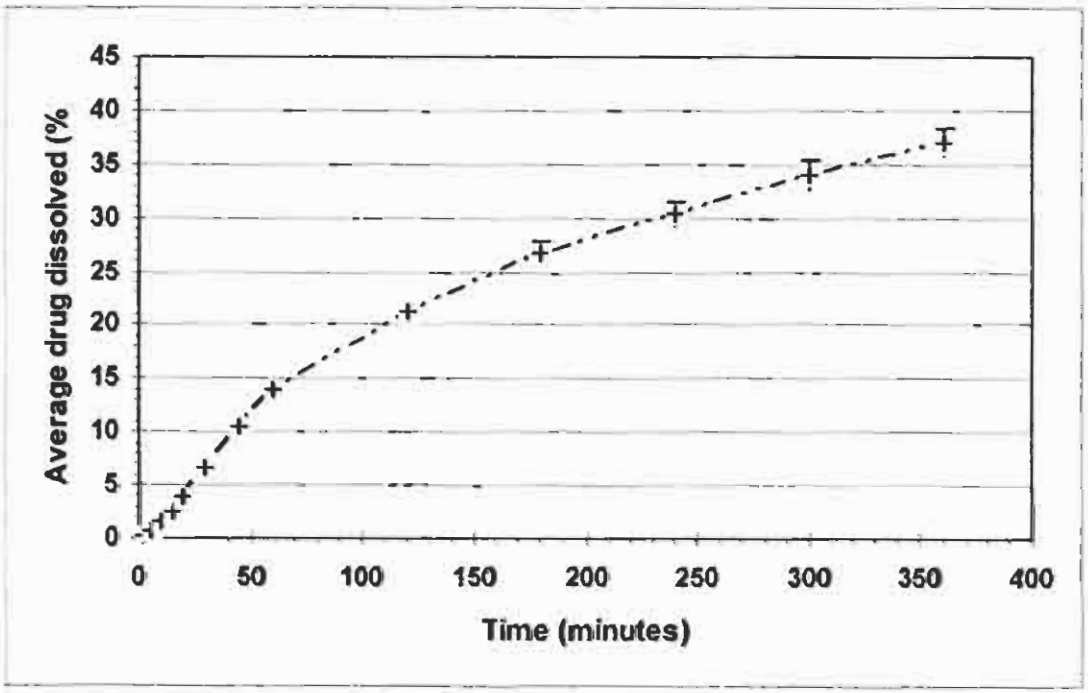


Figure 4.2: Indomethacin release from pure chitosan beads at pH 7.4.

4.3.2 Discussion

The control group gave a 37.03% release of indomethacin from the chitosan beads after six hours. From the ascending curve in Figure 4.2 it can be seen that six hours was not the final release time of indomethacin and that indomethacin will be released after six hours. After approximately 60 minutes the burst effect was completed followed by a gradual drug release. The dissolution profile was basically characterized by two variables: the slope of the upward portion of the curve during the first few minutes of dissolution (DR_i) and the percentage drug dissolved at any time during the dissolution test (C_t). These two variables could be related to each other through the area under the dissolution curve AUC, between any two predetermined dissolution time points. The AUC and DR_i values were calculated during the dissolution studies (see section

2.3.5) to compare the different excipient formulations with each other. The AUC value of the standard ICB formulation was 8665.03 (%drug dissolved.minute^{-1}) and the Dr_i value was calculated at 0.18 (%drug dissolved.minute).

4.4 Indomethacin release from chitosan/SPE beads

The effect of the single pharmaceutical excipients on the rate and extent of drug release from the ICB's will be studied and compared to the ICB's without excipients. In section 3.5 the effects of the SPE on the morphology, drug loading and swelling of the ICB's were studied and discussed. The ICB beads containing the single excipient were prepared as described in section 2.2.1.

4.4.1 Results

The release of indomethacin from SPE chitosan beads were determined as described in section 2.3.4. The results are presented in Table 4.1, Figure 4.3 and annexure C2.

Table 4.1: The AUC, AUC_n, DRi and DRi_n values of ICB's containing SPE.

	AUC	AUC_n	Dri	Dri_n
Indomethacin beads	8665.03	1.00	0.18	1.00
Explotab®	11329.27	1.31	0.21	1.16
Ac-Di-Sol®	13425.07	1.55	0.48	2.68
Vitamin C	13851.64	1.60	0.38	2.13

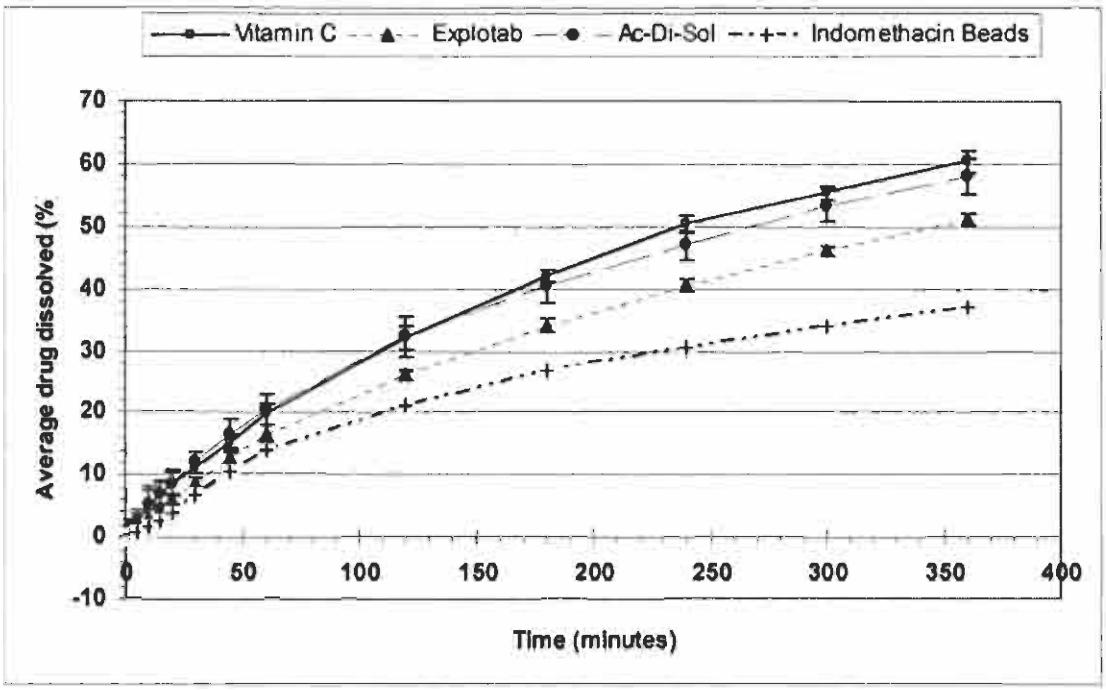


Figure 4.3: Indomethacin release from chitosan/SPE beads at pH 7.4 after 6 hours.

4.4.2 Discussion

As can be seen from Figure 4.3 the inclusion of excipients dramatically increased the release of indomethacin from the chitosan beads. The advantage of the AUC value is that it compares the total dissolution profile of the drug. In addition to allow the researcher to compare the dissolution profiles of different formulations, the dimensionless AUC parameter, AUC_n, relates the AUC's of the various formulations to a common denominater, i.e. the standard formulation. DRi_n, was calculated similarly to the AUC_n to compare the DRi values with one another (see section 2.3.5). Vitamin C presented with the highest percentage drug release after 6 hours. Ac-Di-Sol® presented with the most rapid drug release for the first 15 minutes as can be seen in Table 4.1. The curves of all the excipient-ICB's were even more ascending than the ICB's curve. Thus indometacin will still be released after 6 hours for a more prolonged time than the ICB's. In comparison with the standard ICB's, the beads containing a SPE presented with an increase in initial drug release as well as an increase in the drug release over 6 hours.

4.5 Indomethacin release from chitosan/MPE beads

The effect of MPE in ICB's have been studied in section 3.6 according to the morphology, drug loading and swelling capabilities.

4.5.1 Results

The release of indomethacin from MPE chitosan beads was determined as described in section 2.3.4 and 2.3.5 and the results are presented in Table 4.2, Figure 4.4 and annexure C3.

Table 4.2: The AUC, AUC_n, DRi and DRi_n values of ICB's containing MPE.

	AUC	AUC_n	Dri	Dri_n
Indomethacin beads	8665.03	1.00	0.18	1.00
Explotab®/Ac-Di-Sol®	16373.36	1.89	0.38	2.16
Ac-Di-Sol®/Vitamin C	15080.41	1.74	0.38	2.15
Vitamin C/Explotab®	17109.96	1.97	0.25	1.38

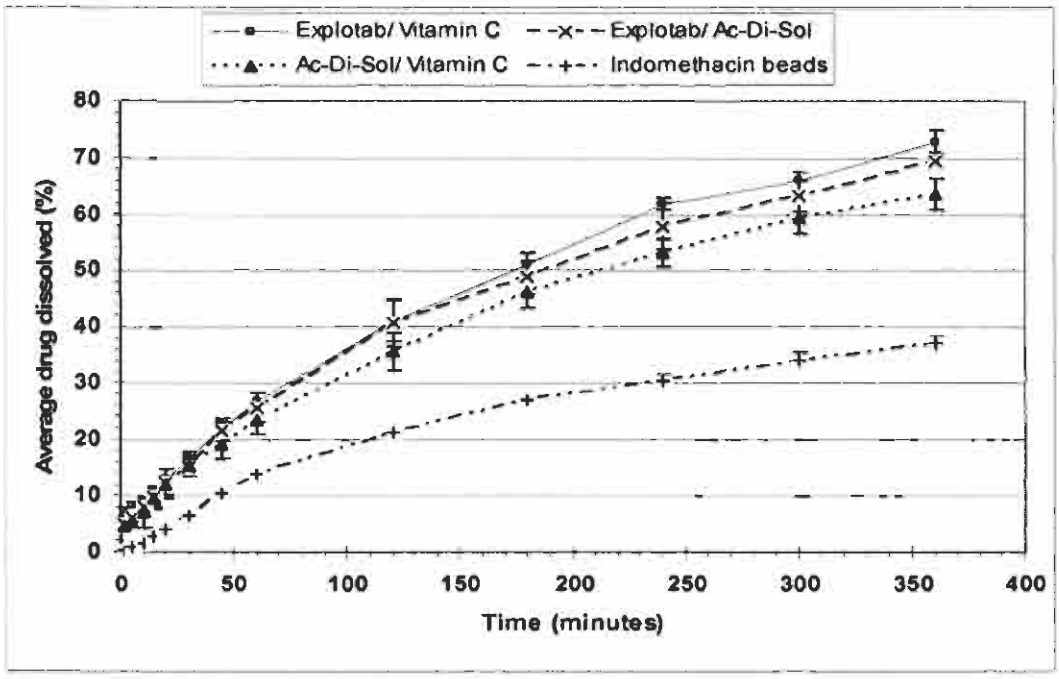


Figure 4.4: Indomethacin release from chitosan/MPE beads at pH 7.4 after a period of 6 hours.

4.5.2 Discussion

The release of the indomethacin increased even more when MPE were added to the formulation. The burst effect continued for the first 2 hours compared to the burst effect of the first 60 minutes of the ICB's without any excipients. The burst effect (indicated as the Dri and DRi_n) can be described as the sudden increase in drug release when dissolution studies are started. The burst effect is an important factor to consider since it can cause undesirable effects like systemic toxicity but in some cases it can also be a favorable occurrence. The burst effect should however always be predictable. The Explotab®/Vitamin C combination presented

with the best drug release. The MPE presented with even better drug release than the SPE. This can be explained by the better swelling capability of the ICB's containing multiple excipients compared to the ICB's containing the single excipient as discussed in section 3.6.4. Thus there is a definite connection between the degree of swelling and the drug release rate of the beads. Although Vitamin C didn't present with the best degree of swelling, the combination with Explotab® proved to be the best MPE combination according to the percentage drug released. All the curves of the MPE were still sharply ascending which indicates that the indomethacin will still be released after 6 hours. The Ac-Di-Sol®/Explotab® combination presented with the best initial drug release as can be seen in table 4.2 and figure 4.6.

Figures 4.5 and 4.6 give an overall summary of the AUC_n and DRi_n values of all the formulations.

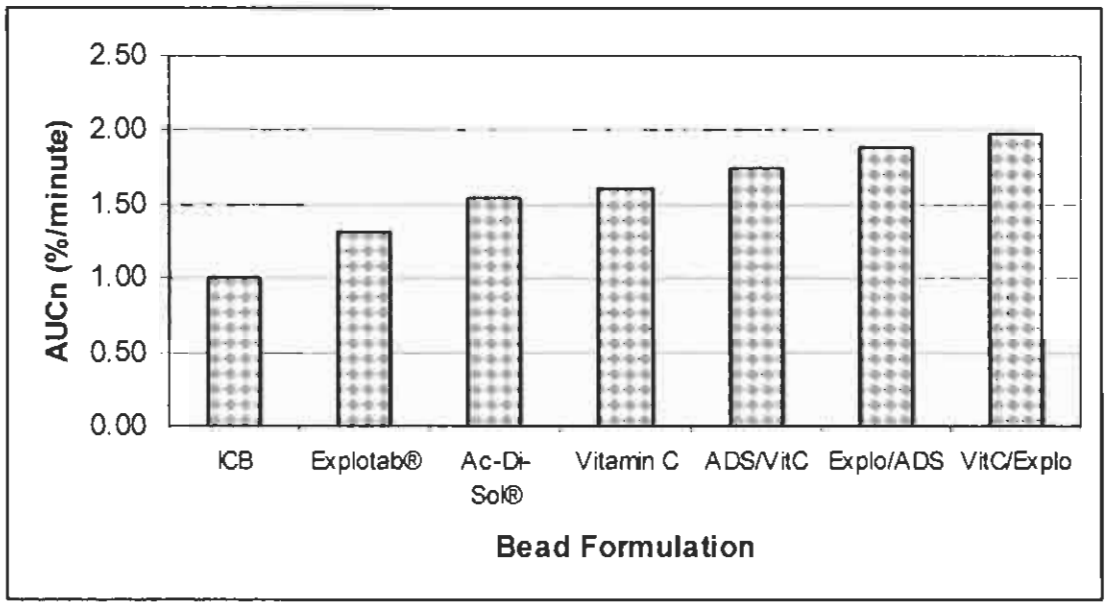


Figure 4.5: AUC values from indomethacin loaded beads at pH 7.4 after a period of 6 hours.

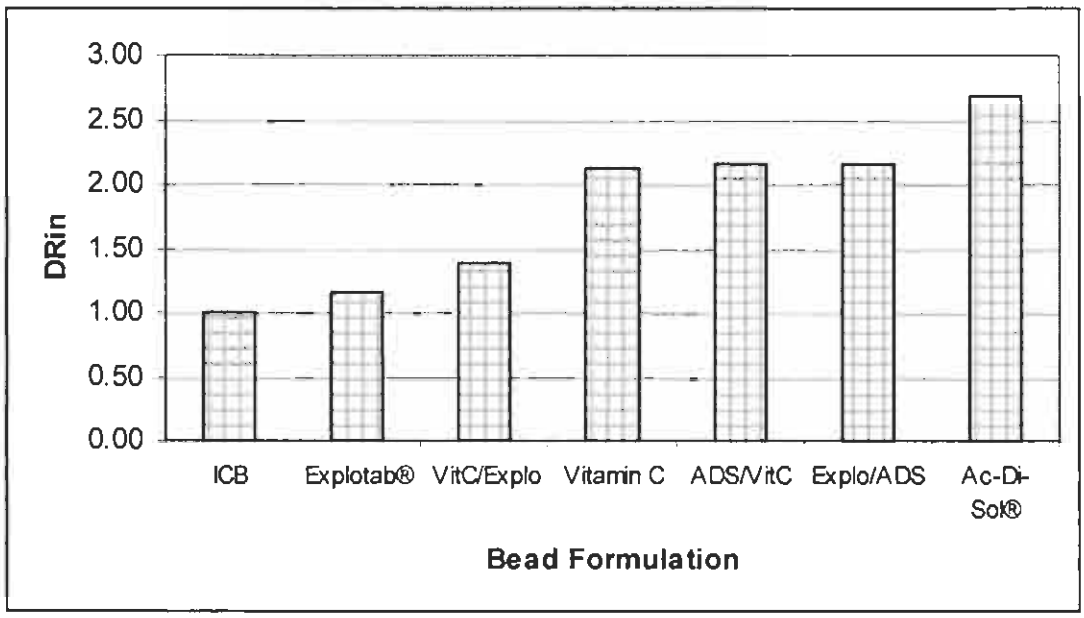


Figure 4.6: Slope values of the first 15 minutes of dissolution from indomethacin loaded beads at pH 7.4 after a period of 6 hours.

The SPE Ac-Di-Sol® presented with the highest slope value with the Ac-Di-Sol® combinations presenting with the overall highest initial drug release. This may be attributed to the presence of indomethacin on the outer surface of the bead and the cylindrical shape of the Ac-Di-Sol® that increased the swelling capability of the ICB's. In the dissolution tests of this study it can be concluded that the Explotab® combinations presented with the best indomethacin release over the period of 6 hours and the Ac-Di-Sol® combinations presented with the best initial drug release for the first 15 minutes, also described as the burst effect.

The results indicated that pharmaceutical excipients definitely increased the drug release from chitosan beads. When the excipients were used in combination with each other the chitosan beads displayed even better indomethacin release.

4.6 Summary

The aim of this study was to determine the effect of pharmaceutical excipients on the release of indomethacin from chitosan beads. But it was also determined that there is a void in pharmaceutical dosage forms for indomethacin to be released in the colon as to bypass the gastrointestinal side effects that conventional dosage forms cause. Colon delivered indomethacin will also be useful in treating colon specific diseases such as ulcerative colitis to name but a few. This study explored chitosan beads as a viable drug delivery vehicle

for indomethacin. The dissolution tests showed that chitosan beads are not only useful as a drug delivery vehicle but also provide controlled drug release of indomethacin for periods over six hours. Pharmaceutical excipients proved to be beneficiary to the formulation of ICB's as it improved the release of indomethacin from the beads. It was also determined that different system variables (pH of the TPP solution, TPP and indomethacin concentration) as well as the formulation excipients definitely have an effect on the morphology, drug loading and swelling capability of the beads. It was concluded that the release rate of indomethacin was dependant on the swelling capability of the beads which in turn were dependant on the porosity of the chitosan matrix. ICB's containing formulation excipients could definitely be an asset to the pharmaceutical industry in the future.

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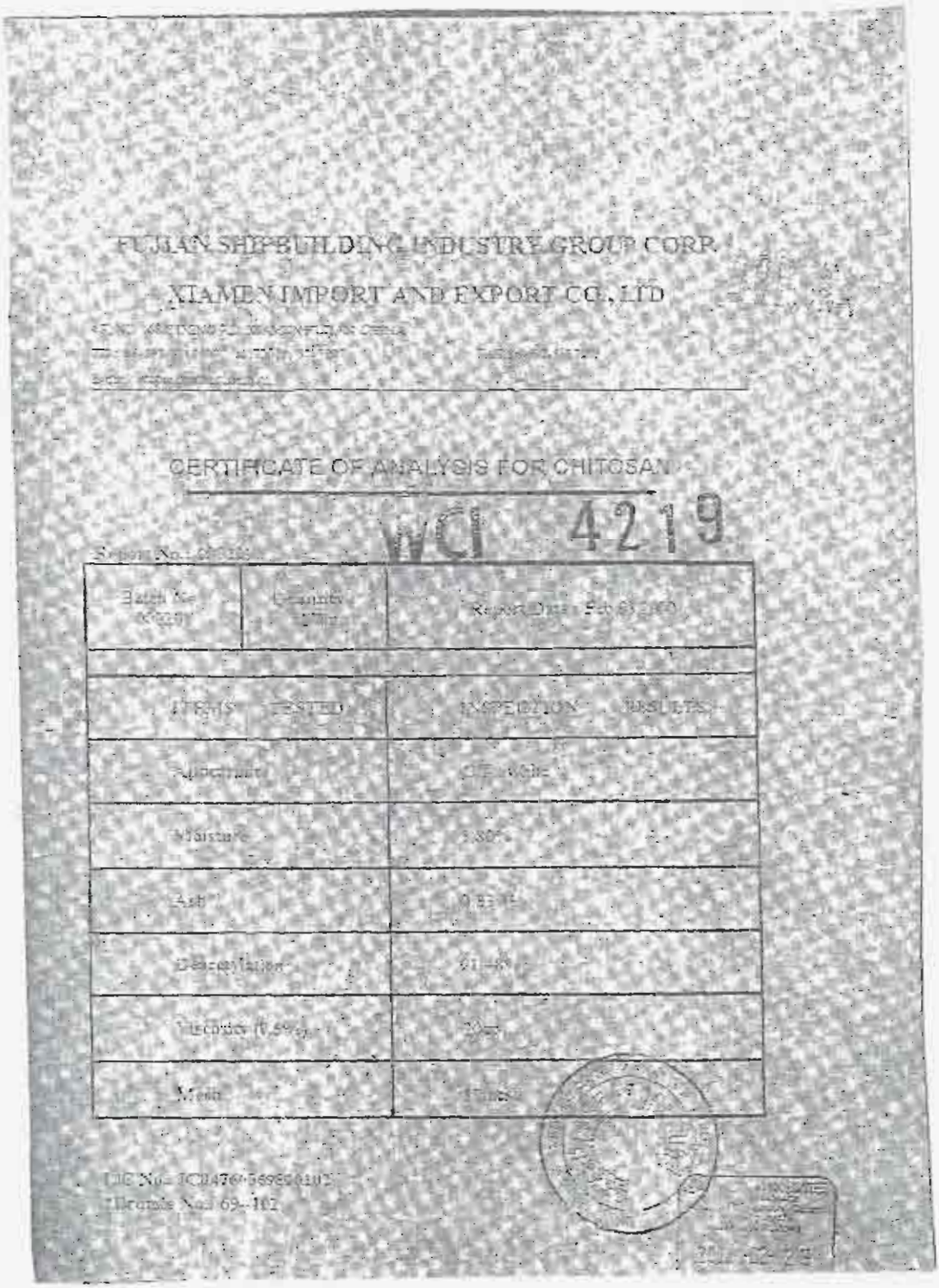
Appendix A: Certificate of Analysis for Chitosan



FUJIAN SHIPBUILDING INDUSTRY GROUP CORP.

XIAMEN IMPORT AND EXPORT CO., LTD

CERTIFICATE OF ANALYSIS FOR CHITOSAN

WCI 4219

ITEMS TESTED	INSPECTION RESULTS
Appearance	Off-white
Moisture	7.80%
Ash	0.85%
Deacetylation	91.4%
Viscosity (1.5%)	20cp
Mesh	80mesh

Appendix B: Certificate of Analysis for Indomethacin

Certificate of Analysis

Supplier: Huzhou Beigang Imp.& Exp.Co.,Ltd.
Manufacturer: Huzhou Konch Pharmaceutical Co.,Ltd.
Tel: 86 572 2033500 Fax: 86 572 2033600 E-Mail: info@beigang.com

PARTICLE SIZE:80%LESS THAN 80UM

PRODUCT NAME	INDOMETHACIN	QUANTITY	500KGS
BATCH NO.	0408010	MANUF. DATE	2004.08.18
INSPECT STANDARD	BP2002	EXPIRY DATE	2008.08.18
PACKING	25KG/DRUM	REPORT NO.	04150

KIRSCH PHARMA SOUTH AFRICA(PTY) LTD.
GEWEL STREET,ISANDO
KPSA2368/09/2004

EXAMINATION

CONTENTS	SPECIFICATION	RESULTS
Characteristics	A White or yellow crystalline powder, Practically insoluble in water, sparingly soluble in alcohol.	Complies
Identification	Infrared absorption: obtained spectrum consistent with indometacin CRS	Comply
	Melting point: 158-162℃	160.0-161.0 ℃
Sulphated Ash	<=0.10 %	0.03 %
Heavy Metals	<=0.002%	<0.001%
Loss on Drying	<= 0.50 %	0.10 %
Related substances	<= 0.50 %	<0.5%
Residual solvents	<= 0.50 %	<0.10%
Assay	98.5-100.5%(on the dried substance)	100.3%

CONCLUSION: IT COMPLIES WITH THE REQUIREMENTS OF *BP2002.*

Inspector: Zheng Qihua 郑其华

Approver: Shen Xueming 沈学明

REPRESENTED BY:
D B Fine Chemicals (Pty) Limited
P.O Box 786
RIVONIA 2128
Johannesburg
10 YEARS 1995 2005

APPENDIX C 1 : Dissolution data of standard ICB's

Table C1: Amount of indomethacin released from pure chitosan beads at pH 7.4 after 6 hours.

	Indomethacin/ Chitosan beads	
Time (min)	% Release	Standard Deviation
0	0.00	0.00
2	0.26	0.07
5	0.78	0.53
10	1.50	0.22
15	2.61	0.27
20	3.82	0.15
30	6.53	0.21
45	10.47	0.05
60	13.87	0.30
120	21.21	0.79
180	26.82	1.11
240	30.33	1.19
300	34.02	1.30
360	37.03	1.26

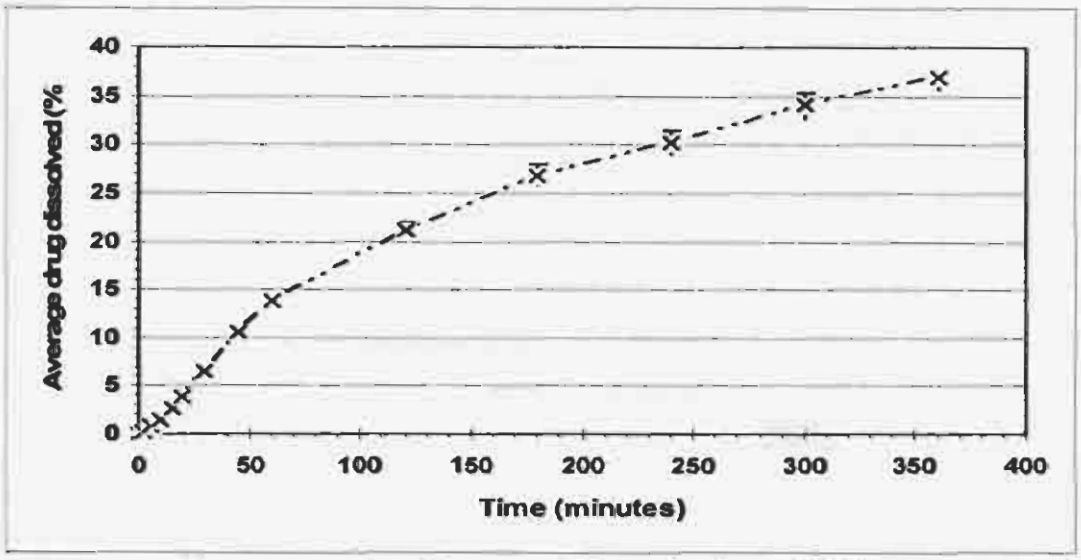


Figure C.1: Indomethacin release from pure chitosan beads at pH 7.4.

APPENDIX C 2 : Dissolution data of ICB's containing SPE's

Table C2: Amount of indomethacin released from chitosan/Explotab®, chitosan/ Vitamin C and chitosan/ Ac-Di-Sol® beads at pH 7.4 after 6 hours.

	Explotab®		Vitamin C		Ac-Di-Sol®	
Time (min)	% Release	Standard Deviation	% Release	Standard Deviation	% Release	Standard Deviation
0	0.00	0.00	0.00	0.00	0.00	0.00
2	1.83	0.95	1.89	0.93	1.21	0.39
5	2.19	1.51	2.93	1.36	1.96	0.10
10	3.17	1.11	4.71	2.71	5.49	2.84
15	4.48	0.86	6.81	2.21	6.97	1.85
20	6.02	0.81	8.62	1.99	8.61	1.71
30	8.59	0.85	11.02	1.00	11.85	1.80
45	12.51	1.11	15.07	1.27	16.43	2.35
60	16.47	1.34	19.65	1.72	20.46	2.44
120	25.99	0.78	31.97	1.91	32.21	3.35
180	33.98	1.14	42.08	1.03	40.41	2.59
240	40.44	0.89	50.46	1.44	47.01	2.39
300	46.01	0.91	55.48	1.13	53.41	2.65
360	51.15	0.84	60.44	1.70	58.10	2.75

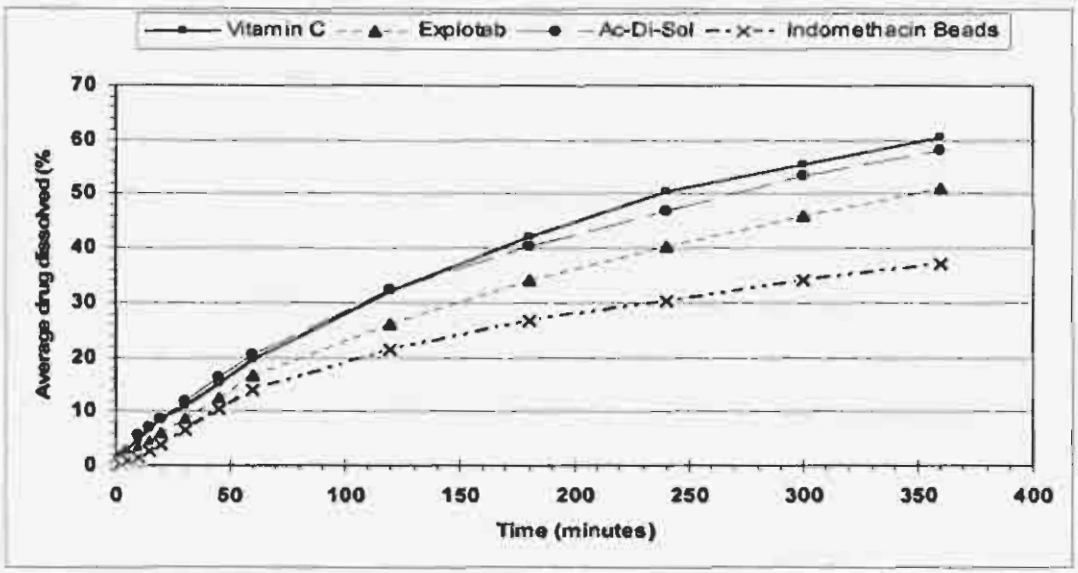


Figure C.2: Indomethacin release from chitosan/SPE beads at pH 7.4 after 6 hours.

APPENDIX C 3 : Dissolution data of ICB's containing MPE's

Table C3: Amount of indomethacin released from chitosan/Explotab®/Vitamin C , chitosan/Vitamin C/Ac-Di-Sol® and chitosan/Ac-Di-Sol®/Explotab® beads at pH 7.4 after 6 hours.

	Explotab®/Vitamin C		Vitamin C/Ac-Di-Sol®		Ac-Di-Sol®/Explotab®	
Time (min)	% Release	Standard Deviation	% Release	Standard Deviation	% Release	Standard Deviation
0	0.00	0.00	0.00	0.00	0.00	0.00
2	7.18	0.74	4.45	0.64	4.86	0.84
5	8.37	0.41	5.18	1.20	5.97	0.47
10	9.08	0.54	7.02	0.23	7.84	1.31
15	10.59	0.60	9.38	0.29	9.84	1.71
20	12.63	0.82	11.86	0.73	12.15	2.57
30	16.59	1.22	15.21	1.39	15.02	1.45
45	21.67	1.36	18.80	0.54	21.41	2.02
60	26.43	1.75	23.13	0.54	25.49	2.51
120	40.91	3.76	35.39	0.53	40.57	4.25
180	50.94	2.19	46.04	0.38	48.75	2.92
240	61.72	1.10	53.13	1.05	57.80	4.18
300	65.84	1.73	59.27	0.49	63.09	2.72
360	72.58	2.36	63.63	0.69	69.35	1.36

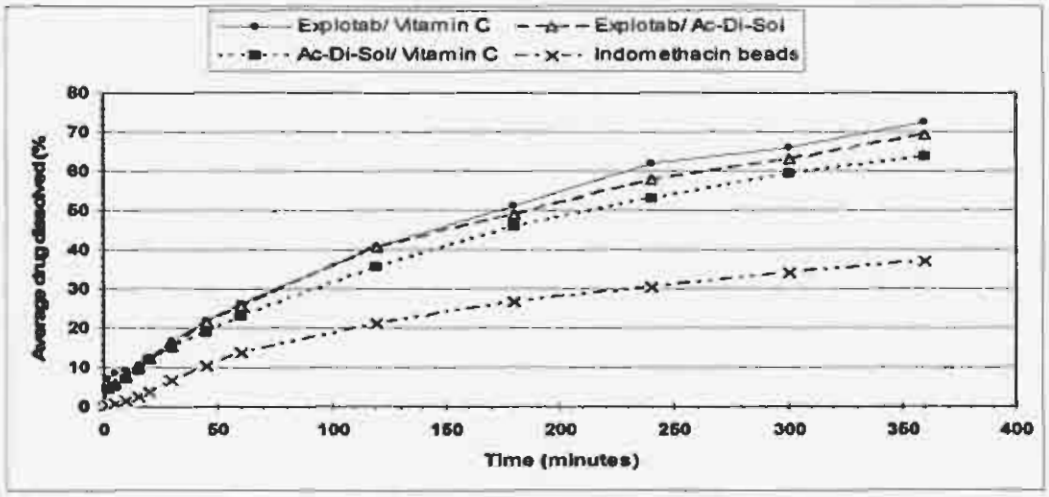


Figure C.3: Indomethacin release from chitosan/MPE beads at pH 7.4 after a period of 6 hours.