# CHITOSAN BEADS AS A DELIVERY VEHICLE FOR THE ANTITUBERCULOSIS DRUG PYRAZINAMIDE

**J.B. Havenga**
(B. Pharm)

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Supervisor: Mr. J. H. Steenekamp

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# Introduction and Aim of Study

The need for a healthy lifestyle and health awareness has increased dramatically over the past few years. With improving technology and some ongoing innovations, a better lifestyle becomes even more desirable despite the increasing cost thereof. We live in a competitive world where the importance of effectiveness is undeniable. It is essential to achieve the desired goals with the least amount of effort and as cost effective as possible. In pharmaceutical sciences the desired therapeutic effect must be achieved with the least amount of drug to make the product as cost effective as possible, especially in underdeveloped parts of the world with limited funding to their disposal.

In recent years there has been increasing interest in controlled release dosage forms. The main interest is to improve patient compliance. These dosage forms have increasingly gained popularity over other dosage forms in treating disease mainly because of the numerous benefits over conventional dosage forms. These formulations not only improve compliance but also significantly reduce the cost of administration, reduce the frequency of drug administration and may even improve or completely avoid certain side-effects commonly encountered with conventional dosage forms.

Beads have become an interesting area of research in as far as controlled release studies are concerned. Beads show various advantages over single unit dosage forms and they are very flexible in dosage form development. Because beads disperse freely in the gastrointestinal tract, they maximize drug absorption, reduce peak plasma fluctuation and minimize potential side-effects without lowering drug bio-availability.

Tuberculosis is a very complex socio-economic disease that is characterized by an alarming death rate and a significant impact on economic development. Tuberculosis treatment consists of combinations of drugs, since resistance is a very common problem. Tuberculosis treatment, with established fixed dose combinations, is getting popular, considering a better patient compliance and a decreased risk of monotherapy and

therefore development of resistance. By additionally extending the dosage interval, patient compliance will also improve. Therefore it would be of great value to consider the possibility of controlled release of TB medicine to determine whether the dosage interval can be prolonged.

Chitosan is a derivate of chitin that is naturally found in abundant quantities. Chitosan contains absorption advancement properties as well as properties that change the release of the drug from the chitosan matrix.

The aim of the study was to prepare and characterize pyrazinamide loaded chitosan beads and to incorporate several pharmaceutical excipients into the beads to determine the influence it has on the release of the drug. The beads were characterized with respect to morphology, solubility, friability, drug loading capacity and swelling behaviour, as well as drug release (dissolution properties).

By incorporating pyrazinamide into the beads, with the motivation that *mycobacterium species* is so notorious to monotherapy, the study aims at achieving pronounced drug levels in both the gastric and intestinal environment and it involves the following as its main objectives:

- To conduct a literature study into:
    - The development, effects, and mechanism of drug release from controlled release formulations.
    - Effectiveness and uses of polymers as drug carriers.
    - The synthesis, uses and safety of chitosan.
    - Type and characteristics of modern controlled release polymers.

- The different preparation methods of beads and their advantages as controlled release drug delivery systems.

- To prepare and characterize chitosan beads with a reliable and reproducible method and to investigate the effect of pharmaceutical excipients on the properties of the beads.

- To determine the effectiveness of the beads as a drug carrier and delivery system for pyrazinamide.

Conventional dosage forms are compared to controlled release dosage forms and the classification and different mechanisms of controlled release dosage forms are discussed in chapter 1. Chapter 2 describes different methods that have been used to prepare and characterize beads. In chapter 3 the preparation and characterization of pyrazinamide loaded chitosan beads, containing several pharmaceutical excipients, are discussed while chapter 4 describes the release of pyrazinamide from the beads.

# Abstract

Controlled release systems aim at achieving a predictable and reproducible drug release profile over a desired time period. These controlled release formulations offer many advantages over conventional dosage forms. These advantages include: reduced dosing intervals, constant drug levels in the blood, increased patient compliance and decreased adverse effects. Complex controlled release formulations such as those with sustained release properties, often require additional steps during the production phase. The cost and economic impact associated with these complex controlled release dosage formulations often outweigh the short term benefits. Thus the development of an economic method to produce controlled release particles is of great importance especially in third world countries.

In controlled release formulations the drug is often equally dispersed throughout a polymer matrix. In the presence of a thermodynamically compatible solvent, swelling occurs and the polymer releases its content to the surrounding medium. The rate of drug release can be controlled by interfering with the amount of swelling and rate of diffusion by manipulating the viscosity of the polymer matrix.

Chitosan is an ideal candidate for controlled drug delivery through matrix release systems. It is a biodegradable polymer with absorption-enhancing properties. Cross-linking chitosan with different cross-linking agents allow the preparation of beads. Beads are frequently used in controlled release dosage forms as they are very flexible in dosage form development and show various advantages over single unit dosage forms. Because beads disperse freely in the gastrointestinal tract they maximize drug absorption, reduce fluctuation in peak plasma, and minimize potential side-effects without lowering drug bio-availability.

Chitosan beads and excipient containing chitosan beads were prepared and investigated as possible controlled release formulations. Pyrazinamide was chosen as the model drug. Chitosan beads and excipient containing chitosan beads were prepared by ionotropic gelation in tripolyphosphate. In this study chitosan/pyrazinamide beads containing pharmaceutical excipients (Ascorbic acid, Explotab® and Ac-Di-Sol®) were produced. The excipients were added individually and in combinations to the chitosan/pyrazinamide dispersion and the beads were characterized on the basis of their morphology, solubility, friability, drug loading capacity and swelling behaviour, as well as drug release (dissolution properties).

The drug loading of the pyrazinamide loaded chitosan beads, was 52.26 ± 0.57%. It was noted that the inclusion of excipients in the beads resulted in an increase in drug loading with the combination of Ascorbic acid and Ac-Di-Sol® giving the highest drug loading of 67.09 ± 0.22%.

It was expected that the addition of the pharmaceutical excipients would lead to a sustained release of pyrazinamide. Dissolutions studies, however, revealed a burst release in both phosphate buffer solutions (PBS) pH 5.60 and 7.40 over the first 15 minutes and the curve reached a plateau after 30 minutes. Thus, apparently the inclusion of the pharmaceutical excipients did not contribute to a sustained release of pyrazinamide over the tested period of six hours. In future studies the dissolution time can possibly be extended to a period of 24 hours. It might be possible for the remaining drug (approximately 40%) in the beads to be released over the extended period. Other polymers can also be investigated to control the release of pyrazinamide. Further studies are, however, necessary to investigate this possibility in the future.

**Key words:** Beads; Chitosan; Ionotropic gelation; Controlled release; Pyrazinamide; Ascorbic acid; Explotab®; Ac-Di-Sol®.

# Uittreksel

Gekontroleerde vrystellingsdoseervorme word hoofsaaklik gebruik om voorspelbare en herhaalbare geneesmiddelvrystelling oor tyd te verseker. Hierdie gekontroleerde vrystellingsdoseervorme bied verskeie voordele oor konvensionele doseervorme. Hierdie voordele sluit die volgende in: verminderde doseringsintervalle, konstante plasmavlakke van die geneesmiddel, verbeterde pasiëntmeewerkendheid en vermindering van newe-effekte. Komplekse gekontroleerde vrystellingsdoseervorme soos dié met verlengde vrystellingseienskappe benodig dikwels addisionele stappe tydens die vervaardigings fase. Die koste en ekonomiese impak geassosieer met hierdie komplekse gekontroleerde vrystellingsdoseervorme oortref dikwels die korttermyn voordele. Dus is die ontwikkeling van 'n ekonomiese metode vir die produksie van gekontroleerde vrystellingsdoseervorme van groot belang, veral in derde wêreldlande.

In gekontroleerde vrystellingsdoseervorme is die geneesmiddel dikwels eweredig versprei in 'n polimeermatriks. Hierdie tipe gekontroleerde vrystellingsdoseervorme swel in die teenwoordigheid van 'n gepaste termodinamiese oplosmiddel. Tydens swelling stel die polimeer/jel matriks die geneesmiddel vry. Die vrystellingstempo van die geneesmiddel kan deur die mate van swelling en/of die tempo van diffusie beheer word deur die viskositeit van die polimeermatriks te manipuleer.

Chitosan is 'n goeie kandidaat vir gekontroleerde vrystellingsdoseervorme. Dit het absorpsiebevorderende eienskappe en word ook biologies afgebreek deur die liggaam. Chitosan word maklik met ione gekruisbind wat dit ideaal maak vir die bereiding van krale. Krale word algemeen gebruik in gekontroleerde vrystellingsdoseervorme omdat dit verskeie voordele bo enkeleenheidsdoseervorme het en baie manipuleerbaar is in doseervormontwikkeling. Omdat krale maklik versprei in die gastrointestinale kanaal verhoog dit die geneesmiddel absorpsie, verlaag piek plasmavlakke en verminder potensiële newe-effekte sonder om die geneesmiddel bio-beskikbaarheid te verlaag.

Chitosankrale en hulpstof bevattende chitosankrale is berei en ondersoek as 'n moontlike verlengde vrystellingsdoseervorm. Pirasinamied is gekies as modelgeneesmiddel. Chitosankrale en hulpstof bevattende chitosankrale is berei deur deur ionotropiese jelering in tripolifosfaat. Chitosan/pirasinamiedkrale wat farmaseutiese hulpstowwe (Askorbiensuur, Explotab® and Ac-Di-Sol®) bevat is in hierdie studie berei. Die hulpstowwe is individueel en in kombinasies by die chitosan/pirasinamied suspensie gevoeg. Die gevormde krale is gekarakteriseer op grond van hul morfologie, oplosbaarheid, breekbaarheid, geneesmiddelinhoud, swellingsgedrag sowel as die geneesmiddel vrystelling.

Die geneesmiddelinhoud van die chitosan/pirasinamiedkrale was 52.26 ± 0.57%. Dit is opgemerk dat die byvoeging van die farmaseutiese hulpstowwe bygedra het tot die verhoging van die persentasie geneesmiddelinhoud. Die kombinasie van Askorbiensuur en Ac-Di-Sol® het die hoogste geneesmiddelinhoud gelewer naamlik, 67.09 ± 0.22%.

Daar is verwag dat die byvoeging van farmaseutiese hulpstowwe sal bydra tot verlengde vrystelling van pirasinamied. Dissolusie studies het egter 'n "bars effek" vertoon in beide PBS 5.60 en 7.40 oor die eerste 15 minute en die kurwe het 'n plato bereik na 30 minute. Dus het die insluiting van die farmaseutiese hulpstowwe geen bydra gelewer tot 'n verlengde vrystelling van pirasinamied nie. In toekomstige studies kan die dissolusie tyd moontlik verleng word na 24 uur. Dit is moontlik dat die oorblywende geneesmiddel (ongeveer 40%) in die krale vrygestel kan word oor die verlengde tydperk. Ander polimere kan ook ondersoek word om die vrystelling van pirasinamied te reguleer. Toekomstige studie is egter nodig om hierdie moontlikhede te ondersoek.

**Sleutelwoorde:** Krale; Chitosan; Ionotropiese jelering; Gekontroleerde vrystelling; Pirasinamied; Askorbiensuur; Explotab®; Ac-Di-Sol®.

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# Chapter 1

# Formulation of controlled release dosage forms: focus on polymers

## 1.1 Introduction

Controlled release dosage forms (CRDF) have been developed for over three decades. They have increasingly gained popularity over conventional dosage forms in treating diseases. Now, they are the focus of pharmaceutical dosage form technology (Saks & Gardner, 1997:237). Controlled drug delivery occurs when a polymer, whether natural or synthetic, judiciously combined with a drug or active agent in such a way that the active agent is released from the material in a pre-designed manner. The release of the active agent may be constant over a long period, it may be cyclic over a long period, or it may be triggered by the environment or other external events. In any case, the purpose behind controlling the drug delivery is to achieve more effective therapies while eliminating the potential for both under- and overdosing (Brannon-Peppas, 1997:1).

One of the first practically used controlled release oral dosage forms was the Spansule® capsule, which was introduced in the 1950s. Spansule® capsules were manufactured by coating a drug onto nonpareil particles and further coating with glyceryl stearate and wax. Subsequently, ion exchange resins were proposed for application as sustained release delivery systems of associable drug (Saunders, 1961:36). Since then numerous products based on various mechanisms and manufacturing techniques have been developed for the treatment of various diseases and conditions. Transdermal patches delivering scopolamine and nitroglycerin were developed for motion sickness and angina, respectively. The oral osmotic pump tablet (OROS®) was introduced and commercialized to deliver phenylpropanolamine HCl for weight control (Accutrim®) (Theeuwes, 1975:1987). Lately, a variety of nicotine transdermal patches were marketed

to help people quit smoking. Controlled release even entered the parenteral arena of drug delivery. Examples include a system delivering a synthetic analog of luteinizing hormone releasing hormone (LHRH), leuprolide acetate (Lupron Depot®). This product is administered for prostatic cancer, endometriosis, and central precocious puberty monthly and quarterly. These above mentioned products have been developed in order to enhance clinical efficacy and reduce total disease management cost, thereby providing economic merit to the society (Saks & Gardner, 1997:237).

## 1.2 Conventional versus Controlled Release Dosage Forms (CRDFs)

Controlled release systems provide numerous benefits over conventional dosage forms. Conventional dosage forms, which are still predominant for pharmaceutical products are not able to control either the rate of drug delivery or the target area of drug administration, and as a result provide an immediate or rapid drug release. This necessitates frequent administration in order to maintain therapeutic drug levels. As a result, drug concentration in the blood and tissues fluctuates widely. As seen in Figure 1-1a the initial drug concentration may be high, which can cause toxic and/or side-effects, then quickly fall down below the minimum therapeutic level as time elapses. The duration of therapeutic efficacy is dependent upon the frequency of administration, the half-life of the drug, and the release rate of the dosage form (Kim, 2000:1).

In contrast, controlled release dosage forms are not only able to maintain therapeutic drug levels with narrow fluctuations but they also make it possible to reduce the frequency of drug administration. The serum concentration of a drug released from controlled release dosage forms fluctuates within the therapeutic range for a longer period of time as seen in Figure 1-1b. The serum concentration profile depends on the manufacturing technology, which may generate different release kinetics, resulting in different pharmacological and pharmacokinetic responses (Kim, 2000:1-2).

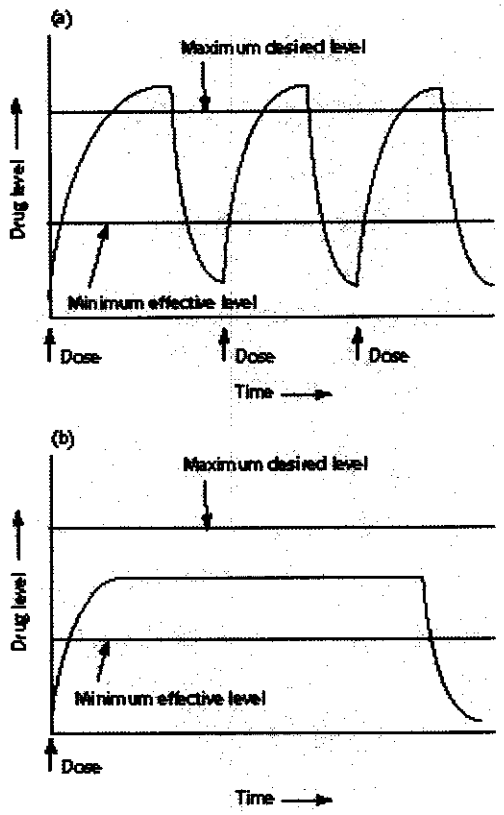


**Figure 1-1:** Drug levels in the blood with (a) traditional drug dosing and (b) controlled delivery dosing (Brannon-Peppas, 1997:1).

Although drug levels can be maintained in the therapeutic range for longer periods of time by giving larger drug doses with conventional dosage forms, this is not usually a suitable approach, especially when such doses may produce toxic levels. An alternative approach is to provide drug at frequent intervals of time, resulting in oscillating drug levels, the so-called peak and valley effect. A second, third, etc., dose of drug will add to whatever drug remains in the body from the preceding dose. This will cause accumulation of drug and perhaps push the level into the toxic region unless adjustments in the dose are made on subsequent doses, clearly an impractical approach for patients. Fortunately, most drugs exhibit first-order accumulation upon repeated dosing at equal intervals so that a plateau in drug level is reached. The level reached, and the time to achieve this level, is dependent on the dose and dosing interval (Notari, 1975:51).

Several potential problems are immediately evident from frequent drug dosing (Notari, 1975:51):

1. Unless the dosing interval is relatively short, depending on the biological half-life of the drug, large peaks and valleys in the drug level will occur. (Oscillations in drug levels may be undesirable in some disease conditions.)

2. Success by this approach is dependent on patient compliance with the dosing regimen. Numerous studies (Steward, 1972:108; Berry & Latiolais, 1969:270) have documented that lack of compliance is an important reason for a failure in drug therapy or inefficient therapy.

3. During the early periods of dosing there may be insufficient drug to generate a favourable biological response, which may be a significant problem in certain disease states.

4. For drugs with a short biological half-life, frequent dosing is needed to maintain relatively constant therapeutic drug levels.

Nevertheless, despite these limitations, drugs given in conventional dosage forms can produce the desired drug levels providing that the proper dose and dosing interval is employed. However, based on the points raised above, drug therapy in conventional dosage forms is often undesirable, impractical and inconvenient. In addition, conventional dosage forms frequently require large amounts of drug to achieve a given therapeutic response and this increases local and systemic toxicity problems. Finally, conventional dosage forms possess a number of other undesirable features, many of which can be overcome or minimized by using controlled action dosage forms, as will be discussed below (Notari, 1975:51; Robinson & Lee 1987:126).

## 1.3 Advantages and Disadvantages of Controlled Release Dosage Forms

There is an attitude shared by many that controlled drug delivery systems are convenient items or at best, they accomplish more efficiently what conventional dosage forms have accomplished with few real clinical benefits. In actuality, controlled drug delivery offers many real and documented advantages over conventional dosage forms including the following (Krowcynski 1987:12; Ritchel 1989:1073):

- Improvement in patient compliance.
- Decrease in total drug use.
- Reduction in local or systemic side-effects.
- Minimization of drug accumulation (with chronic dosage).
- Reduction in potential loss of drug activity (with chronic use).
- Improvement in treatment efficiency.
- Improvement in speed of control of medical conditions.
- Reduction in drug blood level fluctuation.
- Improvement in bio-availability for some drugs.
- Improvement in the ability to provide special effects e.g. morning relief of arthritis.
- Reduction in cost of administration and specialized hospital personnel.

It is also important to mention that controlled release dosage forms possess certain disadvantages that can outweigh the benefits of using these formulations. Such disadvantages include (Ritchel 1989:1073; Kim, 2000:4):

- dose dumping,
- less accurate dose adjustment,
- increased potential for first-pass metabolism,
- dependence on residence time in gastrointestinal tract and
- delayed onset.

Not all drugs are good candidates for incorporating into controlled release dosage forms. The limitations of controlled release dosage form formulation are as follows (Krowcynski, 1987:12):

- There is a risk of drug accumulation in the body if the administered drug has a long half-life, causing the drug to be eliminated at a slower rate than it is absorbed. The half-life of a CRDF drug candidate should be 2-8 hours to avoid this problem.
- Some drugs have a narrow therapeutic index, requiring the serum drug level to be maintained within a narrow range. Such drugs are difficult to prepare as CRDFs.
- If the gastro-intestinal tract limits the absorption rate of the drug, the effectiveness of the CRDF is limited.
- High dose formulations containing more than 500 mg of active ingredient are difficult to manufacture.

- The cost of controlled release formulation technology may be substantially higher than conventional formulation processes.

- If a CRDF is required (especially when new polymers are employed for CRDFs), the cost of obtaining government approval is high.

- If a drug undergoes extensive first-pass clearance, the drug bio-availability may be reduced.

## 1.4 Classification of Controlled Release Dosage Forms

Over the years, there have been several attempts to classify long-acting oral dosage forms. One classification system of such products proposes that there are basically three types: (1) sustained release, (2) prolonged action, and (3) repeat action dosage forms (Ballard & Nelson, 1975:1618).

Ideally, a sustained release oral dosage form is designed to release rapidly, some predetermined fraction of the total dose into the gastrointestinal tract (see Figure 1-2). This fraction (loading dose) is an amount of the drug which will produce the desired pharmacological response as promptly as is consistent with the drug's intrinsic availability for absorption from gastrointestinal absorption sites. The remaining fraction of the total dose (maintenance dose) is then released as rapidly as is required to maintain constant the initial maximum intensity of pharmacological activity for some desirable periods of time in excess of the time expected from the usual single dose of the drug. Thus, the rate of drug absorption from the maintenance dose into the body, should equal the rate of drug elimination from the body by all processes over the time the desired intensity of pharmacological response is required (Rowland & Beckett, 1964:156).

Prolonged action oral dosage forms initially make the drug available to the body in amounts sufficient to produce the desired pharmacological response (see Figure 1-2).

Such dosage forms also provide for replenishing the supply of drug to the body at some rate, which extends the duration of the pharmacological response obtained, when compared to the conventional single dose of the drug. In contrast with sustained release dosage forms, constant drug levels are not maintained (Rowland & Beckett, 1964:156).

A repeated action oral dosage form is designed to release initially the equivalent of a usual single dose of drug, and then another single dose of the drug at some time later (see Figure 1-2).

When the intensity of a drug's pharmacological activity at a point in time is directly proportional to the drug concentration in the blood, the drug concentration in the blood may be substituted for the drug's pharmacological activity into the definition of the three basic types of long-acting oral dosage forms mentioned above.

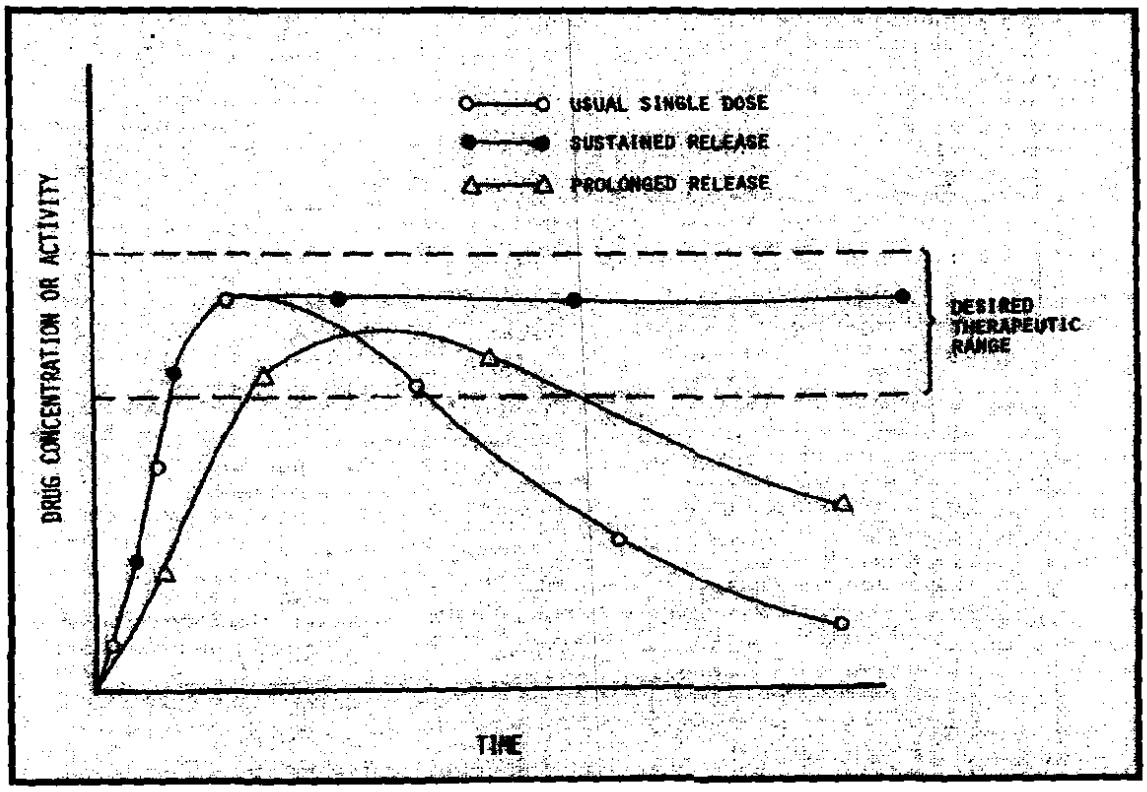


**Figure 1-2:** Relationship between drug concentration or activity and time (Ballard & Nelson, 1975:1618).

Figure 1-2 shows the relationship between drug activity and time for the usual single dose of a drug, a sustained release formulation, and a prolonged action formulation (Ballard & Nelson, 1975:1618).

In practice, it is most difficult to prepare a true sustained release oral dosage form as defined above. One model system which would mimic the curve for sustained release formulations shown in the figure, would involve the administration of the usual single oral dose followed by a continuous intravenous infusion of the drug, started at the time when the peak blood drug concentration or intensity of pharmacological activity, is attained. The rate of infusion should just equal the rate of drug elimination from the body by all processes over the desired time period. The problem of designing an oral maintenance dosage form that would release the drug, akin to the continuous intravenous infusion system just described, is formidable, particularly if it must be economically competitive with similar commercial products. The practical result is that few, if any, dosage forms behave as true sustained release dosage forms, but rather as prolonged action dosage forms.

Unfortunately there is no general agreement as to the use of some standard nomenclature for these specialized drug delivery systems. Often, just because a commercial drug product is labeled as a prolonged release or prolonged action dosage form, there is no assurance that it in fact behaves in a definite pharmacokinetic manner, without adequate supporting data (Rowland & Beckett, 1964:156).

## 1.5 Controlled Release Mechanisms

There are three primary mechanisms by which active agents can be released from a delivery system: diffusion, degradation, and swelling followed by diffusion. Any or all of these mechanisms may occur in a given release system. A discussion of the three mechanisms follows (Brannon-Peppas, 1997:5; Skiens *et al.*, 1980:48).

### 1.5.1 Diffusion Controlled Release Systems

Diffusion occurs when a drug or active agent passes through the polymer that forms the controlled release device. The diffusion can occur on a macroscopic scale - as through pores in the polymer matrix - or on a molecular level, by passing between polymer chains. Examples of diffusion-release systems are shown in Figures 1-3 and 1-4.

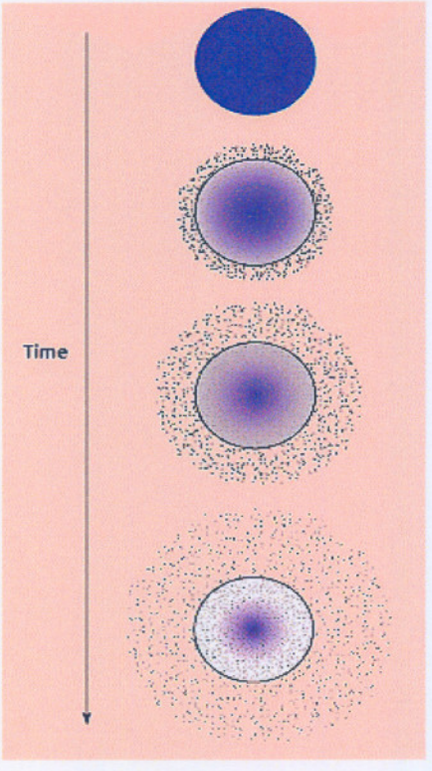


**Figure 1-3:** Drug delivery from a typical matrix drug delivery system (Brannon-Peppas, 1997:5).

In Figure 1-3, a polymer and active agent have been mixed to form a homogeneous system, also referring to as a matrix system. Diffusion occurs when the drug passes from the polymer matrix into the external environment. As the release continues, its rate normally decreases with this type of system, since the active agent has a progressively

longer distance to travel and therefore requires a longer diffusion time to release (Skiens *et al.*, 1980:48).

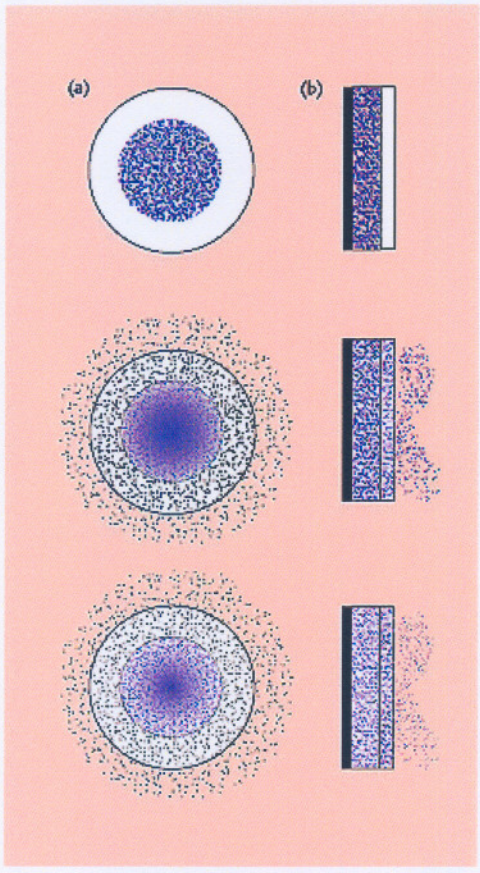


**Figure 1-4:** Drug delivery from typical reservoir devices: (a) implantable or oral systems and (b) transdermal systems (Brannon-Peppas, 1997:6).

For the reservoir systems shown in Figure 1-4a and 1-4b, the drug delivery rate can remain fairly constant. In this design, a reservoir - whether solid drug, dilute solution or highly concentrated drug solution within a polymer matrix – is surrounded by a film or membrane of a rate-controlling material. The only structure effectively limiting the release of the drug, is the polymer layer surrounding the reservoir. Since this polymer coating is essentially uniform and of a non-changing thickness, the diffusion rate of the active agent can be kept fairly stable throughout the lifetime of the delivery system (Yolles & Sartori, 1980:84).

The system shown in Figure 1-4a is representative of an implantable or an oral reserve delivery system, whereas the system shown in Figure 1-4b illustrates a transdermal drug delivery system in which only one side of the device will actually be delivering the drug (Brannon-Peppas, 1997:6).

Once the active agent has been released into the external environment, one must assume that any structural control over drug delivery has been relinquished. For the diffusion-controlled systems described thus far the drug delivery device is fundamentally stable in the biological environment and does not change its size either through swelling or degradation. In these systems, the combinations of polymer matrices and bioactive agents chosen, must allow for the drug to diffuse through the pores or macromolecular structure of the polymer upon introduction of the delivery system into the biological environment, without inducing any change in the polymer itself (Brannon-Peppas, 1997:6).

### 1.5.2 Swelling Controlled Release Systems

It is also possible for a drug delivery system to be designed so that it is incapable of releasing its agent or agents until it is placed in an appropriate biological environment. Swelling controlled release systems are initially dry and when placed in the body will absorb water or other body fluids and swell. The swelling increases the aqueous solvent content within the formulation as well as the polymer mesh size, enabling the drug to diffuse through the swollen network into the external environment (Higuchi, 1963:1145). Examples of these types of devices are shown in Figures 1-5a and 1-5b for reservoir and matrix systems respectively. Most of the materials used in swelling controlled release systems are based on hydrogels, which are polymers that will swell without dissolving when placed in water or other biological fluids. These hydrogels can absorb a great deal of fluid and, at equilibrium, typically comprise 60–90% fluid and only 10–30% polymer (Brannon-Peppas, 1997:8).

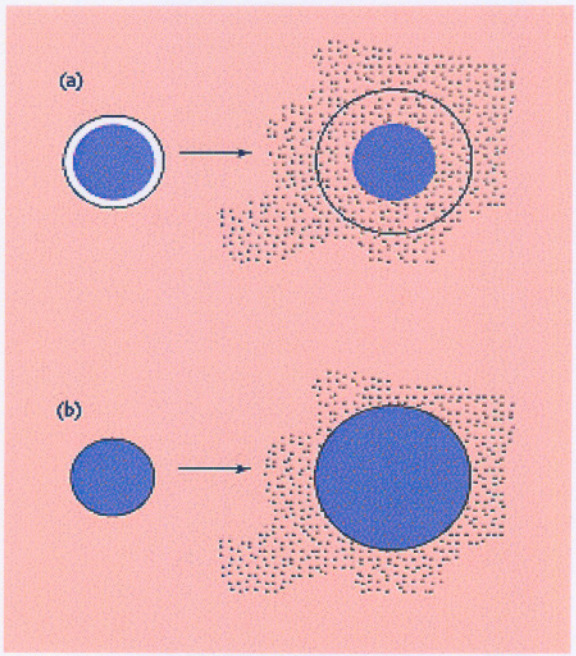


**Figure 1-5:** Drug delivery from (a) reservoir and (b) matrix swelling controlled release systems (Brannon-Peppas, 1997:8).

One of the most remarkable and useful features of a polymer's swelling ability, manifests itself when swelling can be triggered by a change in the environment surrounding the delivery system. Depending upon the polymer the environmental change can involve pH, temperature, or ionic strength, and the system can either shrink or swell upon a change in any of these environmental factors (Kim, 1996:126).

The diagrams in Figure 1-6 illustrate the basic changes in structure of these sensitive systems. Once again, for this type of system the drug release is accomplished only when the polymer swells. Because many of the potentially most useful pH-sensitive polymers swell at high pH-values and collapse at low pH-values, the triggered drug delivery occurs upon an increase in the pH of the environment. Such materials are ideal for systems such as oral delivery in which the drug is not released at low pH values in the stomach but rather at high pH-values in the upper small intestine, for example enteric coated tablets (Brannon-Peppas, 1997:9).

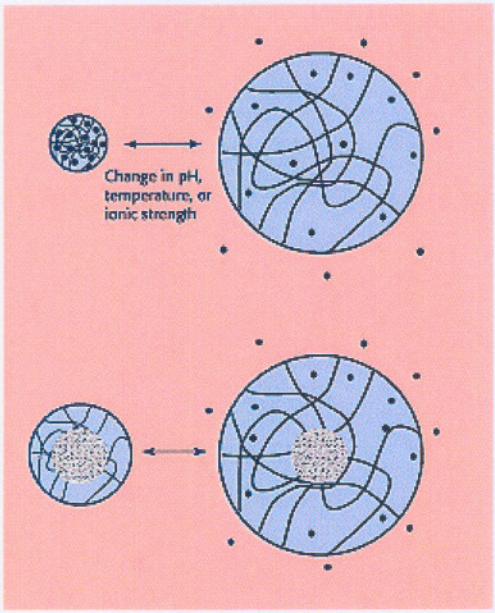


**Figure 1-6:** Drug delivery from environmentally sensitive release systems (Brannon-Peppas, 1997:9).

### 1.5.3 Biodegradable Controlled Release Systems

All of the previously described systems are based on polymers that do not change their chemical structure beyond what occurs during swelling. However, great deals of attention and research effort are being concentrated on biodegradable polymers. These materials degrade within the body as a result of natural biological processes, eliminating the need to remove a drug delivery system after release of the active agent has been completed (Brannon-Peppas, 1997:9).

Most biodegradable polymers are designed to degrade as a result of hydrolysis of the polymer chains into biologically acceptable and progressively smaller compounds. In some cases, as for example polylactides, polyglycolides and their co-polymers the polymers will eventually break down to lactic acid and glycolic acid, enter the Kreb's cycle and further break down into carbon dioxide and water, and excreted through normal processes. Degradation may take place through bulk hydrolysis, in which the polymer degrades in a fairly uniform manner throughout the matrix, as shown in Figure 1-7a. For some degradable polymers, most notably the polyanhydrides and polyorthoesters, the degradation occurs only at the surface of the polymer, resulting in a release rate that is proportional to the surface area of the drug delivery system (see Figure 1-7b) (Heller, 1985:167-177).

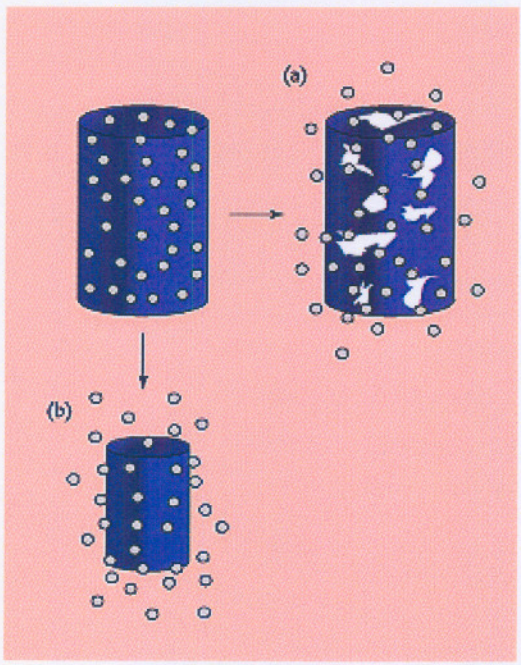


**Figure 1-7:** Drug delivery from (a) bulk-eroding and (b) surface-eroding biodegradable systems (Brannon-Peppas, 1997:10).

The most common means of formulation for these biodegradable materials is that of microparticles, which have been used in oral delivery systems and even more often, in

subcutaneously injected delivery systems. Given appropriate manufacturing methods, microparticles of poly(lactide-co-glycolide) (PLGA) can be prepared in a fairly uniform manner to provide essentially nonporous microspheres as shown in Figure 1-8. These particles will degrade through bulk hydrolysis in water or body fluids, yielding polymer fragments over time. The polymer fragments shown in Figure 1-9, for example, are of PLGA microparticle (75:25 lactide:glycolide) after 133 days of degradation in water (Heller, 1985:167-177).

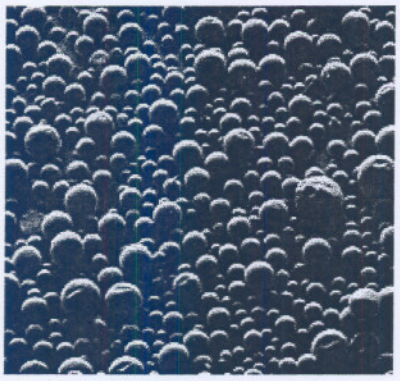

**Figure 1-8:** Biodegradable PLGA microparticles (60:40 lactide:glycolide). (Photo courtesy of T. Tice, Southern Research Institute, Birmingham, AL).

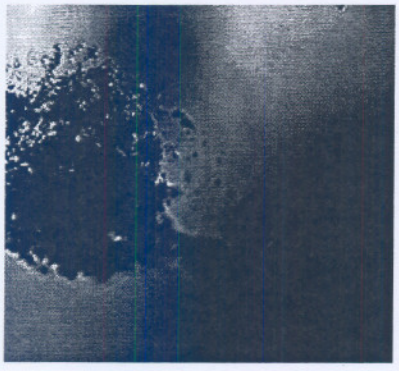

**Figure 1-9:** Biodegradable PLGA microparticle (75:25 lactide:glycolide) after 133 days of degradation in water.

## 1.6 Polymers as Drug Carriers

Polymers, one of the most versatile classes of materials, have changed our day-to-day lives over the past several decades. However, the distinction between temporary and permanent biomedical applications of polymers was made only 30 years ago (Uhrich *et al.*, 1999:3181-3198). Generally, natural and synthetic polymers are used as the structural backbone for both controlled release and conventional drug delivery systems. These polymers may be swollen, non-swollen, porous, non-porous, semi-permeable, erodible, degradable, bio-adhesive, etc. The main difference, between the CRDFs and the conventional systems, is that conventional dosage forms disintegrate. CRDFs normally do not disintegrate and if it does disintegrate, the disintegration is carefully controlled to maintain its rigidity for a long period of time (Passil, 1989:629-677).

The amalgamation of polymer science with pharmaceutical science led to a quantum leap in terms of 'novelty' (flexibility in physical state, shape, size and surface) design and development of novel drug delivery systems (DDSs). Polymeric delivery systems are mainly intended to achieve either a temporal or spatial control over drug delivery (Li & Vert, 1999:71-93). The introduction of the first synthetic polymer based (polyglycolic acid) DDS led to a heightened interest in the design and synthesis of a novel biodegradable polymer that obviated the need to remove the DDS, unlike the non-degradable polymeric systems. Recognizing that intimate contact between a delivery system and an epithelial cell layer will improve the residence time, as well as the efficacy of the DDS, resulted in the design of bioadhesive polymers (Mathowitz *et al.*, 1999:9-45). Further advancements in polymer science led to 'smart' polymeric hydrogel systems that can self-regulate delivery of a bioactive agent in response to a specific stimulus.

Polymers selected in the preparation of the dosage form must comply with the following requirements (Passil, 1989:629-677):

1. *Biocompatibility*: Harmful/toxic impurities must be removed from polymers before their inclusion in the CRDFs. The residual monomers, initiator, and other chemicals used in the synthesis or modification of polymers, must be removed after the polymerization/modification. The chemicals employed in the polymer fabrication processes (i.e. additives, stabilizers, plasticizers and catalyst residues) must be carefully selected to meet regulatory requirements.

2. *Physical and mechanical properties:* The polymers must possess the necessary mechanical properties required for the dosage form design such as: elasticity, compactability, resistance to tensile, swelling and shear stresses, and resistance to tear and fatigue.

3. *Pharmacokinetic properties:* Chemical degradation of the polymer matrix should not occur, and if it does, the degradation products must be non-toxic, non-immunogenic and non-carcinogenic.

There are many ways to synthesize new polymers and modify existing polymers. Different monomers (for addition polymerization or condensation polymerization) may be used, or existing polymers may be modified. However, only a handful of polymers are used in pharmaceutical drug delivery systems due to their commercial availability, established biocompatibility and government registration (Passil, 1989:629-677). Most polymers used in pharmaceutical dosage forms were not originally designed for this purpose. However, the production of new life-saving, genetically engineered drugs (peptides and proteins) which have characteristically short half-lives, presents an opportunity for significant research in the area of polymer development in order to prolong their therapeutic effects in the human body (Langer, 1989:18).

### 1.6.1 Consideration for Selection of Polymers

The selection and design of a polymer is a challenging task because of the inherent diversity of structures and require a thorough understanding of the surface and bulk properties of the polymer that can give the desired chemical, interfacial, mechanical and biological functions. The choice of polymer, in addition to its physico-chemical properties is dependent on the need for extensive biochemical characterization and specific preclinical tests to prove safety. Recently, Angelova and Hunkeler (1999:409-421) have proposed a flow chart for rational selection of polymers for biomedical applications. Table 1 gives a representative list of polymers that have been investigated for drug delivery applications and can be broadly classified into biodegradable and non-biodegradable polymers. A discussion of the most important polymers follows the table. Chitosan will be discussed as a natural polymer, biodegradable polymers will be discussed as a group and cellulose derivates and acrylic polymers will be discussed as non-biodegradable polymers.

**Table 1-1:** Representative list of polymers used in drug delivery (Angelova & Hunkeler, 1999:409-421)

| | |
|---|---|
| **Natural polymers** | |
| Protein-based polymers | Collagen, albumin, gelatin |
| Polysaccharides | Agarose, alginate, carrageenan, hyaluronic acid, dextran, chitosan, cyclodextrins |
| **Synthetic polymers** | |
| ***Biodegradable*** | |
| Polyesters | Poly(lactic acid), poly(glycolic acid), poly(hydroxy butyrate), poly(ε-caprolactone), poly(β-malic acid), poly(dioxanones) |
| Polyanhydrides | Poly(sebacic acid), poly(adipic acid), poly(terphthalic acid) and various copolymers |
| Polyamides | Poly(imino carbonates), polyamino acids |
| Phosphorous-based polymers | Polyphosphates, polyphosphonates, polyphosphazenes |
| Others | Poly(cyano acrylates), polyurethanes, polyortho-esters, poly-dihydropyrans, polyacetals |
| ***Non-biodegradable*** | |
| Cellulose derivatives | Carboxymethyl cellulose, ethyl cellulose, cellulose acetate, cellulose acetate propionate, hydroxypropyl methyl cellulose |
| Silicones | Polydimethylsiloxane, colloidal silica |
| Acrylic polymers | Polymethacrylates, poly(methyl methacrylate), poly hydro(ethyl-methacrylate) |
| Others | Polyvinyl pyrrolidone, ethyl vinyl acetate, poloxamers, poloxamines |

## 1.6.1.1 Applications and Properties of Chitosan

### 1.6.6.1.1 Introduction

Chitosan is a polysaccharide obtained by deacetylating chitin which is the major constituent of the exoskeleton of crustaceous water animals. This biopolymer was traditionally used in the Orient for the treatment of abrasions and in America for the healing of machete gashes (Allan *et al.*, 1984:119-133). A recent analysis of the varnish on one of Antonio Stradivarius's violins showed the presence of a chitinous material. Chitosan was reportedly first discovered by Rouget in 1859 when he boiled chitin in a concentrated potassium hydroxide solution. This resulted in the deacetylation of chitin. Fundamental research on chitosan did not start in earnest until about a century later. In 1934, Rigby obtained two patents, one for producing chitosan from chitin and the other for making films and fibers from chitosan (Rigby, 1934).

In the same year, the first X-ray pattern of a well-oriented fiber made from chitosan was published by Clark and Smith (1936:863-879). Since then, knowledge about chitosan has been greatly advanced by the work of pioneers such as Muzzarelli. The main driving force in the development of new applications for chitosan lies in the fact that the polysaccharide is not only naturally abundant, but it is also non-toxic and biodegradable. Unlike oil and coal, chitosan is a naturally regenerating resource (e.g., crab and shrimp shells) that can be further enhanced by artificial culturing.

It was reported that chitosan is contained in cell walls of fungi (Hadwiger & Backman, 1980:205-211). Chitin, however, is more widely distributed in nature than chitosan and can be found in mushrooms, yeasts, and the hard outer shells of insects and crustaceans. It was reported for example, that about 50-80% of the organic compounds in the shells of crustaceans and the cuticles of insects consist of chitin. At present, most chitosan in practical and commercial use comes from the production of deacetylated chitin, with the shells of crab, shrimp and krill (the major waste by-product of the shellfish-processing industry) being the most available source of chitosan.

### 1.6.1.1.2 Structure and Chemistry of Chitosan

Chitosan is a hydrophilic, cationic polyelectrolyte prepared by N-deacetylation of chitin (Figure 1-10). Chitin is the most abundant natural polymer next to cellulose and is obtained from crab and shrimp shells (Bodmeier *et al.*, 1989:1476). Crustacean shells, a by-product of the shellfish processing industry, exhibit surface binding specificity towards a range of heavy metal ions. The metal ion binding ability of crustacean shell is attributed to the presence of chitin and its deacetylated derivative chitosan in its exoskeletons. The processed waste of crustacean shells contains approximately 10 to 55% chitin on a dry weight basis (Chu, 2002:78).

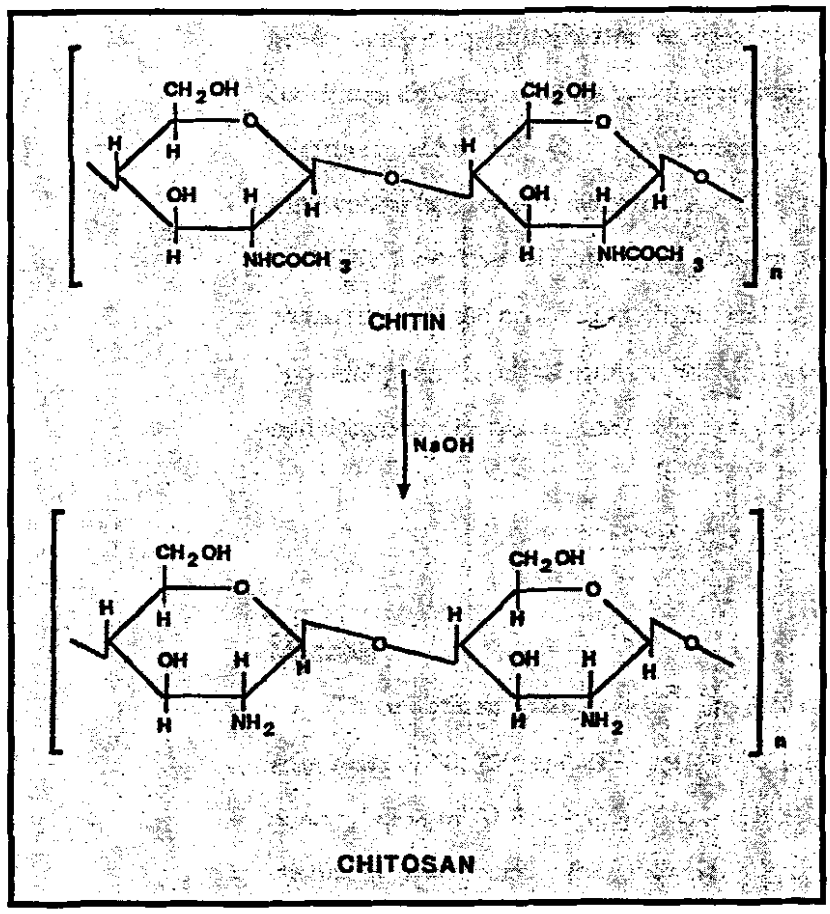


**Figure 1-10:** Chemical structure of chitosan and chitin (Bodmeier *et al.*, 1989:1476).

Chitosan is a collective name given to a group of polymers deacetylated from chitin. The difference between chitin and chitosan lies in the degree of deacetylation. Generally, the

reaction of deacetylating chitin in an alkaline solution cannot reach completion even under harsh treatment conditions. The degree of deacetylation usually ranges from 70% to 95%, depending on the method used (Muzzarelli 1973:83-95).

If the degree of deacetylation is less than 50%, the molecule is chitin and if the degree of deacetylation is more than 50%, the molecule is chitosan. These methods have been thoroughly reviewed by Muzzarelli (1973:83-95). The technique of Horowitz for example, where chitin is treated with solid potassium hydroxide for 30 minutes at 180 °C results in the highest removal (95%) of acetyl groups (Muzzarelli 1973:83-95). Recently, Kobayashi *et al.* (1988:1465) published a procedure for preparing chitosan from mycelia of absidia strains. A chitosan product with 79-91% deacetylation and a molecular weight of 1,200,000 was obtained. Most publications use the term chitosan when the degree of deacetylation is more than 70%. The process is also explained through Paul and Sharma's flow chart (See Figure 1-11) (Paul & Sharma, 2000:5-22).

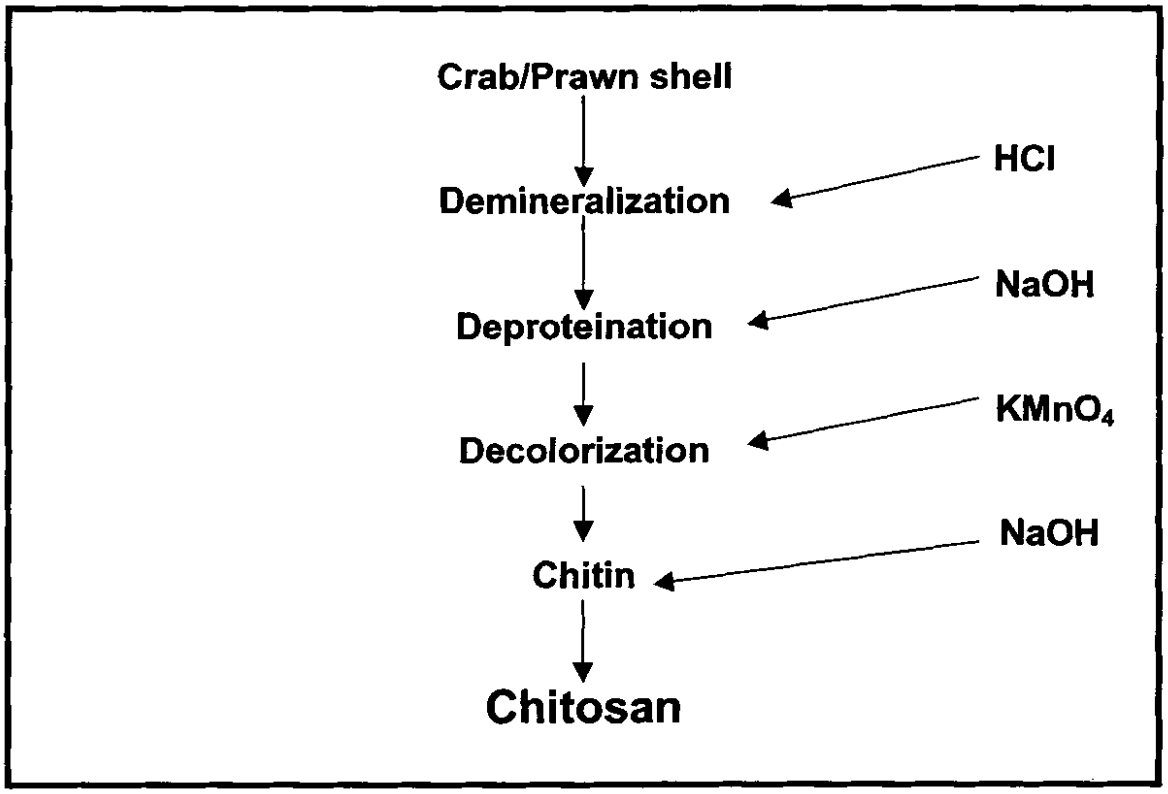


**Figure 1-11:** Chitosan production flow chart (Paul & Sharma, 2000:5-22).

### 1.6.1.1.3 Applications of Chitosan

Chitosan offers a unique set of characteristics as a functional material: hydrophilicity, biocompatablity, biodegradability, antibacterial properties and remarkable affinity to proteins. It is biologically inert, safe for humans and the natural environment. These characteristics make chitosan suitable for application as a supporting material in systems constructed to be functional in biological environments. Applications include biomedical and pharmaceutical applications such as: an excipient in various forms (tablets, beads, etc.) for a variety of delivery systems (oral, nasal etc.); a component in haemodialysis membranes and surgical dressing materials, contact lenses, enzyme and cell encapsulation and immobilization, coating of seeds and leaves to improve plants' resistance to diseases, and coating of fertilizers and pesticides for their controlled release to soil (Krajewska, 2001:38).

Chitosan is also inexpensive and digestible, which makes it a promising vehicle for the development of drug delivery systems. The use of chitosan in the development of drug delivery preparations is based on experience with chitosan intragastric tablets and studies of chitosan coated drug delivery systems. Drugs dispersed in chitosan were found to be released at a constant rate, thus highlighting its potential as a sustained release matrix.

Kneading low molecular weight chitosan with drugs increased the dissolution of several poorly soluble drugs. Enhanced bioavailability of phenytoin in Beagle dogs was reported on administration of the kneaded mixture. The significant increase in dissolution rates may be due to improved wettability, crystallinity or crystal size and shape (Paul & Sharma, 2000:5-22).

Nagai *et al.*, (1984:21-40) and Tian *et al.*, (1988:318-321) used chitosan and its derivatives as additives with other materials such as lactose and starch in the preparation of compressed tablets. The release of drugs from these tablets was found to be related in part to the loading of the chitosan additives and followed a zero-order profile. Bodmeier *et al.*, (1989:413-417) entrapped microparticles of drugs in chitosan beads formed by ionotropic gelation of chitosan in tripolyphosphate solution. On eluting with 0.1 N HCl, the chitosan beads disintegrated and released the microparticles.

The disintegration time appeared to be a function of the polysaccharide viscosity, gelation time and drying method. In another report, Kanke *et al.*, (1989:523) formed three types of chitosan films containing a model drug, prednisolone, for retarded release studies. The three types of films; monolayer, double layer and *N*-acetylated chitosan double layer were prepared by air drying. They found that the *N*-acetylated film gave a slower release rate than the other films. Applications of chitosan in cosmetics were reviewed by Muzzarelli (1983:53) several years ago. He indicated that the use of chitosan could help to remove leftover starch contained in shampoos. Its use would also have the effect of conferring shine and strength to hair due to interactions between the polysaccharide and hair proteins.

### 1.6.1.2 Biodegradable Polymers

Traditional polymers are inert and resist degradation. Other polymers such as acrylic resins are dependent on pH and solubility. In contrast, biodegradable (bioabsorable) polymers undergo depolymerization to absorbable molecules in an organism's tissues. Some polymers undergo microbiological degradation. Biodegradable polymers are very important in manufacturing implants and other parenteral extended release dosage forms. The polyesters, like poly(glycolic acid) (Dexon®) or glycolic/lactic acid copolymers (Vicryl®), employed as synthetic absorbable sutures, were utilized in the manufacture of implantable drug delivery systems (Wood, 1980:1).

Poly(DL-lactic acid), a glassy non-crystalline aliphatic polyester, may be prepared by slowly heating D,L-lactic acid with 0.1% antimony trioxide to 180 °C with a pressure reduction to 5 mm Hg for up to 10 hours, and then to 255 °C and 1 to 2 mm Hg for up to 6.5 hours. The resulting D,L-lactide is recrystallized and heated for 4 to 6 hours at 170 to 200 °C with 0.03% stannous octoate or 0.02% tetraphenyltin at 0.3 to 1 mm Hg. Refluxing in acetone and precipitation of the filtered solution in water gave poly(lactic acid) (Rak *et al.*, 1983:107).

In another study, Yolles *et al.*, (1975:115) prepared films from poly(lactic acid) and tributylcitrate containing 20 % of Cytoxan® for implantation. The method consisted of evaporation of a solution containing the polymers, plasticizers and drug in methylene chloride and compression of the resulting films at 170 °C. Powdered mixtures of poly(lactic acid) with different anticancer agents were also prepared. After injection of a suspension, extended action was observed in mice. *In vitro* such a powder released drug at a constant rate for 92 days (Yolles *et al.*,1978:382). Poly(lactic acid) was also used to produce cyclozocine microcapsules which were injected into rats in the form of suspension in sesame oil. About 90% of drug was released in 8 days (Manson, 1976:847).

Poly(hydroxybutyric acid) can also be used in the formulation of extended release parenteral implants by compressing the drug-polymer mixture. Such implants release the drug for up to 30 days; if they are compressed with raised temperature, for up to 2 to 3 months. By choosing suitable conditions for production, release can be extended to 1 year (Hirano, 1979:1125).

Poly(ε-caprolactone) is a semicrystalline polymer (mp 63°C) which after subcutaneous implantation in rabbits or rats, undergoes non-enzymatic degradation by hydrolytic cleavage of the ester linkages. A copolymer of ε-caprolactone and D,L-dilactide undergoes a similar hydrolysis in tissue. Both polymers are used for the production of extended release implants. The alkyl-2-cyanoacrylate monomers are used as tissue adhesives in surgery (Pitt *et al.*, 1979:497).

Many experiments were carried out with poly(alkyl-2-cyanoacrylate) concerning their application in extended release dosage forms, especially nanoparticles. Drug release rates from poly(alkyl-cyanoacrylate) films were diffusion controlled, proportional to the film thickness. Poly(methyl)- and poly(ethyl)cyanoacrylate were evaluated as suitable for oral extended release dosage forms (EI Egakey, & Speiser, 1982:103). Polymethyl- and polyethylcyanoacrylate nanocapsules, prepared by polymerization, proved to be potential lysosomotropic drug carriers.

Poly(butylcyanoacrylate), being less toxic than poly(methylcyanoacrylate) was employed for intramuscular formulations, among others, for microencapsulated drugs. Using alkyl(2-cyanoacrylate), nanoparticles capable of adsorbing several antineoplastic drugs were formulated. Their tissue distribution after intravenous injection showed specific targeting properties as well as extended action (Couvreur *et al.,* 1980:199). The toxicity of such nanoparticles is low. $LD_{50}$ for mice after intravenous application of free nanoparticles was found to be 196 mg/kg for polyisobutylcyanoacrylate and 230 mg/kg for poly(butylcyanoacrylate). The absence of mutagenity was also shown for both nanoparticles and their degradation products. In experiments with doxorubicin adsorbed on nanoparticles, a significant reduction of mortality and weight loss was recorded in mice. Cardiotoxicity was also decreased due to the prior uptake by the myocardium (Couvreur *et al.,* 1982:790). This indicates that these polymers are very suitable for the production of nanoparticles.

### 1.6.1.3 Derivates of Cellulose

Cellulose is a natural glucose polymer. Pharmaceutical technology utilizes microcrystalline cellulose obtained by the partial depolymerization of α-cellulose by strong acids and mechanical comminuting. Each anhydroglucose molecule has three hydroxyl groups capable of substitution, varying in reactivity. Cellulose is insoluble in water and organic solvents. However, most important for extended release dosage form formulation are various cellulose ethers and esters (Salib *et al.,* 1976:577).

Cellulose esters are obtained by reaction with the proper acid anhydride (e.g., glacial acetic acid and acetic anhydride form cellulose acetate). The cellulose esters: cellulose acetate, acetate butyrate, acetate phthalate, and nitrate are insoluble in water and dissolve in organic solvents. Cellulose acetate films were used in oral osmotic therapeutic systems. Cellulose acetate phthalate solubility in water depends on pH, therefore it is used for enteric coating. Cellulose ethers are obtained using alkyl chloride and sulfate. With the exception of ethyl cellulose, they are water soluble. The viscosity of aqueous solutions of soluble ethers depends on their degree of polymerization.

Methyl cellulose forms micropellets which can prolong the drug release rate 3 to 6 times, (Salib *et al.,* 1976:577) depending on the coating thickness. Acetylsalicylic acid tablets, coated with methyl cellulose, released only 5% of drug after 30 minutes, while analogous tablets coated with hydroxypropyl methyl cellulose, released almost 80% of their drug content. Such a pronounced difference is the result of the thermal gelation (Schwartz & Alvino, 1976:572) of methyl cellulose at 37 °C.

Ethyl cellulose is applied in the formulation of insoluble coatings (Salib & El-Menshawy, 1976:721) or tablets matrix cores (Chandrasekaran & Hillman, 1980:1311), providing for extended release. Fluidized bed coating, using solutions of dehydrated ethanol or a mixture of methylene chloride and methanol (7:3) or methylene chloride and ethanol, (7.5:2.5) is applied. Release rate is determined by the coat or matrix porosity. In the case of water soluble drugs, e.g., potassium chloride, porosity can be modified by the drug/carrier substance ratio. Polyethylene glycol, propylene glycol or methyl cellulose and glycerol can also be added to ethyl cellulose. With increasing amounts of methyl cellulose, the release rate of acetylsalicylic acid increased (Chandrasekaran & Hillman, 1980:1311).

A hydrophobic polymer matrix can be produced among others, from cellulose acetate. It is also a suitable material for membranes in oral osmotic pumps (Oros). The anionic character differentiating carboxymethyl cellulose from other ethers, may be the cause of interactions with cationic drugs and result in derivates with extended release due to decreased solubility.

In the formulation of extended release tablets with antihistamine and potassium chloride, a hydrophilic matrix of carboxymethyl cellulose sodium was applied. Carboxymethyl cellulose sodium forms insoluble salts in the presence of some electrolytes, e.g., aluminium sulfate. This was applied in the formulation of films on chloramphenicol particles (Salib *et al.,* 1976:872). The insoluble aluminium salt of carboxymethyl cellulose precipitates and coats the drug particles.

### 1.6.1.4 Methacrylate Polymers

The glass-like polymer Plexiglass® won the Grand Prix and gold medal at the exhibition held in Paris in 1937. It was polymethacrylate, a hard product, fragile, water insoluble, soluble in chloroform and soluble in hot acetone and benzene. This polymer has been employed in the production of dentures and artificial limbs (Lehmann, 1984:113).

Methacrylic acid is a colorless liquid, soluble in water, alcohol, ether and most organic solvents. It readily undergoes polymerization to give polymers called acrylate resins (acrylic resins). Eudispert®, the commercial preparation is a methacrylate and methacrylic acid copolymer. Therefore it has free carboxylic acid groups which distinguish it from Plexiglass®. The free polyacids are insoluble in water but they form viscous aqueous solutions with alkalis, amines and alkaline earths. Depending on the preparation type (degree of polymerization), solutions of varying viscosity can be made. They are used in suspensions, emulsions, ointments, and pastes (Szigetti, 1965:62). Changing the ester and acid ratio, product solubility in liquids of varying pH can be modified. Using this principle, polymeric coating substances were formulated under the trade name Eudragit® and marketed in 1955 (Lehmann & Dreher, 1969:319).

Applications of ester and acid polymers gave only coatings insoluble in acidic gastric juice, yet dissolving in intestinal fluid. It was not until alkylamine groups were introduced in place of methacrylic acid, that films, also soluble in acidic solution, could be produced (Eudragit® E). The introduction of a quaternary ammonium salt, which is in the ionized form at pH 2 to 8, gives a water insoluble but water permeable film, which is also permeable to dissolved drug molecules (Eudragit® RL and RS).

Neutral polymethacrylates are pharmacologically inactive. Good compatibility with the skin and mucous membranes prompted their use in the formulation of wound sprays and ointment bases. The acrylic resins also have an important role in the formulation of extended release oral dosage forms. There have also been attempts to use them for implant formulation (Lehmann, 1999:2).

Cross-linked copolymers, based on methacrylic acid, serve as ion exchangers for adsorption of active ingredients in the manufacture of sustained release formulations in the form of tablets and suspensions. For sustained release active ingredients can also be embedded in water insoluble polymers, e.g. by compression to tablets, together with polymer powders or by extrusion at the softening temperatures of the polymer, between 120 and 200 °C.

Probably the most important role of polymethacrylates in pharmaceutical manufacture is that of special excipients for coating oral dosage forms and ensuring controlled release of the active ingredient (Lehmann, 1999:2).

Coating of tablets, sugar coated products, capsules, pellets, crystals and other drug loaded cores serve to ensure their physical and chemical stability, to enhance patient compliance and to further improve their therapeutic efficacy. Acknowledging the fact that the efficiency of a pharmaceutical dosage form depends not only on the active ingredient it contains, but also and critically so, on the formulation and processing technique. Scientists and engineers alike have devoted increasing attention to these parameters in recent years (Lehmann, 1999:3).

Due to the ionic characteristics of methacrylate copolymers, they may interact with some drugs. Badawi *et al.* (1980:55) showed that in the solid dispersion of salicylic acid and p-amino-salicylic acid with the cationic methacrylate copolymer, a binding between both drugs and carrier, exists. An anionic copolymer interacts only with p-amino-salicylic acid (Okor, 1982:11).

At first Eudragit® acrylic resins were used in a form of a 12.5% w/v solution in acetone (Eudragit® S) or in an acetone-isopropyl alcohol (4:6) (Eudragit® E) mixture. Due to the flammability and toxicity of these solvents, aqueous polymer suspensions were also introduced (Lehmann & Dreher, 1972:894). Aqueous dispersions of polymers are prepared by emulsion-polymerization. Monomers are dispersed in water with surfactants, which form micelles enclosing a monomer. The addition of initiators induces polymerization. The polymer particles formed have a diameter of 0.01 to 1 μm. Following application on the tablet surface after the water evaporates, the polymer particles bind forming a polymer film.

## 1.7 Conclusion

During the past few decades there were an immense focus on providing modern controlled release dosage forms. The main reason is to improve patient compliance. These dosage forms have increasingly gained popularity over other dosage forms in treating disease mainly because of the numerous benefits over conventional dosage forms. These formulations not only improve compliance but also significantly reduce the cost of administration, reduce the frequency of drug administration and may even improve or completely avoid certain side-effects commonly encountered with conventional dosage forms.

The mechanisms by which active agents can be released from a delivery system have been shown to be divided into three types: diffusion, degradation and swelling followed by diffusion. Any or all of these mechanisms may occur in a given release system.

Diffusion occurs when a drug or other active agent passes through the polymer that forms the controlled release device. The diffusion can occur on a macroscopic scale as through pores in the polymer matrix or on a molecular level, by passing between polymer chains.

Swelling controlled release systems are initially dry, and when placed in the body, will absorb water or other body fluids and swell. The swelling increases the aqueous solvent content within the formulation as well as the polymer mesh size, enabling the drug to diffuse through the swollen network into the external environment.

The two previously described systems are based on polymers that do not change their chemical structure beyond what occurs during swelling. However, great deals of attention and research effort are being concentrated on biodegradable polymers. These materials degrade within the body as a result of natural biological processes, eliminating the need to remove a drug delivery system after release of the drug has been completed.

Whatever the method of controlled release may be, the principle ingredient always seems to be a polymer. Polymers, one of the most versatile classes of materials, have changed our day-to-day lives over the past several decades. These polymers may be synthetic or of a natural origin. The earliest biodegradable controlled drug delivery systems were introduced in the 1970's and were based on polymers formed by lactic acid. Today, polymeric material still provides the most important avenues for research, primarily because of their ease of processing and the ability of researchers to readily control their chemical and physical properties via molecular synthesis.

Polymers must undergo specific preclinical tests to prove their safety before they are used in the preparation of dosage forms. The polymers used as drug carriers need to comply with a wide array of requirements. Firstly, it must be biocompatible; it must possess the necessary physical and mechanical properties and must possess certain pharmacokinetic properties. Furthermore the polymer should not undergo degradation, and if degradation does occur, the by-products must be biocompatible, non-toxic, non-immunogenic and non-carcinogenic.

Chitosan is a polymer of natural origin and has been found by numerous studies to be a safe and bio-compatible polymer suitable for use in controlled release formulations. This polymer offers added effects besides most of the purposes for which it is intended. The mucoadhesive properties of chitosan lead to wide applicability in many dosage formulations. The chemical structure of chitosan also added to its suitability for drug delivery. Improved delivery of several drugs is possible by using chitosan and its derivates in novel drug delivery systems.

# Chapter 2

# Beads for Controlled Drug Delivery: Preparation and Characterization

## 2.1 Introduction

The development of a new drug involves more than the synthesis of the substance that has a particular effect on the body. It must also be considered how to transport the drug to the appropriate part of the body and once there, how to make it available for absorption and distribution. In some cases the development of an appropriate delivery system can be as complex as the development of the drug itself (Thrash, 1999).

According to Brannon-Peppas (1997:5) most controlled release systems fall into one of three categories namely release by diffusion, polymeric degradation or swelling followed by diffusion. Although a number of controlled release systems have been developed in recent years, there is still a big need to optimize drug activity by increasing its bio-availability at the absorption site.

According to Vogelson (2001:49) scientists are attempting to address specific problems that are exhibited by current methods of drug delivery. For example, many drugs' potencies and therapeutic effects are limited or otherwise reduced because of the partial degradation that occurs before they reach a desired target in the body.

Once administered, time release medications deliver treatment continuously, rather than providing relief of symptoms and protection from adverse effects only when necessary. However, this improvement cannot happen until methods are developed to safely shepherd drugs through specific areas of the body, such as the stomach, where low pH can destroy a drug, or through an area where healthy tissue might be adversely affected.

The applications of polymers in controlled drug release systems have become important in the pharmaceutical industry. As discussed in Chapter 1 polymers have several advantages rendering them suitable for controlled drug delivery. Especially polymeric beads have become important as a micro-particulate drug delivery system. The advantages of beads in drug delivery will be reviewed in this chapter as well as the different methods that can be used to prepare and characterize beads for use in drug delivery. Optimization of an ionotropic gelation method for the preparation of beads for this study is discussed and a brief discussion of the active ingredient for the study will also be given.

## 2.2 Model Drug: Pyrazinamide

Pyrazinamide is indicated for the initial treatment of active tuberculosis in adults and children when combined with other antituberculous agents such as isoniazid, rifampicin, ethambutol, streptomycin, cycloserine, ethionamide and para-aminosalicyclic acid. Pyrazinamide is bacteriostatic and is effective only against *Mycobacterium tuberculosis.* Pyrazinamide should always be administered in combination with other effective antituberculous drugs. When used alone, resistance may develop within 6 to 8 weeks (Martindale, 1993:195).

The recommended dose is 20 – 35 $mg.kg^{-1}$ of body weight per day, divided into three or four daily doses, although twice or even once daily, is probably adequate. The standard regimen for the treatment of drug-sensitive tuberculosis is now recommended as isoniazid, rifampin, pyrazinamide for 2 months followed by isoniazid and rifampin for 4 months. Using combination tablets allows three antibiotics in a single tablet to be given in a single daily dose, preferably on an empty stomach half an hour before breakfast (Martindale, 1993:195).

Pyrazinamide is a white, odourless crystalline powder with a slightly bitter taste, sparingly soluble in water, slightly soluble in alcohol and in methylene chloride and very slightly soluble in ether. Pyrazinamide has a melting point of 188 °C to 191 °C (British Pharmacopoeia, Electronic Edition, 2002).

The chemical structure of pyrazinamide is shown in Figure 2-1. The chemical name for Pyrazinamide is Pyrazine-2-carboxamide. Its empirical formula is $C_5H_5N_3O$, and pyrazinamide has a molecular weight of 123.1 g/mol. The drug has a $pK_a$-value of 0.5 (British Pharmacopoeia, Electronic Edition, 2002). From Figure 2-1 it is evident that pyrazinamide, as other amide compounds, is a weak base.

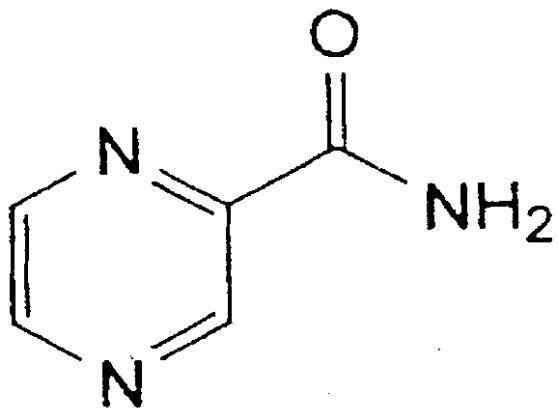


**Figure 2-1:** Structure of Pyrazinamide (British Pharmacopoeia, 2002).

The half-life ($t_{1/2}$) of Pyrazinamide is 9 to 10 hours in patients with normal renal and hepatic function. The plasma half-life may be prolonged in patients with impaired renal or hepatic function. Pyrazinamide is converted in the liver to its major active metabolite, pyrazinoic acid. Approximately 70% of an oral dose is excreted in urine, mainly by glomerular filtration (Goodman-Gilman *et al.*, 1990:1154).

## 2.3 Polymeric Beads

Beads are spherical gel pellets consisting of a biocompatible polymer cross-linked by cross-linking agents. Beads are normally loaded with a drug of choice (Bodmeier *et al.*, 1989:1475; Mi *et al.*, 2002:61). Bead size typically ranges between 0.8 mm and 1.5 mm (Shu & Zhu, 2000:53). It was found that beads, as a novel dosage form have the ability to delay drug release sufficiently to be considered in the manufacture of sustained release products (Bodmeier *et al.*, 1989:1487). The surface and cross-section morphology varies according to the method of drying, either freeze-dried or oven-dried, with freeze-dried beads being more porous (Bodmeier *et al.*, 1989:1487).

Beads show various advantages over single unit dosage forms and they are very flexible in dosage form development and therefore are frequently used in controlled release dosage forms. Because beads disperse freely in the gastrointestinal tract they maximize drug absorption, reduce peak plasma fluctuation and minimize potential side-effects without lowering drug bio-availability. One of the causes for inter- and intra-patient variation, inconsistent gastric emptying, is reduced. The incidences of high local concentrations of bioactive ingredients, which may be irratative or anaesthetic, are decreased significantly. The delivery of two or more bioactive agents that may not be biocompatible is possible. By combination of beads with different release rates, drug delivery at different sites can be achieved (Gehebre-Sellassie, 1989:7).

Aydin *et al.* (1996:101) prepared chitosan beads for the controlled delivery of salmon calcitonin. It was also possible for Anal *et al.* (2003:713) to prepare beads for the sustained release of ampicillin. In another study by Akbuga and Sezer (1995:113-116) controlled release chitosan beads containing piroxicam were successfully prepared.

Gupta and Ravi Kumar (2000:1115) found that not only does a burst effect in drug release occur at an initial pH of 2 but also that the release rate of drug from chitosan beads were much higher in acidic environment than in alkaline environment. El Fattah *et al.* (1998:541) found that the ionic properties of the polymers used in beads and the pH of the dissolution media had an affect on the drug release from beads and that the drug release was dependent on it.

At an acidic value, chitosan beads showed a rapid drug release, whereas a sustained drug release was obtained from Gelrite beads. In contrast, the drug release in phosphate buffer was rapid from Gelrite, and chitosan showed a sustained drug release tendency.

## 2.3.1 Preparation of Beads

Beads can be manufactured by various methods including:

- ionotropic gelation,
- emulsification inotropic gelation,
- extrusion spheronisation,
- melt solidification and
- electric dispersion of polymer solutions.

These techniques usually involve complicated processes utilizing complex apparatus. Of all these methods, preparing beads by ionotropic gelation is the simplest method of preparing beads with favourable characteristics, especially chitosan based beads (Bodmeier *et al*, 1989:1488; Gupta & Ravi Kumar, 2000:1115). Therefore the beads for this study were all prepared by ionotropic gelation. A discussion of the various methods of bead preparation follows.

### 2.3.1.1 Ionotropic Gelation

The ionotropic gelation method used for the preparation of beads is one of the most trusted methods that is widely used and has been described by Bodmeier *et al.*, 1989:1478). Ionotropic gelation is based on the attraction between a positive and a negative charge. The polycationic chitosan form gels with suitable multivalent counter ions like pentasodium tripolyphosphate (TPP), sodium sulfate and sodium citrate.

A solution of the polymer can be prepared with a drug loaded into it. Interactions between the positively charged amino groups and the negatively charged phosphate, are responsible for the gelling, forming the beads (Bodmeier *et al.*, 1989:1483).

Different cross-linking agents can be used depending on the charge carried by the polymer. Calcium chloride can be used with negatively charged polymers like sodium alginate. Sulfhates and citrates can be used with positive charged polymers (Shu & Zhu, 2001: 218). Bodmeier *et al.* (1989:1478) prepared chitosan beads loaded with different drugs (quinidine or sulfadiazine), dissolved or dispersed, (depending on the solubility of the drug) in an aqueous glacial acetic acid (1 % v/v) solution of chitosan (1 % w/w). The polymer-drug solution or dispersion, are dropped through a disposable syringe into a gently agitated tripolyphosphate solution. The bead formed on contact with the tripolyphosphate solution, entrapping the drug within a three-dimensional network of ionically linked polymer.

### 2.3.1.2 Emulsification Ionotropic Gelation

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

Ionotropic gelation can be use in conjunction with certain emulsion techniques. An adaptation of such a method involves a hot carrageenan/oil emulsion dropped into cold water. The method involves high temperatures, which may be incompatible with thermally labile material and have certain disadvantages including uncontrollable particle size and the coagulation of beads into large masses before hardening (Poncelet *et al.*, 1999:171).

To improve on the method, an internal gelation method was proposed in which calcium ions are slowly liberated within the ionic polysaccharides via spontaneous breakdown of gluconolactone, resulting in acidification of an alginate slurry containing a calcium salt. Inconveniences of this method include the cast of the calcium alginate gel since it takes up to 5 minutes for the solution to harden. The gradual hardening renders this method unsuitable for gelation within an emulsion due to aggregation (Poncelet *et al.*, 1999:172).

Poncelet *et al.* (1999:172) presented a method where gelation of a solution is achieved through the rapid release of calcium ions from an insoluble dispersed calcium complex in the aqueous phase. Calcium ion release is initiated via gentle acidification with an oil-soluble acid. Calcium will then be liberated *in situ* from the complex, allowing gelation of alginate. Small beads can be produced under soft conditions at a large scale.

### 2.3.1.3 Extrusion Spheronization

The extrusion spheronization technique is gaining popularity and increasing acceptance in the pharmaceutical industry. The number of excipients available for successful spheronization by this technology is limited (Gosanda *et al.*, 1993:916).

So far, microcrystalline cellulose (MCC) has been used as a universal filler and binder for the extrusion spheronization process. However, alternative materials are being investigated for the purpose to substitute MCC. Chitosan as an alternative filler/binder offers the following advantages: It is as well analyzed as MCC and it is a biodegradable compound from a natural source that has been established in the pharmaceutical and cosmetic industry. Furthermore, chitosan has the potential to be degraded by the colon microflora and could therefore be used for colon targeted delivery of the drugs incorporated in enteric-coated pellets (Berthold *et al.*, 1996:20).

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

The main processing steps of the extrusion spheronization technique are:

- dry powder blending,
- wet mixing,
- extrusion of the wet mass and
- spheronization of the cylindrical extrudate (Neau *et al.*, 2000:130).

For the preparation of chitosan pellets several techniques have been used up to now. In a study conducted by Berthold *et al.*, (1996:20) chitosan beads were produced by dissolving chitosan in an acidic medium and adding a salt containing solution to precipitate chitosan pellets. In another study, Tapia *et al.* (1993:215) dissolved chitosan in diluted acetic acid and added it as granulation liquid to the powder mixture. The mass was pressed through a ram extruder and afterwards spheronized to pellets.

Goskonda *et al.* (1993:917) described the production of chitosan pellets, using the extrusion spheronization with colloidal MCC, which is a spray-dried mixture of 89% microcrystalline cellulose and 11% carboxymethyl cellulose sodium. The quantity of chitosan in the pellets did not exceed 40% of the total solid content. They also noticed that the production of pellets with pure MCC was not possible with the extrusion spheronization technique.

### 2.3.1.4 Melt Solidification

The melt solidification/melt dispersion technique basically involves emulsification of the molten mass in the aqueous phase followed by its solidification by chilling. Wax, a common carrier in various melt techniques, contains a wide group of chemicals such as glycerides, fatty acids, fatty alcohols and their esters. These are widely used as release retardants in the design of sustained release beads, tablets, suspensions, implants and microcapsules. The advantages of waxes include good stability at varying pH-values and moisture levels, well-established safe application in humans due to their non-swellable and water insoluble nature, minimal effect on food in the gastrointestinal tract and no dose dumping (Obaidat & Obaidat, 2001:231).

The melt solidification technique was used by Maheshwari *et al.* (2003:57) and Paradkar *et al.* (2003:34) for the preparation of ibuprofen-cetylalcohol beads and for the preparation of ibuprofen beads, respectively. The drug or drug complex was melted and stirred on a water bath, maintained at 80 °C, to form a uniform molten mass. The melt was then poured into 100 ml water, maintained at 5 °C, and was then stirred continuously using a constant speed stirrer with a propeller blade to obtain beads. The beads formed in this process were separated by filtration and dried at room temperature.

### 2.3.1.5 Electric Dispersion of Polymer Solutions

A method that uses electrostatic potential to prepare beads has been described by Poncelet *et al.* (1999:218). This method has been widely exploited in the industry and has been used for the production of microcapsules on the same principle that micro beads are prepared.

This method involves the dropwise addition of a polymer solution into a solidifying bath. By applying an electrostatic potential between the droplet formation device and the collecting solution, it is possible to obtain smaller droplets which are desirable for many applications. Droplet formation may be divided into two phases. Under a certain critical

value for the electrical potential ($U_c$), liquid exits the nozzle as droplets. The surface tension decreases with increasing potential, resulting in a reduction of droplet diameter to approximately 200 µm. At a higher electric potential, the liquid exits the nozzle as a jet stream which subsequently breaks into droplets which are smaller than 200 µm. In this case, droplet size is mainly determined by the jet instability (theory of Rayleigh) (Poncelet *et al.*, 1999:218).

## 2.4 Characterization of Beads

Beads can be characterized by means of drug loading capacity, morphology studies, friability, swelling and degradation, solubility and drug release profiles.

### 2.4.1 Drug Loading Capacity

Only a certain amount of drug can be incorporated into beads. Thus for beads to be of pharmacotherapeutic advantage as drug carriers, they must be able to incorporate adequate amounts of the drug. It is also important to know the amount of drug incorporated or loaded into such particles in order to determine the amount of product needed per prescribed dose (Gupta & Ravi Kumar, 2000:1115).

Drug loading capacity tests, also called entrapment studies (Alsarra *et al.*, 2002:639) or drug incorporation studies (Mi *et al.*, 2002:61), are mostly conducted by employing a UV spectrophotometer. Gupta & Ravi Kumar (2001:641) described a method whereby a sample of drug loaded beads was accurately weighed and kept in aqueous glacial acetic acid solution (2% v/v) at 30 °C for 48 hours. After centrifugation, the drug concentration 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:

$$\%DLC = \frac{\text{Total amount of drug} - \text{Free amount of drug}}{\text{Weight of the beads}} \quad (2\text{-}1)$$

Lubbe (2002:67) used the following method to determine the drug loading of rifampicin containing chitosan beads. After removal of the beads the water used to wash the excess TPP from the beads was added to the TPP solution used in the preparation of the beads. A sample was taken and the rifampicin concentration was determined by UV spectrophotometric analysis. The amount of drug lost in the TPP solution and during the washing process was calculated. By subtracting the amount of drug lost to the TPP solution during the preparation of the beads from the initial amount of drug in the polymer solution, the percentage drug loading was calculated with the following equation:

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

where $M_0$ is the original amount of drug in the prepared solution and $M_s$ is the amount of drug determined in the TPP solution and water used to wash the beads.

## 2.4.2 Morphology: Scanning Electron Microscopy

Lubbe (2002:48) was able to examine the surface topography of chitosan beads and with the cross-sectional views he was able to describe the pore nature of the bead matrix with scanning electron microscopy (SEM). Cross-sections were obtained by cutting the samples with a razor blade, much in the same way as Bodmeier *et al.* (1989:1479).

Samples in this study were also examined by scanning electron microscopy. Samples were mounted on aluminium sample mounts and coated with gold palladium alloy to minimize surface charging. A Philips FEI Quanta 200 scanning electron microscope (Philips, Netherlands) was used.

### 2.4.3 Friability

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

#### 2.4.3.1 Method

Friability studies on bead samples were conducted as follows for the study. Samples of the beads were taken from each batch, dusted with compressed air and accurately weighed. The samples were placed in the drum of a Roche® friabilator, which was then operated for 4 minutes at 25 rpm, thus for a total of 100 revolutions. The samples were dusted and weighed again. The percentage friability was determined using the following equation:

$$\% \text{ Friability} = [(W_0 - W_t)/W_0] \times 100 \qquad (2\text{-}3)$$

where $W_0$ is the weight of the sample before testing and $W_t$ the weight of the sample after testing. Tests were run in triplicate and the average value was taken as the percentage friability value.

## 2.4.4 Solubility

Gupta *et al.* (2001:641) described a method by which solubility of chitosan beads can be determined by weighing a sample of beads. The samples were weighed and then immersed in a 2 % v/v aqueous solution of acetic acid for 24 hours and the percentage solubility **(%S)** was then determined using the following equation:

$$\%S = [(W_0 - W_t)/W_0] \times 100 \qquad (2\text{-}4)$$

where $W_0$ is the initial weight of the beads and $W_t$ is the weight of vacuum-dried beads after immersion for 24 hours in the acetic acid solution. All solubility testing was done in triplicate and the average value was taken as the percentage solubility value.

## 2.4.5 Swelling and Degradation

The swelling and degradation studies were conducted in much the same manner as Gupta *et al.* (2001:641) and Mi *et al.* (2002:63). A known amount of sample (100 mg) was weighed and placed in 10 ml of the media (PBS pH 5.60 and PBS pH 7.40 at 37 °C) for the required time. The swollen beads were collected at time intervals of 10, 60 and 360 minutes. The net weight of the sample was determined by first blotting the sample with filter paper to remove additional water on the surface of the sample, and then weighing immediately on an electronic balance.

The degree of swelling of the beads in the media was then calculated using the following formula:

$$\textbf{Degree of swelling } (E_{sw}) = [(W_t - W_0)/W_0] \quad (2\text{-}5)$$

where $E_{sw}$, is the degree of swelling of the bead sample at a specific time; $W_t$, is the weight of the beads at equilibrium swelling and $W_0$ is the initial weight of the sample. Each experiment was conducted in triplicate and the average value was taken as the percentage swelling value.

## 2.4.6 Dissolution

The dissolution studies on all the bead formulations were performed in phosphate buffered solution (PBS) at a pH of 5.60 and a pH of 7.40 at 37 °C. PBS was prepared according to the USP (2002:XXI). The dissolution method used to determine the drug release consisted of *apparatus 1* in the USP (2002:1243). The basket stirring element was used.

An Erweka DT6R (Erweka® Apparatebau GmbH, Germany) dissolution apparatus was used. An amount of 25 mg of the beads was weighed (Precisa 240A, Precisa balances, Zürich, Switzerland) and placed in the basket. The basket was lowered into 1000 ml dissolution medium and rotation was started after the basket was immersed into the medium. The rotation speed was kept constant at 50 revolutions per minute.

Samples of 10 ml were withdrawn through 3 μm membrane filters (Millipore, Bedford, Marlborough, England) to eliminate any suspended particles. Samples were withdrawn at time intervals of 2, 5, 10, 15, 20, 30, 45, and 60 minutes after which samples were taken at 2, 3, 4, 5, and 6 hours. After each withdrawal 10 ml of fresh preheated dissolution medium was added to the dissolution medium in the dissolution bath.

All samples were run in triplicate and assayed spectrophotometrically, using a Unicam® spectrophotometer (Unicam, Cambridge, UK) at a wavelength of 268 nm. The following equation was used to correct for dilution of the dissolution media after each sample withdrawal and addition of pure medium:

$$Y_n^* = Y_n + \frac{V_s}{V_m}\sum^{n-1} Y^* \qquad (2\text{-}6)$$

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

The drug concentration of the measured samples was determined using a standard curve.

### 2.4.6.1 Standard Curve

A standard curve was constructed before each dissolution study as well as before each drug loading capacity study (See Figure 2-2). Before every drug loading capacity study two stock solutions of different concentration were prepared. The first stock solution was prepared by dissolving an amount of 25 mg pyrazinamide in 250 ml deionised water, giving a concentration of 100 µg/ml. The second stock solution was prepared by dissolving an amount of 50 mg pyrazinamide in 250 ml deionised water giving a concentration of 200 µg/ml. The two stock solutions were then placed in an ultrasonic bath for 10 minutes after which dilutions were made. Through dilution, standard solutions with concentrations of 6, 10 and 14 µg/ml from the first stock solution and concentrations of 4, 8, 12, 16 and 20 µg/ml from the second stock solution, were prepared. The UV-absorbencies were analyzed spectrophotometrically using a Helios α Unicam spectrophotometer (Unicam, Cambridge, UK) at a wavelength of 268 nm with deionised water as blank.

An example of a standard curve is shown in Figure 2-2:

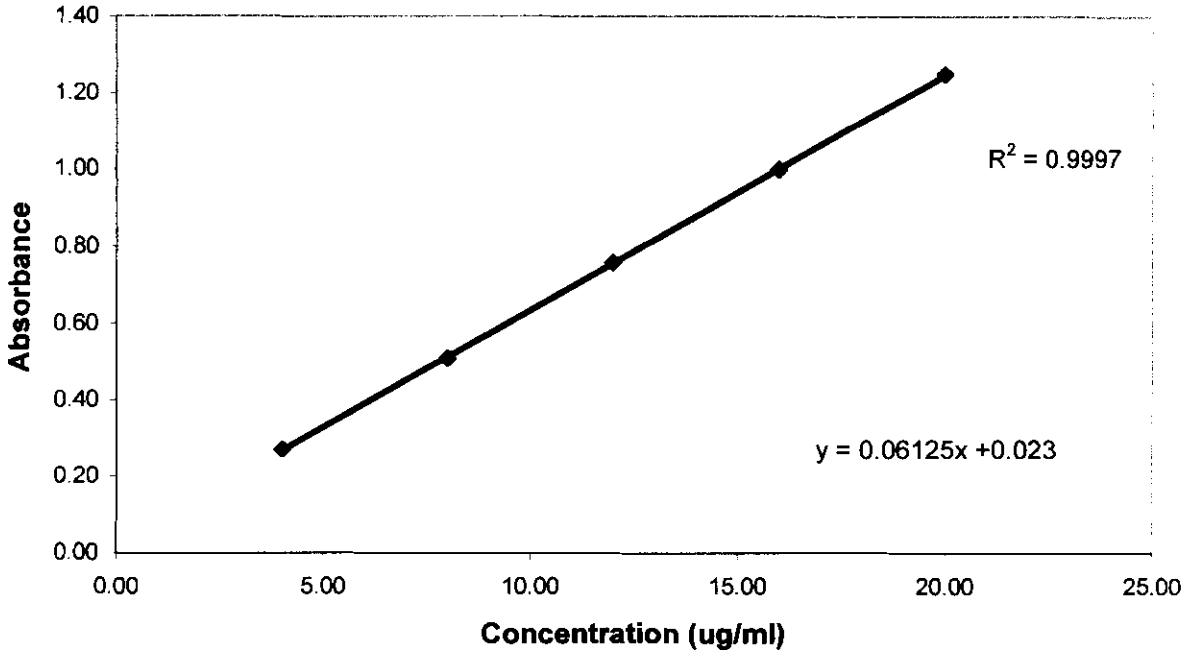


**Figure 2-2:** Example of a standard curve plotted for pyrazinamide in deionised water used in drug loading studies.

Before each dissolution study stock solutions were prepared in both PBS pH 5.60 and PBS pH 7.40. A concentration of 200 µg/ml was prepared by dissolving an amount of 50 mg pyrazinamide in 250 ml of each of the mediums. The stock solutions were then placed in the ultrasonic bath for 10 minutes after which dilutions were made. Standard solutions with concentrations of 4, 8, 12, 16 and 20 µg/ml were prepared for the PBS pH 5.60 and PBS pH 7.40. The UV-absorbencies were then determined spectrophotometrically at a wavelength of 268 nm with either one of the applicable pure mediums as blank.

Graphs of absorbencies versus concentration were plotted and the best straight line through the data points was selected using linear regression (See Figure 2-2). The employed absorbency range obeyed Beer's law over the entire concentration range with a correlation coefficient of $(r^2) \geq 0.9997$ for pH 5.60 and PBS pH 7.40. The slope (m) and the y-intercept (c) were used to calculate the pyrazinamide concentration in each sample using linear regression.

the y-intercept (c) were used to calculate the pyrazinamide concentration in each sample using linear regression.

### 2.4.7 Calculations

All calculations during the study were done using Microsoft® Excel 2003 for Windows (Microsoft® Corporation, Seattle, Washington, USA).

## 2.5 Peparation of Beads for the Study

### 2.5.1 Manipulation of the Method to Achieve Optimal Drug Loading.

For the preparation of pyrazinamide-chitosan controlled release beads, the method based on that of Bodmeier *et al.* (1989:1478) was used. During the development of an optimal method, some problems were encountered. Drug loading was too low, the beads had a high friability and the appearance of the beads did not meet the requirements. Therefore the method had to be adjusted in order to produce beads sufficient for further study. The pH of the TPP solution, TPP-solution to chitosan drug dispersion ratio and the concentration of the chitosan, pyrazinamide and TPP were all investigated to obtain the optimal method to prepare pyrazinamide-chitosan beads.

### 2.5.2 Optimal Concentration of Chitosan

The effect of different concentrations of chitosan had to be evaluated. The chitosan-drug dispersion were prepared by dispersing 3% w/v pyrazinamide in different chitosan solutions (1, 2, 3 and 4% w/v). The chitosan solutions were prepared in deionised water containing 2% v/v glacial acetic acid. Glacial acetic acid was added in order to dissolve the chitosan. The solutions were then stirred until complete dissolution of the chitosan and left overnight until it was bubble free.

Beads were initially prepared by pumping 10 ml of the chitosan-drug dispersion through a gauge 21 needle into 100 ml of a 5% w/v gently agitated TPP-solution using a Watson-Marlow 205S peristaltic pump (Watson-Marlow Ltd., England). Beads formed spontaneously on contact with the TPP-solution. The beads were stirred gently for 30 minutes, to allow for cross linking to occur and then separated by filtration. The beads were then washed twice with a total quantity of 50 ml deionised water to remove any excess TPP on the surface. The beads were then freeze-dried in a BT 2 K freeze-dryer (Virtis Gardiner, U.S.A).

It was found, however, that beads prepared using 1 and 2% w/v chitosan, had irregular shapes and inadequate resistance to mechanical force. Furthermore, severe difficulties were encountered when beads were prepared from the 4% w/v chitosan solution. The solution had a very high viscosity and the pressure needed to prepare beads from this solution was very high, thus the needle became clogged. It was therefore decided to eliminate these chitosan concentrations from subsequent studies. A concentration of 3% w/v chitosan was consequently selected for subsequent studies.

### 2.5.3 Optimal Concentration of Pyrazinamide

The effect of different concentrations of pyrazinamide was investigated to prepare beads with a sufficient drug loading. Chitosan-drug dispersions containing 1, 2, 3, 4, 5 and 6% w/v pyrazinamide were prepared. The chitosan-drug dispersions were prepared by dispersing the pyrazinamide and chitosan in deionised water containing 2% v/v glacial acetic acid. For al the solutions a chitosan concentration of 3% w/v and a total solution volume of 100 ml were used. The solutions were stirred until complete dissolution of the chitosan and left overnight until it was bubble free. Beads were prepared by pumping the chitosan-drug dispersion through a gauge 21 needle into 100 ml of a 5% w/v gently agitated TPP-solution using a peristaltic pump (Watson-Marlow Ltd., England). Beads formed spontaneously on contact with the TPP-solution. The beads were then stirred, washed and freeze-dried in the same way as mentioned under point 2.5.2.

It was found that the percentage drug loading capacity increased as the concentration of pyrazinamide increased (Figure 2-3). The 6% w/v pyrazinamide dispersion produced beads with the highest drug loading, however, the viscosity of the solution was very high and the needle became clogged too often. It was therefore decided to select the 5% w/v pyrazinamide concentration for further study.

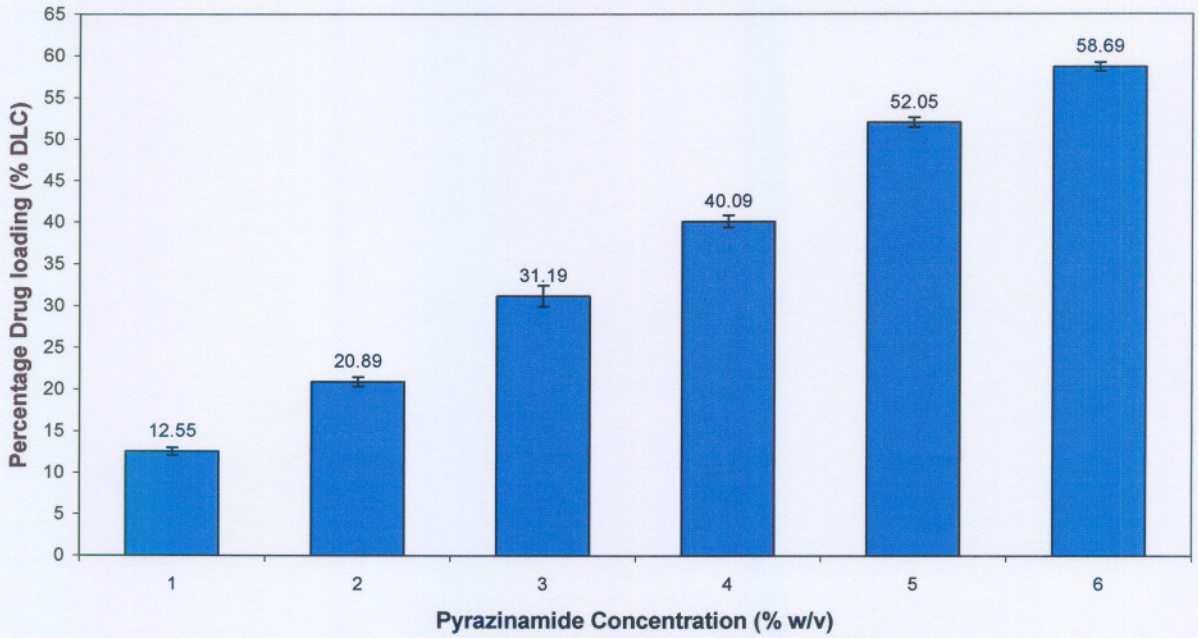


**Figure 2-3:** Drug loading capacity values for different Pyrazinamide concentrations.

## 2.5.4 Determining the Optimal pH and Concentration of TPP

Bodmeier *et al.* (1989:1485) stated that with an increase in TPP concentration during the preparation of sulfadiazine beads, the drug content within the beads decreased. They attributed this observation to the fact that, with an increase in TPP concentration, the solubility of the sulfadiazine increased in the external phase and thus more of the drug leached from the beads. On the basis of this result it was decided to investigate the influence of TPP-concentration in the external solution on drug loading of the pyrazinamide beads.

A dispersion containing 5% w/v pyrazinamide and 3% w/v chitosan in 100 ml deionised water, containing 2% v/v acetic acid, was prepared. The solutions were stirred until complete dissolution of the chitosan and left overnight until it was bubble free. A 3, 4 and 5% w/v TPP-solution was prepared. The chitosan-pyrazinamide dispersion was then added dropwise into the different TPP solutions. Beads formed spontaneously on contact with the TPP-solution. The beads were then stirred, washed and freeze-dried as mentioned under point 2.5.2.

The beads were analyzed with respect to drug loading to determine the optimal pH and TPP-concentration. There was only a slight difference in the drug loading capacity of the beads, prepared at the different TPP-concentrations, as seen in Table 2-1. It was, however, noted that with a decrease in TPP-concentration, beads were formed with irregular shapes and inadequate resistance to mechanical force. The beads formed in the 5% w/v TPP-solution exhibited the highest resistance to mechanical stress. After the surface and cross-sections of the beads were investigated with the scanning electron microscope (SEM) (images not shown), it was clear that an increase in the TPP concentration also led to an increase in the coating of pyrazinamide particles with the chitosan. Beads formed in the 5% w/v TPP-solution had the smoothest surface and was the most spherical without cracks. Therefore the TPP concentration of 5% w/v was selected for further experiments.

**Table 2-1:** Drug loading capacity values for different TPP-concentrations.

| TPP concentration (%w/v) | DLC (%) |
|---|---|
| 3 | 54.68 |
| 4 | 53.23 |
| 5 | 51.85 |

The influence of the pH of the TPP–solution was also investigated. An aqueous TPP-solution generally has a pH (WTW pH 320 SET, Wissenschaftlich-technische werkstätten, Germany) of between 8.89 and 9.10.

Different TPP-solutions over a pH-range of 2-9 were prepared. The pH was adjusted with 0.1 M HCl. The pH of the solutions was measured with a WTW pH 330 pH meter (Weilheim, Germany). A chitosan-pyrazinamide dispersion, containing 3% w/v chitosan and 5% w/v pyrazinamide, was prepared as mentioned in section 2.5.2 and dropped into a 5% w/v TPP-solution, ranging from pH 2 to 9. The beads were stirred gently for 30 minutes, to allow for cross-linking to occur, separated by filtration, washed with deionised water and freeze-dried. The drug loading was determined and the results are shown in Figure 2-4.

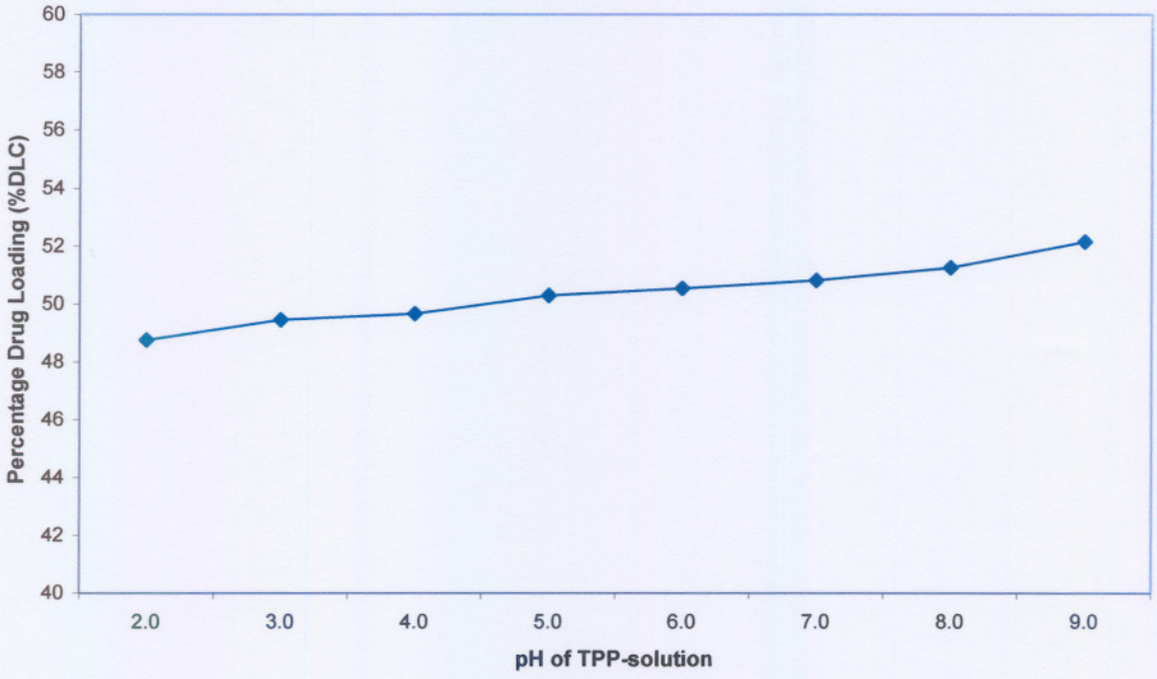


**Figure 2-4:** Effect of TPP-solution pH on percentage drug loading (%DLC).

As seen in Figure 2-4, there is only a slight increase in the drug loading capacity of the beads prepared at different pH-values. However, after the surface and cross-sections of the beads were investigated with the scanning electron microscope (SEM) (images not shown), it was clear that an increase in the pH of the TPP-solution led to an increase in the coating of pyrazinamide particles with the chitosan.

The appearance of the beads also improved with an increase in the pH of the TPP-solution. Therefore it was decided to leave the pH of the solution at the normal pH (pH 8.89 to 9.10) for further experiments.

### 2.5.5 TPP to Chitosan Drug Ratio

Bodmeier *et al.* (1989:1483) also stated that the ratio of chitosan-drug mixture to TPP-phase could affect the drug loading. Therefore the effect of chitosan-drug mixture to TPP-phase ratio was investigated. Five chitosan-pyrazinamide dispersions containing 3% w/v chitosan and 5% w/v pyrazinamide, were prepared as mentioned in section 2.5.2. The solutions were then added dropwise to TPP solutions in ratios of 1:1, 1:2.5, 1:5, 1:7.5 and 1:10 (Chitosan-drug solution:TPP-phase). Beads formed spontaneously on contact with the TPP-solution. The beads were stirred gently for 30 minutes, to allow for cross-linking to occur, and then separated by filtration, washed and freeze-dried as under point 2.5.2. The results are shown in Figure 2-5.

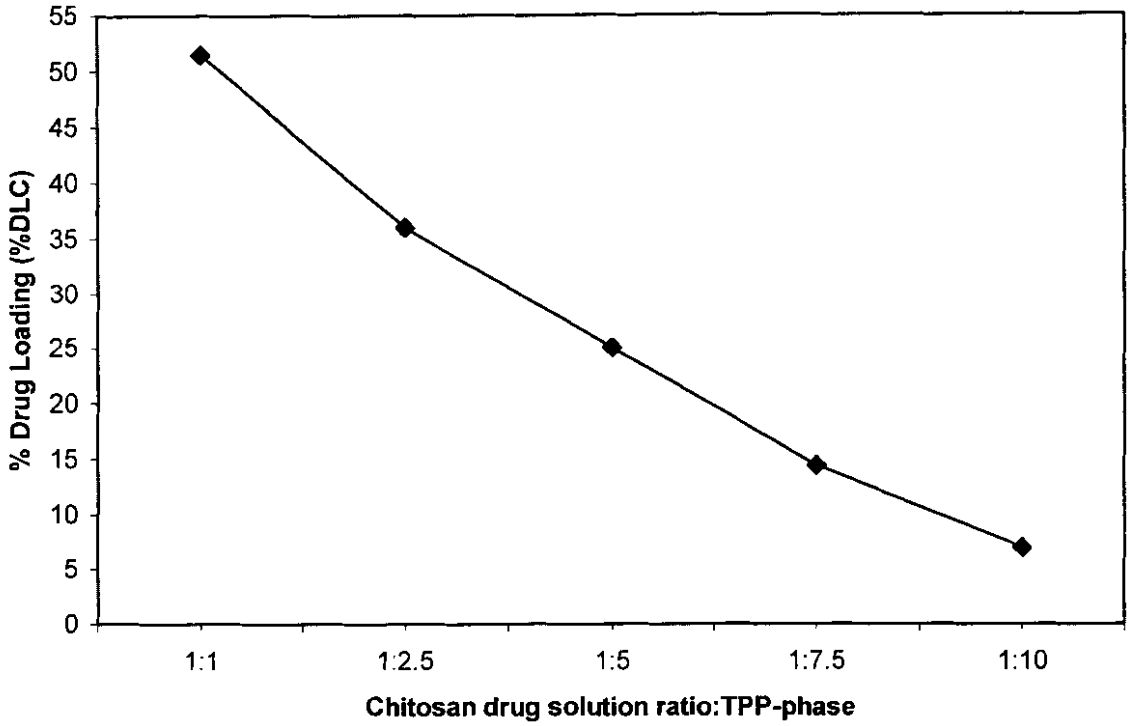


**Figure 2-5:** Effect of chitosan-drug dispersion to TPP-phase ratio on the percentage drug loading (%DLC).

As seen from Figure 2-5, optimal drug loading was achieved at a ratio of 1:1 (chitosan-drug dispersion:TPP-phase) with a %DLC of 51.45%. Therefore the chitosan-drug dispersion: TPP-phase ratio of 1:1 was selected for the optimized preparation method.

The optimize method was as follows: a chitosan concentration of 3% w/v, pyrazinamide concentration of 5% w/v and a TPP concentration of 5% w/v. The pH of TPP was kept at normal pH of TPP (pH 8.89 to 9.10) and a chitosan-drug dispersion: TPP-phase ratio of 1:1 was selected.

## 2.6 Conclusion

The model drug pyrazinamide is well suited for formulation into chitosan beads. By formulating pyrazinamide into chitosan beads it might be possible to achieve controlled release, thereby minimizing the gastrointestinal side-effects of the drug.

Several techniques are used for the preparation of beads which include ionotropic gelation, emulsification inotropic gelation, extrusion spheronisation, melt solidification and electric dispersion. These methods can be modified to give a desired type of bead formulation for a specific pharmaceutical purpose. Ionotropic gelation proves to be a very reliable and simple method for the preparation of beads and is frequently used in the preparation of drug loaded beads.

The method of Bodmeier *et al.* (1998:1478), however, had to be adjusted in order to produce optimal pyrazinamide loaded beads. It was seen that initial problems with low drug loading, high friability and irregular shapes of the beads could be overcome by simple manipulation of the method. A study of the concentration of the chitosan revealed that equally sized round beads, with a suitable mechanical strength, could be easily produced with a concentration of 3% w/v chitosan.

Although this concentration yielded beads with low friability, the drug loading was still not high enough to justify the use of these beads in practice. Therefore the effect of variables, such as the concentration of TPP, the pH of the TPP-solution and the external TPP-phase to internal chitosan drug dispersion phase ratio were evaluated. It was found that these variables had a substantial effect on drug loading and that by simple manipulation of these variables the drug loading could be increased to 52.05%. The optimal conditions were a TPP concentration of 5% w/v with a pH between 8 and 9, (normal pH of TPP) and a ratio of 1:1 for TPP-solution to chitosan drug dispersion.

Methods of characterisation and evaluation of the prepared formulations were also discussed. Beads can be characterized by means of drug loading capacity, morphology studies, friability, swelling and degradation, solubility and drug release profiles. These characteristics are important in order to establish the viability of such formulations as acceptable pharmaceutical dosage forms.

# Chapter 3

# Formulation and Characterization of Chitosan-Pyrazinamide Beads

## 3.1 Introduction

Beads possess some properties that make them a convenient dosage form for optimal drug delivery. These properties of beads and the advantages over conventional dosage forms have been discussed in chapter 1 and 2. In this chapter the formulation and characterization of pyrazinamide loaded chitosan beads containing different pharmaceutical excipients, are discussed.

In a previous study by Mohlala (2004:37), the influence of pharmaceutical excipients (Ascorbic acid, Ac-Di-Sol® and Explotab®) were investigated on chitosan (Chit) beads. Chitosan beads were prepared containing single pharmaceutical excipients (SPE) with different concentrations. After the chitosan/SPE containing beads were characterized by morphology and swelling behaviour, it was found that the pharmaceutical excipients produced beads with increased pore sizes and improved swelling behaviour. Based on these results, a single concentration of each excipient was selected for the preparation of chitosan/multiple pharmaceutical excipient beads (MPE).

The study in producing the MPE beads was continued with 0.25% w/v Ascorbic acid (ASC) and Explotab (EXPL), and 0.5% w/v Ac-Di-Sol (ADS). In the chitosan/MPE containing beads, an inclusion of a combination of excipients led to a higher porosity of the beads compared to the chitosan/SPE beads, although a decrease in the swelling behaviour was observed for the chitosan/MPE beads. Thereafter, rifampicin loaded Chitosan/SPE and Chitosan/MPE beads were prepared with the above mentioned concentrations of the excipients. The rifampicin loaded beads were characterized on the basis of their morphologhy, swelling behaviour and drug loading capacity. The SEM

micrographs showed that rifampicin had an effect on the morphology of the beads. The outer surface of the beads seemed to have changed due to the presence of drug particles on the outer surface of the beads. The swelling behaviour values of rifampicin loaded beads appeared lower compared to drug free beads.

Based on this study by Mohlala (2004:37), it was decided to use the concentrations of the excipients in his study to produce chitosan/SPE and chitosan/MPE beads loaded with pyrazinamide and characterise them on the basis of their morphology, solubility, friability, drug loading capacity and swelling behaviour. If promising results could be obtained with pyrazinamide, it should be possible to combine rifampicin and pyrazinamide in chitosan beads and it could be investigated in future studies in the treatment of tuberculosis.

## 3.2 Study Design

The ionotropic gelation method of Bodmeier *et al.* (1989:1478) was used in this study to prepare blank chitosan beads, chitosan/pyrazinamide beads and both chitosan/SPE and chitosan/MPE beads loaded with pyrazinamide. Tripolyphosphate (TPP) was used as a cross-linking agent. The pharmaceutical excipients and their concentrations used in this study are given in Table 3-1.

**Table 3-1:** Composition of Pyrazinamide loaded chitosan/SPE and chitosan/MPE beads.

| **Chitosan/SPE** | | **Chitosan/MPE** | | | |
|---|---|---|---|---|---|
| **Excipient** | **(%w/v)** | **Excipient** | **(%w/v)** | **Excipient** | **(%w/v)** |
| ASC | 0.25 | EXPL | 0.25 | ADS | 0.5 |
| EXPL | 0.25 | ASC | 0.25 | ADS | 0.5 |
| ADS | 0.5 | ASC | 0.25 | EXPL | 0.25 |

## 3.3 Preparation of Beads for the Study

### 3.3.1 Materials

The following materials were used in the preparation of the different beads: Pyrazinamide (Aventis, Waltloo), Chitosan (91.4% deacetylated) [For COA see Annexure A], glacial acetic acid 100% (Merck, South Africa), tripolyphosphate (Sigma, South Africa), Ac-Di-Sol® (FMC, Ireland), Explotab® (Mendell, England) and Ascorbic acid (Merck, South Africa). All the chemicals used in the study were of analytical grade and were used as received.

### 3.3.2 Method

Chitosan drug loaded beads were produced by dispersing 5% w/v pyrazinamide and 3% w/v chitosan in deionised water, containing 2% v/v acetic acid, to render a total volume of 100 ml. The solutions were stirred until complete dissolution of the chitosan and left overnight until it was bubble free. The chitosan/SPE and chitosan/MPE beads loaded with pyrazinamide were produced much in the same way as the excipient free beads. The pharmaceutical excipients were dissolved/dispersed in the chitosan/pyrazinamide dispersion in the specified concentrations (Table 3-1).

Beads were prepared by pumping the chitosan-drug dispersion through a gauge 21 needle into 100 ml of a 5% w/v gently agitated TPP-solution, using a Watson-Marlow 205S peristaltic pump (Watson-Marlow Ltd., England). Beads formed spontaneously on contact with the TPP-solution. The beads were stirred gently for 30 minutes to allow for cross-linking to occur and then separated by filtration. The beads were then washed twice with a total volume of 50 ml deionised water to remove any excess TPP on the surface. The beads were then freeze-dried in a BT 2 K freeze-dryer (Virtis Gardiner, U.S.A). The freeze-dried beads were then characterized.

## 3.4 Characterization of Beads

Beads were characterized with respect to morphologhy, drug loading, friability, solubility and swelling studies.

## 3.4.1 Morphologhy

### 3.4.1.1 Method

Morphology studies were done with a scanning electron microscope (SEM) as described in Chapter 2 section 2.4.2.

### 3.4.1.2 Results

The different micrographs of the prepared beads obtained with the SEM are represented in Figures 3-1 to 3-14.

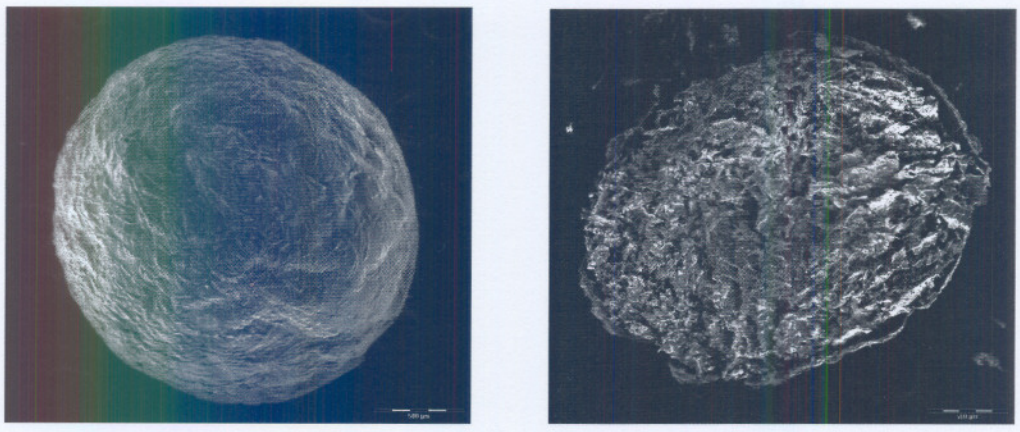

**Figure 3-1:** Full view and cross-section of a plain pyrazinamide (5% w/v) loaded chitosan (3% w/v) bead.

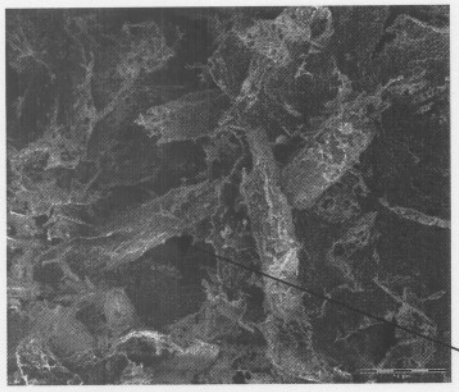


**Figure 3-2:** Microscopic view of drug particles covered with chitosan, inside a cross-cut chitosan (3% w/v) bead.

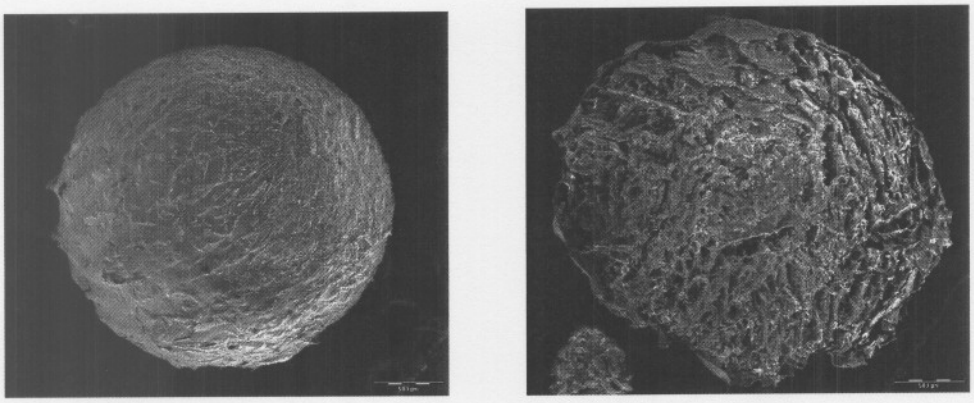

**Figure 3-3:** Full view and cross-section of a pyrazinamide (5% w/v) loaded Chit/ASC bead.

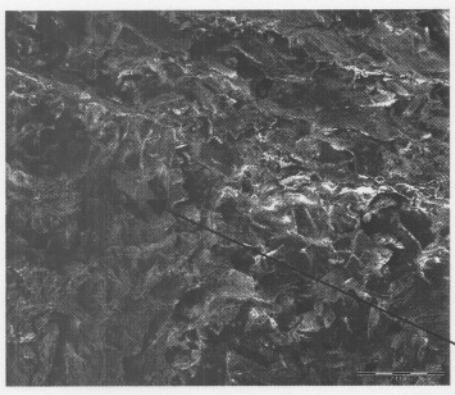


**Figure 3-4:** Microscopic view of drug particles inside a cross-cut Chit/ASC bead.

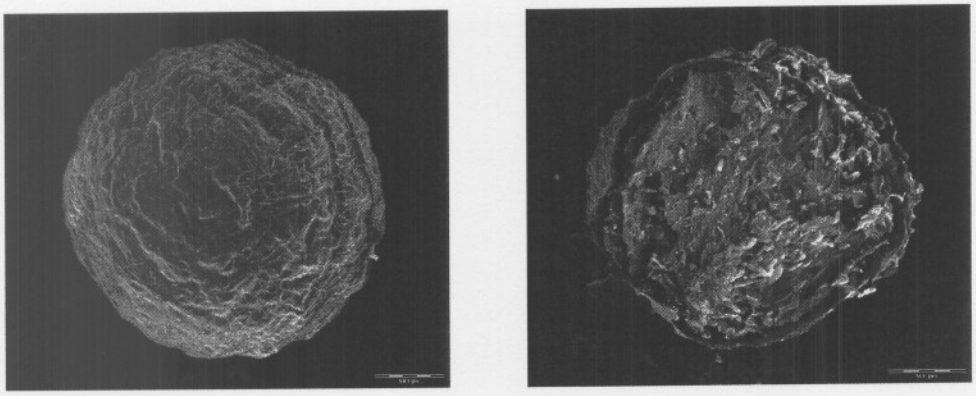

**Figure 3-5:** Full view and cross-section of a pyrazinamide (5% w/v) loaded Chit/EXPL bead.

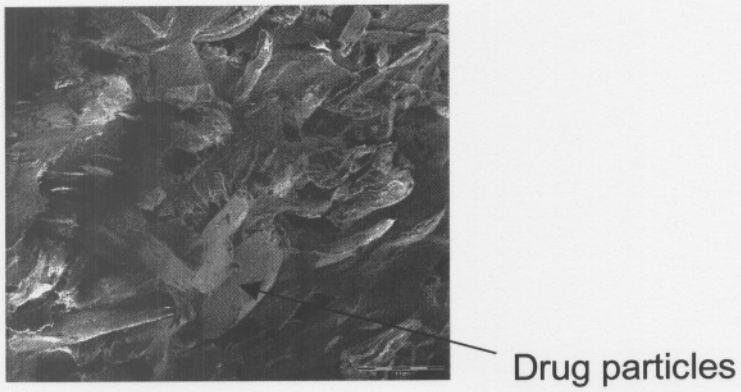


**Figure 3-6:** Microscopic view of drug particles inside a cross-cut Chit/EXPL bead.

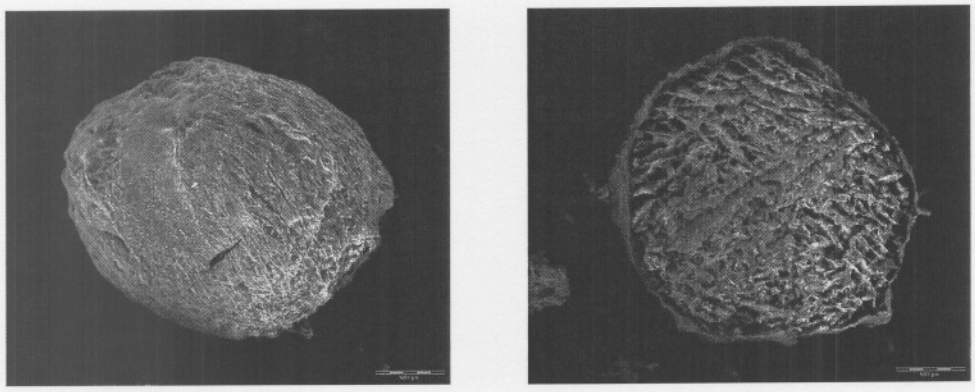

**Figure 3-7:** Full view and cross-section of a pyrazinamide (5% w/v) loaded Chit/ADS bead.

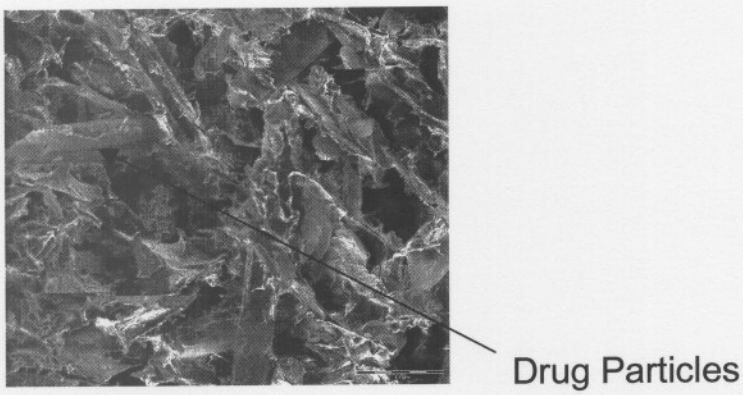


**Figure 3-8:** Microscopic view of drug particles inside a cross-cut Chit/ADS bead.

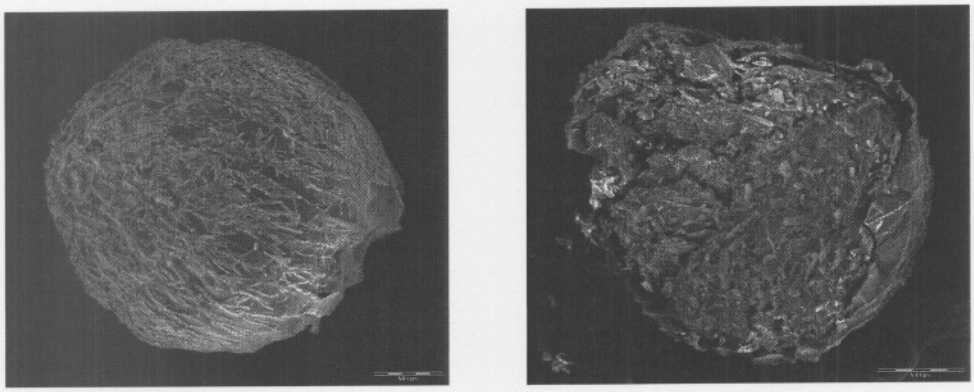

**Figure 3-9:** Full view and cross-section of a pyrazinamide (5% w/v) loaded Chit/ASC/EXPL bead.

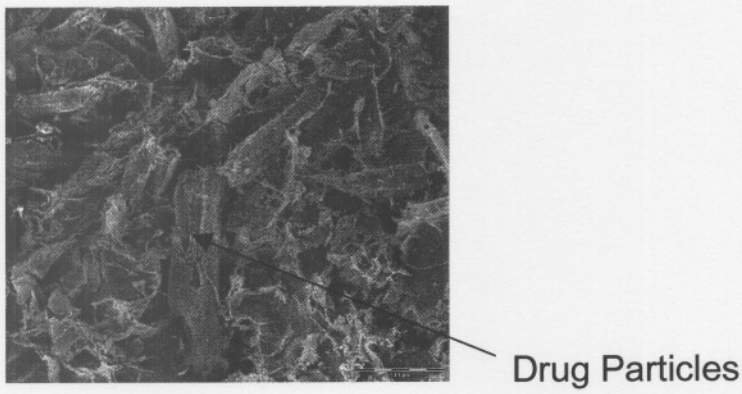


**Figure 3-10:** Microscopic view of drug particles inside a cross-cut Chit/ASC/EXPL bead.

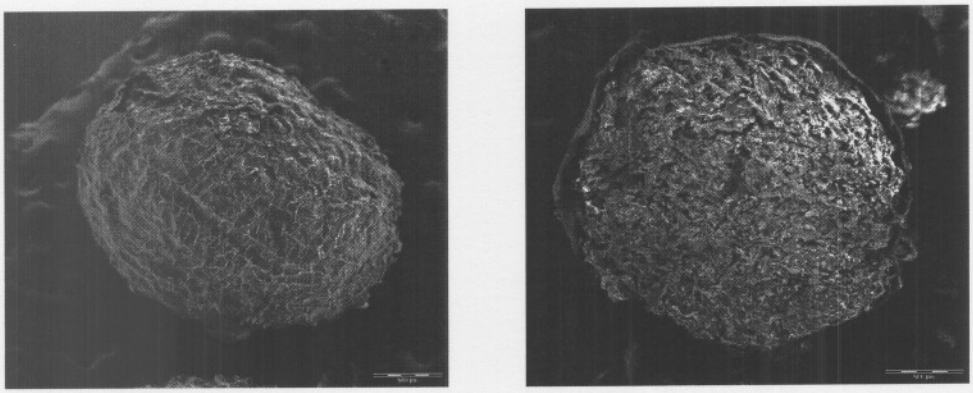

**Figure 3-11:** Full view and cross-section of a pyrazinamide (5% w/v) loaded Chit/ASC/ADS bead.


**Figure 3-12:** Microscopic view of drug particles inside a cross-cut Chit/ASC/ADS bead.

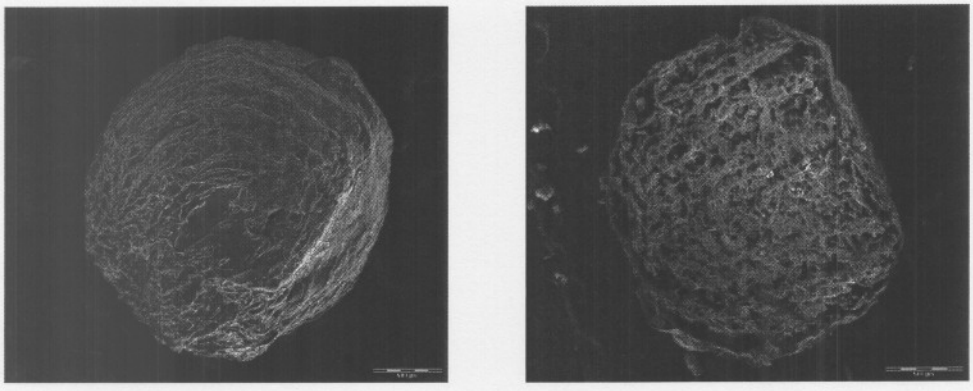

**Figure 3-13:** Full view and cross-section of a pyrazinamide (5% w/v) loaded Chit/EXPL/ADS bead.

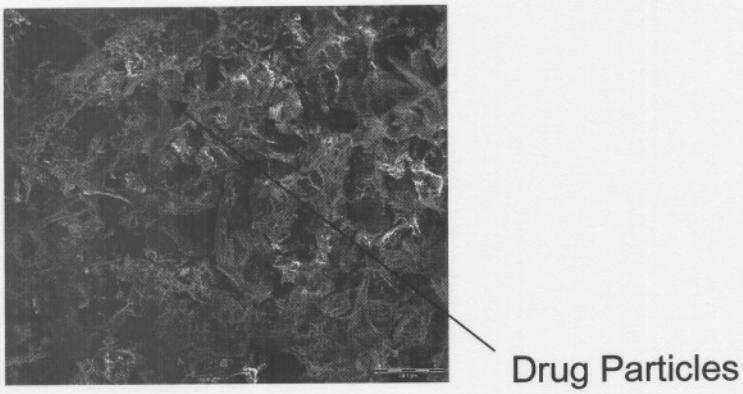


**Figure 3-14:** Microscopic view of drug particles inside a cross-cut Chit/EXPL/ADS bead.

### 3.4.1.3 Discussion

All of the bead formulations prepared for the study, had a diameter of between 1-2 mm and the shape of the beads remained almost spherical. The outer surface views of the different bead formulations differed from one formulation to the other. The surface views of all of the bead formulations revealed the presence of crystals on the surface of the beads. After closer investigation it was found that the crystals were in fact remaining TPP-crystals. This can be the result of using not enough water to wash the beads after filtration at the time of preparation of the beads. The plain pyrazinamide loaded chitosan beads (Figure 3-1) and the Ascorbic acid loaded chitosan beads (Figure 3-3) had the smoothest outer surfaces compared to the other bead formulations. The inclusion of Explotab produced beads with a wavy surface, as seen in Figure 3-5, Figure 3-9 and Figure 3-13 and the inclusion of Ac-Di-Sol led to beads with a cracked surface, as seen in Figure 3-7, Figure 3-11 and Figure 3-13.

The cross-section of the beads also differed from bead formulation to bead formulation. The internal structure of the different bead formulations were all porous with some more porous than the other. As seen in the SEM photos, all the bead formulations that contained Ascorbic acid, produced beads with a denser structure compared to beads produced with Ac-Di-Sol and Explotab. The denser structure may lead to a lower degree of swelling as water penetration could be more difficult in the case of a denser bead.

Bead formulations containing Ac-Di-Sol and Explotab, revealed beads with a more porous nature and this can be favourable for swelling and dissolution studies. The dissolution medium can penetrate the beads easier, reaches the drug particles quickly and this can lead to quicker dissolution of the drug. It also seems like the inclusion of Ac-Di-Sol gave the beads a flaky appearance compared to beads with Explotab where the beads have a plate-like structure.

From the microscopic view it can be seen that the drug particles are the best coated with the chitosan in the pure pyrazinamide/chitosan beads and the bead formulations containing Explotab are coated fairly. The drug particles are coated to a small extend with the chitosan in the bead formulations containing Ac-Di-Sol and Ascorbic acid. Good matrix forming was however noticed for the solid parts of all the beads with good dispersion of the different particles throughout the bead structure.

## 3.4.2 Drug Loading

### 3.4.2.1 Method

The quantity of pyrazinamide incorporated in the beads was determined by spectrophotometric analysis. An amount of each bead formulation was powdered in a mortar. An accurately weighed amount of 20 mg of each sample was then transferred to a 100 ml volumetric flask after which the flasks were made up to volume with deionised water. The mixtures were then stirred for 2 hours on a magnetic stirrer (Labcon, Laboratory marketing services, Maraisburg, South Africa) and then sonicated for a further 120 minutes. From every sample 10 ml was extracted and made up to volume in a 100 ml volumetric flask. Samples were then taken from the flask, filtered and analysed spectrophotometrically, using a Helios α Unicam spectrophotometer (Unicam, Cambridge, UK) at a wavelenght of 268 nm.

The absorbance values were used to calculate the quantity of pyrazinamide (experimental value) in each sample of the beads. This experimental values were then compared to the theoretical values of the beads to obtain the % drug loading of the bead formulations. The percentage drug loading was calculated according to the following equation:

$$\% \text{ Drug Loading} = \frac{\text{Experimental value}}{\text{Theoretical value}} \times \frac{100}{1} \qquad (3\text{-}1)$$

### 3.4.2.2 Results

The results of the drug loading (%DLC) of the bead formulations are given in Table 3-2 and graphically depicted in Figure 3-15.

**Table 3-2:** Drug loading values (average ± SD) of the bead formulations.

| Bead Formulation | % Drug Loading |
|---|---|
| Chitosan | 52.26 ± 0.57 |
| Chit/ASC | 59.60 ± 0.53 |
| Chit/EXPL | 58.06 ± 0.29 |
| Chit/ADS | 60.25 ± 0.45 |
| Chit/EXPL/ADS | 66.95 ± 0.78 |
| Chit/EXPL/ASC | 65.31 ± 0.52 |
| Chit/ADS/ASC | 67.09 ± 0.22 |

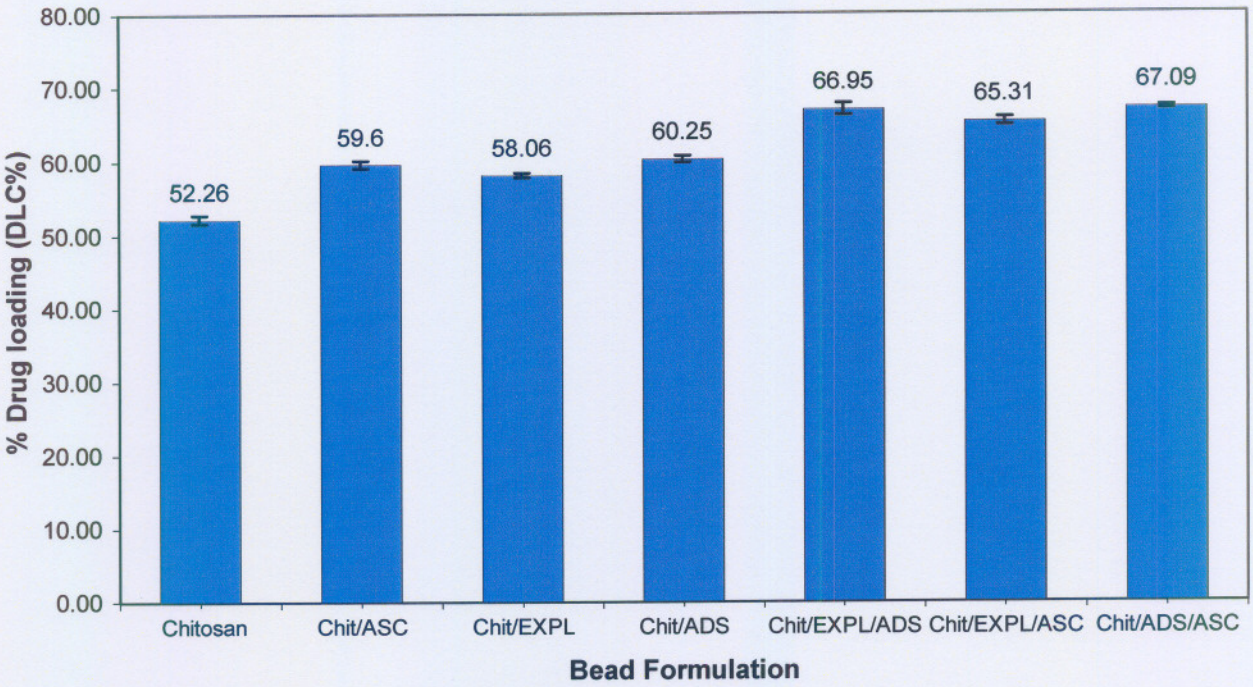


**Figure 3-15:** Graphic representation of percentage drug loading (%DLC) (average ± SD) of the bead formulations.

### 3.4.2.3 Discussion

The drug loading of the plain pyrazinamide loaded chitosan beads was 52.26 ± 0.57%. It was noted that the inclusion of excipients in the beads resulted in an increase in drug loading. The increase was, however, dependent on the type and combination of the excipients added. As seen in Table 3-2 and Figure 3-15, the chitosan/SPE beads showed an increase in the drug loading but the highest drug loading were obtained with chitosan/MPE beads.

The increased drug loading, seen in the case of the SPE and MPE beads, could possibly be explained by the increase in the viscosity of the chitosan/pyrazinamide dispersion, as was seen by visual examination. The combination of the pharmaceutical excipients in the MPE bead formulations possibly resulted in a more pronounced increase in the viscosity of the chitosan/pyrazinamide dispersion, causing a slower leaking of the drug into the TPP-phase. This slower leaking is the probable reason for the higher drug loading of the MPE bead formulations, compared to the SPE bead formulations.

## 3.4.3 Friability

### 3.4.3.1 Method

Friability studies were conducted as a measurement of mechanical strength of the beads and to evaluate the friability of the formulations as dosage forms. The tests were performed as described in chapter 2 section 2.4.3 and the values determined using equation 2-3.

### 3.4.3.2 Results

The friability results of the bead formulations are presented in Figure 3-16.

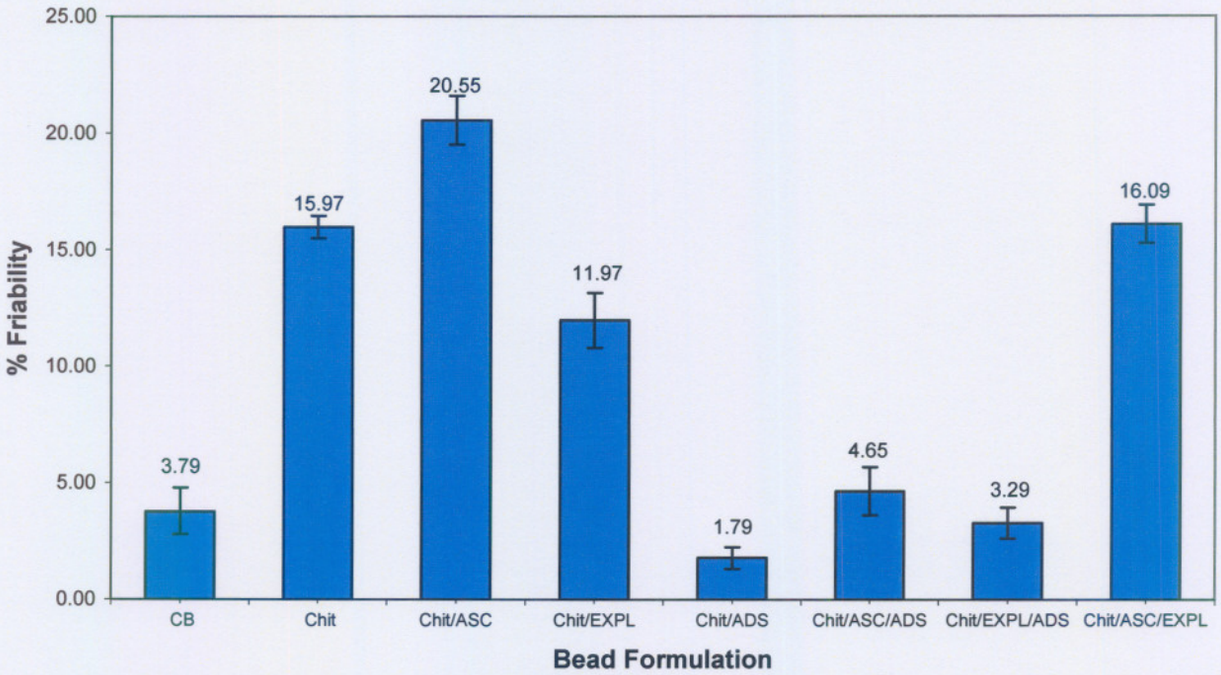


**Figure 3-16:** Percentage friability of bead formulations.

### 3.4.3.3 Discussion

According to the USP (2002:39), friability values of ≤ 1 % are ideal for tablets. There is, however, no official friability value for beads. Blank chitosan (CB) beads (containing no pyrazinamide) were quite hard and had a friability of 3.79 ± 0.99%. The friability of the pyrazinamide loaded chitosan (Chit) beads was 15.97 ± 0.48%. These beads were quite soft and broke easily when handled. It seems like the inclusion of pyrazinamide into the chitosan bead led to a softening in the beads. (The softer bead structure could be attributed to less cross-linking as a result of the pyrazinamide content). The inclusion of the different excipients into the bead structure resulted in a varying effect, dependent on the type and combination of excipients used. As seen in Figure 3-16, additions of Ascorbic acid and the combination of Ascorbic acid and Explotab to the beads led to an increase in the friability of 20.55 ± 1.05% and 16.09 ± 0.82% respectively. This level of friability is quite unacceptable and it was noticed that a large number of beads broke during handling.

The addition of Ac-Di-Sol, Explotab, Ac-Di-Sol/Ascorbic acid and Ac-Di-Sol/Explotab, however led to a decrease in the friability of the beads. The low friability values of the beads containing Ac-Di-Sol are difficult to explain and need further investigation in future studies. These beads were quite hard and minimal breakage appeared during handling. The lowest friability of 1.79 ± 0.47% was obtained with the addition of Ac-Di-Sol to the pyrazinamide/chitosan beads.

## 3.4.4 Solubility

### 3.4.4.1 Method

Solubility of the beads was determined according to the method of Gupta *et al.* (2001:641) as described in chapter 2 section 2.4.4 and the values were determined, using equation 2-4.

### 3.4.4.2 Results

The solubility results of the different bead formulations are presented in Figure 3-17.

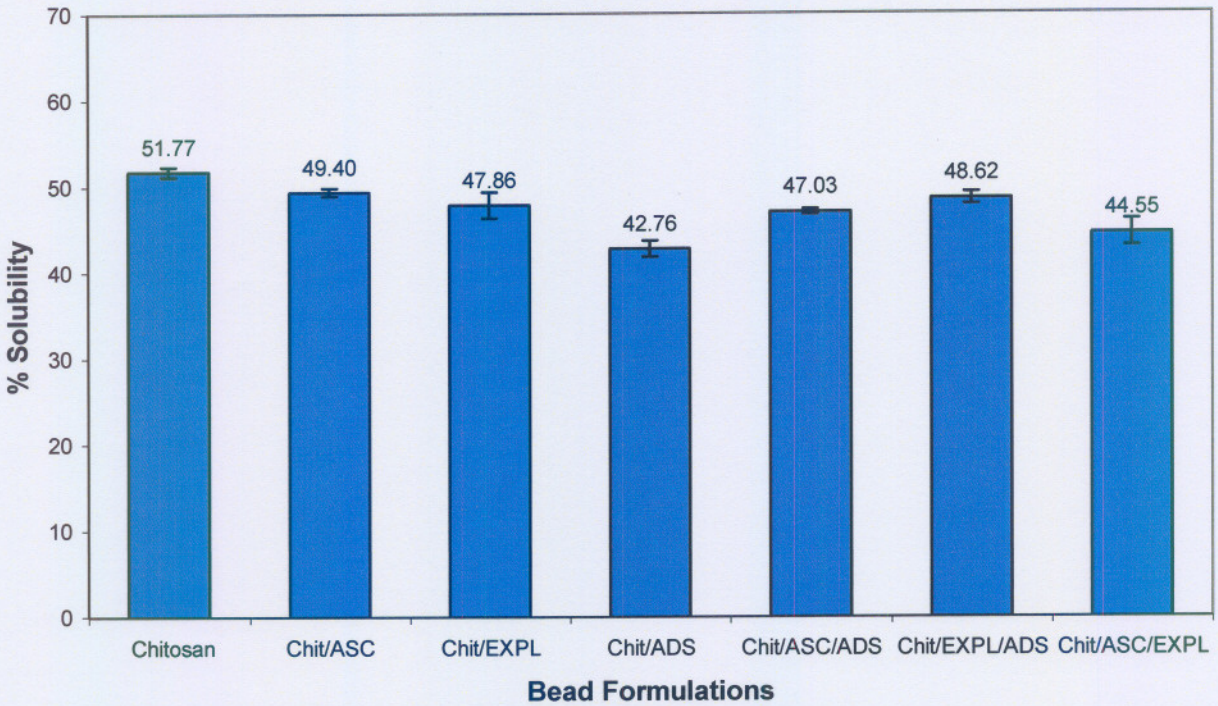


**Figure 3-17:** Percentage solubility (average ± SD) of bead formulations.

### 3.4.4.3 Discussion

According to Gupta *et al.* (2001:641) the solubility of beads is an indication of the degree of cross-linking, with better cross-linked beads having lower solubility than poorly cross-linked beads. Thus, from Figure 3-17 it is evident that the high solubility is probably the result of poor cross-linking of the beads. From Figure 3-17 it also can be seen that the solubility of the beads is dependent on the type and combination of excipients added. Chitosan/pyrazinamide beads had the highest solubility (51.77 ± 0.57%), compared to beads loaded with excipients.

The solubility of the Chit/ASC bead formulation was comparable to the solubility of the plain chitosan bead formulation (49.40 ± 0.49% vs 51.77 ± 0.57%). The inclusion of Explotab caused a small decrease in the solubility, but this decrease was, however, not statistically significant (Tukey HSD test, $p > 0.05$) (See Annexure B). The inclusion of Ac-Di-Sol caused a more pronounced decrease in the solubility of the bead formulations with the lowest solubility exhibited by the Chit/ADS bead formulation (42.76 ± 0.92%). The higher concentration of the Ac-Di-Sol compared to the Explotab (0.5 vs 0.25%) could account for the lower solubility of the Chit/ADS formulation, compared to the Chit/EXPL formulation. Although the combination of Ac-Di-Sol and Explotab caused a decreased bead solubility, the decreased solubility was not synergistic as one would expect from the solubility results from the single Chit/ADS and Chit/EXPL formulations.

Explotab (sodium starch glycolate) is a modified starch and Ac-Di-Sol is a cross-linked form of sodium carboxymethylcellulose (croscarmellose sodium). Both of these excipients have a high affinity for water, which results in rapid tablet disintegration, and Ac-Di-Sol is referred to as a super-disintegrant (Lieberman *et al.,* 1989:109). The addition of these two excipients also led to an increase in the viscosity of the pyrazinamide/chitosan dispersion. Thus, the lower bead solubility of Ac-Di-Sol/Chitosan beads (42.76 ± 0.92%) could be the result of the inclusion of the viscosity increasing excipients.

The addition of Ascorbic acid to Ac-Di-Sol (Chit/ASC/ADS beads) enhanced the bead solubility and led to a higher solubility of 47.03 ± 0.33% compared to the 42.76 ± 0.92% of chit/ADS beads. Ascorbic acid lowers the pH of the dispersion. Chitosan is soluble in a medium with a low pH. As mentioned poor cross-linking causes a higher bead solubility. As a result of this poor cross-linking it is possible that the lowering in pH, caused by the Ascorbic acid in the Ascorbic acid containing formulas, could result in the higher bead solubility of the ascorbic acid formulas. This increased bead solubility was statistically significant (Tukey HSD test, $p < 0.05$) (See Annexure B).

Explotab containing chitosan beads (Chit/EXPL beads) led to a decrease in the solubility but this decrease was not statistically significant (Tukey HSD test, $p > 0.05$). The

combination of Explotab and Ac-Di-Sol lowered the solubility, but this decrease was not statistically significant (Tukey HSD test, $p > 0.05$).

## 3.4.5 Swelling Behaviour

### 3.4.5.1 Method

Swelling tests were performed according to the method described by Gupta *et al.* (2001:641) discussed in chapter 2 section 2.4.5. The tests were conducted in triplicate over 6 hours in a phosphate buffer solution (PBS) at pH 5.60 and 7.40 at 37 °C. Samples of beads (100 mg) were weighed into test tubes and 5 ml PBS was introduced into each test tube. The test tubes were then immersed and left in a water bath set at 37 °C. The weight of the beads after removing the media and excess PBS droplets around the beads, was recorded as $W_t$, the weight of the beads after time $t$. The weight of the beads was determined after 10, 60 and 360 minutes. The swelling behaviour was then expressed as the degree of swelling (Esw) by the following equation:

$$\textbf{Degree of swelling } (E_{sw}) = [(W_t - W_0)/W_0] \qquad (3\text{-}1)$$

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

### 3.4.5.2 Results

The results obtained in the swelling behaviour studies are given in Table 3-3 and depicted graphically in Figure 3-18 and Figure 3-19.

**Table 3-3:** Degree of swelling (Esw) (average ± SD) of bead formulations at pH 5.60 and 7.40.

| Pyrazinamide Loaded Formulations | Degree of Swelling (Esw) | | | | | |
|---|---|---|---|---|---|---|
| | 10 min | | 60 min | | 360 min | |
| | pH 5.60 | pH 7.40 | pH 5.60 | pH 7.40 | pH 5.60 | pH 7.40 |
| CB | 2.44 ± 0.14 | 2.55 ± 0.07 | 2.46 ± 0.18 | 2.55 ± 0.06 | 2.51 ± 0.15 | 2.61 ± 0.08 |
| Chit | 1.89 ± 0.14 | 1.86 ± 0.08 | 1.85 ± 0.04 | 1.70 ± 0.08 | 1.83 ± 0.04 | 1.52 ± 0.11 |
| Chit/ASC | 1.42 ± 0.08 | 1.49 ± 0.08 | 1.50 ± 0.18 | 1.41 ± 0.11 | 1.47 ± 0.04 | 1.38 ± 0.14 |
| Chit/EXPL | 1.75 ± 0.25 | 1.58 ± 0.15 | 1.84 ± 0.06 | 1.75 ± 0.01 | 1.93 ± 0.04 | 1.94 ± 0.04 |
| Chit/ADS | 1.89 ± 0.03 | 1.93 ± 0.08 | 2.00 ± 0.08 | 2.00 ± 0.06 | 2.04 ± 0.07 | 2.08 ± 0.30 |
| Chit/EXPL/ADS | 1.89 ± 0.13 | 2.00 ± 0.14 | 2.00 ± 0.22 | 2.07 ± 0.08 | 2.07 ± 0.13 | 2.09 ± 0.07 |
| Chit/EXPL/ASC | 1.65 ± 0.06 | 1.66 ± 0.08 | 1.59 ± 0.01 | 1.58 ± 0.03 | 1.46 ± 0.04 | 1.50 ± 0.03 |
| Chit/ADS/ASC | 1.78 ± 0.06 | 1.92 ± 0.14 | 1.85 ± 0.04 | 1.82 ± 0.14 | 1.76 ± 0.03 | 1.71 ± 0.13 |

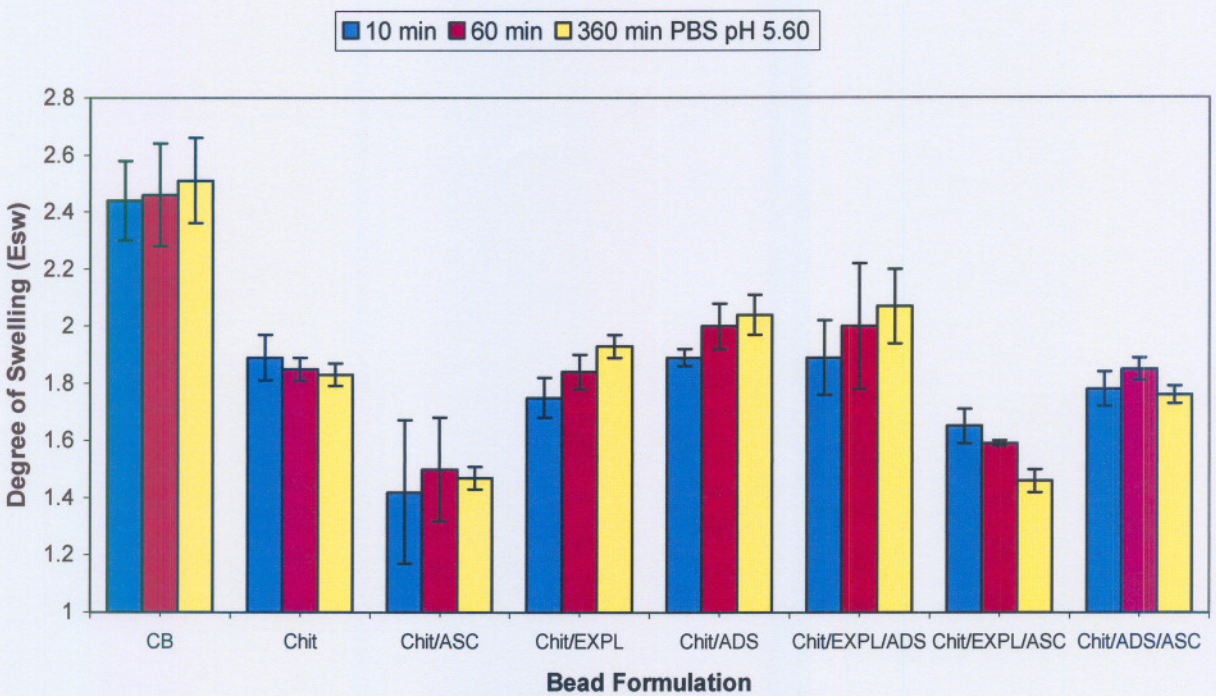


**Figure 3-18:** Degree of swelling of the bead formulations in PBS pH 5.60.

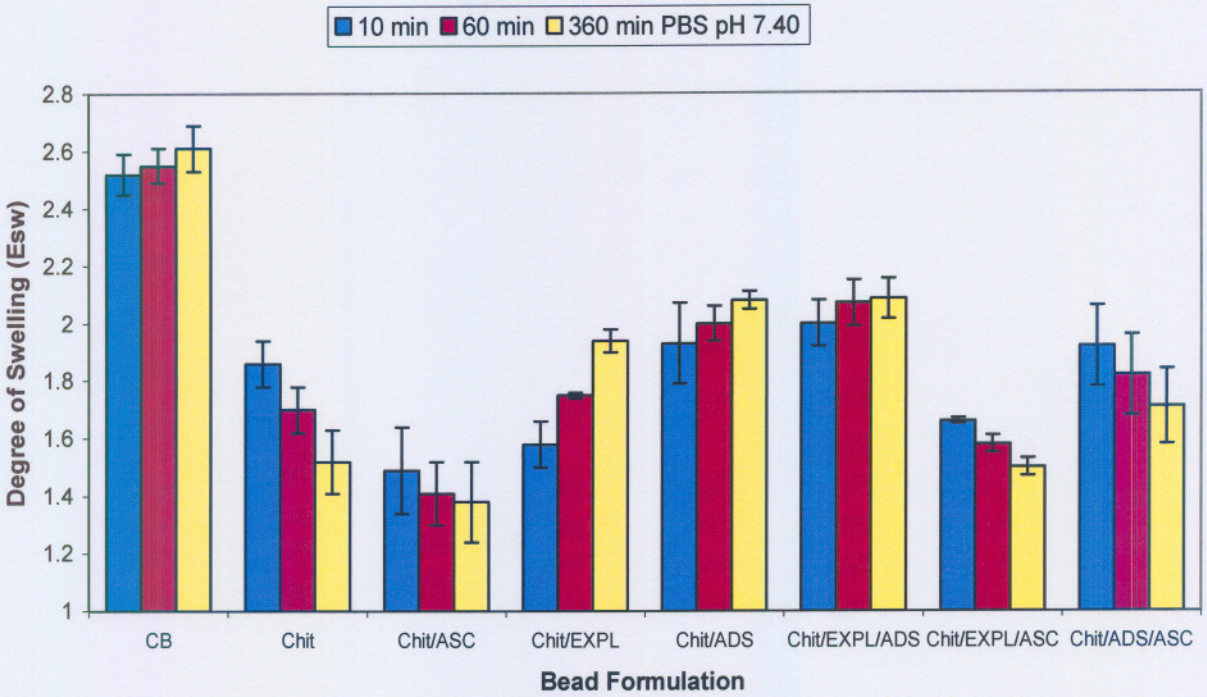


**Figure 3-19:** Degree of swelling of the bead formulations in PBS pH 7.40.

### 3.4.5.3 Discussion

From Figure 3-18 and Figure 3-19 it is evident that blank chitosan beads (CB) showed the best swelling of all the different bead formulations. It is also the only bead formulation with an average degree of swelling above 2.4 in both PBS pH 5.60 and 7.40.

Blank chitosan beads exhibited a degree of swelling of 2.44 ± 0.14 after 10 minutes in PBS pH 5.60. The degree of swelling did not increase statistically significant after the initial swelling of 10 minutes, as the formulation exhibited a degree of swelling of 2.51 ± 0.15 after 6 hours. This tendency is exhibited by all the bead formulations at pH 5.60.

In PBS pH 7.40 the blank chitosan beads exhibited a swelling degree of 2.55 ± 0.07 after the first 10 minutes. The degree of swelling also did not increase statistically significant after the initial swelling of 10 minutes, as the formulation exhibited a swelling degree of 2.61 ± 0.08 after 6 hours.

As seen in Figure 3-18 and 3-19 the swelling behaviour of excipient included beads were dependent on the type and combination of the excipients added. Once again the different type and combinations had different effects on the swelling of the beads. Generally the inclusion of Ascorbic acid into the bead formulations led to shape losing and dissolving of the beads after the first 10 minutes, which resulted in a lower degree of swelling.

The inclusion of Ac-Di-Sol produced a degree of swelling comparable to the degree of swelling exhibited by the chitosan/pyrazinamide bead formulations. This was evident at both pH 5.60 and 7.40. On the SEM photographs in section 3.4.1 it could be seen in general, that the internal structure of the beads containing Ascorbic acid, was very dense and the beads containing Ac-Di-Sol had a very porous structure. Therefore we can say that this is a possible explanation why Ascorbic acid, containing beads exhibited a lower degree of swelling compared to the degree of swelling of the Ac-Di-Sol containing bead formulations.

The degree of swelling of the pyrazinamide/chitosan beads containing only Ascorbic acid (Chit/ASC) was not comparable to the swelling of pure pyrazinamide/chitosan beads in both PBS pH 5.60 and pH 7.40. The degree of swelling was only 1.42 ± 0.08 after 10 minutes in the PBS pH 5.60 and increased only slightly to 1.50 ± 0.08 and after 60 minutes started to lose shape. The same tendency was seen for the formulation at pH 7.40.

The pyrazinamide/chitosan beads containing only Explotab (Chit/EXPL), showed comparable swelling to that of pure pyrazinamide/chitosan beads. There was an increase in swelling up to 6 hours for both PBS pH 5.60 and pH 7.40.

As mentioned earlier, the degree of swelling of the pyrazinamide/chitosan beads containing only Ac-Di-Sol (Chit/ADS), was comparable to the swelling of plain pyrazinamide/chitosan beads over the first 10 minutes in both PBS pH 5.60 and pH 7.40. But up to 6 hours the degree of swelling of the Ac-Di-Sol containing beads, was higher than the pure pyrazinamide/chitosan beads reaching a maximum of 2.04 ± 0.07 in PBS pH 5.60 and 2.08 ± 0.30 in PBS pH 7.40.

The pyrazinamide/chitosan beads containing the combination of Ac-Di-Sol and Explotab, (Chit/EXPL/ADS) showed also comparable swelling to that of pure pyrazinamide/chitosan beads. There was an increase in swelling for both PBS pH 5.60 and pH 7.40 up to 6 hours. The combination of Ac-Di-Sol and Explotab produced the highest degree of swelling of all the pyrazinamide containing bead formulations in both PBS 5.60 and PBS pH 7.40 with a maximum swelling degree of 2.07 ± 0.13 and 2.09 ● 0.07 respectively.

The degree of swelling of the pyrazinamide/chitosan beads containing the combination of Ascorbic acid and Explotab (Chit/ASC/EXPL), was not comparable to the swelling of the pure pyrazinamide/chitosan beads in both mediums. The maximum degree of swelling in both mediums was obtained after the first 10 minutes and thereafter the swelling decreased up to 6 hours.

Most of the excipients containing beads compared good with the swelling standard laid down by the plain chitosan/pyrazinamide beads. Some even increased the swelling degree, but the inclusion of Ascorbic acid did not result in a degree of swelling comparable to the plain chitosan/pyrazinamide beads. It seemed like the beads weakened with the inclusion of Ascorbic acid. In general, all the bead formulations containing Ascorbic acid, lost shape and started to dissolve after 10 - 60 minutes.

In conclusion it can be said that the inclusion of Ascorbic acid in the bead formulations led to a decrease in swelling. The inclusion of Ac-Di-Sol and Explotab produced beads with better swelling abilities. This can be of an advantage in dissolution studies. The increase in swelling of the Ac-Di-Sol and Explotab formulations can possibly be linked to the swelling properties of these excipients in water (both function as swelling disintegrants).

## 3.5 Conclusion

Morphology, drug loading, friability, solubility and swelling studies were all used to characterize the chitosan/SPE and chitosan/MPE beads loaded with pyrazinamide. The morphology studies revealed that the inclusion of Ascorbic acid in the different bead formulations produced beads with a denser internal structure. The inclusion of Ac-Di-Sol and Explotab produced beads with a more porous structure.

The inclusion of the Ascorbic acid (beads with a denser structure) in the different bead formulations, resulted in beads with a lower degree of swelling than the pure chitosan/pyrazinamide beads. In general, the beads swelled significantly over the first 10 minutes.

The inclusion of Ac-Di-Sol (beads with a more porous structure) in the different bead formulations produced beads with a degree of swelling that compared well with the degree of swelling of the plain chitosan/pyrazinamide beads. Over the first 10 minutes the swelling of all of the Ac-Di-Sol containing bead formulations were almost identical to the swelling of the pure chitosan/pyrazinamide beads, but after 10 minutes the swelling of the Ac-Di-Sol containing beads increased to a maximum, higher than the maximum of the plain chitosan/pyrazinamide beads.

In general, the inclusion of Explotab into the bead formulations resulted in good swelling. Blank chitosan beads (CB) however, showed the best swelling of all the different bead formulations in PBS pH 5.60 and 7.40.

Drug loading studies showed that the inclusion of the pharmaceutical excipients into the chitosan-drug matrix, improved the drug loading. The best improvement was obtained by the inclusion of the combination of Ac-Di-Sol and Ascorbic acid, with an increase of 14.83 % in the drug loading. The increase in drug loading is of economic importance for the pharmaceutical industry as less drug is wasted during the preparation of drug loaded beads.

Friability studies showed that plain pyrazinamide loaded chitosan beads produced beads with a very high friability in comparison to blank chitosan beads (containing no drug). The inclusion of the different excipients into the bead structure resulted in a varying effect dependent on the type and combination of excipients used. The addition of Ascorbic acid and the combination of Ascorbic acid and Explotab to the beads led to an increase in the friability. The addition of Ac-Di-Sol, Explotab, Ac-Di-Sol/Ascorbic acid and Ac-Di-Sol/Explotab however led to a decrease in the friability of the beads. The low friability values of the beads containing Ac-Di-Sol can be the result of the higher ratio of Ac-Di-Sol to Ascorbic Acid and Explotab.

The solubility of the beads was dependent on the type and combination of excipients added. The inclusion of pharmaceutical excipients all led to a decrease in the solubility of the beads.

Dissolution studies on all samples are discussed in Chapter 4.

# Chapter 4

# Pyrazinamide Release from Chitosan/SPE and Chitosan/MPE Beads

## 4.1 Introduction

Dissolution is defined as a process by which a solid substance enters into a solvent to yield a solution (Banakar, 1991:1). The dissolution rate is the amount of solid substance that goes into solution per unit time under standardized conditions (Abdou, 1989:11). In biological systems, drug dissolution in an aqueous medium is an important prerequisite for systemic absorption. The rate at which drugs with poor aqueous solubility dissolve from an intact or disintegrated solid dosage form in the gastrointestinal tract often controls the rate of systemic absorption of the drug (Shargel & Yu, 1991:132).

Drug release plays an important role during the development of new solid pharmaceutical dosage forms. The pharmaceutical industries and registration authorities focus on dissolution studies to ensure that drug release occur in an appropriate manner. Under certain conditions dissolution profiles can serve as a surrogate for the assessment of bioequivalence. Several theories or kinetic models have been developed to help describe drug dissolution from immediate and modified release dosage forms. These models consist of a function of time over the amount of drug dissolved from the pharmaceutical dosage form. The quantitative interpretation of the values obtained in the dissolution assay, is facilitated by a generic equation that mathematically translates the dissolution curve in functions of some parameters related to the specific dosage forms. These equations can be deducted from theoretical analysis of the process, for example, in zero order kinetics or in some cases where a theoretical fundament does not exist in prolonged

release forms or coated forms, more adequate empirical equations are used (Costa & Lobo, 2001:123).

The preparation and characterization of pyrazinamide loaded chitosan/SPE and chitosan/MPE beads were described in chapter 2 and 3. This chapter will look at the release rate and dissolution profiles of formulations used in the study to determine whether a viable controlled release dosage form could be manufactured. Drug loading studies have been described as part of the characterization of beads in chapter 3 and these results have been used during the dissolution studies to determine the amount of pyrazinamide released from the beads. Dissolution studies were conducted at pH 5.60 and 7.40 over a period of 6 hours.

## 4.2 Characterisation of Dissolution Profiles

Quantitative interpretation of values obtained in dissolution assays is easier, using mathematical equations which describe the release profile in functions of some parameters related to the pharmaceutical dosage forms. In some dosage forms, the rate of drug release may be influenced by stages resulting from different chemical or physical reactions. This uncontrollable situation makes it sometimes impossible to derive a mathematical model that describes the drug release out of such a system (Costa & Lobo, 2001:123).

There are many mathematical models describing drug dissolution in the literature, for example the zero order model, mean dissolution time, difference and similarity factor, Higuchi model, Weibull model and Korsmeyer-Peppas, to mention a few (Costa & Lobo, 2001:123). Some of these models that could be used for the interpretation of simple drug release systems, are discussed here.

### 4.2.1 Mean Dissolution Time

Mean dissolution time (MDT) is the mean time used to characterize drug release rate from a dosage form under *in vitro* dissolution conditions (Reppas and Nicolaides, 2000:231). MDT is the mean ratio of the first zero moment of the dissolution rate-time curve and is expressed by the following equation (4-1) (Figure 4-1 defines the symbols):

$$MDT = \frac{\int_0^\infty tX_d(t)dt}{\int_0^\infty X_d(t)dt} = \frac{ABC}{X_{d,\max}} \tag{4-1}$$

On a practical basis, MDT can be estimated from the cumulative mass dissolved vs time profile, by using the following equation (Reppas and Nicolaides, 2000:231):

$$MDT = \frac{\sum_{i=1}^{n} t_{mid}\Delta X_d}{\sum_{i=1}^{n} \Delta X_d} \tag{4-2}$$

where $i$ is the sample number, $n$ is the total number of sample times, $t_{mid}$ is the time at the midpoint between $i$ and $i-1$, and $\Delta X_d$ is the additional mass of drug dissolved between $i$ and $i-1$.

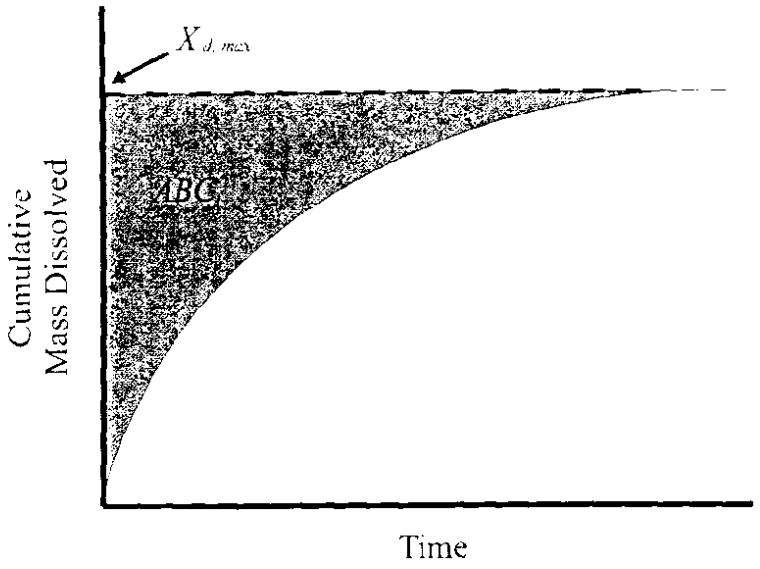


**Figure 4-1:** Graphic representation of the parameters used to estimate the mean dissolution time (MDT): $X_{d,max}$ is the actual maximum cumulative mass dissolved and ABC is the shaded area (Reppas and Nicolaides, 2000:231).

## 4.3 Comparison of Dissolution Profiles

### 4.3.1 Difference factor ($f_1$) and Similarity factor ($f_2$)

In recent years, the Food and Drug Agency (FDA) has placed more emphasis on a dissolution profile comparison in the area of post-approval changes and biowaivers. Under appropriate test conditions, a dissolution profile can characterize the product more precisely than a single point dissolution test. A dissolution profile comparison between pre-change and post-change products for SUPAC related changes, or with different strengths, helps assure similarity in product performance and signals bioinequivalence (Shah *et al.*, 1998:889).

Among several methods investigated for dissolution profile comparison, $f_2$ is the simplest. Moore & Flanner, (1996:64) proposed a model independent mathematical approach to compare the dissolution profile using two factors, $f_1$ which is the difference factor and $f_2$ which is the similarity factor.

The difference factor ($f_1$) calculates the percentage difference between the two curves at each time point and is a measurement of the relative error between the two curves (Moore & Flanner, 1996:64):

$$f_1 = \left\{\left[\sum_{t=1}^{n}\left|R_t - T_t\right|\right] / \left[\sum_{t=1}^{n} R_t\right]\right\} \bullet 100 \qquad (4\text{-}3)$$

where $n$ is the number of time points, $R_t$ is the dissolution value of the reference (pre-change) batch at time $t$, and $T_t$ is the dissolution value of the test (post-change) batch at time $t$.

The similarity factor ($f_2$) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves (Moore & Flanner, 1996:64):

$$f_2 = 50\log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n} w_t (R_t - T_t)^2\right]^{-0.5} \bullet 100\right\} \qquad (4\text{-}4)$$

where $R_t$ is the reference assay at time point $t$, $T_t$ is the test assay at time point $t$, $n$ is the number of pull points. And $w_t$ is an optional weight factor.

In dissolution profile comparisons, especially to assure similarity in product performance, regulatory interest is in knowing how similar the two curves are, and to have a measure, which is more sensitive to large differences at any particular time point. For this reason, the $f_2$ comparison has been the focus in Agency guidances (Moore & Flanner, 1996:64).

For curves to be considered similar, $f_1$ values should be close to 0, and $f_2$ values greater than 50 (50-100), ensure sameness or equivalence of the two curves and thus, of the

performance of the test (post-change) and reference (pre-change) products (Moore & Flanner, 1996:64; Shah *et al.* 1998:889).

## 4.4 Burst effect

When dissolution studies are started there is sometimes a sudden increase in the drug release. This phenomenon is typically referred to as "burst release". In many of the controlled release formulations, immediately upon placement in the release medium, an initial large bolus of drug is released before the release rate reach a stable profile. The burst effect can be viewed from two perspectives, a positive and negative perspective. The burst effect is often regarded as a negative consequence of creating long-term controlled release devices, causing non-desirable problems such as local or systemic toxicity. One of the current difficulties with burst release is that it is unpredictable, and even when the burst is desired, the amount of burst cannot be significantly controlled (Huang and Brazel, 2001:122).

Depending on the intended use of a pharmaceutical dosage form, this initial high release may also be favourable. Such is the case with wound treatment, where an initial high concentration of drug is required for relief, followed by a constant release of drug for wound healing. Other instances where burst release might be favourable include targeted drug delivery systems and pulsatile release systems (Huang and Brazel, 2001:122).

According to Huang and Brazel (2001:123) there are several potential reasons why burst effect can occur. Some of the potential reasons include process conditions, the surface characteristics of the host material, the formulation geometry, some host/drug interactions, and morphology and porous structure of the dry material. Burst effects are not uncommon in drug release from beads.

Gupta and Ravi Kumar (2000:1117) reported a burst effect in the drug release of diclofenac sodium containing chitosan beads during the first hour of dissolution. They attributed it to the initial swelling of the beads. Better swelling resulted in better release. Bodmeier *et al.* (1989:1486) also reported a burst effect in drug release. They attributed

the burst effect to sulfadiazine crystals that formed on the exterior of the beads they prepared, without relation to swelling.

Understanding of the burst effect during controlled release is still limited but knowledge continues to grow as researchers realise both the economic and therapeutic importance of the burst period. For many applications small quantities may be acceptable, as long as the burst effect is predictable (Huang and Brazel, 2001:134).

## 4.5 Method

The dissolution studies were performed according to the method described in chapter 2 section 2.4.6. Although the same amount of beads, for the different bead formulations, was weighed for every dissolution test performed, the amount of drug differed slightly, due to the differences in drug loading. Therefore the amount of the pyrazinamide had to be calculated for every bead formulation based on the results obtained from the drug loading (See Table 4-1).

**Table 4-1:** Amount of pyrazinamide (in mg) per 25 mg of dissolution formulation as calculated from the drug loading capacity (DLC).

| Bead formulation | DLC (%) | Average mass (mg) | Pyrazinamide (mg) |
|---|---|---|---|
| Chit/Pyr | 52.26 | 25.9 | 13.53 |
| Chit/ASC | 59.60 | 25.3 | 15.07 |
| Chit/EXPL | 58.06 | 25.2 | 14.63 |
| Chit/ADS | 60.25 | 25.6 | 15.42 |
| Chit/EXPL/ADS | 66.95 | 25.6 | 17.13 |
| Chit/EXPL/ASC | 65.31 | 25.5 | 16.65 |
| Chit/ADS/ASC | 67.09 | 25.6 | 17.17 |

Two standard curves were constructed before every dissolution study. The method used to plot the standard curves was already discussed in chapter 2 section 2.4.6.1. Examples of standard curves are given in Figure 4-2 and Figure 4-3.

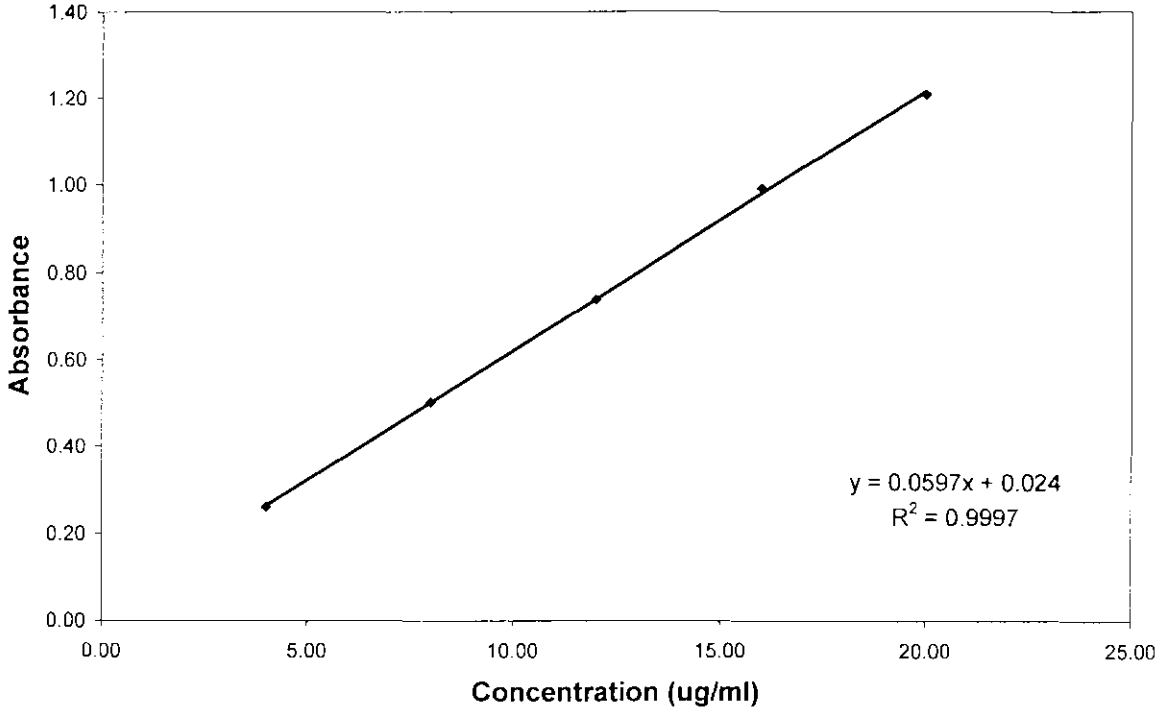


**Figure 4-2:** Example of a standard curve plotted for pyrazinamide in PBS pH 5.60.

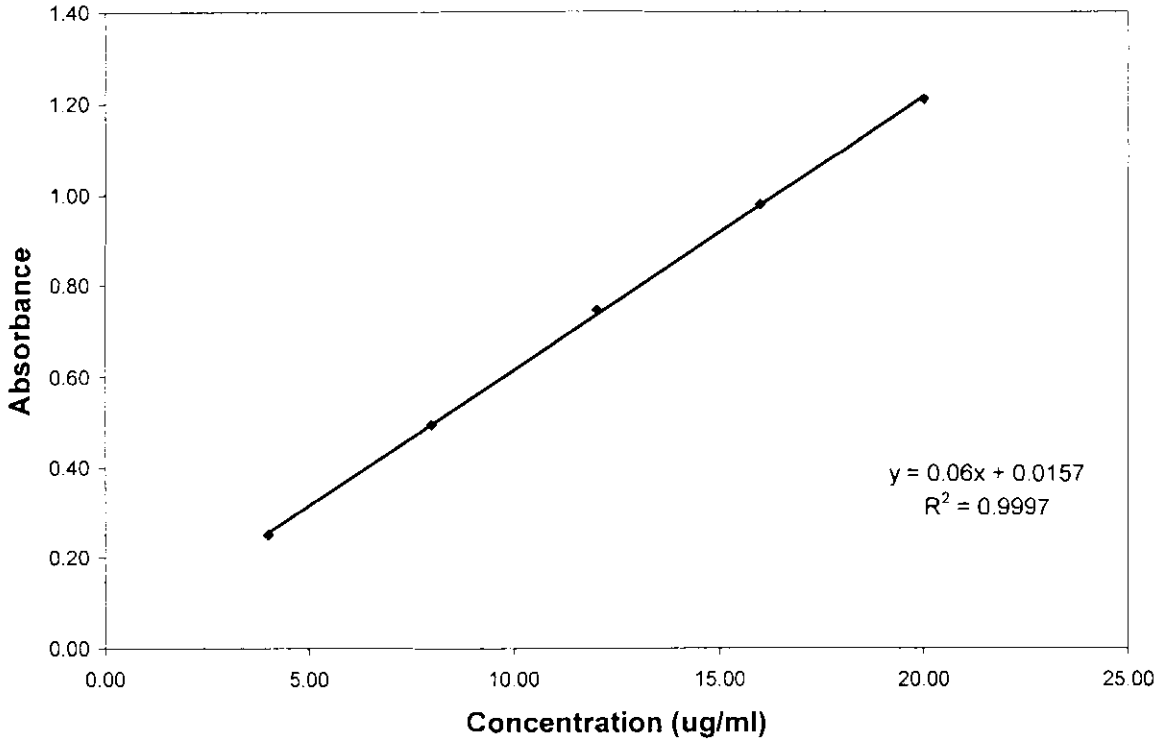


**Figure 4-3:** Example of a standard curve plotted for pyrazinamide in PBS pH 7.40.

## 4.6 Results and Discussion

### 4.6.1 Mean Dissolution Time

Calculated mean dissolution times (MDT) and average mean dissolution times (Ave MDT) for formulations in PBS pH 5.60 and 7.40 for time 0 – 360 minutes, are reported in Tables 4-2 and 4-3.

**Table 4-2:** Calculated mean dissolution times (MDT) and average mean dissolution times (Ave MDT) for formulations in PBS pH 5.60 for time 0 – 360 minutes.

| PBS pH 5.60 | | | | | |
|---|---|---|---|---|---|
| | | MDT 1 | MDT 2 | MDT 3 | Ave MDT |
| 1 | Chit | 15.27 | 12.80 | 12.04 | 13.37 ± 1.69 |
| 2 | Chit/ASC | 12.11 | 8.43 | 13.27 | 11.27 ± 2.53 |
| 3 | Chit/EXPL | 10.26 | 10.90 | 14.43 | 11.86 ± 2.25 |
| 4 | Chit/ADS | 10.14 | 10.05 | 12.12 | 10.77 ± 1.17 |
| 5 | Chit/ASC/EXPL | 6.87 | 7.76 | 9.85 | 8.16 ± 1.53 |
| 6 | Chit/ASC/ADS | 6.12 | 4.60 | 5.25 | 5.33 ± 0.76 |
| 7 | Chit/EXPL/ADS | 11.37 | 8.16 | 14.27 | 11.27 ± 3.06 |

**Table 4-3:** Calculated mean dissolution times (MDT) and average mean dissolution times (Ave MDT) for formulations in PBS pH 7.40 for time 0 – 360 minutes.

| PBS pH 7.40 | | | | | |
|---|---|---|---|---|---|
| | Bead formulations | MDT 1 | MDT 2 | MDT 3 | Ave MDT |
| 1 | Chit | 13.57 | 9.39 | 9.83 | 10.93 ± 2.30 |
| 2 | Chit/ASC | 9.03 | 10.87 | 10.47 | 10.12 ± 0.97 |
| 3 | Chit/EXPL | 8.11 | 9.68 | 9.54 | 9.11 ± 0.87 |
| 4 | Chit/ADS | 11.27 | 16.66 | 8.13 | 12.02 ± 4.32 |
| 5 | Chit/ASC/EXPL | 5.79 | 6.13 | 5.82 | 5.91 ± 0.19 |
| 6 | Chit/ASC/ADS | 9.56 | 10.01 | 11.12 | 10.23 ± 0.80 |
| 7 | Chit/EXPL/ADS | 11.99 | 9.46 | 11.46 | 10.97 ± 1.34 |

## 4.6.2 Similarity factor ($f_2$)

Calculated similarity factors ($f_2$) for bead formulations vs plain chitosan beads in PBS pH 5.60 and 7.40 are reported in Table 4-4.

**Table 4-4:** Similarity factor-values for bead formulations vs plain chitosan beads in PBS pH 5.60 and 7.40.

| Similarity Factor ($f_2$) | | | |
|---|---|---|---|
| | Bead Formulations | ($f_2$) vs CB | |
| | | PBS 5.60 | PBS 7.40 |
| 1 | Chit/ASC | 44.93 ± 4.87 | 43.91 ± 6.34 |
| 2 | Chit/EXPL | 47.01 ± 4.32 | 44.04 ± 4.87 |
| 3 | Chit/ADS | 43.86 ± 1.62 | 48.24 ± 0.94 |
| 4 | Chit/ASC/EXPL | 44.92 ± 4.69 | 41.77 ± 3.87 |
| 5 | Chit/ASC/ADS | 41.30 ± 3.30 | 42.68 ± 4.83 |
| 6 | Chit/EXPL/ADS | 41.81 ± 0.33 | 42.08 ± 6.29 |

### 4.6.3 Dissolution in PBS pH 5.60

#### 4.6.3.1 Results

The calculated % dissolution values for all the chitosan beads containing single and multiple pharmaceutical excipients, are reported in Table B-1 to B-7 (Annexure B) and graphically depicted in Figures 4-4 and 4-5.

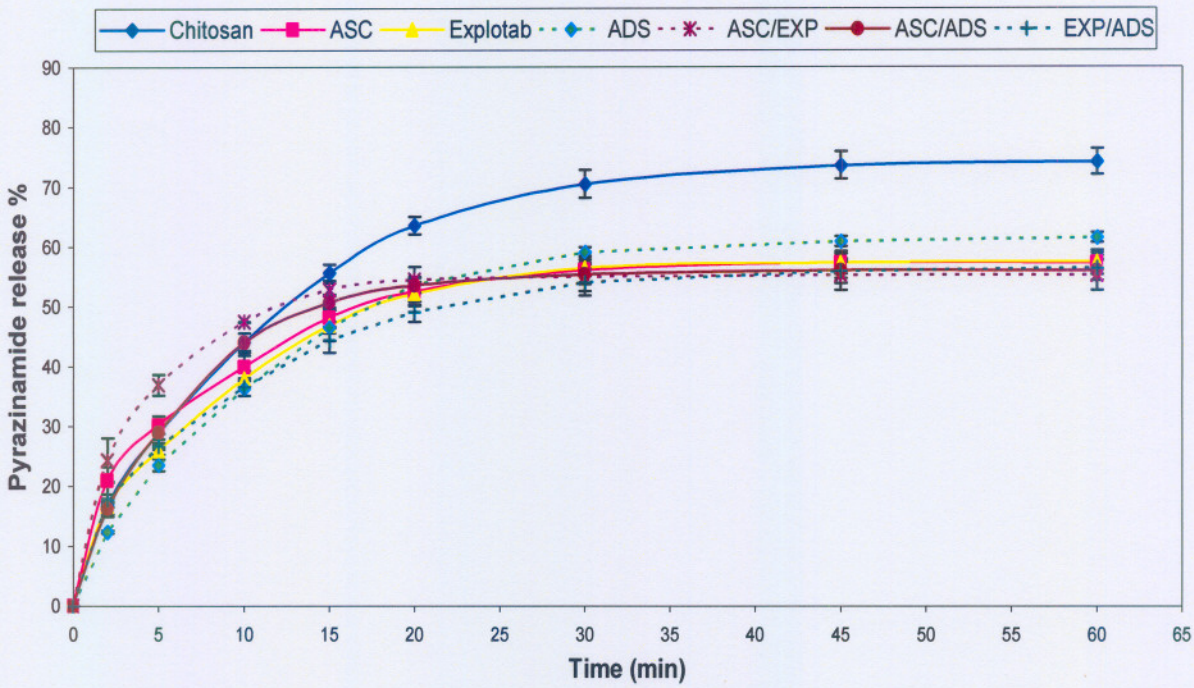


**Figure 4-4:** Pyrazinamide release from bead formulations in PBS pH 5.60 over the first 60 minutes.

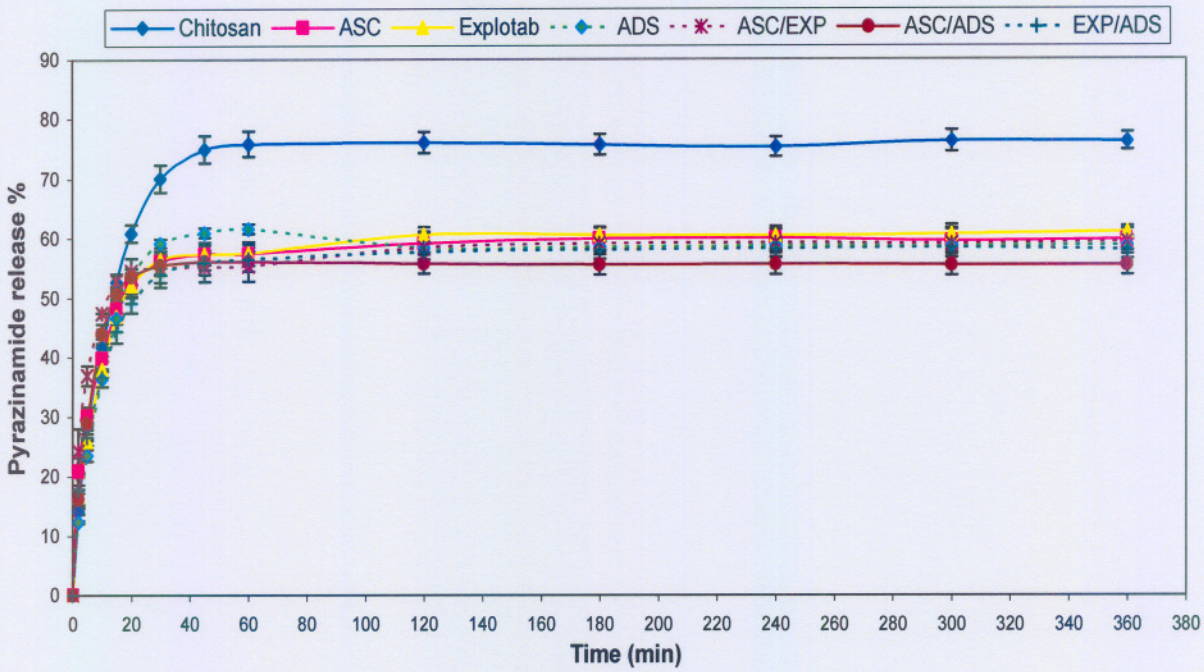


**Figure 4-5:** Pyrazinamide release from bead formulations in PBS pH 5.60 over 360 minutes.

### 4.6.3.2 Discussion

The effect of pharmaceutical excipients on the release of pyrazinamide from the different bead formulations, appeared to be in contradiction to what was expected. It was expected that the addition of these excipients would lead to a sustained release of pyrazinamide over a period of 6 hours. Observation of Figures 4-4 and 4-5 indicates that all the bead formulations exhibit a burst release in PBS pH 5.60 during the first 15 minutes.

Plain chitosan/pyrazinamide beads (Table 4-2, formulation 1) exhibited the longest mean dissolution time (MDT=13.37 ± 1.69) of all the bead formulations. Inclusion of the pharmaceutical excipients all led to a decrease in MDT. The inclusion of Ascorbic acid resulted in the most pronounced decrease in MDT (MDT=5.33) (formulation 6). This decrease can possibly be attributed to the basicity of pyrazinamide and the pH in the micro-environment (in the diffusion layer). Ascorbic acid is a week acid and will decrease the pH of the diffusion layer and as a result increases the dissolution of pyrazinamide, causing a faster MDT. This was seen for all the Ascorbic acid containing

formulas (formulation 2, 5 and 6). Although the decrease in MDT was seen for all the Ascorbic acid containing formulations, it was only statistically significant (Tukey HSD test, $p < 0.1$) for formulae 5 and 6 (see Annexure B for results of Tukey test). It seems that the combination of Ascorbic acid with Ac-Di-Sol (formulation 5) and Explotab (formulation 6) caused a more pronounced decrease in MDT than Ascorbic acid as a single excipient. Although the combination of Ascorbic acid and Ac-Di-Sol resulted in a faster MDT than Ascorbic acid and Explotab, this difference was not statistically significant. Explotab and Ac-Di-Sol function as disintegrants by swelling as a result of water uptake (Lieberman *et al.,* 1989:109). This swelling causes an increase in viscosity within the micro-environment. It is possible that the increase in viscosity could cause a longer time for pyrazinamide to be exposed to the pH-lowering effect of Ascorbic acid, causing the shorter MDT, as compared to the formulation containing Ascorbic acid as a single excipient (formulation 2). Pyrazinamide is a weak base and therefore the dissolution rate will increase in a medium with a low pH.

The average similarity factor ($f_2$) for all the bead formulations containing pharmaceutical excipients, was less than 50 ($f_2 < 50$) in comparison to the plain chitosan/pyrazinamide beads (Table 4-4). For the bead formulations to be considered similar, the similarity factor must be greater than 50 ($f_2 > 50$). From Table 4-4 it is evident that there was no similarity between chitosan/pyrazinamide beads and bead formulations containing excipients.

Plain chitosan/pyrazinamide beads released more than 75 % of the drug content within 30 minutes, so there was no significant delayed release. The curve reached a plateau at approximately 30 minutes. Although the beads containing excipients exhibited faster MDT values, it is evident from Figure 4-6 that all the formulations released less drug than the plain chitosan/pyrazinamide beads after 360 minutes. After 360 minutes there was still about 40 % of drug left for all the formulations, compared to the less than 24 % of drug left in the plain chitosan/pyrazinamide beads (see Table 4-5).

Calculated surface area under the curve values for the dissolution curves are reported in Table 4.6.

**Table 4-5:** Percentage pyrazinamide (%) left in beads after 360 minutes in PBS pH 5.60 and 7.40.

| | Bead formulations | % Drug left in Beads after 360 min | |
|---|---|---|---|
| | | PBS 5.60 | PBS 7.40 |
| 1 | Chit | 23.78 | 24.99 |
| 2 | Chit/ASC | 40.23 | 42.17 |
| 3 | Chit/EXPL | 38.31 | 41.95 |
| 4 | Chit/ADS | 41.19 | 37.77 |
| 5 | Chit/ASC/EXPL | 40.61 | 43.99 |
| 6 | Chit/ASC/ADS | 44.49 | 43.27 |
| 7 | Chit/EXPL/ADS | 41.97 | 43.09 |

**Table 4-6:** Average surface area under the curve (AUC) (average ± SD) for bead formulations in PBS pH 5.60 and 7.40.

| | Bead formulations | Average AUC | |
|---|---|---|---|
| | | PBS 5.60 | PBS 7.40 |
| 1 | Chit | 24422.58 ± 617.27 | 26260.40 ± 1250.88 |
| 2 | Chit/ASC | 20842.62 ± 677.21 | 20233.04 ± 394.32 |
| 3 | Chit/EXPL | 21261.40 ± 488.67 | 21648.90 ± 706.10 |
| 4 | Chit/ADS | 20538.11 ± 269.27 | 20372.70 ± 1222.64 |
| 5 | Chit/ASC/EXPL | 20898.21 ± 819.72 | 19833.12 ± 710.32 |
| 6 | Chit/ASC/ADS | 19686.58 ± 601.21 | 19843.37 ± 105.86 |
| 7 | Chit/EXPL/ADS | 20236.46 ± 573.39 | 19861.02 ± 872.54 |

The average surface area under the curve (AUC) was the largest for the chitosan/pyrazinamide beads (AUC=24422.58 ± 614.27) compared to excipient containing beads (see Table 4-6). This also shows that more of the drug was released from the chitosan/pyrazinamide beads over 360 minutes than from the other bead formulations. This also explains why the curves similarity factor ($f_2$) values are smaller than 50 in comparison to the plain chitosan/pyrazinamide beads.

Chitosan/pyrazinamide beads exhibited no sustained release because more than 75% of the drug was already released within 30 minutes. The inclusion of the pharmaceutical excipients did not contribute to a sustained release of pyrazinamide in PBS pH 5.60. Although there was no sustained release over 6 hours, there was still 38-45% of pyrazinamide in the excipient containing beads left (depending on the formulation). It is possible that 38-45% of pyrazinamide that is still left might still be released if the dissolution is done over a period of 24 hours. Further studies are, however, necessary to investigate this possibility in the future.

### 4.6.4 Dissolution in PBS pH 7.40

#### 4.6.4.1 Results

The calculated % dissolution values for all the chitosan bead formulations containing single and multiple pharmaceutical excipients, are reported in Table B-1 to B-7 (Annexure B) and graphically depicted in Figures 4-6 and 4-7.

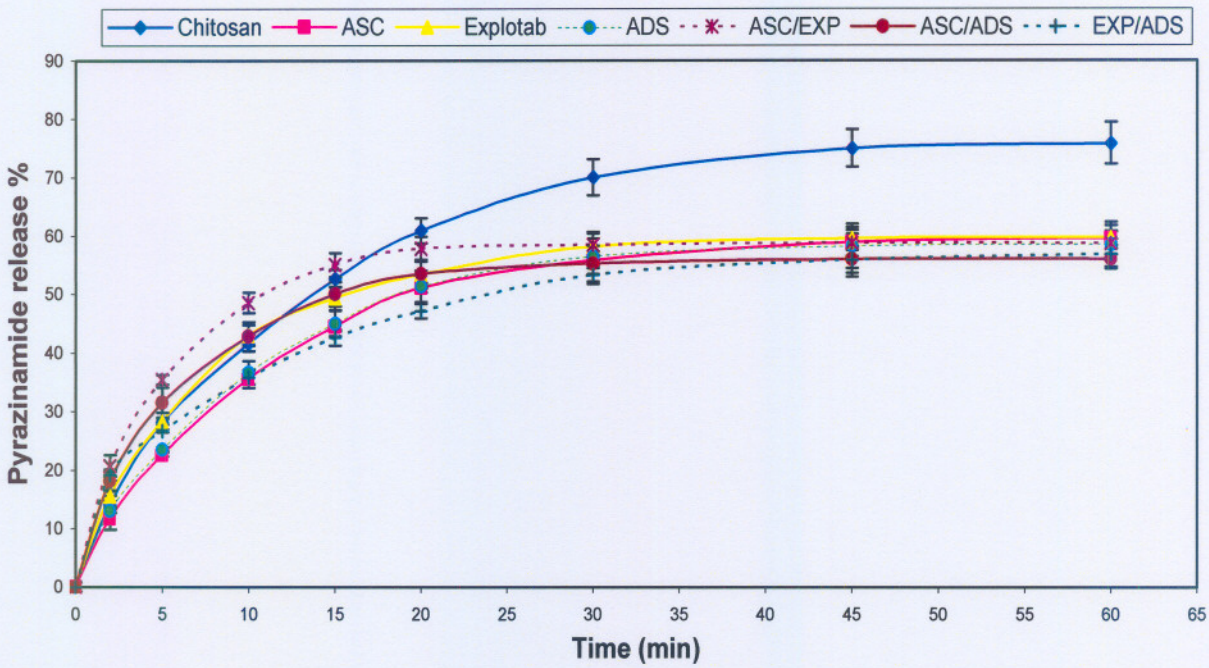


**Figure 4-6:** Pyrazinamide release from bead formulations in PBS pH 7.40 over the first 60 minutes.

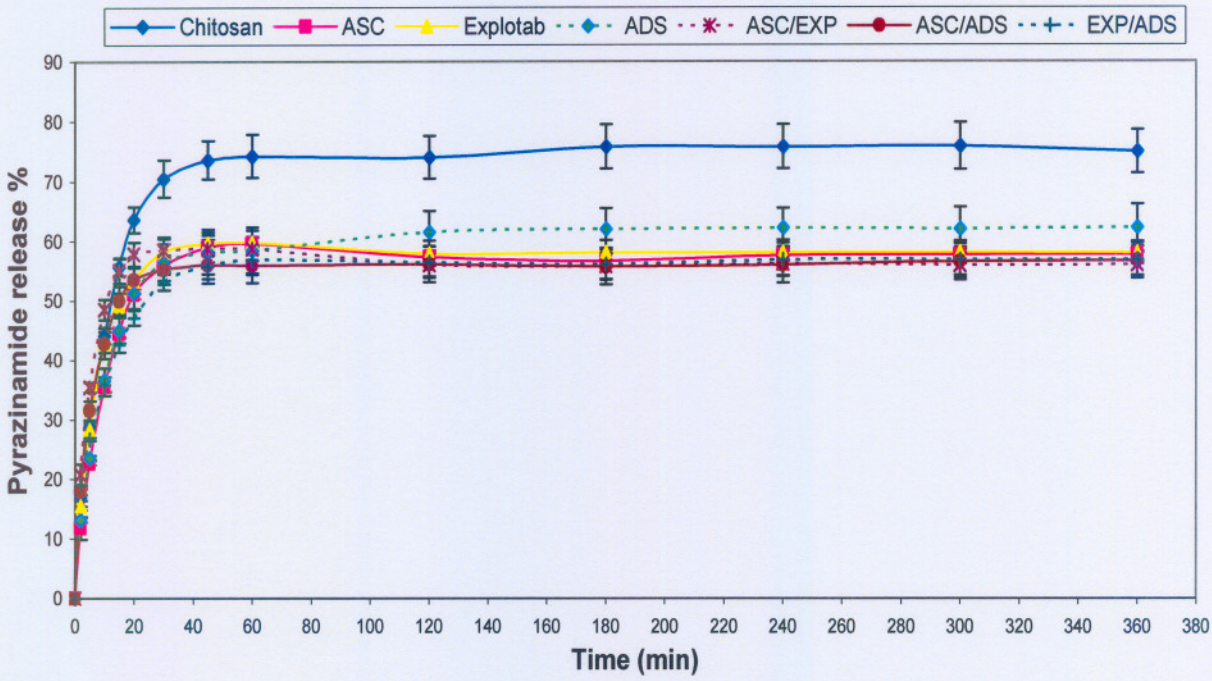


**Figure 4-7:** Pyrazinamide release from bead formulations in PBS pH 7.40 over 360 minutes.

### 4.6.4.2 Discussion

Dissolution studies in PBS pH 7.40 in general, resulted in the same tendency as in PBS pH 5.60. Observation of Figures 4-6 and 4-7 indicates that all the bead formulations in PBS pH 7.40 also exhibited a burst release during the first 15 minutes. The ranking of the mean dissolution time (MDT) of the bead formulations is basically the same as in PBS pH5.60 but the MDT is only shorter than in PBS pH 5.60. The longest mean dissolution time (MDT=12.02 ± 4.32) was observed with chitosan/Ac-Di-Sol beads (formulation 4). There is also no statistically significant difference in the MDT of the chitosan/pyrazinamide beads compared to the excipients containing beads (see Annexure B for results of Tukey-test) (Tukey HSD test, $p > 0.1$).

The average similarity factor value ($f_2$) for all the bead formulations containing pharmaceutical excipients, was less than 50 ($f_2 < 50$) in comparison to the plain chitosan/pyrazinamide beads (Table 4-3). From Table 4-4 it is evident that there was no

similarity between chitosan/pyrazinamide beads and bead formulations containing excipients.

Plain chitosan/pyrazinamide beads also released more than 75 % of the drug content within 30 minutes in PBS pH 7.40, so there was no significant sustained release and the curve reached a plateau at approximately 30 minutes. Figure 4-7 exhibits less drug released from the excipient containing beads than the plain chitosan/pyrazinamide beads after 360 minutes. After 360 minutes there was still about 38-45 % of drug left in the excipient containing beads, compared to the less than 25 % of drug left in the plain chitosan/pyrazinamide beads (see Table 4-5).

Chitosan/pyrazinamide beads exhibited the largest average surface area under the curve, (AUC=26260.40 ± 1250.88) compared to excipients containing beads (see Table 4-6). This also shows that more of the drug was released from the chitosan/pyrazinamide beads over 360 minutes, than from the other beads. There is no similarity between the curves of plain chitosan/pyrazinamide beads and excipient containing beads and this explains why the curves similarity factor ($f_2$) is smaller than 50 in comparison to the plain chitosan/pyrazinamide beads.

The inclusion of the pharmaceutical excipients did not contribute to a sustained release of pyrazinamide in PBS pH 7.40. After a period of 6 hours there was still 37-44% of pyrazinamide in the excipient containing beads left. It is possible that the 37-44% of pyrazinamide that is still left, can be released if the dissolution is done over a period of 24 hours. Further studies are, however, necessary to investigate this possibility in the future.

## 4.7 Conclusion

Pyrazinamide release from the beads in PBS pH 5.60 and 7.40 was studied over a period of 6 hours at 37 °C. The effect of pharmaceutical excipients on the release of pyrazinamide from the different bead formulations appeared to be in contradiction to what was expected. It was expected that the addition of these excipients would lead to a sustained release of pyrazinamide over a period of 6 hours. Observation of the dissolution data indicated that all the bead formulations exhibit a burst release in PBS pH 5.60 and 7.40 during the first 15 minutes. Chitosan/pyrazinamide beads showed no sustained release because 75% of the drug was already released within 30 minutes. The inclusion of the pharmaceutical excipients did not contribute to a sustained release of pyrazinamide. After a period of 6 hours in PBS pH 5.60 and 7.40, there was still 37-45% of pyrazinamide in the excipient containing beads left.

In future studies the dissolution time can be extended to a period of 24 hours. It might be possible for the remaining drug in the beads to be released over the extended period. Other polymers can also be investigated to control the release of pyrazinamide. Further studies are, however, necessary to investigate this possibility in the future.

# Chapter 5

# Summary and Future Prospects

## 5.1 Summary

Controlled release dosage forms are of increasing importance in the pursuit for patient compliance. Specialized controlled release dosage forms with the ability to shield drugs from the harsh environment of the stomach, often require additional steps in the production of the formulation, thereby increasing the cost associated with production. This high cost of production is of economic importance and often outweighs the immediate benefits to the patients, especially in third world countries. It would therefore be favorable if a method could be devised by which such specialized controlled release dosage forms can be produced by simple means.

Chitosan is a polymer of natural origin, with excellent biodegradable and biocompatible characteristics and is commonly used in the pharmaceutical industry. Chitosan also has excellent adsorption properties and is non-toxic. These characteristics make chitosan suitable for application as a supporting material in systems constructed to be functional in biological environments. Chitosan also find application in biomedical and pharmaceutical processes, in food industry, in agricultural and wastewater treatment. Due to its unique polymeric cationic character and its gel and film forming properties, chitosan has been extensively examined in the pharmaceutical industry for its potential in the development of controlled release drug delivery systems.

It is evident that when chitosan is compared to traditional excipients, it appears to have superior characteristics and is especially flexible in its use. Many experimental drug delivery systems that are chitosan based, have been designed, developed and optimised for desired drug release profiles. The desire to develop chitosan based microparticulate drug delivery systems led to the formulation of microparticles and nanoparticles.

The literature study demonstrated that the ability of chitosan to provide several advantages in drug delivery was not limited to micro- and nanoparticles alone. Beads are also part of microparticulate drug delivery systems and it has been demonstrated in many studies that beads are a versatile dosage system for controlled drug delivery. Beads show various advantages over single unit dosage forms and they are very flexible in dosage form development. Because beads disperse freely in the gastrointestinal tract, they maximize drug absorption, reduce peak plasma fluctuation and minimize potential side-effects without lowering drug bio-availability. A number of techniques can be used to prepare beads but the ionotropic gelation method is the most reproducible method and was used in this study for the preparation of chitosan beads. This method also provides easy entrapment of the drug inside the beads. Enforced by cross-linking, bead formation takes place with ease due to the cationic nature of chitosan which allows drug entrapment within a three-dimensional network of ionically linked polymer. In this way the drug is locked within the matrix of the bead.

Pyrazinamide is indicated for the initial treatment of active tuberculosis in adults and children. Pyrazinamide should always be administered in combination with other effective antituberculous drugs such as isoniazid, rifampin, ethambutol, streptomycin, cycloserine, ethionamide and para-aminosalicyclic acid. When used alone, resistance may develop within 6 to 8 weeks. Pyrazinamide was chosen as study drug for incorporation into chitosan beads. The conventional treatment regimen for tuberculosis has led to incompliance in most patients and resulted in many deaths over decades. Incorporating pyrazinamide into chitosan beads can improve its absorption and bioavailability. In this study the ability of pharmaceutical excipients such as Ac-Di-Sol®, Explotab® and Ascorbic acid, was investigated to enhance the swelling behaviour of the beads to increase the rate and extent of pyrazinamide release over 6 hours at pH 5.60 and 7.40. The excipients were suspended or dissolved in the polymer solution prior to drug loading.

Beads were produced by dispersing pyrazinamide in an aqueous glacial acetic acid solution of chitosan. Varying concentrations of different types and different combinations of pharmaceutical excipients were added to the dispersion. The dispersion was dropped through a needle into an agitated solution of tripolyphosphate (TPP). Beads formed on contact with the TPP-solution. The beads were stirred gently for 30 minutes to

allow for cross-linking to occur, separated by filtration, washed with deionised water and freeze-dried.

Beads were then characterized by means of drug loading capacity, morphology studies, friability, swelling and degradation, solubility and drug release profiles. A scanning electron microscope (SEM) was used to investigate the effect of the excipients, either single or in combination, on the structure of the beads, with emphasis on their porosity. After close investigation it was found that bead formulations containing Ascorbic acid, produced beads with a denser structure. Bead formulations with Ac-Di-Sol and Explotab resulted in beads with a more porous structure.

Studies on the drug loading showed that the addition of the pharmaceutical excipients to the bead formulations, improved the drug loading capacity of the pyrazinamide containing chitosan beads. The increase in drug loading was dependent on the type and combination of the excipients added, with the combination of Ac-Di-Sol and Ascorbic acid having the most significant effect.

Friability of the beads was also tested. The inclusion of the different excipients into the bead structure resulted in a varying effect dependent on the type and combination of excipients used. In general, the inclusion of Ascorbic acid weakened the beads and Ac-Di-Sol and Explotab strengthened the beads' resistance to friability.

Swelling behaviour studies were also conducted by immersing a pre-weighed sample of the beads in phosphate buffer solution (PBS) of pH 5.60 and 7.40 at 37 °C. The weight increase, after removing the beads from the PBS and blotting any excess droplets, was used to calculate the degree of swelling (Esw). In general, the inclusion of Ascorbic acid in the bead formulations led to a decrease in swelling and the inclusion of Ac-Di-Sol and Explotab produced beads with better swelling abilities.

The solubility of the beads was dependent on the type and combination of excipients added. The inclusion of pharmaceutical excipients all led to a decrease in the solubility of the beads.

Pyrazinamide release from the beads in PBS was studied over a period of 6 hours at pH 5.60 and 7.40 at 37 °C. The effect of pharmaceutical excipients on the release of pyrazinamide from the different bead formulations, appeared to be in contradiction to what was expected. It was thought that the addition of these excipients would lead to a sustained release of pyrazinamide over a period of 6 hours. Observation of the dissolution data indicated that all the bead formulations exhibit a burst release in PBS pH 5.60 and 7.40 during the first 15 minutes. Chitosan/pyrazinamide beads showed no sustained release because 75% of the drug was already released within 30 minutes. The inclusion of the pharmaceutical excipients did not contribute to a sustained release of pyrazinamide. After a period of 6 hours in PBS pH 5.60 and 7.40 there was still 37-45% of pyrazinamide in the excipient containing beads left.

## 5.2 Future Prospects

During this study a number of variables were identified which may have had an influence on the characteristics of the prepared beads, thus emphasizing the need to optimise certain techniques in this study. The variables include the following:

- The viscosity of the polymer used should be taken into consideration. The viscosity of the polymer/drug mixture had a direct effect on the diffusion rate of the drug out of the beads and will influence the drug loading and drug release. A more viscous solution might lead to a better drug suspension and a better drug loading. An increase in the polymer viscosity, especially chitosan, might improve control over the drug release rate.

- The extend of cross-linking time has an effect on the quality of the beads in terms of shape. Cross-linking increases shape and structural integrity as well as the ability of the bead to maintain roundness. The time allowed for cross-linking appeared not to be sufficient. With an increase in cross-linking time, the TPP could diffuse deeper into the bead formulations, thereby increasing the mechanical strength of the beads and decreasing the solubility.

- Another factor that may influence pyrazinamide release from the beads is the chemical interaction of the pyrazinamide with the chitosan and the pharmaceutical excipients added. More attention can be focused in the future on the chemical interactions of the excipients on pyrazinamide. Possible interactions can be assessed by DSC analysis of pure chitosan beads and beads containing the pharmaceutical excipients prior to drug loading and release studies.

- A deeper investigation into the dissolution of the bead formulations may prove useful. Dissolution studies revealed that almost 25% of pyrazinamide was left in the chitosan/pyrazinamide beads and 38-45% was left in excipient containing beads after a period of 6 hours. Extending the dissolution time up to 24 hours may lead to releasing of the remaining pyrazinamide.

# Annexure A
# Certificates of Approval

CERTIFICATE OF ANALYSIS FOR CHITOSAN

**XIAMEN JIANG YUAN IMPORT AND EXPORT COMPANY**

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

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## CERTIFICATE OF ANALYSIS FOR CHITOSAN

Original

**Report No.:** 021009

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

厦门江源经济发展公司 进出口单证章

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

WCI 7109

Aventis Pharma

Aventis

# ANALYTICAL REPORT – RAW MATERIAL

MATERIAL: PYRAZINAMIDE
CODE: NRP 108
SUPPLIER: AVENTIS, WALTLOO
SPECIFICATION: 2002 - 07 - 03

MANUFACTURE DATE: 30/09/2003
CONTROL NO.: 2830
DATE RECEIVED: 07/10/2003
QUANTITY: 418.10 kg

| TEST | LIMITS | RESULTS |
|---|---|---|
| DESCRIPTION | A white, crystalline powder. | Complies ✓ |
| SOLUBILITY | Text | Complies ✓ |
| IDENTIFICATION<br>a) IR | Positive | Complies ✓ |
| b) UV<br>I. Absorptivities at 268 nm | Difference < 3.00 % | 2.27% ✓ |
| II. Specific absorption | 640 - 680 | 655 ✓ |
| III. Absorbance maximum | About 310 nm | 312 nm ✓ |
| c) Melting point | 188.0 – 191.0 °C | 190.1° C ✓ |
| d) Reaction with Ferrous Sulphate | The solution becomes dark blue | Complies ✓ |
| e) Reaction with sodium hydroxide | Odour of ammonia is perceptible | Complies ✓ |
| MELTING RANGE | 188.0 – 191.0° C | 189.5 – 190.3° C ✓ |
| APPEARANCE OF SOLUTION | Solution S is clear and colourless | Complies ✓ |
| ACIDITY AND ALKALINITY | a) Solution is red | Complies ✓ |
| | b) Solution is colourless | Complies ✓ |
| | c) Solution is red | Complies ✓ |
| WATER Karl Fischer | NMT 0.500 % | 0.045% ✓ |
| HEAVY METALS | a) NMT 0.001 % (USP) | N/A ✓ |
| | b) NMT 10 ppm (BP) | < 10ppm ✓ |
| ROI / SULPHATED ASH | NMT 0.100 % | 0.009% ✓ |
| RELATED SUBSTANCES TLC | NMT 0.2 % | <0.2% ✓ |
| ASSAY<br>(Calculated on anhydrous basis) | a) 99.0 – 100.5 % (BP) | 100.0% ✓ |
| | b) 99.0 – 101.0 % (USP) | N/A ✓ |
| PARTICLE SIZE | NMT 2.00 % > 250 µm (60 mesh) | 0.87% ✓ |

ANALYST: _____ DATE: 09/10/2003 REF NO.: — ANALYSIS TIME: 1.6 day

MATERIAL STATUS: RELEASED ✓ PASSED REJECTED

17/10/03

REMARKS: _____

RELEASE AUTHORITY: Stead ✓ DATE: 9.10.03

REVIEWED BY QA: _____ DATE: 9.10.2003

FINAL RELEASE BY QA: _____ DATE: 9/10/2003

Marking sheet unsigned. Corrected 09/10/2003

PROTECTED

OK Stead 13/9/02

# Annexure B
# Experimental Data

**Table B-1:** Dissolution data for pyrazinamide release from pure chitosan beads at pH 5.60 and 7.40 over a period of 6 hours.

| | pH 5.60 | | pH 7.4 | |
|---|---|---|---|---|
| Time (min) | % Release | Standard Deviation | % Release | Standard Deviation |
| 0 | 0 | 0 | 0 | 0 |
| 2 | 14.19658 | 0.4681526 | 16.97164 | 1.4896191 |
| 5 | 28.3426 | 2.0585765 | 28.93891 | 0.4819884 |
| 10 | 41.7272 | 1.4523689 | 44.14496 | 1.4061643 |
| 15 | 52.75035 | 1.3514012 | 55.73763 | 1.4925757 |
| 20 | 60.92083 | 1.4782132 | 63.64904 | 2.1482244 |
| 30 | 70.11276 | 2.3142232 | 70.54448 | 3.085351 |
| 45 | 75.03969 | 2.2794275 | 73.65844 | 3.1722487 |
| 60 | 75.91031 | 2.1182241 | 74.29626 | 3.568603 |
| 120 | 76.13093 | 1.7421451 | 74.08001 | 3.5389493 |
| 180 | 75.72559 | 1.7164065 | 75.87606 | 3.6805504 |
| 240 | 75.31001 | 1.6076948 | 75.82339 | 3.6802607 |
| 300 | 76.31766 | 1.7962813 | 75.92668 | 3.9140955 |
| 360 | 76.22434 | 1.4861718 | 75.00929 | 3.6084709 |

**Table B-2:** Dissolution data for pyrazinamide release from pure chitosan/Ascorbic acid beads at pH 5.60 and 7.40 over a period of 6 hours.

| | pH 5.60 | | pH 7.4 | |
|---|---|---|---|---|
| Time (min) | % Release | Standard Deviation | % Release | Standard Deviation |
| 0 | 0 | 0 | 0 | 0 |
| 2 | 11.82687 | 2.30063789 | 20.95091 | 1.93425674 |
| 5 | 22.69502 | 1.47168216 | 30.22301 | 0.32600167 |
| 10 | 35.62905 | 1.96757922 | 39.96351 | 1.53064861 |
| 15 | 44.57135 | 1.58732531 | 48.30468 | 0.83380177 |
| 20 | 51.18761 | 2.24024947 | 52.64786 | 0.82489726 |
| 30 | 55.91648 | 2.29605076 | 56.19972 | 1.4093505 |
| 45 | 59.00164 | 1.90731989 | 57.459 | 1.36126306 |
| 60 | 59.63246 | 2.0211065 | 57.47642 | 1.21149628 |
| 120 | 59.19076 | 1.93649809 | 57.34545 | 1.12460105 |
| 180 | 59.95489 | 1.89655929 | 56.7411 | 1.09440154 |
| 240 | 60.0202 | 1.97440057 | 57.66397 | 1.04438729 |
| 300 | 59.56992 | 2.06304257 | 57.73121 | 1.17330381 |
| 360 | 59.76739 | 1.91221713 | 57.83066 | 1.22327156 |

**Table B-3:** Dissolution data for pyrazinamide release from pure chitosan/Explotab beads at pH 5.60 and 7.40 over a period of 6 hours.

| | pH 5.60 | | pH 7.4 | |
|---|---|---|---|---|
| Time (min) | % Release | Standard Deviation | % Release | Standard Deviation |
| 0 | 0 | 0 | 0 | 0 |
| 2 | 15.58264 | 1.275972 | 16.6673 | 0.809152 |
| 5 | 28.42935 | 0.615872 | 25.92579 | 1.443754 |
| 10 | 43.02834 | 1.344641 | 38.0324 | 1.751499 |
| 15 | 49.34063 | 2.592282 | 46.97329 | 2.012339 |
| 20 | 53.44959 | 2.054049 | 52.19398 | 2.159605 |
| 30 | 58.28493 | 1.421549 | 56.51874 | 2.446319 |
| 45 | 59.66751 | 1.457876 | 57.45299 | 1.862976 |
| 60 | 59.84498 | 1.461536 | 57.63172 | 2.00594 |
| 120 | 60.70382 | 1.183942 | 57.99547 | 2.087912 |
| 180 | 60.6141 | 1.205393 | 58.13161 | 2.061058 |
| 240 | 60.40496 | 1.114979 | 58.11301 | 2.05086 |
| 300 | 60.71387 | 1.484365 | 58.08774 | 1.921858 |
| 360 | 61.06811 | 0.914673 | 58.05693 | 1.873296 |

**Table B-4:** Dissolution data for pyrazinamide release from pure chitosan/Ac-Di-Sol beads at pH 5.60 and 7.40 over a period of 6 hours.

| | pH 5.60 | | pH 7.4 | |
|---|---|---|---|---|
| Time (min) | % Release | Standard Deviation | % Release | Standard Deviation |
| 0 | 0 | 0 | 0 | 0 |
| 2 | 13.16388 | 0.240137 | 12.42637 | 0.415423 |
| 5 | 23.60523 | 0.973685 | 23.56891 | 0.36213 |
| 10 | 36.73192 | 1.292419 | 36.38629 | 1.944371 |
| 15 | 45.06973 | 0.973148 | 46.62848 | 2.143585 |
| 20 | 51.39312 | 1.258977 | 53.6794 | 2.697822 |
| 30 | 56.5328 | 0.755266 | 59.20465 | 3.043954 |
| 45 | 58.30111 | 0.808098 | 60.98614 | 3.754491 |
| 60 | 58.55902 | 0.812253 | 61.64895 | 3.847043 |
| 120 | 58.52831 | 0.822211 | 61.56739 | 3.500906 |
| 180 | 58.59786 | 0.874832 | 62.01376 | 3.467614 |
| 240 | 58.69944 | 0.937367 | 62.209 | 3.303241 |
| 300 | 58.75532 | 0.71426 | 62.07953 | 3.558974 |
| 360 | 58.81123 | 0.928208 | 62.23415 | 3.932558 |

**Table B-5:** Dissolution data for pyrazinamide release from pure chitosan/Ascorbic acid/Explotab beads at pH 5.60 and 7.40 over a period of 6 hours.

| | pH 5.60 | | pH 7.4 | |
|---|---|---|---|---|
| Time (min) | % Release | Standard Deviation | % Release | Standard Deviation |
| 0 | 0 | 0 | 0 | 0 |
| 2 | 20.754 | 3.700521 | 24.29587 | 1.815221 |
| 5 | 35.47106 | 1.719601 | 36.94382 | 0.804862 |
| 10 | 48.58809 | 0.051097 | 47.505 | 1.719872 |
| 15 | 55.05828 | 1.088956 | 52.89177 | 2.058446 |
| 20 | 57.86678 | 2.132356 | 54.55004 | 2.009337 |
| 30 | 58.52042 | 2.403312 | 55.04943 | 1.901796 |
| 45 | 58.80111 | 2.512271 | 55.28642 | 2.250849 |
| 60 | 58.74111 | 2.565225 | 55.41216 | 1.927931 |
| 120 | 58.50189 | 2.489641 | 56.03814 | 1.922323 |
| 180 | 59.10068 | 2.589686 | 55.80221 | 2.169647 |
| 240 | 59.22544 | 2.358124 | 56.22053 | 2.088892 |
| 300 | 59.07475 | 2.306203 | 55.90225 | 2.04261 |
| 360 | 59.39084 | 2.079236 | 56.01139 | 1.982256 |

**Table B-6:** Dissolution data for pyrazinamide release from pure chitosan/Ascorbic acid/Ac-Di-Sol beads at pH 5.60 and 7.40 over a period of 6 hours.

| | pH 5.60 | | pH 7.4 | |
|---|---|---|---|---|
| Time (min) | % Release | Standard Deviation | % Release | Standard Deviation |
| 0 | 0 | 0 | 0 | 0 |
| 2 | 18.18494 | 0.985649 | 16.25629 | 0.733465 |
| 5 | 31.61634 | 1.064247 | 28.93516 | 1.587639 |
| 10 | 42.82384 | 1.628287 | 44.0109 | 2.555987 |
| 15 | 50.05932 | 1.297921 | 50.73902 | 2.421738 |
| 20 | 53.54621 | 1.374497 | 53.6604 | 2.117638 |
| 30 | 55.28407 | 1.645289 | 55.4775 | 2.467442 |
| 45 | 56.06072 | 1.392874 | 56.14551 | 3.06473 |
| 60 | 56.03324 | 1.65088 | 56.1397 | 2.991153 |
| 120 | 55.77512 | 1.685442 | 56.15928 | 3.054593 |
| 180 | 55.54039 | 1.693595 | 55.75386 | 2.99135 |
| 240 | 55.68499 | 1.805088 | 55.98521 | 2.99858 |
| 300 | 55.50307 | 1.834191 | 56.5676 | 3.085 |
| 360 | 55.50739 | 1.74298 | 56.73156 | 3.003833 |

**Table B-7:** Dissolution data for pyrazinamide release from pure chitosan/Explotab/Ac-Di-Sol beads at pH 5.60 and 7.40 over a period of 6 hours.

| | pH 5.60 | | pH 7.4 | |
|---|---|---|---|---|
| Time (min) | % Release | Standard Deviation | % Release | Standard Deviation |
| 0 | 0 | 0 | 0 | 0 |
| 2 | 19.23664 | 2.9431284 | 17.86192 | 0.9460902 |
| 5 | 26.74758 | 0.5834486 | 26.66311 | 0.2593306 |
| 10 | 35.92361 | 1.5086707 | 36.59965 | 1.2626222 |
| 15 | 42.66746 | 2.0524547 | 44.46267 | 1.3398255 |
| 20 | 47.18713 | 1.6880903 | 49.2097 | 1.2085735 |
| 30 | 53.35708 | 2.2653276 | 54.13267 | 1.5379298 |
| 45 | 55.93499 | 2.0539054 | 56.00478 | 2.2575219 |
| 60 | 56.94494 | 2.0603886 | 56.54815 | 2.516985 |
| 120 | 57.77306 | 1.3455317 | 56.51595 | 2.6181617 |
| 180 | 58.07453 | 1.5300441 | 56.19049 | 2.6360314 |
| 240 | 58.27726 | 1.6102594 | 56.9099 | 2.7500935 |
| 300 | 58.41271 | 1.6893488 | 56.89617 | 2.6305668 |
| 360 | 58.02827 | 1.552374 | 56.90987 | 2.7074651 |

**Table B-8:** Statistical interpretation of solubility (Tukey HSD test).

| Cell No. | Tukey HSD test; variable Solubility (JBHavenga_ Solubility.sta)<br>Approximate Probabilities for Post Hoc Tests<br>Error: Between MS = .93821, df = 7.0000<br>Bead_formulations | {1}<br>51.770 | {2}<br>49.400 | {3}<br>47.860 | {4}<br>42.760 | {5}<br>47.030 | {6}<br>48.615 | {7}<br>44.550 |
|---|---|---|---|---|---|---|---|---|
| 1 | Chit/Pyr | | 0.301815 | 0.045998 | 0.000549 | 0.017641 | 0.115992 | 0.001681 |
| 2 | Chit/ASC | 0.301815 | | 0.693435 | 0.002707 | 0.301815 | 0.976029 | 0.015632 |
| 3 | Chit/EXPL | 0.045998 | 0.693435 | | 0.011940 | 0.968939 | 0.980089 | 0.095723 |
| 4 | Chit/ADS | 0.000549 | 0.002707 | 0.011940 | | 0.030072 | 0.005554 | 0.560128 |
| 5 | Chit/ASC/ADS | 0.017641 | 0.301815 | 0.968939 | 0.030072 | | 0.669384 | 0.265204 |
| 6 | Chit/EXPL/ADS | 0.115992 | 0.976029 | 0.980089 | 0.005554 | 0.669384 | | 0.038246 |
| 7 | Chit/ASC/EXPL | 0.001681 | 0.015632 | 0.095723 | 0.560128 | 0.265204 | 0.038246 | |

**Table B-9:** Statistical interpretation of Mean Dissolution Time at PBS pH 5.60. (Tukey HSD test).

| Cell No. | Tukey HSD test; variable MDT (MDT stats.sta)<br>Approximate Probabilities for Post Hoc Tests<br>Error: Between MS = 3.9875, df = 14.000<br>Include condition: pbs='5_60'<br>Bead Formulation | {1}<br>13.370 | {2}<br>11.270 | {3}<br>11.863 | {4}<br>10.770 | {5}<br>8.1600 | {6}<br>5.3233 | {7}<br>11.267 |
|---|---|---|---|---|---|---|---|---|
| 1 | Chit | | 0.846585 | 0.962208 | 0.687813 | 0.073667 | 0.003298 | 0.845680 |
| 2 | Chit/ASC | 0.846585 | | 0.999749 | 0.999907 | 0.506706 | 0.033013 | 1.000000 |
| 3 | Chit/EXPL | 0.962208 | 0.999749 | | 0.992357 | 0.320984 | 0.017098 | 0.999741 |
| 4 | Chit/ADS | 0.687813 | 0.999907 | 0.992357 | | 0.684305 | 0.057114 | 0.999911 |
| 5 | Chit/ASC/EXPL | 0.073667 | 0.506706 | 0.320984 | 0.684305 | | 0.603532 | 0.507861 |
| 6 | Chit/ASC/ADS | 0.003298 | 0.033013 | 0.017098 | 0.057114 | 0.603532 | | 0.033134 |
| 7 | Chit/EXPL/ADS | 0.845680 | 1.000000 | 0.999741 | 0.999911 | 0.507861 | 0.033134 | |

**Table B-10:** Statistical interpretation of Mean Dissolution Time at PBS pH 7.40. (Tukey HSD test).

| Cell No. | Tukey HSD test; variable MDT (MDT stats.sta)<br>Approximate Probabilities for Post Hoc Tests<br>Error: Between MS = 4.0057, df = 14.000<br>Include condition: pbs='7_40'<br>Bead Formulation | {1}<br>10.930 | {2}<br>10.123 | {3}<br>9.1100 | {4}<br>12.020 | {5}<br>5.9133 | {6}<br>10.230 | {7}<br>10.970 |
|---|---|---|---|---|---|---|---|---|
| 1 | Chit | | 0.998568 | 0.913615 | 0.992568 | 0.091501 | 0.999364 | 1.000000 |
| 2 | Chit/ASC | 0.998568 | | 0.994956 | 0.897633 | 0.205267 | 1.000000 | 0.998127 |
| 3 | Chit/EXPL | 0.913615 | 0.994956 | | 0.579631 | 0.479463 | 0.991434 | 0.905469 |
| 4 | Chit/ADS | 0.992568 | 0.897633 | 0.579631 | | 0.028084 | 0.919441 | 0.993900 |
| 5 | Chit/ASC/EXPL | 0.091501 | 0.205267 | 0.479463 | 0.028084 | | 0.185433 | 0.087732 |
| 6 | Chit/ASC/ADS | 0.999364 | 1.000000 | 0.991434 | 0.919441 | 0.185433 | | 0.999129 |
| 7 | Chit/EXPL/ADS | 1.000000 | 0.998127 | 0.905469 | 0.993900 | 0.087732 | 0.999129 | |

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