INFLUENCE OF MODIFIED RELEASE EXCIPIENTS ON KETOPROFEN RELEASE FROM CHITOSAN PARTICLES

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To God who carries me in his arms every day and exposes me to the greatness of the world.

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CONTENTS

INTRODUCTION AND AIM OF STUDY ..... **V**

ABSTRACT ..... **VII**

UITREKSEL ..... **IX**

Chapter 1

FORMULATION OF A SUSTAINED RELEASE DOSAGE FORM **1**

1.1 INTRODUCTION 1

1.2 CONTROLLED RELEASE 3

1.2.1 Characteristics *3*

1.2.2 Mechanism of release *5*

1.3 POLYMERS AS DRUG CARIERS 8

1.4 CHITOSAN A NATURAL POLYMER 11

1.4.1 Properties of chitosan *12*

1.4.2 Synthesis of chitosan from chitin rich bio-material *14*

1.4.3 Structure of chitosan *15*

1.4.4 Pharmaceutical applications of chitosan *16*

1.4.5 Safety of chitosan *17*

1.5 SEMI-SYNTHETIC POLYMERS FOR SUSTAINED RELEASE 18

1.5.1 The Polymers Used in the study *20*

1.5.1.1 Cellulose and its derivatives 20

1.5.1.2 Methacrylates 21

1.5.1.3 Polyvinylpyrrolidone 23

1.6 CONCLUSION 24

Chapter 2

PREPARATION AND CHARACTERISATION OF BEADS AND GRANULES FOR CONTROLLED DRUG DELIVERY **26**

2.1 INTRODUCTION 26

2.2 MODEL DRUG: KETOPROFEN 27

2.3 BEADS 28
2.3.1 Preparation of beads *29*
2.3.2 Preparation of beads for the study *34*
2.4 GRANULES 35
2.4.1 Preparation of granules *35*
2.4.2 Preparation of granules for study *37*
2.5 CHARACTERISATION OF BEADS AND GRANULES 38
2.5.1 Dissolution and drug release *38*
2.5.2 Drug loading capacity *41*
2.5.3 Friability *42*
2.5.4 Morphology: Scanning electron microscopy *42*
2.5.5 Solubility *43*
2.5.6 Swelling and degradation *43*
2.5.7 Calculations *44*
2.6 CONCLUSION 44

Chapter 3

RESULTS AND DISCUSSION OF CHARACTERIZATION TESTS 46

3.1 INTRODUCTION 46
3.2 MORPHOLOGY 46
3.2.1 Method *46*
3.2.2 Results *46*
3.2.3 Discussion *51*
3.2.3.1 Beads 51
3.2.3.2 Granules 52
3.3 DRUG LOADING CAPACITY 54
3.3.1 Method *54*
3.3.2 Results *54*
3.3.3 Discussion *55*
3.4 FRIABILITY 56
3.4.1 Method *56*
3.4.2 Results *57*
3.4.3 Discussion *57*
3.5 SOLUBILITY 58
3.5.1 Method *58*

3.5.2 Results *59*
3.5.2.1 Beads 59
3.5.2.2 Granules 59
3.5.3 Discussion *60*
3.5.3.1 Beads 60
3.5.3.2 Granules 61
3.6 SWELLING BEHAVIOUR 63
3.6.1 Method *63*
3.6.2 Results *63*
3.6.3 Discussion *65*
3.7 CONCLUSION 67

Chapter 4

DISSOLUTION STUDIES OF CHITOSAN AND CHITOSAN-CONTROLLED RELEASE POLYMER BEADS 70

4.1 INTRODUCTION 70
4.2 MODELING AND CHARACTERISATION OF DISSOLUTION PROFILES 71
4.2.1 Zero order *71*
4.3 MEAN DISSOLUTION TIME 72
4.4 COMPARISON OF DISSOLUTION PROFILES 73
4.4.1 Similarity factor *73*
4.5 BURST EFFECT 74
4.6 METHODOLOGY 75
4.7 RESULTS AND DISCUSSION 77
4.7.1 Mean dissolution time *77*
4.7.2 Similarity factor (f_2) *79*
4.7.3 Formulations of 1% w/v and 14.29% w/w CR polymer *79*
4.7.3.1 Results 79
4.7.3.2 Discussion 81
4.7.4 Formulations of 2.5% w/v and 29.41% w/w CR polymer *84*
4.7.4.1 Results 84
4.7.4.2 Discussion 86
4.7.5 Formulations of 5% w/v and 45.45% w/w CR polymer *88*
4.7.5.1 Results 88
4.7.5.2 Discussion 90
4.8 SUMMARY AND CONCLUSION 92

Chapter 5

SUMMARY AND FUTURE PROSPECTS **95**

5.1 SUMMARY 95

5.2 FUTURE PROSPECTS 98

ANNEXURE A **100**

ANNEXURE B **106**

ANNEXURE C **118**

RESEARCH ARTICLE: THE INFLUENCE OF POLYVINYLPYRROLIDONE ON THE RELEASE PROFILE OF KETOPROFEN FROM CHITOSAN MATRIX PARTICLES 119

LIST OF FIGURES **142**

LIST OF TABLES **145**

REFERENCES **146**

INTRODUCTION AND AIM OF THE STUDY

In recent years there has been increasing interest in controlled release dosage forms. These controlled release dosage forms have many advantages over conventional dosage forms, many of these advantages such as less frequent dosage intervals, and better control over drug plasma concentration fluctuation are for the patient. Controlled release polymers not only benefits the patient, but drug side-effects like gastric irritation or drug stability problems like acidic degradation can also be minimized or overcome by using these polymers. The production of controlled release formulations that can overcome these obstacles is many times not as simple as the formulation of conventional dosage forms, and may involve complex steps such as production and coating with several layers of different polymers. With each additional step in the production of dosage forms the costs multiply and there is a risk of some wastage of materials. In third world counties these formulations could significantly enhance the quality of life of many patients, but often production costs outweigh the immediate advantages.

Beads are frequently utilized in controlled release formulations, as they are often simple to prepare and show various advantages over conventional dosage forms. In this study an attempt will be made to produce beads according to a simple method, capable of delayed and controlled release. The flexibility of chitosan gel beads allows for the incorporation of many different drugs, but often problems of low drug loading is experienced when a drug with a high solubility in alkaline regions is incorporated. This study will therefore also attempt to create a method of bead production capable of overcoming this obstacle. To achieve the desired goals, the effect of modern controlled release polymers on the release rate of the drug from the chitosan beads will be studied. By incorporating these polymers the creation of an optimal controlled release dosage form will be studied.

To study the effectiveness of the bead samples produced in the study ketoprofen was chosen as the drug of choice. Ketoprofen is a non-steroidal anti-inflammatory drug with a short half-life and high solubility in alkaline environments and, like most of the non-steroidal anti-inflammatory drugs, can cause severe gastric irritation which can lead to gastric ulceration.

The objectives of the study are:

- To conduct a literature study into:
  - The development, effects, and mechanism of drug release form 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.

- To formulate a method for the economic and simple production of an enteric release dosage form with controlled release characteristics in the intestinal environment.

- To determine the effectiveness of these samples as drug delivery systems.

ABSTRACT

Controlled release formulations offer many advantages over conventional dosage forms. These include reduced plasma fluctuations and improved patient compli: nce. Complex controlled release formulations such as those with enteric release properties, often require additional steps in the production phase. The costs and economic impact associated with these complex controlled release dosage formulations often outweigh the immediate 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 generally dispersed throughout a polymer matrix. The rate of drug release is often determined by the viscosity or complexity of the polymer matrix through which the drug needs to diffuse in order to be released. With enteric release the polymer coating, insoluble in an acidic environment is often applied in the final phase of production.

Chitosan is a versatile polymer of natural origin with many favorable characteristics. These include its safety, biocompatibility, and biodegradability. Simple methods can be applied and modified to produce controlled release particles form chitosan. The effect of modern controlled release polymers such as Aqoat® AS-HF, Eudragit® S100 and Kollidon® SR was investigated.

Chitosan beads and chitosan-polymer beads, as well as chitosan granules and chitosan-polymer granules, were prepared and investigated as possible controlled release formulations. Ketoprofen was chosen as the model drug. Chitosan beads and chitosan-polymer beads were prepared by inotropic gelation in tripolyphosphate. Chitosan

granules and chitosan-polymer matrix granules were prepared by binding chitosan with an acetic acid solution as a granulating system. The beads and granules appeared differed in appearance as well as in the results obtained from various experiments. Granules prepared in the study did not appear to be effective with regards to enteric and controlled release. Beads prepared form Kollidon SR appeared to be effective with regards to enteric and controlled release, with Kollidon 1% and 5% w/v chitosan beads achieving good drug loading of up to 73.13% and releasing less than 15 % of the total drug content in 0.1 M HCl after 60 minutes. Drug release continued steadily for up too 360 minutes in pH 7.2. It was concluded that Kollidon® SR loaded chitosan beads may be a viable controlled release dosage form with enteric release properties, and that future experiments, possibly with lower polymer concentrations, are worthwhile.

Key words: Beads; Granules; Chitosan; Inotropic gelation; Controlled release; Ketoprofen; Aqoat® AS-HF; Eudragit® S100; Kollidon® SR.

UITTREKSEL

Gekontroleerde vrystellingsdoseervorme bied verskeie voordele oor konvensionele doseervorme. Hierdie voordele sluit in verminderde plasmafluktuasies en verbeterde pasiëntmeewerkendheid. Komplekse gekontroleerde vrystellingsdoseervorme soos die met enteriese vrystellingseienskappe benodig dikwels addisionele stappe tydens vervaardiging. Die kostes en ekonomiese impak geassosieër 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 wêreldslande.

In gekontroleerde vrystellingsdoseervorme is die geneesmiddel dikwels eweredig versprei in 'n polimeermatriks. Die tempo van geneesmiddelvrystelling word meestal bepaal deur die viskositeit of kompleksiteit van die polimeermatriks waardeur die geneesmiddel moet diffundeer om vrygestel te word. Vir enteriese vrystelling word 'n addisionele polimeerbedekking dikwels aangewend tydens die finale vervaardigingsvase.

Chitosan is 'n veeldoelige polimeer van natuurlike oorsprong met vele gunstige karaktereienskappe. Hierdie eienskappe sluit in veiligheid, bio-verenigbaarheid, en bio-afbreekbaarheid. Eenvoudige metodes kan aangewend en aangepas word om gekontroleerde vrystellingsdeeltjies van chitosan te berei. Die effek van moderne gekontoleerde vrystellingspolimere, soos Aqoat® AS-HF, Eudragit® S100 en Kollidon® SR is ondersoek.

Ketoprofien is gekies as modelgeneesmiddel. Chitosankrale en chitosan-polimeer matrikskrale is berei deur inotropiese jelering in tripolifosfaat. Chitosan granules en

chitosan polimeer matriks granules is berei deur binding met chitosan wat benat was deur 'n asynsuuroplossing as granuleringsisteem. Die krale en granules het betekenisvol verskil in voorkoms, en in die resultate verkry vanuit verskeie eksperimente. Granules wat berei is in die studie was nie suksesvol in enteriese of gekontroleerde vrystelling nie. Dit was moontlik om die vrystelling van ketoprofien te kontoleer met Kollidon ® SR. Kollidon ® SR 1% en 5% m/v chitosan krale het goeie geneesmiddellading getoon, met waardes so hoog as 73.13% en het minder as 15% geneesmiddel vrygestel na 60 minute in 0.1 M HCl. Verder het die vrystelling van die geneesmiddel toegeneem tot op 360 minute in 'n medium met pH 7.2. Daar is tot die gevolgtrekking gekom dat Kollidon® SR belaaide chitosan krale moontlik 'n lewensvatbare gekontroleerde vrystelling doseervorm is met enteriese vrystellingseineskappe, en dat toekomstige eksperimente, moontlik met laer polimeerkonstrasies, die moeite werd sal wees.

Sleutelwoorde: Krale; Granules; Chitosan; Inotropiese Jelering; Gekontroleerde vrystelling; Ketoprofien; Aqoat® AS-HF; Eudragit® S100; Kollidon® SR;

CHAPTER 1

Formulation of a sustained release dosage form

1.1 INTRODUCTION

The past three decades saw a development of controlled release dosage forms. These dosage forms have increasingly gained popularity over other dosage forms in treating disease. Controlled release dosage forms have a significant potential to provide economic value by enhancing efficacy and decreasing treatment costs. Controlled release systems provide numerous benefits over conventional dosage forms. Conventional dosage forms are still a predominant pharmaceutical dosage form. These dosage forms are not able to control either the rate of drug delivery or the area of drug release and provide an immediate or rapid drug release. Thus frequent administration is needed to maintain therapeutic drug levels over any treatment period. This causes drug levels to fluctuate widely in the blood and tissues. The concentration may initially be high and cause side-effects, and then later decline below therapeutic levels (Saks & Gardner, 1997:237).

Chitosan, a natural polymer, is obtained from the alkaline deacetylation of chitin which is widely distributed in nature as the principal component of the exoskeletons of crustaceans and insects as well as the cell walls of some bacteria and fungi (Şenel & McClure, 2004:1). Chitosan has several properties of interest to the pharmaceutical industry. The simplest way of achieving slow release of drugs by means of chitosan is to employ it as a gel-forming excipient in matrix-type formulations. Kawashima *et al.* (1985:2107) found that chitosan retarded release of aspirin from tablets during dissolution testing in an acidic environment. Chitosan has been shown to be a viable excipient for controlled release bead formulation. Win *et al.* (2003:305) suggested that chitosan gel beads may be useful as a controlled drug delivery system in oral

administration and may avoid drug release in the highly acidic gastric fluid region of the stomach.

Chitosan has also been used extensively in formulations of granulates. Chitosan acts as a controlled release excipient in these formulations. Miyazaki *et al.* (1998:642) showed the potential of chitosan granules as an oral controlled release dosage form more than a decade ago. When a commercial capsule was administered orally, peak plasma concentrations were reached within 30 min. The granules did not give a sharp peak in the plasma concentration, but produced a sustained plateau of the drug. This was due to the slow release rate and a longer residence time of chitosan granules in the stomach. Sustained release was observed in this dosage form. Tarıncı and Ermiş (1997:71) studied the effect of chitosan granules as a means of achieving sustained release of ketoprofen in suppositories. It was found that the release rate could be controlled by variation in the mixing ratio of the drug and chitosan.

Chandy and Sharma (1992:949 and 1993:939) drew a comparison between chitosan matrix formulations, chitosan beads and chitosan granules. It was found, in two separate studies, using nifedipine and ampicillin, that chitosan matrix formulations rendered slower release than chitosan beads and granules. However, it was speculated that by simple manipulations to the formulations it would be possible to obtain the desired controlled release from chitosan products.

Mi *et al.* (1997:2495) found that the mechanism of action of chitosan in controlled release matrix-type formulations, are based on its ability to act as a gel forming excipient. Ritger and Peppas (1987:37) found that chitosan formed swellable and degradable gels in controlled release tablets, and that the mechanism of release was typical of hydrogel-based systems, namely non-Fickian diffusion. Release kinetics was almost zero-order. Gel formation was more significant in acidic environments (pH 1.2) than in alkaline environments. The retardation effect of chitosan on drug

release was therefore found to be most marked in acidic environments (Goskonda and Upadrastha, 1993:915).

Säkinen (2003:44) incorporated small amounts of enteric polymers in either a matrix formulation or as a coating. This was done to reinforce the gels formed by chitosan and to control the release at acidic pH values. Small amounts of these enteric polymers considerably prolonged drug absorption. This study suggests that a combination of chitosan, which acts as a gel forming excipient, and a polymer insoluble in acidic environments could be valuable in the development of controlled release formulations.

1.2 CONTROLLED RELEASE

1.2.1 CHARACTERISTICS

Controlled release dosage forms attempt to alter drug absorption by modifying the release rate. This leads to reduction in fluctuations in plasma drug concentration, increased apparent half-life, and sustained drug effects. Assuming certain absorption and metabolism characteristics, a constant release rate may result in constant blood levels and maximize the duration for which drug levels are in the therapeutic range. Much of the research in this area has been influenced by the desire to achieve a constant release rate or zero-order delivery (Thrombre, 1991:156). In recent years controlled drug delivery formulations and the polymers used in these systems have become much more sophisticated, with the ability to do more than simply extend the effective release period of a drug. Current controlled release systems can respond to changes in the biological environment and deliver, or cease to deliver drugs, based on these changes. In addition, materials have been developed that should lead to targeted delivery systems, in which a particular formulation can be directed to the specific cell, tissue, or site where the drug it contains, is to be delivered (Brannon-Peppas, 1997:2). Thus a number of features distinguish controlled release dosage forms form conventional pharmaceutical dosage forms. See Table 1-1.

Table 1-1: Features unique to controlled release dosage forms (Adapted from Thrombre, 1991:156).

Altered pharmacokinetics because of modified release (input) rates:

- Lower peak-to-through blood level fluctuations.
- Longer apparent half-life.

Multi-disciplinary research and development teams are important.

Emphasis on device performance and reliability:

- Medical rationale for controlled release *in vitro* tests vs. *in vivo* performance.
- Effect of food and/or pH of gastro-intestinal tract on performance.
- Device reliability and reproducibility.
- Safety of novel dosage forms.
- Bioavailability and bioequivalence.

Manufacture relies on advances in polymer science and technology.

Controlled release dosage forms have certain advantages to conventional dosage forms, these include (Krowcynski 1987:12):

- Improved patient compliance.
- Decrease in total drug dose.
- Reduction in local or systemic side-effects.
- Reduction in potential loss of drug activity.
- Improvement in treatment efficiency.
- Improvement in speed of control of medical conditions.

- Decreased blood level fluctuations.
- Improved bioavailability of some drugs.
- Reduction in costs of administration and specialized hospital personnel.

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

- Dose dumping.
- Less accurate dose adjustment (tablets can't be halved).
- Increased potential form first-pass metabolism.
- Delayed onset of drug action.

Not all drugs are good candidates for incorporation into controlled release dosage forms; these include drugs with long half-lives, drugs with a narrow therapeutic index, drugs with limited gastro-intestinal absorption and drugs with a high dosage. Furthermore, the cost of manufacturing controlled release technology may be exceptionally higher than that of conventional dosage forms (Krowcynski, 1987:12).

1.2.2 MECHANISM OF RELEASE

Controlled release dosage forms can easily be classified according to the mechanism that controls the release of the drug. The most common mechanism is diffusion. Two types of diffusion-controlled systems have been developed. Firstly a reservoir device in which the drug forms a core surrounded by an inert diffusion barrier (Figure 1-1). Examples of these systems include:

- Membranes
- Capsules
- Microcapsules
- Liposomes
- Hollow fibers

The second type is a monolithic device. The drug is dispersed or dissolved in an inert polymer (See Figure 1-2). This acts as a reservoir system. The drug diffusion through the polymer matrix is the rate-limiting step. The release rates can be altered by the choice of polymer (Langer, 1990:1527).

Results in a study by Narasimhan and Peppas (1997:297) indicated that the molecular weight of the polymer can control the rate of drug release. Polymer molecular weight is usually directly proportional to the viscosity of the polymer. An increase in viscosity can lead to slower diffusion rates of the drug through the polymer in sustained release dosage forms. With increasing viscosity grade the average molecular weight of the polymer chains increases, resulting in an increased entanglement of the macromolecules. Thus, the mobility of the polymer chains decreases, leading to a reduced free volume available for diffusion. Hence, the probability for a drug molecule to jump from one cavity to another is decreased, leading to a decreased mass transfer rate. Merkli *et al.* (1994:105) found that the rate of drug release was controlled by changing the molecular weight and indirectly the viscosity of the polymers. Polymers with 3500, 5800, 10100, 15200, 33300 Da molecular weight were synthesized. Drug release ranging from one day for the 3500 Da to 7 days for the 33300 Da molecular weight polymers was obtained.

Chemical control can be achieved using biodegradable polymers. Biodegradable systems are a favourable means of drug delivery because the biodegradable devices are eventually absorbed by the body and thus need not be removed surgically when implanted. Polymer biodegradation can be defined as the conversion of a material that is insoluble in water into one that is water-soluble. The drug is ideally distributed uniformly throughout the biodegradable polymer (Sigma Aldrich, 2004). The erosion process occurs either in bulk (wherein the matrix degrades uniformly) or at the polymer's surface (where by the release rate is related to the polymer's surface area). As the polymer surrounding the drug is eroded, the drug is released (See Figure 1-3) (Vogelson, 2004:49).

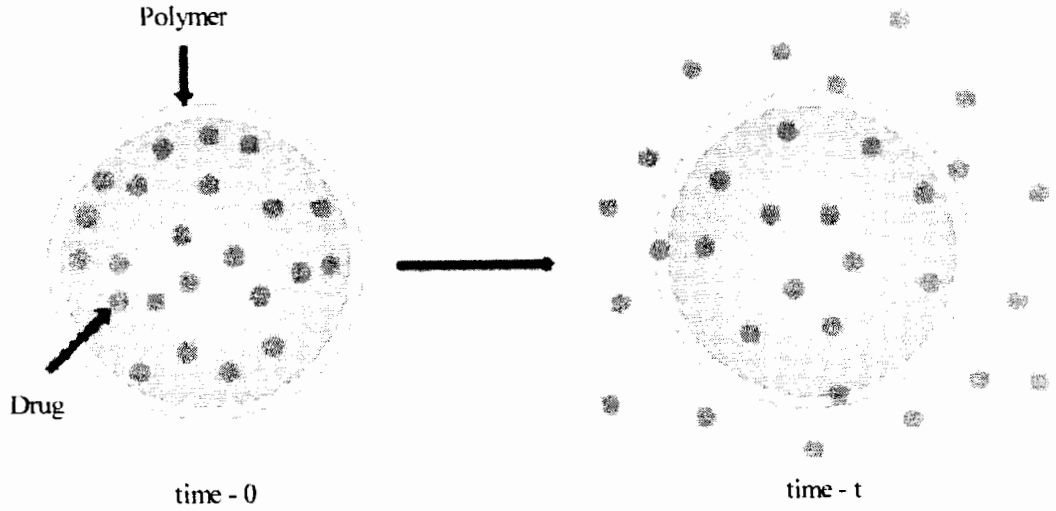


Figure 1-1: Representation of a reservoir diffusion controlled release system (Sigma Aldrich, 2004).

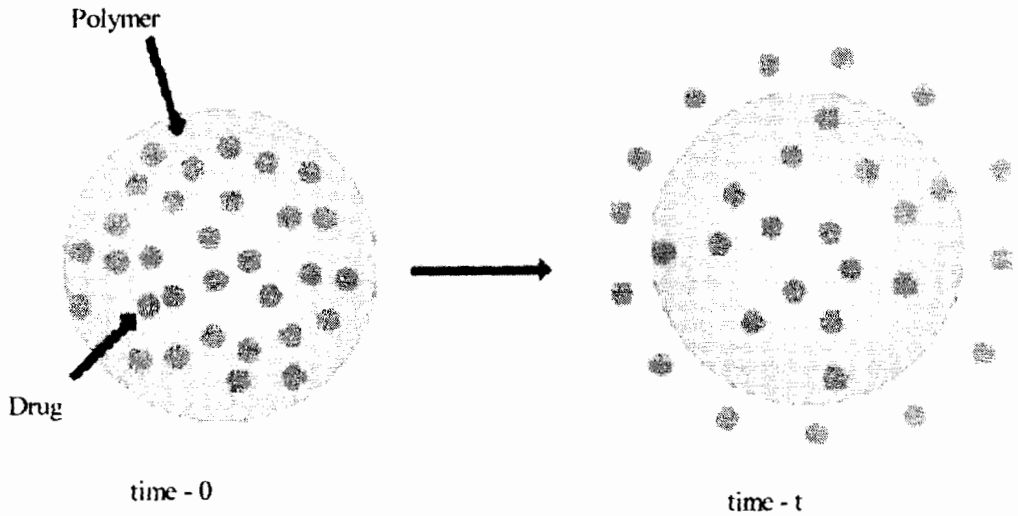


Figure 1-2: Representation of a monolithic diffusion controlled release system (Sigma Aldrich, 2004).

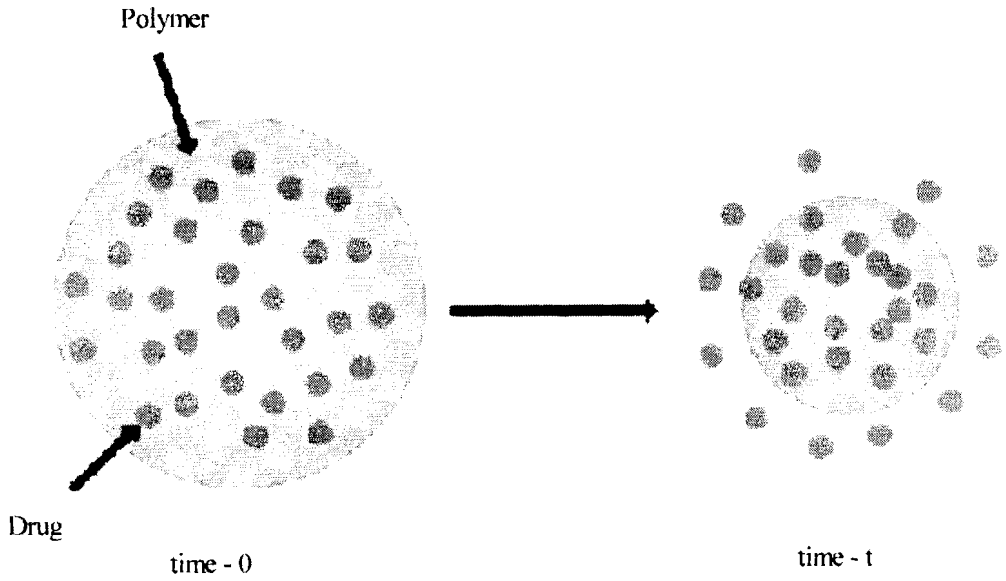


Figure 1-3: Representation of a biodegradable controlled release system (Sigma Aldrich, 2004).

Overall, controlled release dosage forms seem to be an important field of research, as these dosage forms are relatively new.

1.3 POLYMERS AS DRUG CARIERS

The earliest controlled drug delivery systems, introduced in the 1970's, were based on polymers formed by lactic acid. Today, polymeric material still provide 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 (Vogelson, 2001:49). Controlled drug delivery occurs when a polymer, whether natural or synthetic, is sensibly combined with drug or other active agent in such a way that the active agent is released from the material in a predetermined manner (Brannon-Peppas, 1997:1).

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

- swelling
- non-swelling
- porosity
- non-porosity
- degradability
- non-degradability
- bio-adhesion
- semi-permeability.

A range of materials has been used historically to control the release of drugs. The earliest of these polymers were originally selected because of their desirable physical properties. Examples of early biomaterials for drug controlled delivery include (Brannon-Peppas, 2004:3):

- Poly (urethanes) for elasticity
- Poly (siloxanes) or silicones for insulating ability
- Poly (methyl methacrylates) for physical strength and transparency
- Poly (vinyl alcohol) for hydrophilicity and strength
- Poly (ethylene) for toughness and lack of swelling
- Poly (vinyl pyrrolidone) for suspension capabilities

These polymers were not always biologically acceptable, and some can cause severe reactions when used *in vivo*. For a material to be successfully incorporated into the body it must have an appropriate physical structure, minimal undesired aging, and must be readily process able. Some polymers that are currently being used in studies for controlled drug delivery, that posses these characteristics are (Brannon-Peppas, 2004:3):

- Poly (2-hydroxy ethyl methacrylate)
- Poly (N-vinyl pyrrolidone)
- Poly (methyl methacrylate)
- Poly (vinyl alcohol)
- Poly (acrylic acid)
- Polyacrylamide
- Poly (ethylene-co-vinyl acetate)
- Poly (ethylene glycol)
- Poly (methacrylic acid)

In recent years additional polymers designed primarily for medical applications have entered the arena of controlled release. Many of these materials are designed to degrade within the body, among them (Brannon-Peppas, 2004:3):

- Polylactides (PLA)
- Polyglycolides (PGA)
- Poly (lactide-co-glycolides) (PLGA)
- Polyanhydrides
- Polyorthoesters

Early research into biodegradable systems focused on naturally occurring polymers such as collagen, cellulose, etc. Recent research moved more to the area of chemical synthesis, as these polymers are more easily manipulated by changes in their chemistry. Examples of such polymers include polyanhydrides, polyesters, polyacrylic acids, polymetylachrylates, polymetachrylates, and polyurethanes (Vogelson, 2004:50).

The main difference between controlled release dosage forms and conventional dosage forms lies in the disintegration of the conventional dosage form, where as the controlled release system seldom disintegrates. Even if the system eventually

disintegrates the dissolution of the matrix is usually carefully controlled to maintain its rigidity for a long period of time (Kim, 2000:7).

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

1.4 CHITOSAN A NATURAL POLYMER

Chitosan is a partially deacetylated polymer obtained from the alkaline deacetylation of chitin which is a glucose-based unbranched polysaccharide widely distributed in nature as the principal component of the exoskeletons of crustaceans and insects as well as of cell walls of some bacteria and fungi (Şenel & McClure, 2004:1). Rouget (1859:792) reportedly first discovered Chitosan in 1859 when he boiled chitin in a concentrated potassium hydroxide solution. This resulted in the deacetylation of chitin.

One reason why chitosan has become of interest is undoubtedly because it can be obtained from the second most abundant natural polymer, chitin. Academic and industrial scientists are faced with a vast challenge to find new and practical applications for this material (Goosen M.F, 1997: XI). During the past 20 years, a substantial amount of work has been published on this polymer and its potential use in various applications. To mention some areas of investigation: one of the most useful properties of chitosan is its chelating ability. Chitosan has been considered for pharmaceutical formulation and drug delivery applications in which attention has

been focused on its absorption-enhancing, controlled release and bio adhesive properties (Dodane & Vilivalam, 1998:246). Chitosan has also been shown to be a safe natural polymer and to improve HDL- and LDL- cholesterol levels in human trials (Ausars et al., 2003:397). Kofuji *et al.* (2004:65) observed that chitosan gel beads are a promising biocompatible and biodegradable vehicle for treatment of local inflammation. Şenel and McClure (2004:1) showed chitosan to be a very attractive substance for diverse applications such as a biomaterial in, pharmaceutical and medical fields, and that it has been used for systemic and local delivery of drugs and vaccines. It is for all the above reasons that chitosan was chosen to be evaluated in conjunction with, other new and well-known polymers, as an excipient for controlled release.

1.4.1 PROPERTIES OF CHITOSAN

Chitosan is insoluble at neutral and alkaline pH values, and forms water-soluble salts with inorganic and organic acids including glutamic, hydrochloric, lactic and acetic acids (Kristl *et al.*, 1993:13). Chitosan dissolves in acidic media. Upon dissolution, the amino groups of the polymer become protonated rendering the molecule positively charged. Recently, importance has been attached to chitosan derivatives with a defined degree of deacetylation and depolymerization because of their significantly different physicochemical properties. The degree of acetylation represents the proportion of N-acetyl-D-glucosamine units with respect to the total number of units (Singla & Chawla, 2001:1047). The properties of chitosan, such as pKa-value and solubility, can be modified by changing the degree of deacetylation and formulation properties such as the pH and ionic strength. In neutral pH solutions, most chitosan molecules will lose their charge and precipitate from solution (Şenel & McClure, 2004:2).

Chitosan can be manipulated by means of chemical derivatization to promote new biological activities and to modify its mechanical properties. The molecule contains primary amino groups that are reactive and provide a mechanism for side group

attachment using a variety of mild reaction conditions. In general, the addition of a side chain disrupts the crystal structure of the material and increases the amorphous fraction. This creates a material with lower stiffness and altered solubility. The precise nature of changes in chemical and biological properties of chitosan depends on the nature of the side group. In addition, characteristic features of chitosan, such as being cationic, haemostatic and insoluble at high pH, can be completely reversed by a sulfation process, rendering the molecule anionic and water-soluble, and also introduce anticoagulant properties (Shu & Matthew, 2000). The variety of groups that can be attached as a side chain to chitosan is almost unlimited, and side chain groups can be chosen to provide specific functionality, modify physical properties or alter biological properties.

Due to its high molecular weight and linear unbranched structure, Wang and Xu (1994:149–152) found that chitosan is an excellent viscosity-enhancing agent in acidic environments. It behaves as a pseudo-plastic material exhibiting a decrease in viscosity with increasing rates of shear. The viscosity of chitosan solutions increase with an increase in chitosan concentration; decrease in temperature and with increasing degree of deacetylation, which is a structural parameter also influencing physiochemical properties such as the molecular weight, the elongation at break and the tensile strength.

Chitosan is polycationic in acidic environments and possesses the ability to form gels at acidic pH values, because of its hydrophilic character and ability to retain water in its structure. The acetylation of chitosan in hydro-alcoholic media allows the selective modification of the free amino groups and is responsible for a process of gelation. The charge density of the chain segments is an essential parameter for the formation of gels and all factors that lower this parameter favour deswelling and reversibility (Vachoud *et al.*, 2000:295– 304). These physicochemical and physical properties, the high degree of hydration, as well as the polyelectrolyte behaviour of this kind of gel allow applications such as use as a controlled release drug-delivery system.

1.4.2 SYNTHESIS OF CHITOSAN FROM CHITIN RICH BIO-MATERIAL

The production of chitosan-glucan complexes is associated with a fermentation process, similar to those for the production of citric acid from *Aspergillus niger, Mucor rouxii* and *Streptomyces*, which involves alkaline treatment yielding chitosan-glucan complexes. The alkali removes the proteins and deacetylates chitin simultaneously. Depending on the alkali concentration, some soluble glycans are removed. The processing of crustacean shells mainly involves the removal of proteins and the dissolution of chitin carbonate, which is present in crab shells in high concentrations. The resulting chitin is deacetylated in 40% sodium hydroxide at 120°C for 1 to 3 hours. This treatment produces 70% deacetylated chitosan (Kumar, 2000:1-27). The process is also explained through Paul and Sharma's flow chart (See Figure 1-4).

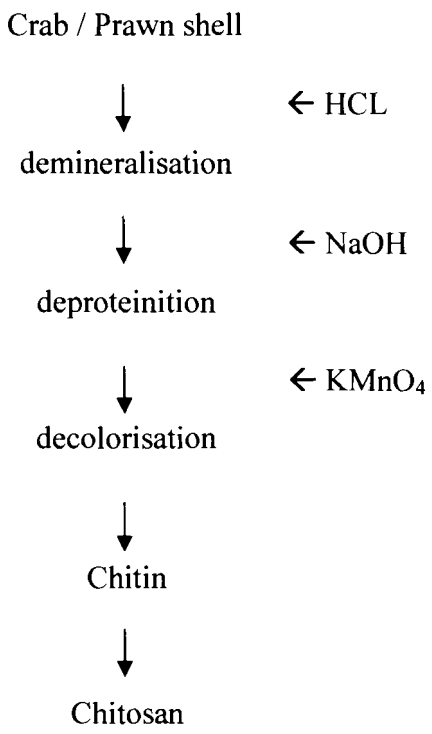


Figure 1-4: Chitosan production flow chart (Paul & Sharma, 2000:18).

1.4.3 STRUCTURE OF CHITOSAN

Chitosan consists of linear 1-4 linked 2-acetamino-2-deoxy-β-D-glucopyranose and 2-amino-β-D-glucopyranose units (Hamman *et al.*, 2000: 35-38). Chitosan has a high molecular weight and its unit formula is $C_6H_{11}O_4N$. There are two free hydroxyl groups and one primary amino group for each C_6 unit (Illum *et al.*, 1994:1187). The chemical structure of chitosan is given in Figure 1-5.

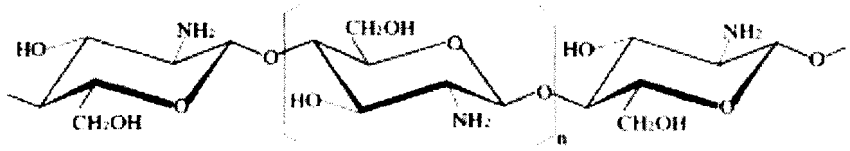


Figure 1-5: The chemical structure of chitosan (Adapted from Egawa *et al.*, 1999:238).

1.4.4 PHARMACEUTICAL APPLICATIONS OF CHITOSAN

Chitosan has attracted much interest in pharmaceutical research as a polymeric drug carrier and as a novel drug carrier enhancer across nasal and intestinal epithelia (Viljoen, 2002:25). Chitosan has also been shown as an excipient for direct tablet compression. Chitosan has been investigated in the pharmaceutical industry as an excipient to be used in, direct tablet compression, as a tablet disintegrant, for the production of controlled release solid dosage forms and for the improvement of drug dissolution. Chitosan has, compared to traditional excipients, been shown to have superior characteristics and especially flexibility in its use. Furthermore, chitosan has been used in the production of controlled release implant systems for the delivery of hormones over extended periods of time. Lately, the transmucosal absorption promoting characteristics of chitosan has been exploited especially for nasal and oral delivery of polar drugs to include peptides, proteins and for vaccine delivery (Illum, 1998:1326).

Säkkinen (2003:48) found that drug release *in vitro* could be controlled by altering the amount and grade of chitosan in the granules. Increasing the chitosan content decreased the release rate after the formation of chitosan gels in an acidic environment. Microcrystalline chitosan had a more marked retardant effect than conventional chitosan, because of its superior gel formation. Retardation of drug release increased with the molecular weight of microcrystalline chitosan but deacetylation had no marked effect. A slowed release was achieved with model drugs that are slightly soluble in acidic environments, such as ibuprofen and furosemide. With a readily water soluble drug, such as paracetamol, retardation of drug release was only slight. All the chitosans studied by Säkkinen, had fairly marked mucoadhesive tendencies *in vitro*. Adhesive strength increased with the molecular weight of chitosan, while other variables, including deacetylation and crystal structure, had no marked effect.

1.4.5 SAFETY OF CHITOSAN

The safety and biocompatibility of chitosan has been evaluated in several studies including studies on humans (Ausars, *et al.*, 2003:397; Ylitalo *et al.*, 2002:1). Because chitinase and chitosanase enzymes are not present in human intestine, chitosan is not specifically degraded; it is therefore not absorbed from the intestine. Chitosan is thus regarded as relatively safe following oral administration (Shahidi *et al.*, 1999:37). Knapczyk *et al.* (1989: 665-670) found that administration of a dose of 15 g/kg chitosan in Wistar rats, in solution or powder form, for 14 days, did not result in any acute toxic effects or pathological changes. The oral LD50% of chitosan in mice has been reported to be over 16 g/kg (Arai *et al.*, 1968: 89-94).

Chitosan acts by forming gels in the intestinal tract which entrap lipids and other nutrients, including fat soluble vitamins and minerals, thus interfering with their absorption. Dietary chitosan may influence calcium metabolism by accelerating its urinary excretion. The reported undesirable effects are a marked decrease in plasma vitamin E level, reduction in bone mineral content and growth retardation. Ascorbic acid enhances gel formation of chitosan, thereby potentiating the plasma cholesterol lowering activity. Bile acid composition and short-chained fatty acid content in the cecum are altered by chitosan which impedes lipid emulsification and chitosan also inhibits the growth of micro-organisms, and therefore may on prolonged ingestion, alter the normal flora of the intestinal tract which may result in the growth of resistant pathogens. Chitin-chitosan, when used as a food supplement, does lower plasma cholesterol and triglycerides and improves the HDL-cholesterol/total cholesterol ratio. Certain medical precautions, such as monitoring of fat soluble vitamin levels, should be observed with long-term ingestion of high doses of chitosan to avoid potential adverse metabolic consequences (Koide, 1998:1091).

In human trials lasting for up to 12 weeks no clinical significant adverse effects have been noted. There was however a significant improvement in LDL- and HDL-cholesterol levels. The amount of chitosan needed for a pharmaceutical formulation is

however very low, less than 1 g/dose. It can be safe to say that at these low concentrations no significant adverse effects due to chitosan can be expected (Ausars, *et al.*, 2003:397). Ylitalo *et al.* (2002:1-7) suggested that care should be taken when chitosan is administered to patients with allergies to crustaceans, as this may lead to allergic reactions, but chitosan products have been freely available the past few years as a weight control product, and no allergies, or allergic reactions to these products have been reported.

1.5 SEMI-SYNTHETIC POLYMERS FOR SUSTAINED RELEASE

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

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

The benefits of including enteric polymers in chitosan formulations, to achieve ideal release profiles, have been shown by several studies. Joo *et al.* (2004:118) developed a system composed of stabilizing granules, which contain mistletoe lectin (VCA), extract or powder, coated by a biodegradable polymer wall. Their results indicated that successful incorporation of VCA into alginate/chitosan microcapsules had been achieved and that the alginate/chitosan microcapsule protected the VCA from degradation at acidic pH values. It was also found that coating the VCA with polyacrylic polymers such as Eudragit, produced outstanding results with ideal release profiles and only minimal losses of cytotoxicity after manufacturing steps. Shi and Tan (2002:4469) prepared a system which consists of chitosan micro cores entrapped

within an enteric polymer. Vitamin D_2 (VD2), used as a model drug, was efficiently entrapped in chitosan microcores using spray drying and was microencapsulated by coating with ethylcellulose. The morphology and release properties of microcapsules were tested and the results of drug release *in vitro* showed that the microcapsules could produce sustained release in intestine juice. Inouye *et al.* (1987:297) used chitosan and mixtures of chitosan with carboxymethylcellulose sodium (CMC-Na) as vehicles for sustained release tablets of water-insoluble drugs. Sustained release of prednisone from tablets prepared by direct compression of chitosan and CMC-Na was noticed. Furthermore, the release rate of PDS could be controlled by changing the relative amounts of chitosan and CMC-Na, or the amount of drug in the tablets. Inouye *et al.* (198:297) found such chitosan/CMC-Na mixtures were a promising basis in the design of sustained release dosage forms of water-insoluble drugs.

Chourasia and Jain (2004:201) prepared cross-linked chitosan microspheres from an emulsion system using liquid paraffin as the external phase and a solution of chitosan in acetic acid as the dispersed phase. The cross-linked chitosan microspheres were coated with Eudragit® L100 and S100 as pH-sensitive polymers. In vitro drug-release studies were performed in conditions simulating stomach-to-colon transit. It was found that by coating the microspheres with Eudragit®, pH-dependant release profiles could be obtained. No release was observed at acidic pH; however, when it reached the pH where Eudragit starts solublizing there was continuous release of drug from the formulation. According to Rhöm Pharma Polymers, Eudragit® L100 and S100, dissolves in pH 6.0 and pH 7.0 respectively (Degussa, 2004). Some relatively new lesser known enteric polymers based on hydroxypropyl methylcellulose namely Aqoat® AS-MG and AS-HF compare well to Eudragit® L100 and S100 with regards to pH sensitivity (SHIN-ETSU: 2004). Little research has been done to compare the effect of these two polymers on the release of drug from chitosan particles. Furthermore, the effect of the above mentioned enteric polymers on delayed release is based on their insolubility in acidic environments. It would therefore be beneficial to compare the effect of these polymers to a well know pH independent polymer such as Kollidon® SR. It is for these reasons that the effect of including these different enteric polymers in the formulation will be studied.

1.5.1 THE POLYMERS USED IN THE STUDY

1.5.1.1 Cellulose and its derivatives

The investigation and the use of cellulose and its derivatives in a wide range of applications have fascinated research, manufacturing, and development teams over many years. Shibuya *et al.* (1985:352) found as early as 1985 that methylcellulose compared favourably with other more well known excipients such as lactose. Franz and Peck (1982:1193) found an early indication that cellulose derivatives could be used to manipulate the dissolution rate of drugs. It was found that the type and particle size of microcrystalline cellulose had a significant effect on the solubility of fluphenazine. Tablet film coatings with hydroxypropyl methyl cellulose films were studied as early as 1978 by Rowe (1978:343). Porter and Ridgway (1982:5) found that cellulose acetate phthalate was not only a suitable enteric polymer coating but found that cellulose acetate phthalate delayed release of the drug longer in simulated intestinal fluid than polyvinyl acetate phthalate.

1.5.1.1.1 Hydroxypropyl methylcellulose acetate succinate

Hydroxypropyl methylcellulose acetate succinate (HPMCAS) is a rather new enteric coating material developed mainly for aqueous coating. Several studies were conducted on the safety of HPMCAS. Hoshi *et al.* (1985:187) concluded that HPMCAS caused no deaths or behavioural abnormalities in an acute toxicity study. Furthermore it was found that there were no deaths or behavioural changes in the subchronic toxicity or the chronic toxicity study group. HPMCAS was approved as a pharmaceutical excipient first in Japan in 1987, and then in the United States and several European countries in 2001. In an aqueous system, a suspension of the HPMCAS fine powder, triethyl citrate as a plasticizer and water is applied for coating.

HPMCAS has the unique character of a low gas transition temperature, which can give a solid dispersion for poorly soluble drugs (SHIN-ETSU Chemical Co. 2004)

Bechtold and Thoma (1999) found HPMCAS to be a quite stable aqueous enteric coating, suitable for controlled release. Säkkinen *et al.* (2003:28) found that HPMCAS was beneficial, when included in either a matrix or when used as a coating, in the production of a controlled release dosage form. HPMCAS is marketed by Shin-Etsu Chemical Company under the name Aqoat® with different varieties requiring different coating mediums and subsequently dissolve in different pH ranges (See Table 1-2). The Aqoat® AS-HF variant will be used in this study. Aqoat AS-HF is soluble at pH 6.8 and is applied as an aqueous dispersion (SHIN-ETSU Chemical Co. 2004).

Table 1-2: Characteristics of available Aqoat® coatings.

	Grade	**pH solubility in McIlvaine's Buffer solution**	**Application From in Coating Operation**
AS	LF	≥5.5	Aqueous Dispersion
	MF	≥6.0	
	HF	≥6.8	
	LG	≥5.5	Organic Solvent
	MG	≥6.0	
	HG	≥6.8	

1.5.1.2 Methacrylates

Methacrylate polymers are anionic polymers based on methacrylic acid esters. The films of this polymer are insoluble below pH 5 and thus resist gastric fluid. By salt formation in the neutral to weakly alkaline medium of the intestinal fluid, the films dissolve step-wise at pH values above 5.5 up to pH 7, making it suitable for drug delivery from the duodenum up to the ileum. Methacrylate polymers can be applied as coatings to all conventional solid oral dosage forms, such as tablets and capsules. These polymers can also be used to manufacture beads, granules, pellets, and sustained-release tablets (Degussa. 2004).

Methacrylate polymers are manufactured by Degussa International and are marketed under the Eudragit® brand name. Eudragit® S100 is a pH dependent anionic polymer powder, solubilizing above pH 7.0, and is suitable for targeted drug delivery in the ileum. Säkkinen *et al.* (2003:47) found that Eudragit® S100 was beneficial, when included in either a matrix or when used as a coating, in the production of a controlled release dosage form. Lorenzo-Lamosa *et al.* (1998:1) found that by coating chitosan microcores with Eudragit® S100 and L100, perfect pH-dependent release profiles could be attained.

Table 1-3: Characteristics of available Eudragit® coatings (Degussa.2004).

Aqueous dispersions	
EUDRAGIT® L 30 D-55	pH dependent anionic aqueous polymer dispersion solubilizing above pH 5.5 for targeted drug delivery in the duodenum.
Solid substances	
EUDRAGIT® L 100-55	Spray dried EUDRAGIT® L 30 D-55 which can be reconstituted for aqueous formulations for targeted drug delivery in the duodenum.
EUDRAGIT® L 100	pH dependent anionic polymer powder solubilizing above pH 6.0 for targeted drug delivery in the jejunum.
EUDRAGIT® S 100	pH dependent anionic polymer powder solubilizing above pH 7.0 for targeted drug delivery in the ileum.
Organic solvents	
EUDRAGIT® L 12,5	pH dependent anionic polymer solution solubilizing above pH 6.0 for targeted drug delivery in the jejunum.
EUDRAGIT® S 12,5	pH dependent anionic polymer solution solubilizing above pH 7.0 for targeted drug delivery in the ileum.

1.5.1.3 Polyvinylpyrrolidone

Polyvinylpyrrolidone also known as polyvinyl acetate / polyvinylpyrrolidone is derived from a polyvinyl acetate-dispersion and appears as a spray dried, non hygroscopic powder consisting of polyvinyl acetate (8 parts w/w) and polyvinylpyrrolidone (2 parts w/w). Kollidon ® SR, an excipient for drug delivery matrices based on polyvinyl acetate, possesses good controlled release properties. According to Fussnegger (2003:6) the release profiles of tablet formulations with Kollidon ® SR were not influenced by different dissolution media.

According to Reza *et al.* (2003:291) they were able to produce sustained release formulations containing high levels of polyvinylpyrrolidone and found in a comparative study that the release kinetics was governed by the type and content of

polymer in their matrix system. Higher polymeric content (75%) in the matrix decreased the drug release rate because of increased tortuosity and decreased porosity. Kollidon SR outperformed hydroxypropyl methylcellulose while Cumba wax retarded drug release even more than Kollidon SR was able to.

1.6 CONCLUSION

The movement during the past few decades towards better patient compliance has placed immense focus on producing modern controlled release formulations. These formulations not only improve patient compliance but also significantly reduce the cost of administration and may even improve or completely avoid certain side-effects commonly encountered with conventional dosage forms. Common side-effects of controlled release formulations such as dosed dumping and problems with dose adjustment can possibly be overcome with formulations such as beads or granules.

The mechanism of drug release from controlled release systems has been shown to be primarily divided into two types. The first being a reservoir device in which the drug forms a core surrounded by an inert diffusion barrier. The inert diffusion barrier usually consists of polymers with a low solubility thereby retarding drug release. The second being a monolithic device in which the drug is dispersed or dissolved in an inert polymer matrix. The release of the drug is limited by the degradation or by the rate of diffusion through the polymer matrix.

Whatever the method of controlled release may be, the main ingredient always seemes to be a polymer, and this has been visible form the very first commercially produced controlled release formulation in the 1970's. These polymers may be synthetic or of a natural origin. The safety of these polymers always need to be investigated with one of the main concerns being bio-degradability, the products of this degradation process being biocompatible, non-toxic, non-carcinogenic and non-immunogenic. 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.

It was also found that formulations of chitosan alone may not be sufficient to produce optimal controlled release formulations. Therefore other modern controlled release formulations were also investigated in this study. These polymers include the cellulose derivatives, such as Hydroxypropyl methylcellulose acetate succinate marketed as Aqoat® AS-HF, the methacrylates, namely Eudragit® S100, and a polyvinyl acetate-dispersion marketed as Kollidon® SR. These polymers have been shown in numerous studies to be safe and viable options in the production of controlled release formulations.

The production of a relatively simple formulation consisting mainly of chitosan and the effect of the above motioned polymers on this formulation will be evaluated in this study. The preparations of such formulations are discussed in the following chapter as well as means for characterization of the samples.

CHAPTER 2

Preparation and characterisation of beads and granules for controlled drug delivery.

2.1 INTRODUCTION

Drugs are rarely administered solely as pure chemical substances but almost always given as formulated preparations. These can vary from relatively simple solutions to complex drug delivery systems through the use of appropriate additives or excipients in the formulations to provide varied and specialised pharmaceutical functions. The principle objective of dosage from design is to achieve a predictable therapeutic response to a drug included in a formulation which is capable of large scale manufacture with reproducible product quality (York, 2002:1).

According to Vogelson (2001:49) current methods of drug delivery exhibit specific problems that scientists are attempting to address. 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 ingested, 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.

Currently the developments of sustained release dosage formulations with the ability to carry as much as possible drug to the distant parts of the digestive system are

receiving a lot of attention. Manufacturing of these formulations usually involve multiple stages of production, including mixing, manufacturing, drying and coating.

In this chapter experimental methods for the development of controlled release dosage forms of ketoprofen based on chitosan and controlled release polymers will be outlined. Two methods will be studied, one based on a bead formulation, and another based on a simple granulation technique. Methods for the characterisation and evaluation of the two formulations will also be discussed.

2.2 MODEL DRUG: KETOPROFEN

Ketoprofen is a nonsteroidal anti-inflammatory drug with analgesic and antipyretic properties. In anti-inflammatory models ketoprofen has been shown to have inhibitory effects on prostaglandin and leucotrine synthesis. Ketoprofen also possesses antibradykinin activity as well as lysosomal membrane-stabilizing activity.

Ketoprofen is a white or almost white, odourless, non-hygroscopic, crystalline powder, practically insoluble in water, freely soluble in acetone, in alcohol and in methylene chloride. Ketoprofen has a melting point of 94°C to 97°C (British Pharmacopoeia, 2002). The chemical name for ketoprofen is 2-(3-benzoylphenyl)-propionic acid (See Figure 2-1). Its empirical formula is $C_{12}H_{12}O_2$, with a molecular weight of 254.29 g/mol. The drug has a pKa-value of 5.94 in methanol:water (3:1) and an n-octanol:water partition coefficient of 0.97 (buffer pH 7.4) (Orudis Online, 2004).

Ketoprofen has a short half-life of about 1.8 ± 0.4 hours. It has a longer half-life in renal failure (3.2 ± 0.5 h) and elderly patients (2.7 ± 0.6 h) but the half-life still remains relatively short (Orudis Online, 2004).

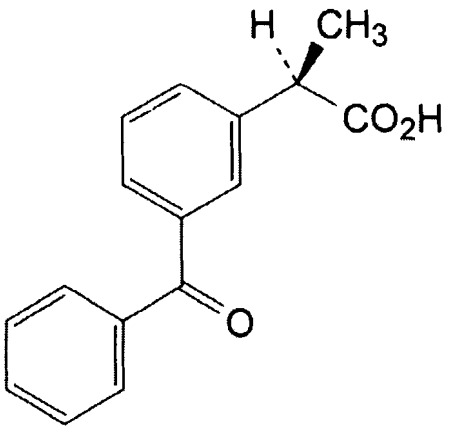


Figure 2-1: Structure of Ketoprofen (British Pharmacopoeia, 2002).

Ketoprofen was chosen as a candidate for formulation in a controlled release dosage from because of its low solubility in acidic environments. Säkkinen (2003:47) found that the solubility of a drug could substantially affect the slow-release characteristics of formulations. Slow release only took place with drugs having low solubility in acidic environments. Secondly, ketoprofen has a short half-life of only 1.8 ± 0.4 hours, which makes it a perfect candidate for controlled release formulations. Furthermore formulations with chitosan may prove to reduce gastric irritation caused by active compounds, like the anti-inflammatory drugs, such as ketoprofen, due to its antacid and anti-ulcer activities (Açikgoz *et al.*, 1995:275). Therefore it would be favourable to produce a dosage from with enteric release properties as well as controlled drug delivery in the alkaline environments of the small intestine.

2.3 BEADS

Beads are spherical drug loaded gel particles. These particles are manufactured by dropping a biocompatible polymer into a solution containing a cross-linking agent of choice (Bodmeier et al., 1989:14475). It was also found that beads, as a novel dosage from, have the ability to delay drug release sufficiently to be considered in the manufacture of sustained release products (Bodmeier, 1989:1487 and Anal, et al., 2003:713). Beads vary in size from 788.1 ± 6.4 μm to 3.4 ± 0.3 mm (Aral and

Akbuğa, 1998:10 and Won *et al.*, 2005:2149). The surface and cross-section morphology varies according to the method of drying, either freeze-dried or oven-dried, with freeze-dried beads being more porous (Bodmeier *et al.*, 1989:1478).

Beads are frequently used in controlled release dosage forms as they are flexible in dosage from development and show various advantages over single unit dosage forms. Because beads disperse freely in the gastrointestinal tract, they maximise drug absorption, reduce peak plasma fluctuation, and minimise potential side-effects without lowering drug bio-availability. By combination of beads with different release rates, drug delivery at different sites can be achieved (Ghebre-Sellassie, 1989:7). Anal *et al.* (2003:713) prepared beads for the sustained release of ampicillin. It was also possible for Aydin *et al.* (1996:101) to prepare chitosan beads for the controlled delivery of salmon calcitonin.

El Fattah *et al.* (1998:541) found drug release from beads to be dependent on the ionic properties of the polymers used in beads and the pH of the dissolution media. In acidic pH, 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. 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 an acidic environment than in an alkaline environment.

2.3.1 Preparation of beads

Beads can be manufactured by various technological methods including:

- emulsification inotropic gelation,
- electric dispersion of polymer solutions,

- extrusion spheronisation technique,
- inotropic gelation and
- melt solidification.

These techniques usually involve complicated processes utilising complex apparatus. Of all these methods, preparing beads by inotropic gelation is the simplest method of preparing beads with favourable characteristics, especially chitosan based beads (Bodmeier, 1989:1488 and Gupta and Ravi Kumar, 2000:1115). Therefore beads were prepared by inotropic gelation.

2.3.1.1 Inotropic gelation method

This method is based on the principle of interaction between two opposite charges. This can be depicted by the interaction between the polycationic chitosan and anionic compounds such as sodium sulphate, sodium citrate and tripolyphosphate (TPP). A viscous solution of the polymer and drug is prepared. The polymer-drug solution is dropped through a disposable needle into a gently agitated TPP-solution. TPP dissolves in water to dissociate into both OH^- and TPP-ions. These ions can completely diffuse into the chitosan droplet, and electrostaticaly attract to the NH_3^+ groups in chitosan, which result in phase-inversion of the chitosan droplets (Mi *et al.* 2002:761). The bead forms on contact of the polymer-drug solution with the TPP-solution, thus entrapping the drug within the three-dimensional network of ionic linked polymer (Bodmeier *et al.*, 1998:1478).

2.3.1.2 Manipulation of the method to achieve optimal drug loading.

The method for preparation of ketoprofen-chitosan controlled release beads was based on that of Bodmeier *et al.* (1989:1478). Initially two main problems were encountered during the development of an optimal method, namely low drug loading and beads with a high friability. Therefore the method had to be adjusted in order to produce beads viable for further study.

2.3.1.2.1 Optimal concentration of chitosan.

The effect of different levels of chitosan had to be evaluated. The chitosan-drug dispersion was prepared by dispersing 3% w/v ketoprofen in solutions of 1, 2, 3 and 4% w/v chitosan in 100 ml of a 2% v/v solution of glacial acetic acid. Beads were initially prepared by pumping 10 ml of the chitosan-drug dispersion though a gauge 21 needle into 100ml of a 3% w/v gently agitated TPP-solution using a peristaltic pump. 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 separated by filtration. The beads were then washed twice with a total amount of 50 ml deionised water to remove any excess TPP on the surface. The beads were then freeze-dried in a Secfroid freeze-dryer (Lausanne, Switzerland)

It was however found 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 a 4% w/v solution of chitosan. The solution has 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 concentrations from subsequent studies. A concentration of 3% w/v chitosan was consequently selected for the method.

2.3.1.2.2 Determining the optimal TPP concentration and pH.

TPP-solutions generally have a pH (WTW pH 320 SET, Wissenschaftlich-technische werkstätten, Germany) of between 8.89 and 9.10 in solutions. The model drug, ketoprofen, used in this study has a pKa-value of 5.94. Therefore most of the drug leached out of the beads into the external TPP-phase, during preparation. It was therefore deducted that in order to achieve optimal drug loading the pH of the external TPP-phase had to be adjusted to below the pKa-value of ketoprofen. Bodmeier *et al.* (1989:1485) also 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 drug

leached from the beads. This was also found to be the case with ketoprofen. It was however noted that with TPP concentrations below 3% w/v beads with adequate resistance to mechanical force could not be produced. Therefore the TPP concentration of 3% w/v was selected for further experiments.

Beads were prepared by dispersing 3% w/v ketoprofen in a 3% w/v chitosan in a 2% v/v glacial acetic acid solution. A volume of 10ml of this chitosan-drug dispersion was dropped into 100ml of a 3% w/v TPP-solution at a pH of between pH 2 and 9. The beads were stirred for 30 minutes, separated by filtration, washed with deionised water and freeze-dried. The drug loading was determined and the results are shown in Figure 2-2.

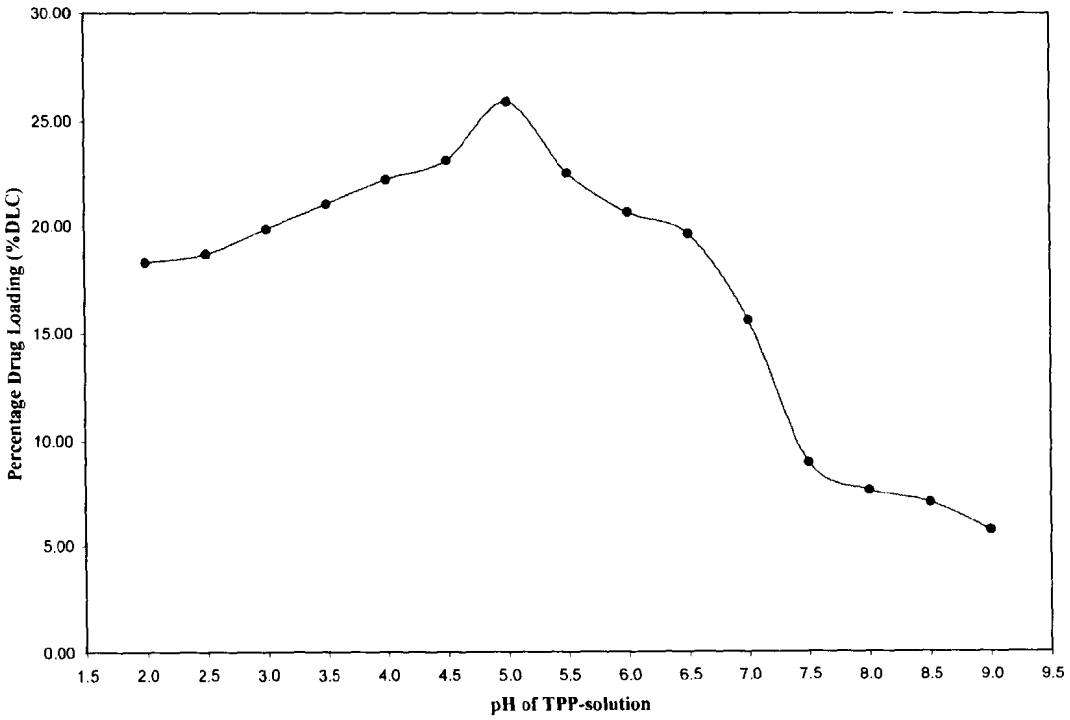


Figure 2-2: Effect TPP-solution pH on percentage drug loading (%DLC).

As seen in Figure 2-2 optimal drug loading could only be achieved with a TPP-phase pH of $\leq$ 5.0. Therefore a TPP-phase with a pH of 5.0 was selected for the method.

The drug loading of 25.89 ± 0.61% however higher at this pH was still not high enough to justify the use of these beads in practice. Further manipulation of the method was required to achieve optimal drug loading.

To decrease the solubility of the ketoprofen in the external phase further, an amount of 150mg ketoprofen was added to 100ml of the TPP-phase. This improved drug loading further to as high as 52.62 ± 0.18%. Bodmeier *et al.* 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. The results are shown in Figure 2-3.

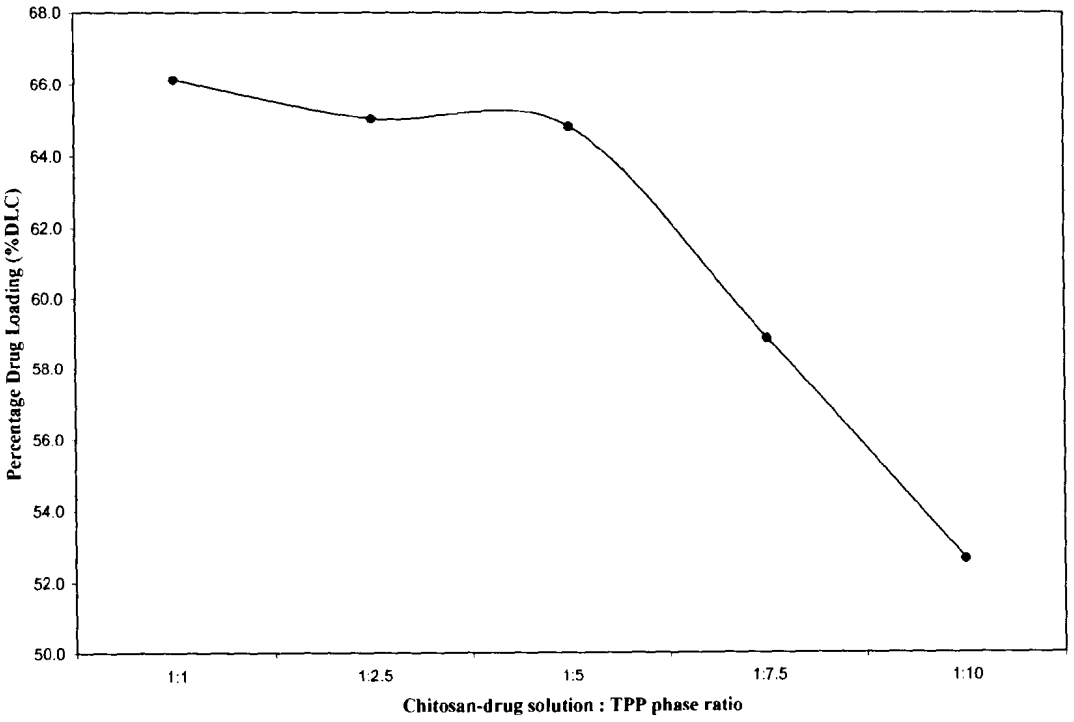


Figure 2-3: Effect of chitosan-drug dispersion to external TPP-phase ratio on the percentage drug loading (%DLC).

As seen from Figure 2-3 optimal drug loading was achieved with ratios lower than 1:5 (chitosan-drug dispersion:TPP-phase) with a %DLC of 64.80 ± 0.68%. However

beads with satisfactory mechanical strength could not be prepared with ratios lower than 1:5 (chitosan-drug solution:TPP-phase). Therefore the chitosan-drug solution:TPP-phase ratio of 1:5 was selected for the optimized preparation method.

2.3.2 Preparation of beads for the study.

2.3.2.1 Materials

The following materials were used during the preparation of chitosan drug loaded beads and chitosan-polymer drug loaded beads. Chitosan (83% deacetylated) [For COA see Annexure A], glacial acetic acid 100% (Merck, South Africa), tripolyphosphate (Sigma, South Africa), Aqoat® grade AS-HF (Synthapharm, Germany) [For COA see Annexure A], Eudragit® grade S100 (Degussa Pharmaceuticals Rhom, Germany), Kollidon® grade SR (BASF, South Africa) and ketoprofen (Boehringer Ingelheim Bidachem, Italy) All chemicals used were of analytical grade and were used as received.

2.3.2.2 Method

Chitosan drug loaded beads were produced by preparing a 3% w/v dispersion of ketoprofen in a solution containing 3% w/v chitosan, prepared in a 2% v/v aqueous solution of acetic acid. The solution was sonicated (Julabo Labortechnik, Swarzwald, Germany) until it was bubble free. Drug loaded chitosan-polymer beads were produced much in the same way as polymer free beads, but amounts of 1, 2.5 or 5% w/v Aqoat® AS-HF, Eudragit® S100 or Kollidon® SR were additionally dispersed in the chitosan acetic acid solution, depending on the polymer investigated.

The beads were formed by dropping the chitosan-drug dispersion or chitosan-drug-polymer dispersion through a gauge 21 needle into a bubble free gently agitated solution of TPP using a peristaltic pump (Watson-Marlow 205S, Watson-Marlow Ltd., England). The pH of the TPP-solution was adjusted to pH 5.0 and an amount of

75mg ketoprofen was added to 50ml of the TPP-solution prior to bead preparation. The ratio chitosan-drug-dispersion to TPP-solution was kept steady at 1:5 to ensure optimal drug loading. Therefore 10ml of the chitosan drug dispersion was added to 50ml of TPP-solution. A stirring time of 30 minutes was allowed for cross-linking to occur, before the beads were removed by filtration. The beads were washed twice with 50ml of deionised water and freeze-dried for 24 hours.

2.4 GRANULES

Granulation can be defined as any process whereby small particles are gathered into larger, permanent masses in which the original particles can still be identified (Perry and Chilton, 1973:57). Pharmaceutical granulations are used primarily for the preparation of materials intended for tabletting. To a lesser extent they are used as a precursor to the encapsulation process. In some applications the process is used to produce spherical granules for modified release formulations or to prepare granules as sprinkles to be used by the patients directly. Successful agglomeration of primary particles depends on proper control of the adhesional forces between particles, which encourage agglomerate formation and growth to provide adequate mechanical strength in the product (Darkin, 1997:5-8). Pharmaceutical granules typically have a size range between 0.2 and 4.0 mm, depending on their subsequent use.

2.4.1 Preparation of granules

Granulation methods can be divided into two types: dry methods in which no liquid is employed and wet granulation in which a liquid is employed in the granulation process.

2.4.1.1 Dry granulation

In dry methods, the primary powder particles are aggregated under high pressure. There are two main processes. Either a large tablet is produced in a heavy-duty

tabletting press or the powder is squeezed between two rollers to produce a sheet like material. In both cases these intermediate products are broken up using suitable milling techniques to produce granular material, which is usually sieved to separate the desired size fraction (Summers and Aulton, 2002:366).

2.4.1.2 Wet granulation

Wet granulation involves the massing of a mix of dry primary powder particles using a granulating liquid. The liquid must be volatile so that it can be removed by drying, and must be non-toxic. Typical liquids include ethanol, isopropanol and water. The granulation liquid can be used alone or, more commonly, a solvent containing a dissolved adhesive or binder is used as the binding liquid to ensure particle adhesion once the granule is dry. In the traditional wet granulation method the wet mass is forced through a sieve to produce wet granules which are then dried. A subsequent screening stage breaks agglomerates of granules and removes the fine material which can then be recycled. Variations of this traditional method depend on the equipment used but the general principle of initial particle aggregation using a liquid remains the same in all the processes (Summers and Aulton, 2002:367).

Chandy and Sharma (1993:939) prepared ampicillin-chitosan granules for sustained release. The results propose the possibility of modifying the formulation in order to obtain the desired controlled release of the drug for a convenient oral sustained delivery system. Säkkinen *et al.* (2003:227) showed that granules prepared by a simple method using chitosan and microcrystalline chitosan were successful in controlling release of ibuprofen and furosemide. Microcrystalline chitosan outperformed chitosan in its ability to control drug release rates, and was successful even at low concentrations such as 40% w/w.

2.4.2 Preparation of granules for study

2.4.2.1 Materials

The following materials were used during the preparation of chitosan drug loaded beads and chitosan-polymer drug loaded beads. Chitosan (83% deacetylated) [For COA see Annexure A], glacial acetic acid 100% (Merck, South Africa), Aqoat® grade AS-HF (Synthapharm, Germany) [For COA see Annexure A], Eudragit® grade S100 (Degussa Pharmaceuticals Rhom, Germany), Kollidon® grade SR (BASF, South Africa) and ketoprofen (Boehringer Ingelheim Bidachem, Italy) All chemicals used were of analytical grade and were used as received.

2.4.2.2 Method

Granules had to be prepared in such a way as to resemble the composition of the beads. Usual granule preparation by means of wet granulation requires the addition of a granulating liquid to the premixed powdered substances used in the granules. However, for the granules to be comparable to the beads in composition the granules had to be prepared using only the main chemical substances of the beads. It could be said that in the case of the beads, cross-linked chitosan acts as the binder in the formula. Therefore granules had to be prepared using chitosan as the binder.

Säkkinen *et al.* (2003:229) prepared granules very similar to the ones required in the study using 40% microcrystalline chitosan and 60% drug. This mixture was granulated using a 2.5% aqueous solution of acetic acid. The masses were moistened with the granulation liquid in a mortar and granulated trough a 2.0 mm sieve. Therefore granules were prepared in very much the same way as Säkkinen *et al.* (2003:229) by means of wet granulation.

Weighed amounts of ketoprofen, chitosan and polymer, similar to the composition of the beads (see Table 2.1), were added to a glass jar. The formulation powder was then

mixed in a Turbula mixer (Model T2C, Wily A. Bachofen Maschinefabrik, Basel, Switzerland) for 5 minutes. After mixing the powder mass was wetted with 2.5% v/v glacial acetic acid in deionised water in a mortar, and granulated trough a 2 mm sieve.

The resulting granulate was dried over night at 35°C in an oven (Term-O-Mat oven, Labotec, South Africa) and dried an additional 8 hours at 35°C under vacuum. The granulate was then broken up by force, and sieved. Only granules of sieve fraction 710 to 850 µm (Fritsch analysette, Germany) were used in the study. This method of preparing chitosan granules was used to eliminate any additional effects that excipients such as binders might have on drug release from the granules.

Table 2-1: Composition of the granules used in the study.

Bead	Composition			Correlating	Composition		
	Chitosan w/v	Drug w/v	Polymer w/v	Granule	Chitosan m/m	Drug m/m	Polymer m/m
CB	3%	3%	0%	CG	50.00%	50.00%	0%
A1B	3%	3%	1%	A1G	42.86%	42.86%	14.29%
A2.5B	3%	3%	2.5%	A2.5G	35.92%	35.92%	29.41%
A5B	3%	3%	5%	A5G	27.27%	27.27%	45.45%
E1B	3%	3%	1%	E1G	42.86%	42.86%	14.29%
E2.5B	3%	3%	2.5%	E2.5G	35.92%	35.92%	29.41%
E5B	3%	3%	5%	E5G	27.27%	27.27%	45.45%
K1B	3%	3%	1%	K1G	42.86%	42.86%	14.29%
K2.5B	3%	3%	2.5%	K2.5G	35.92%	35.92%	29.41%
K5B	3%	3%	5%	K5G	27.27%	27.27%	45.45%

2.5 CHARACTERISATION OF BEADS AND GRANULES

2.5.1 Dissolution and drug release

The release characteristics of all the formulations were studied in 0.1 M HCl with a pH of 1.2 and phosphate buffered solution (PBS) pH 7.2 (USP 2003:XXI) at 37 ± 0.5 °C. The dissolution method used to determine the drug release from the samples

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. A sample amount of 0.140 g (beads or granules) 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 submerged into the medium. The rotation was kept constant at 50 revolutions per minute. Samples of 5 ml were withdrawn through 3 μm membrane filters (Millipore, Bedford, Marlborough, England) to eliminate any suspended particles. For the first 60 minutes the dissolution study was conducted in 0.1 M HCl (pH 1.2). Samples were withdrawn at time intervals of 1, 2, 3, 4, 5, 10, 15, 30, 45 and 60 minutes. After each withdrawal dissolution medium was replenished with fresh preheated dissolution medium.

After 60 minutes in 0.1 M HCl the baskets were removed from the dissolution medium, patted dry with absorbent lint free paper, and immediately placed in PBS (pH 7.2) for the remainder of the dissolution study. Dissolution samples of 5 ml were withdrawn through 3 μm membrane filters at time intervals of 61, 62, 63, 64, 65, 70, 75, 90, 105, 120, 150, 180, 210, 240, 270, 300, 330, 360 minutes, and replaced with fresh preheated dissolution medium.

All samples were run in triplicate and assayed spectrophotometrically, using a Unicam® spectrophotometer (Unicam, Cambridge, UK) at 258 nm wavelength. The following equation was used to correct for dilution of the dissolution media after each sample withdrawal and addition of pure medium:

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

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

2.5.1.1 Standard curve

A standard curve was constructed before each dissolution study as well as before each drug loading capacity study for both mediums used. A stock solution was prepared by dissolving an amount of 50 mg ketoprofen for 0.1 M HCl and 60 mg ketoprofen for PBS pH 7.2, in 10ml absolute ethanol and placed in an ultra sonic bath for 10 minutes. The solute was then diluted to 200ml in a volumetric flask with the desired medium. Standard solutions with concentrations of 5 to 25 $\mu g.cm^{-3}$ for 0.1 M HCl, and 6 to 30 $\mu g.cm^{-3}$ for PBS pH 7.2 were prepared from the stock solution. The UV-absorbencies were analysed spectrophotometrically at a wave length of 258 nm with either one of the applicable pure mediums as control.

Graphs of absorbencies against concentration were plotted and the best straight line through the data points was selected using linear regression. The concentration range obeyed Beer's law with correlation coefficients of $(r^2) \geq 0.9997$ for 0.1 M HCl and $(r^2) \geq 0.9995$ for PBS pH 7.2. The slope (m) and the y-intercept (c) were used to calculate the ketoprofen concentration in each sample.

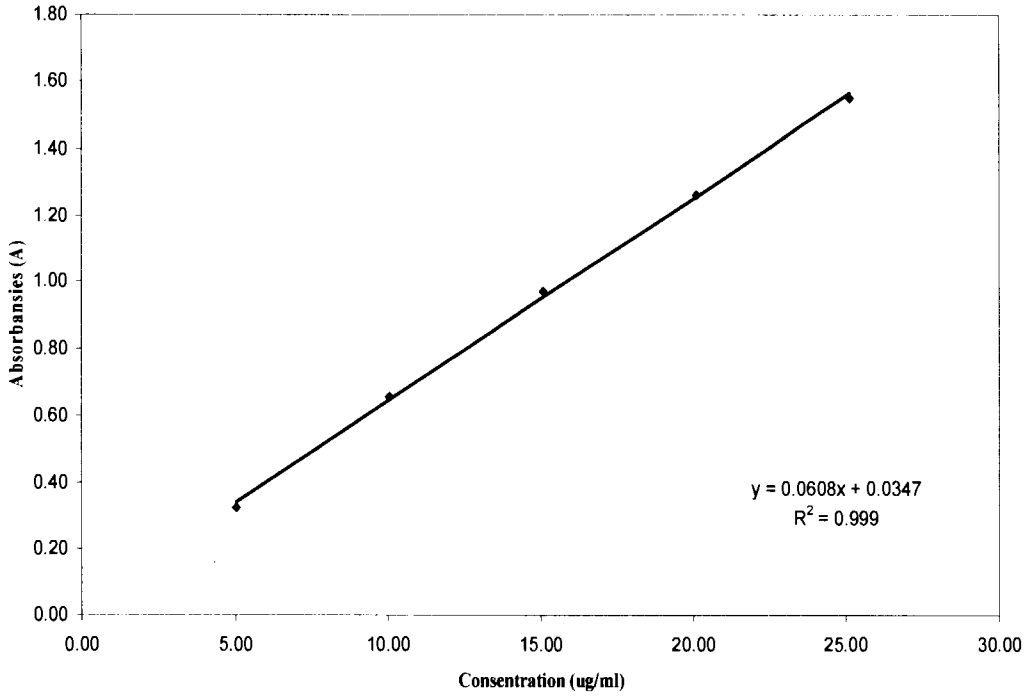


Figure 2-4: Example of a standard curve plotted for ketoprofen in 0.1M HCl.

2.5.2 Drug loading capacity

Beads can only incorporate a certain amount of drug. For pharmaceutical particles to be considered as a viable dosage from, an adequate amount of drug must be incorporated into such particles. 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 *et al.* (2000:1116) and Bodmeier *et al.* (1989:1478) described a method whereby a sample of drug loaded beads or microgranules was accurately weighed and kept in a specific amount of 2% acetic acid at 30 °C for 48 hours, or 0.1 M NaOH at 22 °C for 48 hours, respectively. The samples were filtered and solubilities were obtained by measuring the absorbencies of the samples spectrophotometrically and determining the resulting drug concentration by means of linear regression after appropriate dilution in 0.1 M NaOH. All drug loading capacity tests were run in

triplicate. The drug loading capacity (DLC) of the beads were calculated from the following equations:

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

2.5.3 Friability

Samples of beads and granules were taken from each batch, dusted with compressed air and accurately weighed. The sample was placed in the drum of a Roche® friabulator, 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:

$$\% \text{ Friability} = [(W_0 - Wt)/W_0] \times 100 \quad (2\text{-}4)$$

where W_0 was the weight of the sample before testing and Wt the weight of the sample after testing. Tests were run in triplicate.

2.5.4 Morphology: Scanning electron microscopy

Scanning electron microscopy (SEM) was used to characterise the surface and cross-sections of the samples. Cross-sections were obtained by cutting the samples with a razor blade, much in the same way as Bodmeier *et al.* (1989:1479). Samples were mounted on aluminium sample mounts and coated with gold palladium alloy to minimise surface charging. A Philips XL30DX4i scanning electron microscope (Philips, Netherlands) was used. 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 exactly using the above described method.

2.5.5 Solubility

Gupta *et al.* (2001:641) described a method by which solubility of chitosan beads can be determined. 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:

$$\%S = [(W_0 - W_t)/W_0] \times 100 \qquad (2\text{-}5)$$

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.

2.5.6 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 (0.1 M HCl and PBS pH 7.2 at 37°C) for the required time. 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 percentage swelling of the sample in the media was calculated using the formula:

$$E_{sw} = [(W_e - W_0)/W_0] \times 100 \qquad (2\text{-}6)$$

where E_{sw} is the percentage swelling of the sample at a specific time, W_e 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.

2.5.7 Calculations

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

2.6 CONCLUSION

The model drug ketoprofen is well suited for formulation into chitosan beads. By formulating ketoprofen into chitosan beads it might be possible to achieve controlled release, thereby minimising the gastrointestinal side-effects of the drug. It is specialised that by incorporation of enteric polymers into chitosan granules and beads, it will be possible to produce sustained release chitosan formulation with a degree of enteric release, limiting the initial release of the drug in the stomach.

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

Although this concentration yielded beads with low friability the drug loading efficiency was still not high enough to justify the use of these beads in practice. Therefore the effect of variables such as the optimal concentration of TPP, the optimal pH of the TPP-solution and the optimal external TPP-phase to internal chitosan drug dispersion phase ratio was evaluated. It was found that these variables had a

substantial effect on drug loading efficiency and that by simple manipulation of these variables the drug loading efficiency could be increased to 64.80 ± 0.68%. The optimal situations being a TPP concentration of 3% w/v with a pH of 5.0 and 0.150% w/v ketoprofen dissolved in the TPP-solution and a ratio of 5:1 TPP-solution to chitosan drug dispersion.

Since this is a comparative study of beads to granules, granules had to be prepared to resemble the formulation of the beads as closely as possible. The problem of finding a suitable binder for the granules was investigated and it was found that simple granules containing no additional binders and still primarily bind only with chitosan could be prepared according the method used by Säkkinen *et al.* (2003:229). Granules closely resembling the beads could thus be prepared using only a 2.5% v/v aqueous acetic acid solution as a granulation system.

Methods of characterization and evaluation of the prepared formulations were also discussed. Micro-particulate samples such as the above mentioned, can be characterised by means of drug release profiles, drug loading capacity, friability, morphology, solubility and swelling and degradation studies. These characterisations are important in order to establish the viability of such formulations as acceptable pharmaceutical dosage forms.

CHAPTER 3

Results and discussion of characterization tests

3.1 INTRODUCTION

Beads have several benefits over conventional dosage forms. These advantages have been discussed in chapter 2. In order to evaluate the potential of ketoprofen loaded chitosan beads and granules as well as chitosan-polymer beads and granules prepared with different concentrations of Aqoat® AS-HF, Eudragit® S100 and Kollidon® SR, several characterising tests were preformed. The experimental methods of these tests are fully described in chapter 2. The results of these tests as well as the discussion of the results are presented in this chapter.

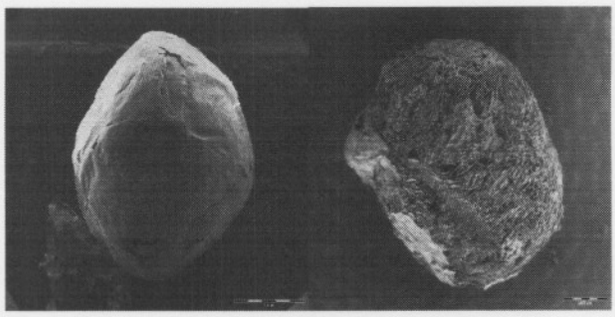
3.2 MORPHOLOGY

3.2.1 Method

Morphology studies were carried out on all samples with scanning electron microscopy (SEM) as described in chapter 2 section 2.5.5.

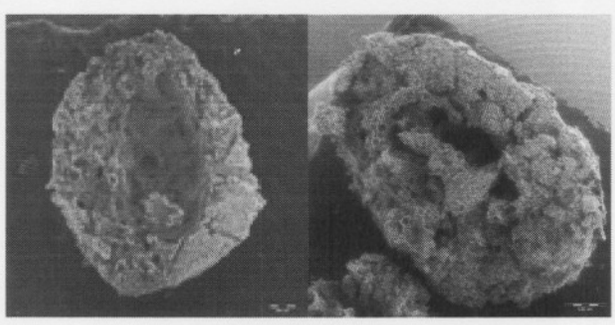
3.2.2 Results

Micrographs of the prepared beads and granules are given in Figures 3-1 to 3-12.



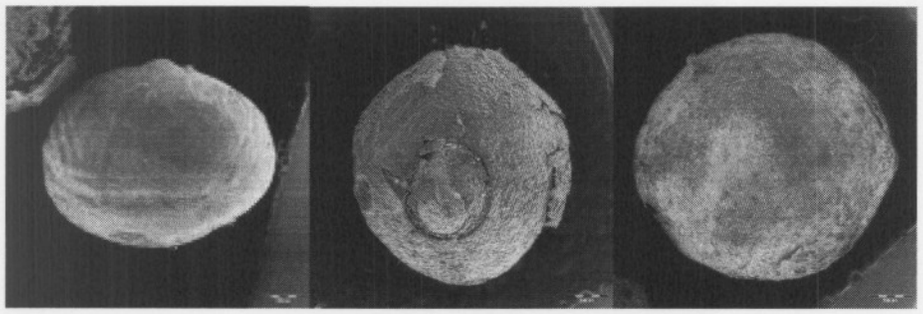
(a) (b)

Figure 3-1: Full view of a ketoprofen loaded bead, prepared in TPP, pH 9.0 (a), and a ketoprofen loaded chitosan bead, prepared in TPP pH 5.0 (b) (CB).



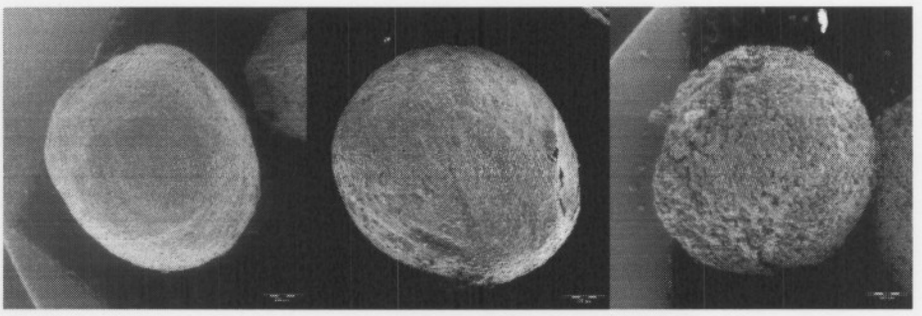
(a) (b)

Figure 3-2: Cross sectional view of a ketoprofen loaded chitosan bead prepared in TPP, pH 9.0 (a), and a ketoprofen loaded chitosan bead, prepared in TPP, pH 5.0 (b) (CB).



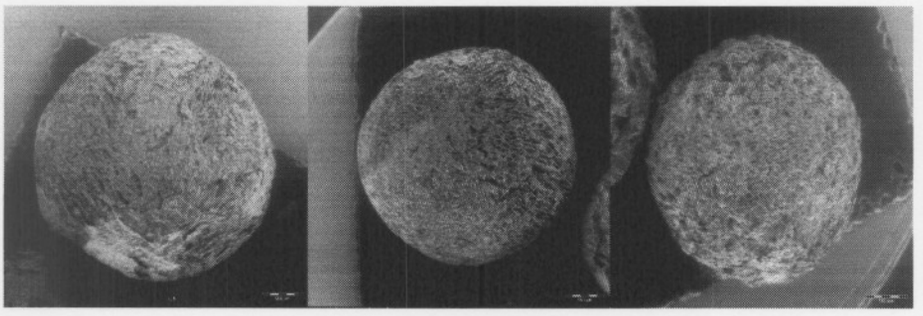
(a) (b) (c)

Figure 3-3 Full view of 1 (a), 2.5 (b) and 5% w/v (c) Aqoat® AS-HF beads (A1B, A2.5B, and A5B).



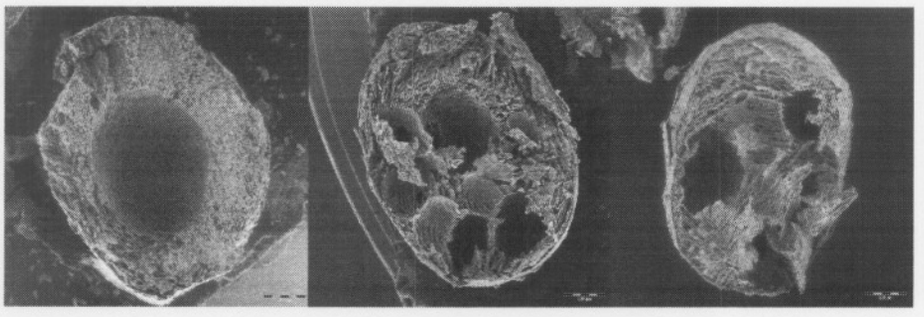
(a) (b) (c)

Figure 3-4: Full view of 1 (a), 2.5 (b) and 5% w/v (c) Eudragit® S100 beads (E1B, E2.5B, and E5B).



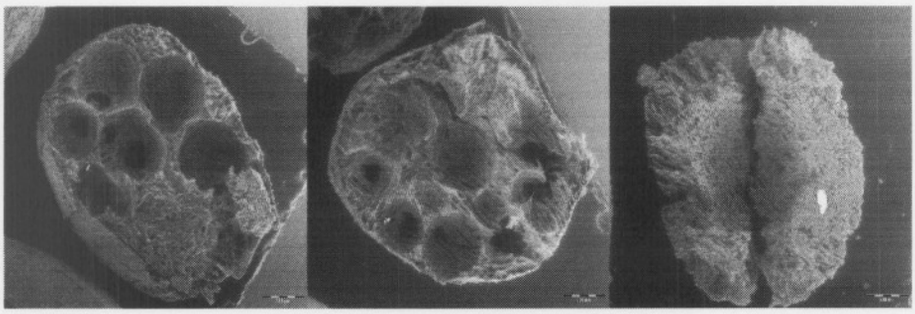
(a) (b) (c)

Figure 3-5: Full view of 1 (a), 2.5 (b) and 5% w/v (c) Kollidon® SR beads (K1B, K2.5B, and K5B).



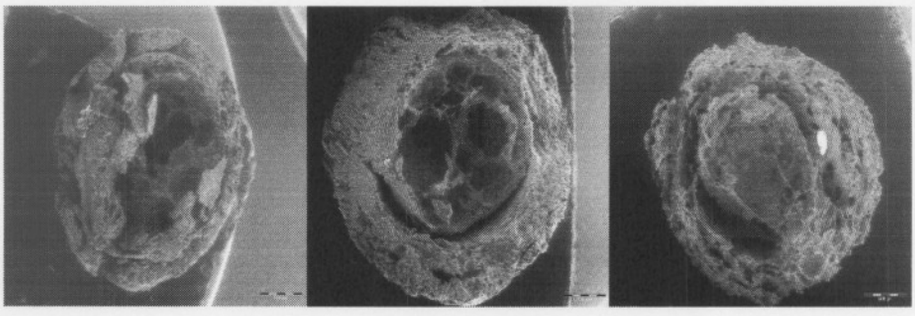
(a) (b) (c)

Figure 3-6: Cross-sectional view of 1 (a), 2.5 (b) and 5% w/v (c) Aqoat® AS-HF beads (A1B, A2.5B, and A5B).



(a) (b) (c)

Figure 3-7: Cross-sectional view of 1 (a), 2.5 (b) and 5% w/v (c) Eudragit® S100 beads (E1B, E2.5B, and E5B).



(a) (b) (c)

Figure 3-8: Cross-sectional view of 1 (a), 2.5 (b) and 5% w/v (c) Kollidon® SR beads (K1B, K2.5B, and K5B).

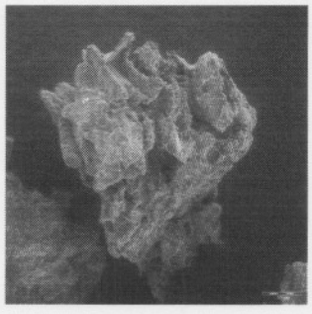
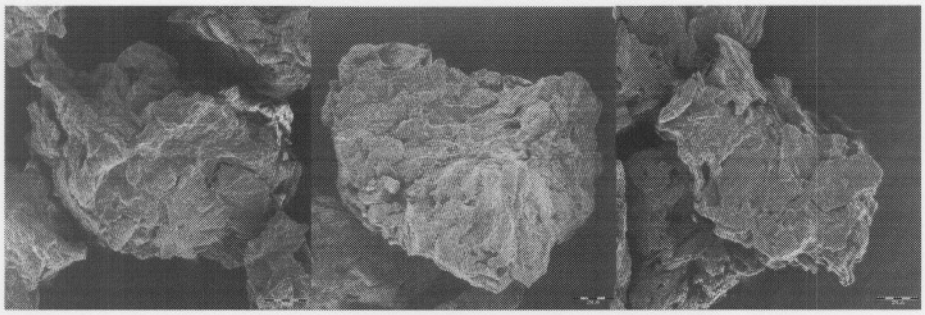
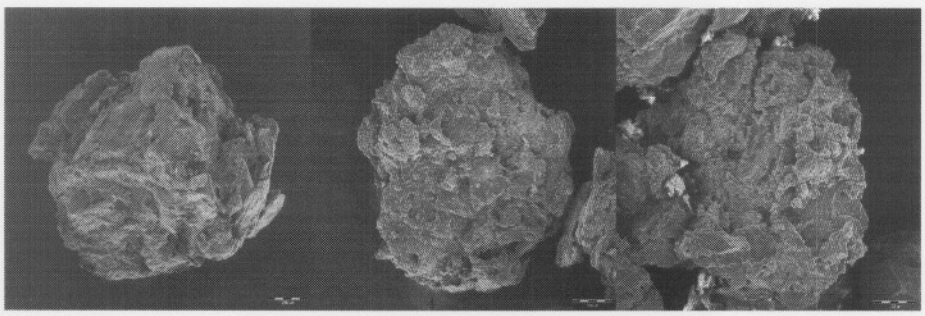


Figure 3-9: Full view of ketoprofen loaded chitosan granule (CG).



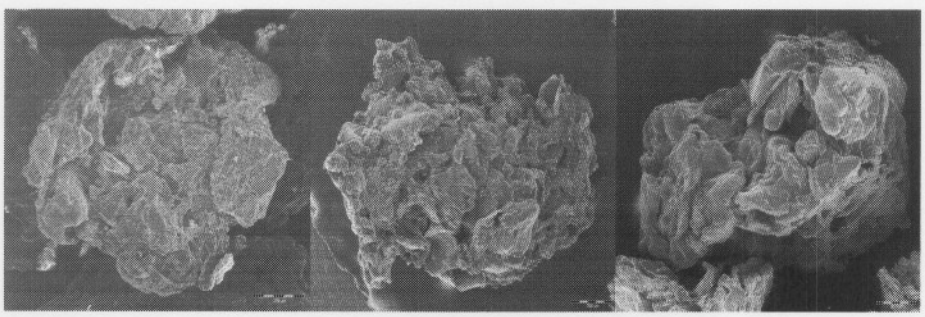
(a) (b) (c)

Figure 3-10: Full view of 1 (a), 2.5 (b) and 5% w/v (c) Aqoat® AS-HF granules (A1G, A2.5G, and A5G).



(a) (b) (c)

Figure 3-11: Full view of 1 (a), 2.5 (b) and 5% w/v (c) Eudragit® S100 granules (E1G, E2.5G, and E5G).



(a) (b) (c)

Figure 3-12: Full view of 1 (a), 2.5 (b) and 5% w/v (c) Kollidon® SR granules (K1G, K2.5G, and K5G).

3.2.3 Discussion

3.2.3.1 Beads

The shape of the beads prepared for the study remained almost spherical with a diameter of between 1-2 mm. The surface view of drug loaded chitosan (See Figure 3-1) beads prepared without adjusting the pH of the external TPP phase seem to be much smoother to that of the ketoprofen loaded chitosan beads, produced in a TPP phase with pH 5.0. This can be explained by the interaction between chitosan and the cross-linking agent TPP. The ability of TPP to cross-link chitosan may decrease with a decrease in pH as found by Mi *et al.* (2001:761). Mi *al.* (2001:761) also found that chitosan beads prepared in acetic TPP-solutions was found to be more porous than the beads prepared in basic TPP-solutions. This is clear in Figure 3-1 and Figure 3-2. From these micrographs it can be deducted that although an increase in drug loading was observed by modifying the method of Bodmeier *et al.* (1998:1478) to that described in chapter 2, the structural integrity of the beads was negatively altered.

Figure 3-3 to Figure 3-5 depict full view micrographs of beads produced by the inclusion of 1, 2.5 and 5% w/v controlled release polymer. It is clear that inclusion of the polymer yielded beads with a better surface structure than that of the polymer free bead. Inclusion of 1, 2.5 and 5% w/v Aqoat® AS-HF produced beads with equally improved surface structure (See Figure 3-3). The circular crack visible in Figure 3-3 may be a result of the preparation technique for SEM micro graphing. Inclusion of 1% w/v Eudragit® S100 produced beads with a smooth surface. Increasing the Eudragit® S100 concentration resulted in a more porous surface. The same tendency is shown in Figure 3-5 for Kollidon® SR beads with the effect also visible at a 1% w/v concentration. The more porous structure could be a result of lower total chitosan concentrations in the bead matrix, consequently producing less surface cross-linking in the TPP-solution. The controlled release polymer included in the beads is visible in all bead produced on both the surface as well as in the cross-sectional views. It can therefore be speculated that good matrix forming occurred.

Evaluation of the internal structure of the beads produced by the inclusion of 1, 2.5 and 5% w/v polymer revealed a more porous nature than that of the polymer free chitosan beads (See Figure 3-6 to Figure 3-8). The porous nature increased with an increase in Aqoat® AS-HF concentration, while it seemed to remain relatively constant in beads produced by the inclusion of Eudragit® S100 and Kollidon® SR. Kollidon® SR beads appeared to have a denser, flakier structure to the outer edges of the beads. This could be a result of the short time allowed for cross-linking. From the micrographs it can be concluded that TPP-ions could only interact on the outer layer of the beads. Mi *al.* (2001:761-763) also stated several reasons for a more porous internal structure. Beads produced by Mi *al.* in an acetic environment were found to be more porous. Short stirring times also yielded bead with not only a more porous internal structure but also a more porous surface layer. It was also found that lower TPP concentrations produced more porous beads with the most porous beads being from 2.5 and 1.5% w/v TPP-solutions. All these factors were eminent in the method used to produce beads in this study and may have been responsible for the internal appearance of the samples. It is also possible that air bubbles may have contributed to the more porous nature. Tiny air bubbles may have stayed trapped in the viscous gel dispersion. Even though attempts were made to remove the bubbles by means of sonication, air bubbles could still be present in the gel dispersion. 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.

3.2.3.2 Granules

Compared to beads, granules (Figure 3-9 to Figure 3-12) had a relatively simple structure. Since granules had to be milled in a mortar to achieve the required size no need was seen to cut the granules, as the surface views and internal views did not differ.

Surface views of the granules were similar for ketoprofen loaded chitosan granules and for granules with polymer included. Due to the fact that the required size fraction of the granules was collected by means of sieving, the size of the granules was

uniform. Polymer free granules appeared as a tightly packed clump with little or no definition between the drug particles and chitosan (See Figure 3-9). This could be attributed to the method used to produce the granules. Chitosan dissolves in acetic acid, to from a gel. When acetic acid was used to granulate the drug-chitosan mixture, chitosan formed a gel which coated all the particles to from the tightly packed particles.

Aqoat® AS-HF containing granules (See Figure 3-11) were very similar in appearance to ketoprofen loaded chitosan granules. It was however visible that Aqoat® AS-HF containing granules had a smoother surface layer for concentrations of 14.29 and 29.41% w/w. It was noticed that 45.45% w/w Aqoat® AS-HF containing beads had a flakier surface appearance. This could be attributed to the lower chitosan concentration of only 27.27% w/w. Since chitosan acts as the binder in the granule formulation, a lower concentration thereof would led to a weaker granule, more prone to cracking and flaking.

The same phenomenon is visible for both Eudragit® S100 and Kollidon® SR granules (See Figure 3-11 and Figure 3-12). Eudragit® S100 granules had a much less flakier surface appearance than Kollidon® SR. This may be a result of a negative interaction between Kollidon® SR and the binder used. Further investigation is needed. Concentrations of 29.41 and 45.45% w/w Eudragit® S100 had similar degrees of flakiness, with high concentrations of the spherical like polymer particles visible on the surface of the granules.

Spherical particles of Kollidon® SR were mostly visible on the surface of granules made with a concentration of 45.45% w/w polymer. There was however a more significant decrease in the denseness of the granules with every increase in polymer concentration, and high concentrations of polymer did not seem to from good tightly packed matrixes. A large hole was visible for granules produce with 45.45% w/w polymer added (See Figure 3-12).

3.3 DRUG LOADING CAPACITY

3.3.1 Method

The drug loading of the samples were determined by powdering the sample in a mortar. An accurately weighed amount of the sample was then transferred to a 100 ml volumetric flask. The flask was made up to volume with PBS pH 7.2. The mixture was then stirred for 24 hours on a magnetic stirrer (Labcon, Laboratory marketing services, Maraisburg South Africa) and then sonicated for a further 120 minutes. Samples were taken from the flask, filtered and analysed spectrophotometrically using a Unicam spectrophotometer at a wavelength of 258 nm.

3.3.2 Results

The drug loading of the granules were found to be a constant value of 92.04 ± 1.78%. The results of the drug loading (%DLC) of the beads are given in Figure 3-13.

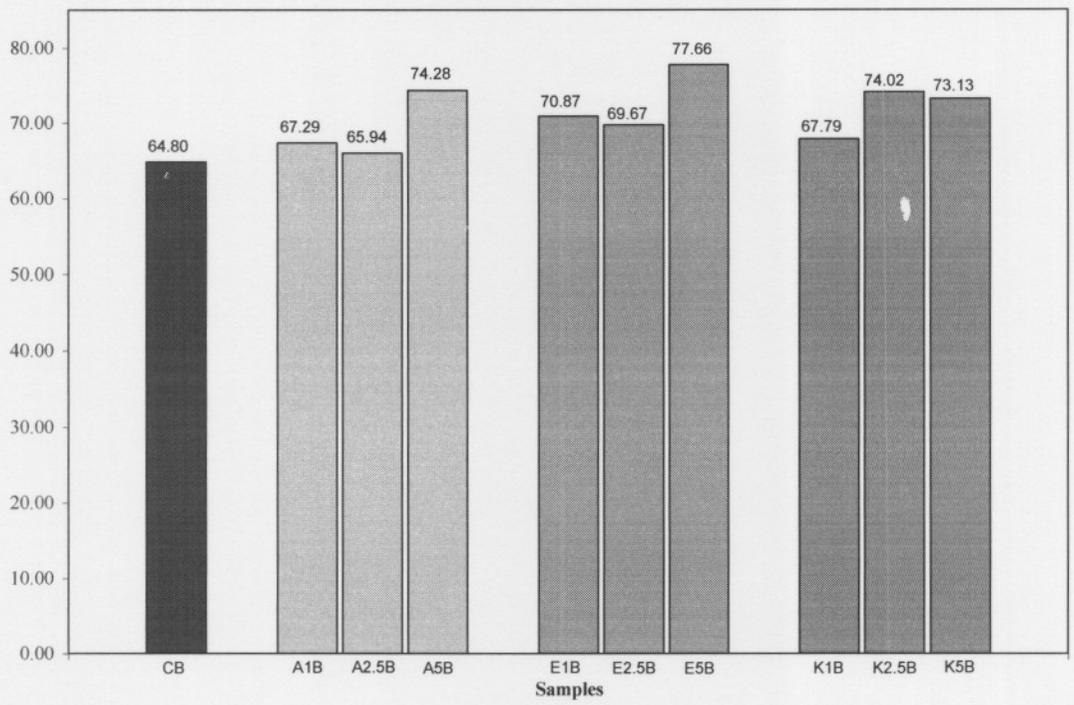


Figure 3-13: Graphic representation of percentage drug loading (%DLC) of bead formulations.

3.3.3 Discussion

It was noted that the inclusion of a polymer in the beads resulted in an increased drug loading. The increase in drug loading was however dependant on the type and concentration of polymer added.

Adding 1 and 2.5% w/v Aqoat® AS-AF respectively to the beads produced beads with slightly higher, though statistically insignificantly (67.29 and 65.94% respectively) drug loading than polymer free chitosan beads (64.80%). Although statistically there was no difference between these values (See Annexure B). The effect of Aqoat® AS-AF on drug loading was a definite increase with the addition of higher concentrations of polymer to the bead matrix, with a statistical increase in drug loading of 74.28% for 5% w/v Aqoat® AS-AF.

A statistically similar effect was observed for Eudragit® S100 1% w/v (%DLC = 70.84) and 2.5% w/v (%DLC = 69.67) Eudragit® S100 beads (See Appendix B). The highest drug loading, of all formulations, was 77.66%, obtained for the beads with the higher polymer concentration of 5% w/v Eudragit® S100.

The effect of Kollidon® SR in the beads appeared to increase the drug loading with an increase in concentration of controlled release polymer. The 1% w/v beads produced a drug loading of 67.79%. Higher concentrations of Kollidon® SR produced statistically similar drug loading (2.5% w/v Kollidon® SR = 74.02 and 5% w/v Kollidon® SR = 73.13%DLC) (See Appendix B).

The improved drug loading efficiency may be due to the matrix forming properties of the polymers added. These polymers started forming a matrix during the production phase of the bead. It could therefore be speculated that the matrix formed during the production phase decreased the drug release from the internal chitosan-polymer-drug dispersion into the external TPP-phase.

3.4 FRIABILITY

3.4.1 Method

Friability studies were conducted as a measurement of mechanical strength of the samples and to evaluate the viability of the formulations as dosage forms. The tests were performed as described in chapter 2 section 2.5.4 and the values determined using equation 2-4.

3.4.2 Results

The friability results of the bead samples are presented in figure 3-14. The results of the friability of the granules were all lower than 1% w/v. The results of the average friability of the granules were found to be 0.46 ± 0.24%.

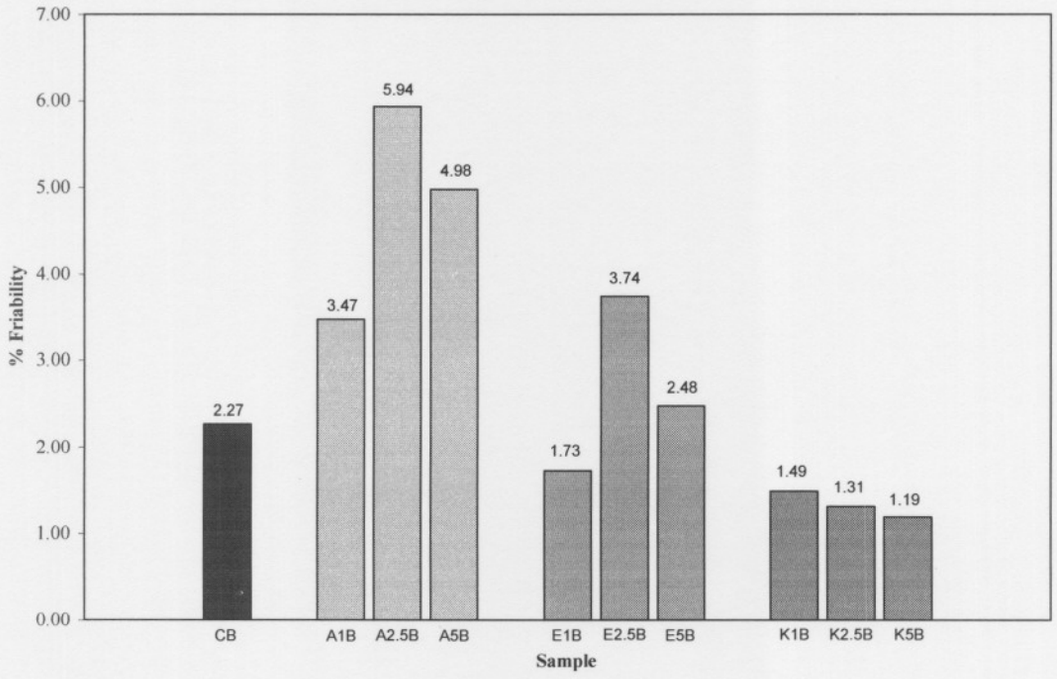


Figure 3-14: Percentage friability of bead samples.

3.4.3 Discussion

According to the USP friability values of ≤ 1 are ideal. The friability of all the granule samples prepared in the study was below 1% with an average value of 0.46 ± 0.24%. From this value it can be concluded that the granules prepared in the study had a suitable mechanical strength. This means that not much granulate would be lost during packaging and transport of the granules to the patient and that the granules would remain intact until usage.

The friability of the beads is high with 2.27 ± 0.14% for ketoprofen loaded chitosan beads. The inclusion of controlled release polymers into the bead structure resulted in a varying effect dependant on the type of polymer used. Additions of 1% w/v Aqoat® AS-HF to the beads led to an increase in friability to 3.47 ± 0.11%. Higher concentrations of Aqoat® AS-HF had a similar effect with statistically different values, increasing the friability of the beads to 5.94 ± 0.06% and 4.98 ± 0.18% respectively for 2.5 w/v and 5% Aqoat ® AS-HF (See Annexure B). This level of friability is quite unacceptable and it was noticed that a large number of beads broke during handling.

The inclusion of 1% w/v Eudragit® S100 however lowered the friability to 1.73 ± 0.03% and beads appeared quite hard, with minimal breakage during handling. Higher concentrations, of the polymer, increased the friability of the beads to 3.74 ± 0.06 and 2.48 ± 0.03%, for 2.5 and 5% w/v Eudragit® S100 respectively. Although the friability was higher for 2.5 and 5% w/v Eudragit® S100 the beads were still manageable. According to the Tukey test preformed there was no statistical difference between the friability of chitosan beads and that of 5% w/v Eudragit ® S100 beads (See Annexure B).

The effect of Kollidon ® SR was a noticeable decrease in friability with an increase in controlled release polymer. The lowest friability of 1.19 ± 0.08% was measured for a Kollidon ® SR concentration of 5% w/v.

3.5 SOLUBILITY

3.5.1 Method

Solubility of beads and granules was determined according to the method of Gupta *et al.* (2001:641) as described in chapter 2 sections 2.5.6, and the values were determined using equation 2-5.

3.5.2 Results

3.5.2.1 Beads

The results for the solubility tests of the bead samples are depicted in Figure 3-15.

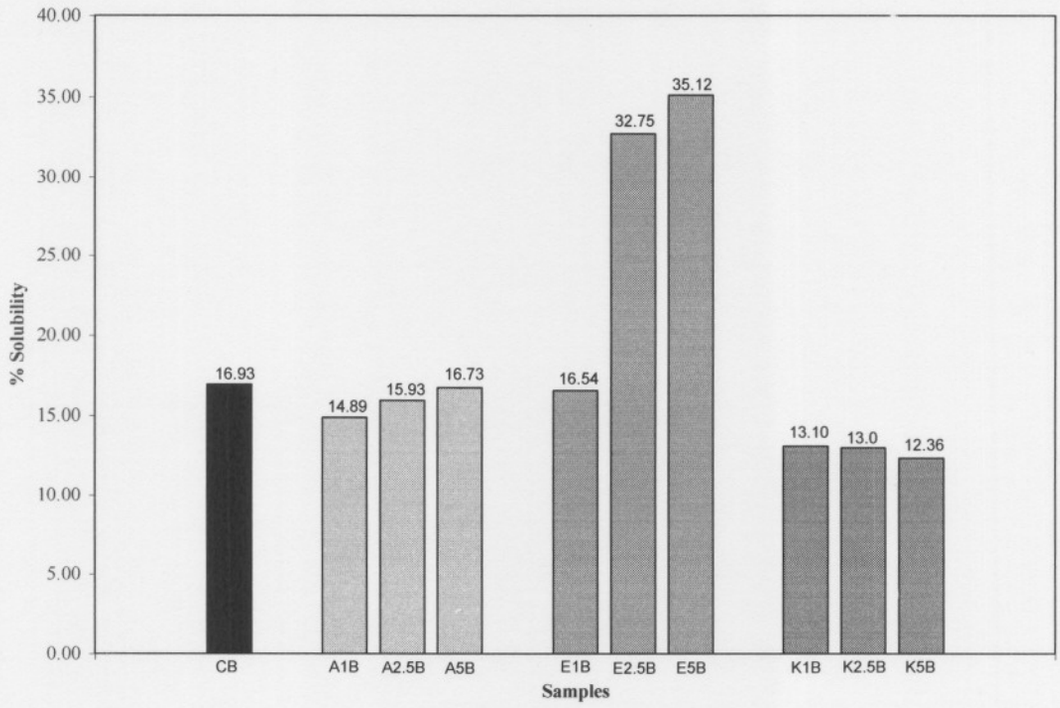


Figure 3-15: Percentage solubility of ketoprofen loaded beads.

3.5.2.2 Granules

The results for the solubility tests of the granules samples are depicted in Figure 3-16.

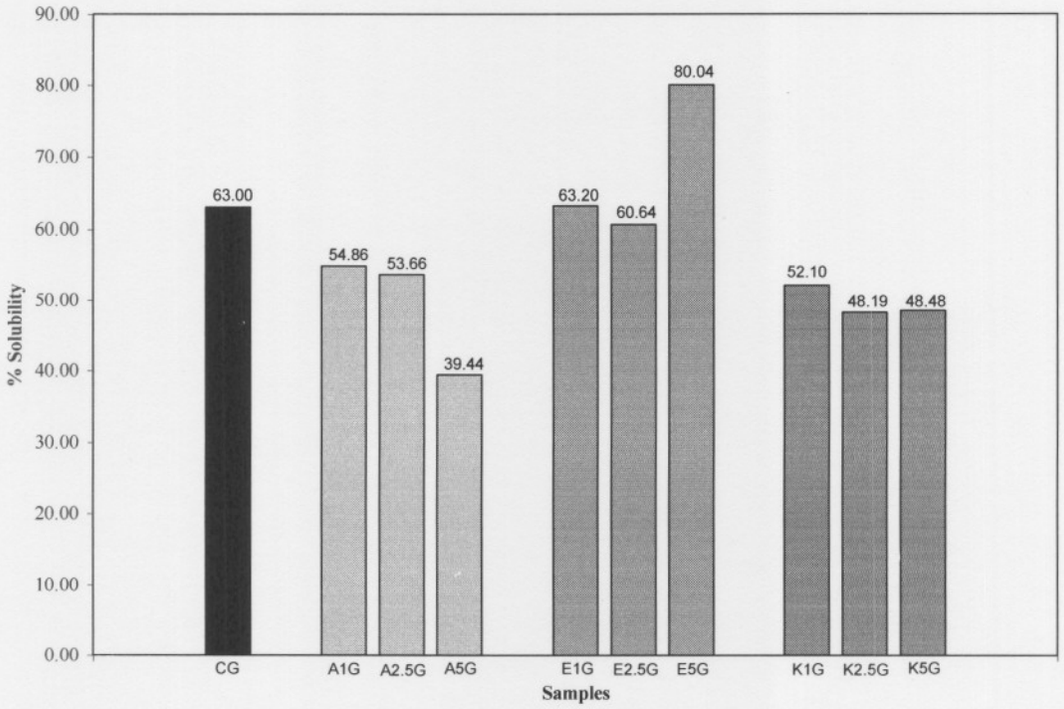


Figure 3-16: Percentage solubility of ketoprofen loaded granules.

3.5.3 Discussion

3.5.3.1 Beads

From Figure 3-15 it is clear that the solubility tests shown that the effect of the controlled release polymer included in the bead matrix to be dependant on the type and concentration of polymer used. Polymer free beads showed an average solubility of 16.93 ± 0.17% when compared to polymer loaded beads. 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. The high solubility of ketoprofen loaded chitosan beads can be attributed to the poor cross-linking due to the changes made to the method described in chapter 2.

Addition of 1% w/v Aqoat® AS-HF to the bead matrix statistically lowered the solubility of the beads to 14.89 ± 0.18%. With higher concentrations of Aqoat® AS-HF improved solubility with respect to 1% w/v was achieved, although for both 2.5

and 5% w/v Aqoat® AS-HF the values of 15.93 ± 0.27 and 16.73 ± 0.06% respectively was not statistically lower than that of ketoprofen loaded chitosan beads (See Annexure B). The higher degree of solubility might be a result of lower total concentrations of chitosan in the bead matrix, as more of the core is taken up by the polymer, and less by the chitosan, the concentration of cross-linked chitosan declines.

The inclusion of Eudragit® S100 into the bead formulation did not decrease the bead solubility. Eudragit® S100 1% w/v beads gave solubility values statistically identical to that of polymer free beads (See Annexure B). Higher Eudragit® S100 concentrations gave a statistical increased solubility when compared to polymer free beads although the effect was more pronounced with 2.5 and 5% w/v Eudragit® S100 yielding solubility values of 32.75 ± 0.6 and 35.12 ± 0.02% respectively (See Annexure B). These values imply that Eudragit® S100 beads might be unsuitable for controlled drug delivery as the integrity of the bead matrix is questionable and dose dumping might occur. Furthermore the high solubility of this the Eudragit® might be related to the solubility of this polymer in an alkaline (PBS buffer) environment.

By including Kollidon® SR in the bead formulation the degree of solubility could statistically significantly decreased ($p<0.05$) be decreased (See Annexure B). With higher concentrations of Kollidon® SR yielding beads with even lower solubility. The effect was most pronounced for 5% w/v Kollidon® SR, with a solubility of 12.36 ± 0.03%. The lower solubility of the Kollidon® SR beads might be related to the pH independent release characteristics of this polymer.

3.5.3.2 Granules

Since the granules produced by the method described in chapter 2, section 2.4.2 does not involve cross-linking of chitosan it was expected that the solubility of granules would be higher than that of cross-linked chitosan beads. This was found to be the case. It is clear from Figure 3-16 that the solubility of ketoprofen loaded chitosan

granules, with a solubility of 63.00 ± 1.01% was higher than that of the beads with a similar compositions (%S = 16.93 ± 0.17%). The inclusion of Kollidon® SR in the granule formulations similar to the bead formulations resulted in a decrease granule solubility. The average decrease in solubility was 52.10 ± 0.12, 48.19 ± 0.0.1 and 48.48 ± 0.60% for the 14.29, 29.41 and 45.45% w/w granule formulations respectively

The effect of controlled release polymer inclusion into the granule formulation was also dependant on the type and concentration of polymer used, with all polymer types and concentrations yielded statistically lower solubility values, compared to polymer free granules, except for 1% Eudragit® S100. The effect of Aqoat® AS-HF was comparable to that of Kollidon® SR. The inclusion of these two polymers into the granule formulations yielded granules with statistically lower solubility with respect to polymer free beads (See Annexure B). The effect of 14.29% w/w polymer added only lowered the solubility of the granules slightly with a solubility of 54.86 ± 0.54% for Aqoat® AS-HF and 52.10 ± 0.12% Kollidon® SR. At higher polymer concentrations Aqoat® AS-HF preformed better than Kollidon® SR with an improved solubility of 53.66 ± 0.43 and 39.44 ± 0.52% for 29.41 and 45.45% w/w respectively. For 29.41 and 45.45% w/w Kollidon® SR the resulting solubility was statistically the same with a solubility of 48.19 ± 0.1 and 48.48 ± 0.60% respectively (See Annexure B).

The inclusion of Eudragit® S100 in the granules did not decrease granule solubility except for the 29.41% w/w concentration. It must however be noted that this decrease was small, only 3.75% with respect to the average solubility values. At a Eudragit® S100 concentration the average increase in solubility was 27.05%

3.6 SWELLING BEHAVIOUR

3.6.1 Method

Swelling tests were performed according to the method described in section 2.5.6 in chapter 2. Due to the high solubility of the granule samples these samples were unsuitable for swelling experiments and the swelling results reflected are only applicable to the bead formulations.

3.6.2 Results

The results obtained during the determination of swelling behaviour are given in Table 3-2 and depicted graphically in figure 3-17 to 3-19.

Table 3-1: Degree of swelling (E_{sw}) of bead samples prepared in the study in 0.1 M HCl and PBS pH 7.2.

Sample	Degree of swelling					
	10 min		60 min		360 min	
	0.1 M HCl	PBS pH 7.2	0.1 M HCl	PBS pH 7.2	0.1 M HCl	PBS pH 7.2
CB	2.80 ± 0.04	2.79 ± 0.56	9.42 ± 0.72	3.05 ± 0.28	17.03 ± 0.11	3.34 ± 0.70
A1B	2.09 ± 0.43	2.45 ± 0.47	2.78 ± 0.29	3.05 ± 0.18	4.69 ± 0.02	4.10 ± 0.87
A2.5B	2.87 ± 0.69	1.61 ± 0.16	4.36 ± 0.41	1.93 ± 0.39	6.07 ± 0.52	3.34 ± 0.07
A5B	1.56 ± 0.69	1.82 ± 0.11	2.12 ± 0.28	1.83 ± 0.11	2.69 ± 0.66	1.94 ± 0.04
E1B	3.06 ± 0.05	2.67 ± 0.01	3.16 ± 0.74	3.42 ± 1.31	2.93 ± 0.01	1.80 ± 0.19
E2.5B	2.54 ± 0.11	2.57 ± 0.89	3.02 ± 0.41	2.93 ± 0.13	4.56 ± 0.24	1.99 ± 0.01
E5B	2.54 ± 0.50	1.86 ± 0.01	3.88 ± 0.88	2.64 ± 0.07	4.88 ± 0.30	1.66 ± 0.76
K1B	4.52 ± 0.11	2.92 ± 0.01	5.12 ± 0.92	3.10 ± 0.21	4.20 ± 0.32	3.28 ± 0.34
K2.5B	3.96 ± 0.66	2.65 ± 0.24	3.67 ± 0.24	3.02 ± 0.07	3.46 ± 0.10	2.88 ± 0.52
K5B	2.79 ± 0.06	1.78 ± 0.18	2.20 ± 0.28	1.79 ± 0.09	2.10 ± 0.02	1.89 ± 0.07

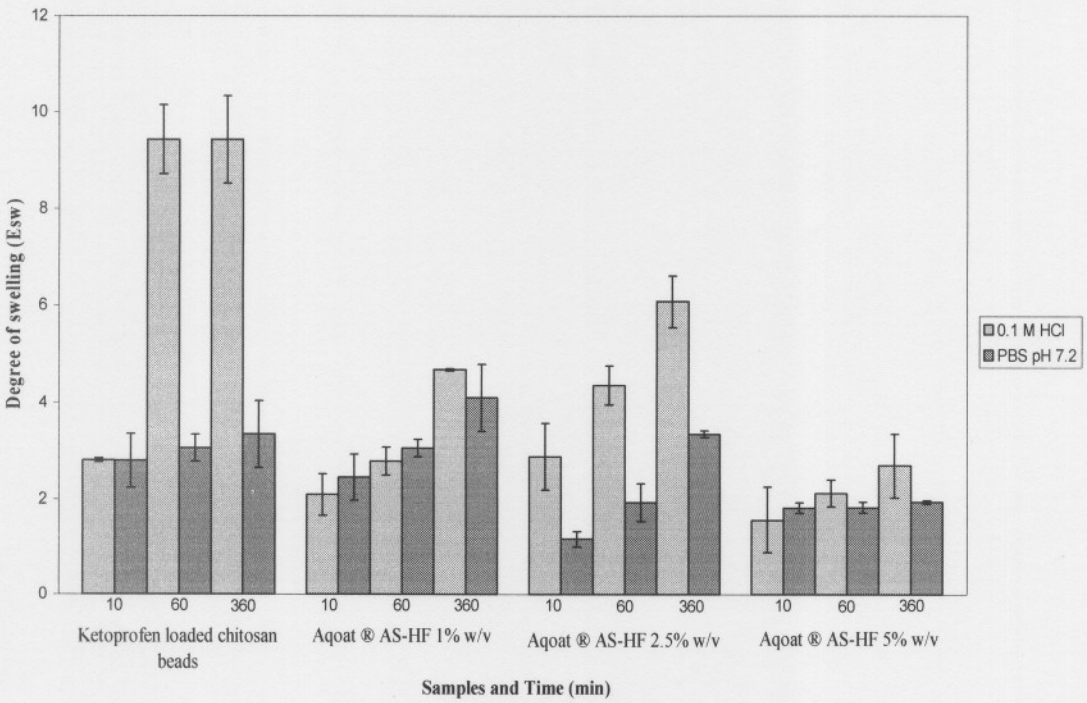


Figure 3-17: Degree of swelling for Aqoat® AS-HF formulations.

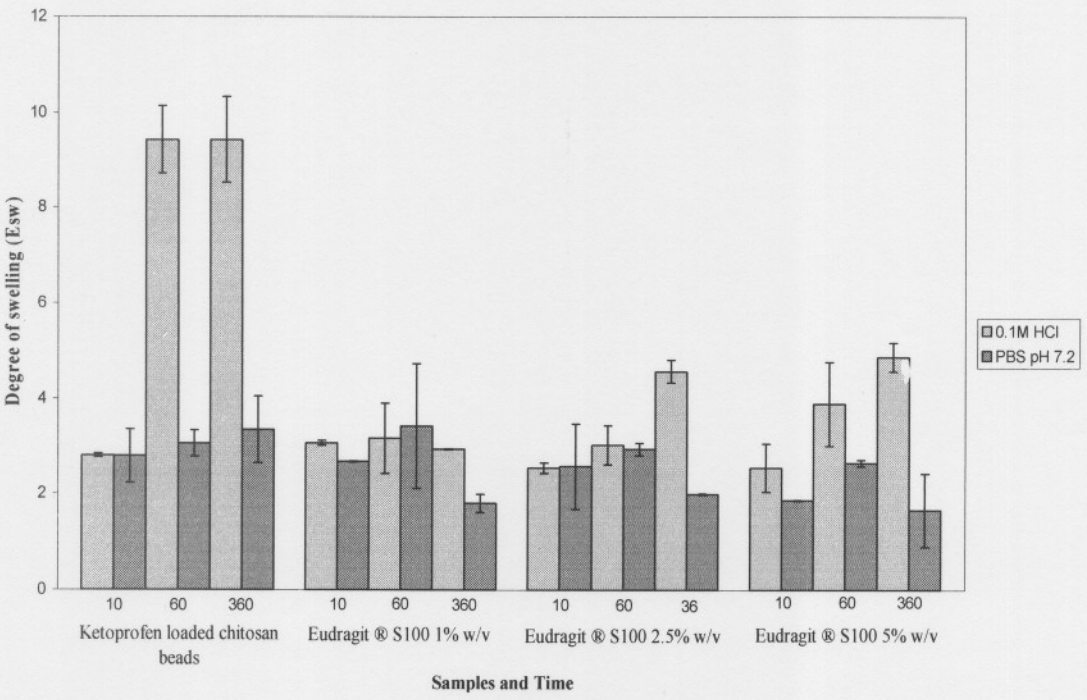


Figure 3-18: Degree of swelling for Eudragit® S100 formulations.

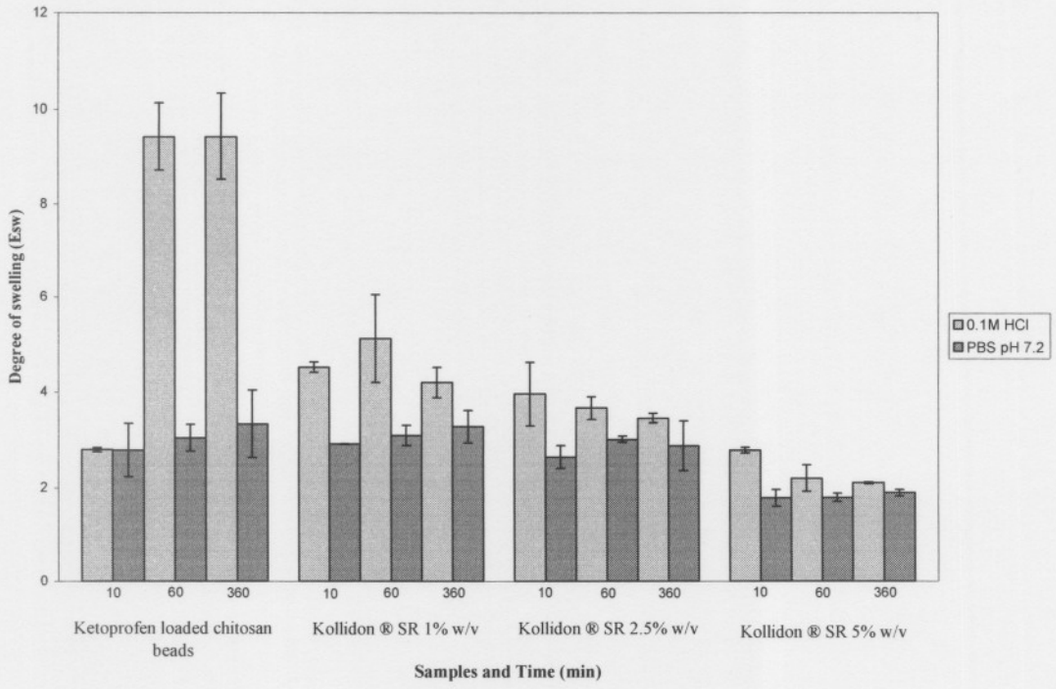


Figure 3-19: Degree of swelling for Kollidon® SR formulations.

3.6.3 Discussion

Due to the poor cross-linking allowed by the modified method described in chapter 2 section 2.3.2, the swelling behaviour of the pure chitosan beads was not adequate for the use in controlled release. Beads made from only chitosan and drug showed good swelling during the first 10 minutes submerged in 0.1 M HCl but started losing shape and dissolving after 60 minutes reaching a maximum Esw of 9.42 ± 0.72. This was expected as results from solubility indicated poor cross-linking had occurred. After 6 hours the beads almost completely dissolved forming a gel. The swelling behaviour of ketoprofen loaded chitosan beads was much weaker in PBS pH 7.2 than in 0.1 M HCl. Beads swelled only a maximum of 4.10 ± 0.87 after 6 hours The rate of swelling seemed to increase only slightly after each time interval. The results was differed from those obtained by Gupta *et al.* (200:641) with their chitosan beads reaching a degree of swelling of between 10 and 12% within the first day, and only starting to disintegrate after approximately two days.

Observation of Figure 3-17 to Figure 3-19 indicates that the swelling behaviour of beads produced by the inclusion of controlled release polymers were dependant on the concentration and type of polymer used, and on the type of swelling medium used. Generally the inclusion of controlled release polymers into the bead formulations led to a decrease in the total degree of swelling for both dissolution mediums when compared to ketoprofen loaded chitosan beads.

In Figure 3-17 it can be seen that an increase in Aqoat® AS-HF concentration led to an increase in swelling for 1 and 2.5% w/v polymer, but that the high concentration of 5% w/v, decreased the degree of swelling in both swelling mediums used. Swelling was mostly hindered by the high concentration of 5% w/v Aqoat® AS-HF, reaching only a maximum degree of swelling of 2.69 ± 0.66% and 1.94 ± 0.04% for 0.1 M HCl and PBS pH 7.2 respectively. It was noticed that the beads produced from Aqoat® AS-HF did not dissolve during the duration of the experiment as is indicated by the steady climb in swelling values in Figure 3-17.

Beads produced with Eudragit® S100 in the formulation yielded less favourable swelling behaviour. The degree of swelling was lower than that of ketoprofen loaded chitosan beads in 0.1 M HCl but remained almost the same for pH 7.2. Eudragit® S100 1% w/v showed a slight increase in swelling after 10 minutes in 0.1 M HCl. The degree of swelling remained almost the same after 60 minutes, but decreased after 360 minutes, indicating that some degree of degradation had started to occur. The same was true for 1, 2.5 and 5% w/v beads in PBS pH 7.2, although the effect appeared more pronounced. This might be because Eudragit® S100 is designed to release actives in a pH range of 6.5 to 7.5 (Deggusa, 2004). Eudragit® S100 beads of 2.5 and 5% w/v did not break down in 0.1 M HCl, but showed a noticeable increase in swelling with every time interval. Although signs of degradation were found during the evaluation of Eudragit® S100 beads, the samples maintained their spherical shape throughout the study.

Kollidon® SR samples had a similar but, more pronounced, tendency as 1% w/v Eudragit® S100 beads to show a decrease in swelling after 6 hours in 0.1 M HCl. The effect was however not noticed in PBS pH 7.2. Beads degree of swelling continued to increase up to 6 hours for 1 and 5% w/v samples but seemed to reach a constant after 60 minutes for 2.5% w/v Kollidon ® SR beads. Swelling was mostly inhibited by 5% Kollidon ® SR with maximum values for Esw of 2.10 ± 0.02% and 1.89 ± 0.07% for 0.1 M HCl and PBS pH 7.2 respectively.

Lastly it should be noted that according to Win *et al.* (2003:308) a successful oral drug carrier must be resistant to the impact of pH gradients due to pH changing from the strongly acidic medium in the stomach to the weakly alkaline medium of the intestine. It is clear from the above mentioned results that the inclusion of controlled release polymers to the weakly cross-linked ketoprofen loaded chitosan bead reduced the impact of the two swelling mediums on the samples. Therefore the inclusion of controlled release polymers into the bead matrixes improved the functionality of the samples. This effect the polymers had on the bead formulation should however be further investigated.

3.7 CONCLUSION

Morphology, drug loading, friability, solubility, and swelling tests revealed that the methods described in chapter 2 could be used to effectively prepare chitosan beads and granules containing modern controlled release polymers.

Morphology studies in general revealed that possible poor cross-linking occurred during the preparation of the beads in TPP. Porous beads with rough surface layers were observed for ketoprofen loaded chitosan beads prepared according to the modified method described in chapter 2. Addition of Aqoat AS-HF, Eudragit® S100 and Kollidon® SR all led to the appearance of a large pore like internal bead structure. Although these pore like structures appeared inside the beads the surface layers

appeared smoothed. Good matrix arrangement was observed for all samples. Only slight differences in the morphology of chitosan granules and chitosan-polymer granules could be observed. High concentrations of Aqoat® AS-HF and Kollidon® SR appeared to weaken the surface appearance of the granules, with more cracks being visible. The controlled release polymers were visible on the surface of all matrix granules prepared.

Results showed that the inclusion of modern controlled release polymers into the chitosan-drug matrix improved the drug loading. The best improvement was due to Eudragit® S100 in 5% w/v concentration with an increase of 12.80% in the drug loading. This increase in drug loading is of economic importance for the pharmaceutical industry as less drug is wasted during the preparation of drug loaded beads. Drug loading remained invariable for all granules and was much higher than that of beads.

Although all samples appeared to be viable dosage forms some tests revealed otherwise. Friability testing showed beads to be quite non-resistant to mechanical force when compared to granules. The addition of Aqoat® AS-HF increased the friability to 5.94%, while Kollidon® SR improved the friability of beads to 1.19%. All granulate samples had acceptably low friability.

Solubility tests showed beads to be much less prone to dissolve than granules. This showed that beads prepared during the study stood a much better chance at being successful controlled release dosage forms. The addition of Kollidon® SR proved to lower the solubility better than the other polymers. Aqoat® AS-HF and Eudragit® S100 gave contradicting results in beads and granules.

According to swelling and degradation studies chitosan beads swelled out of control in 0.1 M HCl after 60 minutes and started to dissolve. This is due to the solubility of

chitosan in acidic solutions. Chitosan beads showed good swelling in PBS pH 7.2. The degree of swelling appeared to be influenced by the pH of the medium used, the type of polymer used, and the concentration of polymer used. A significantly higher degree of swelling was measured for 2.5% w/v Aqoat® AS-HF and 1% w/v Eudragit® S100 in 0.1 M HCl than for PBS pH 7.2. Although all samples produced by the addition of controlled release polymers improved the degree of swelling in 0.1 M HCl when compared to chitosan beads, the higher degree of swelling showed that possible higher release rates might be measured in the acidic regions of the stomach. The best swelling results was achieved for 1% w/v Eudragit® S100 and Aqoat® AS-HF.

Dissolution studies on all samples are discussed in chapter 4.

CHAPTER 4

Dissolution studies of chitosan and chitosan-controlled release polymer beads.

4.1 INTRODUCTION

Dissolution is the process by which a chemical or drug becomes dissolved in a solvent. In biologic systems, drug dissolution in an aqueous medium is an important prerequisite of systemic absorption. The rate at which drugs with poor aqueous solubility dissolve from the intact or disintegrated solid dosage from in the gastrointestinal tract often controls the rate of systemic absorption of the drug. Thus dissolution tests are discriminating of formulation factors that may affect drug bioavailability. (Shargel & Yu, 1999:132)

The preparation and characterisation of formulations were discussed in chapters 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 (CR) dosage from could be manufactured and which type of formulation, beads or granules, is the best. Results from the drug loading study described in chapter 3 section 3.3 was used in the dissolution study to determine the amount of drug released during the study. As described in chapter 2 section 2.5.1 two dissolution mediums were used. Dissolution studies were conducted in 0.1 M HCl (pH 1.2 ± 0.05) for the first hour after which the studies were continued in phosphate buffered solution (pH 7.2 ± 0.05) for the remainder of the studies. As with drug loading tests, ketoprofen concentrations were calculated from absorbencies measured spectrophotometrically at 258 nm wavelength.

4.2 MODELING AND CHARACTERISATION OF DISSOLUTION PROFILES

Although this may not always be possible, it would be ideal if the release of drug from formulations could be explained mathematically. Some models that could be used for interpretation simple drug release systems are discussed here. 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, 2001:128).

4.2.1 Zero order

When it is assumed that the area of the dosage from does not change, and no equilibrium conditions are obtained, drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be presented by the following equation:

$$W_0 - W_t = K_t \qquad (4\text{-}1)$$

Where W_0 is the initial amount of drug in the dosage form, W_t is the amount of drug in the dosage from at time t and K is a proportionality constant. The equation can be simplified by dividing the equation by W_0 to give:

$$F_t = K_0 t \qquad (4\text{-}2)$$

Where $F_t = 1 - (W_t/W_0)$ and F_t represents the fraction of drug dissolved in time t and K_0 the apparent dissolution rate constant or zero order release constant. Therefore if

the previously established conditions are fulfilled, a graphic representation of the drug-dissolved fraction versus time will be linear.

Pharmaceutical dosage forms that follow this profile of release, release the same amount of drug per unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action. The following equation expresses this model:

$$Q_1 = Q_0 + K_0 t \qquad (4\text{-}3)$$

Where Q_1 is the amount of drug dissolved in time t, and Q_0 is the initial amount of drug in the solution. Most of the time $Q_0 = 0$. K_0 is the zero-order release constant (Costa & Lobo, 2001:123-124)

4.3 MEAN DISSOLUTION TIME

Ritger and Peppas (1987:31) define mean dissolution time (MDT) as the mean time for the drug to dissolve under in vitro dissolution conditions. MDT is the mean ratio of the first to zero moments of the dissolution rate-time curve and is expressed by the following equation (4-1) (Figure 4-1 defines the symbols):

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

According to Ritger and Peppas (1987:31) MDT can be estimated from the cumulative mass dissolved *vs.* the time profile using the following equation (4-2):

$$MDT = \frac{\sum_{i=1}^{n} t_{mid}\Delta X_d}{\sum_{i=1}^{n} \Delta X_d} \qquad (4\text{-}2)$$

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 ΔX_d is the additional mass of drug dissolved between i and $i-1$.

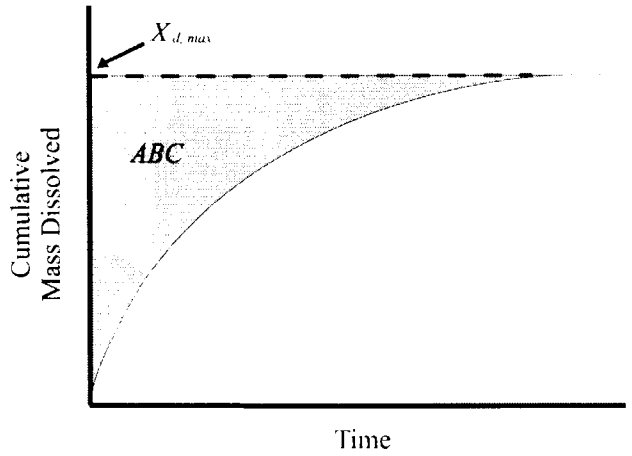


Figure 4-1: Graphic representation of the parameters used to estimate the mean dissolution time (*MDT*): $X_{d,\,max}$ is the actual maximum cumulative mass dissolved, and *ABC* is the shaded area (Ritger and Peppas 1987:31).

4.4 COMPARISON OF DISSOLUTION PROFILES

4.4.1 Similarity factor

The similarity factor (f_2) is used to compare the dissolution profile of each formulation with that of marketed formulation. In this approach, recommended by the FDA Guidance for Industry, a value between 50 and 100 indicates similarity between two dissolution profiles. If the f_2 value is close to 100, the two profiles are nearly identical (Shah et al. 1998:889; Moore & Flanner, 1996:64). The similarity factor can be determined by the following equation (4-3):

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

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 pints, and w_t is an optional weight factor. The equation is a logarithmic transformation of the sum of squared error. It takes the average sums of squares of the difference between test and reference profiles and fits the result between 0 and 100. The similarity factor (f_2) is 100 when the test and reference profiles are identical and approaches zero as the dissimilarity increases.

4.5 BURST EFFECT

Burst effect is the sudden increase in drug release noted when dissolutions studies are started. 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. This phenomenon is typically referred to as burst release. Depending on the intended use of a pharmaceutical dosage form, this initial high release may 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. Often burst release may cause non-desirable problems such as local or systemic toxicity (Lubbe, 2002:31).

According to Huang and Brazel (2001:123) there are several potential reasons why burst effect can occur, these 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.

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 burst quantities may be acceptable, as long as the burst effect is predictable (Huang & Brazel 2001:134).

4.6 METHODOLOGY

The dissolution studies were preformed according to the method described in chapter 2 section 2.5.1. Although the same amount of formulations was weighed for every dissolution test preformed, the amount of drug differed slightly, due to the difference in drug loading. Therefore the amount of active had to be calculated for every formulation based on the results achieved from the drug loading (See Table 4-1).

Table 4-1: Amount of ketoprofen (in μg) per 0.140g of dissolution formulation as calculated from the drug loading capacity (DLC).

Bead Formulations	DLC (%)	Average Mass (g)	Ketoprofen (μg)	Granule Formulations	DLC (%)	Average Mass (g)	Ketoprofen (μg)
CB	64.80	0.1413	91.56	CG	92.18	0.1403	129.33
A1B	67.29	0.1422	95.69	A1G	91.00	0.1402	127.58
A2.5B	65.94	0.1402	92.45	A2.5G	92.61	0.1410	130.58
A5B	74.28	0.1407	104.52	A5G	90.00	0.1399	125.91
E1B	70.87	0.1402	99.36	E1G	90.10	0.1421	128.03
E2.5B	69.67	0.1413	98.44	E2.5G	91.79	0.1403	128.78
E5B	77.66	0.1392	108.11	E5G	95.00	0.1408	133.76
K1B	67.79	0.1399	94.83	K1G	95.00	0.1389	131.96
K2.5B	74.02	0.1412	104.52	K2.5G	90.78	0.1400	127.09
K5B	73.13	0.1422	103.98	K5G	92.00	0.1410	129.72

It was convenient to use the same mediums as the dissolution media used to plot the two standard curves, for the determination of drug loading and the dissolution studies. Two standard curves were constructed before every dissolution study. The method used to plot the standard curves was already discussed in chapter 2 section 2.5.7. Examples are given in Figure 4-2 and Figure 4-3.

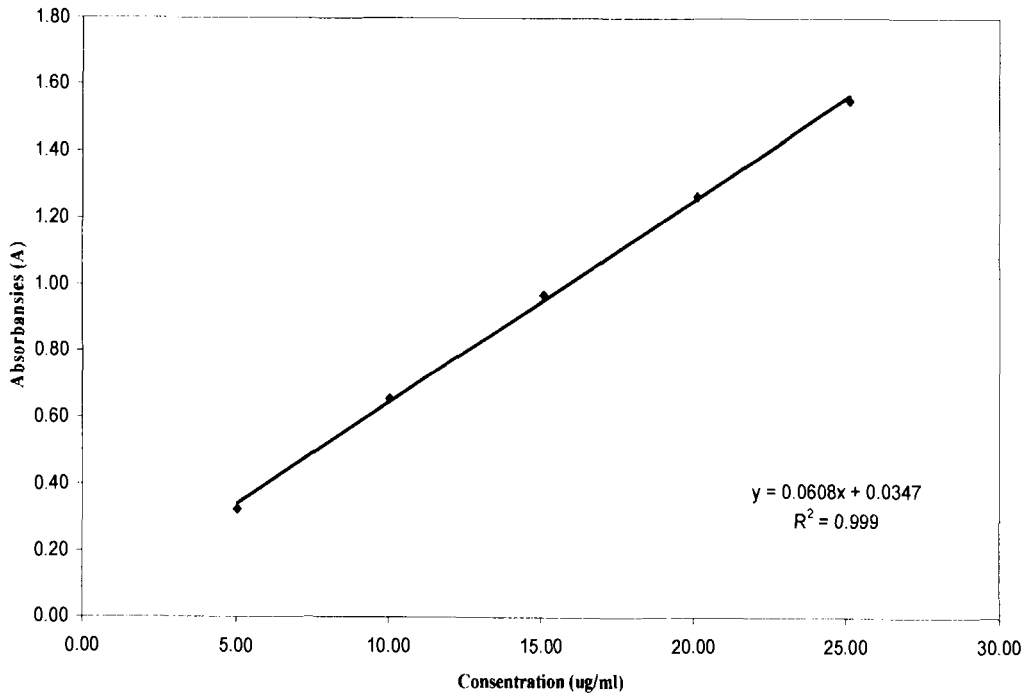


Figure 4-2: Example of a standard curve plotted for ketoprofen in 0.1 M HCl.

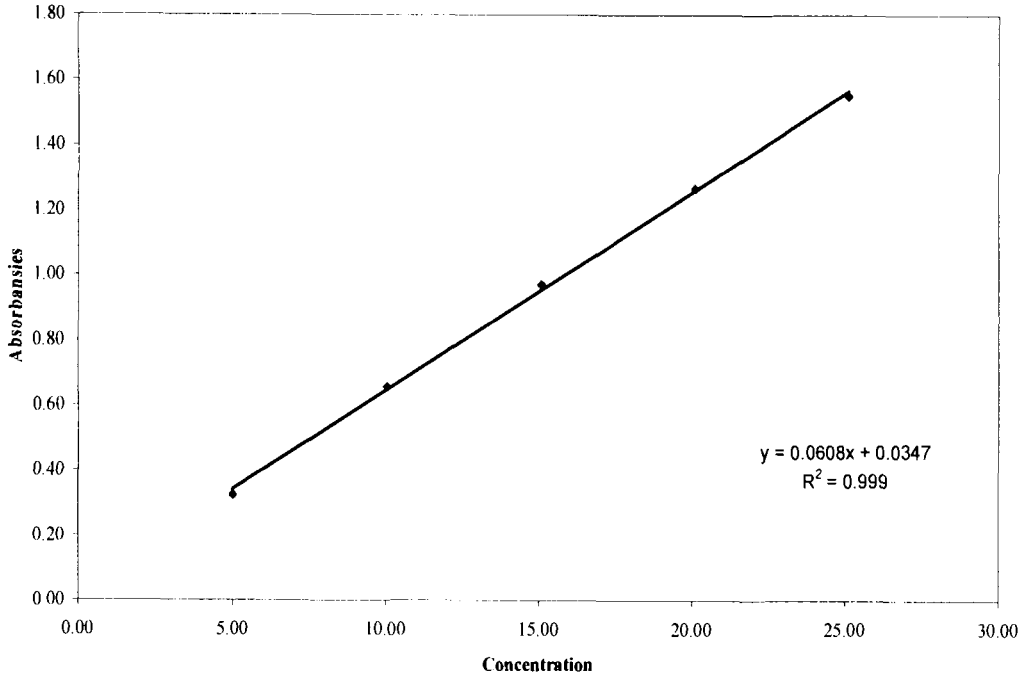


Figure 4-3: Example of a standard curve plotted for ketoprofen in PBS pH 7.2.

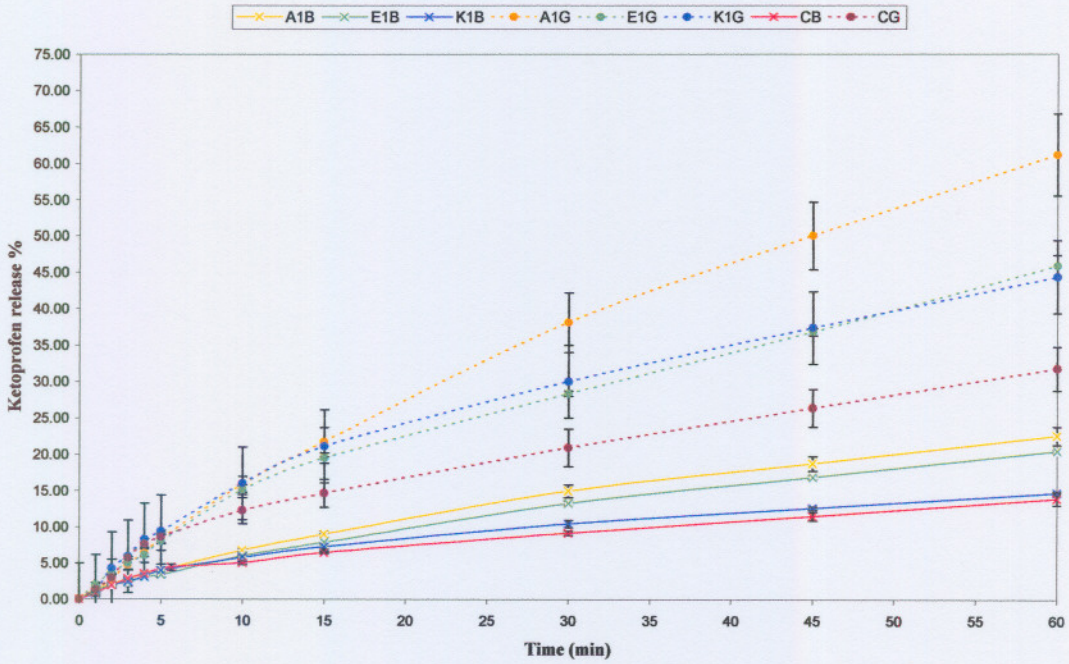


Figure 4-4: Ketoprofen release from beads containing 1% w/v CR polymer and granules containing 14.29% w/w CR polymer during the first 60 minutes in 0.1 M HCl.

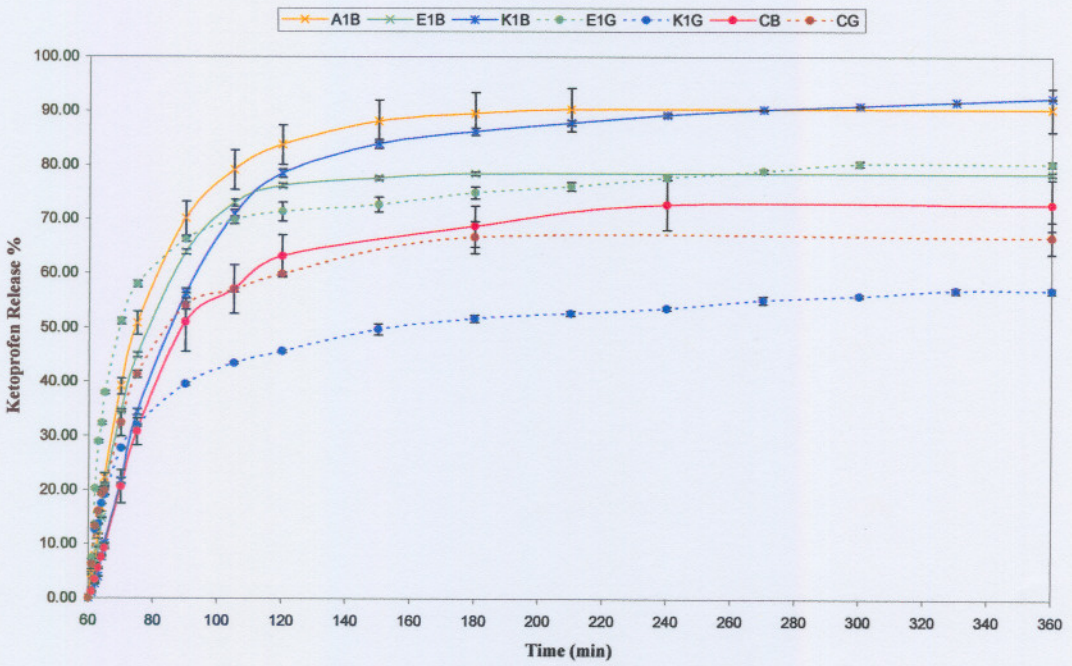


Figure 4-5: Ketoprofen release from beads containing 1% w/v CR polymer and granules containing 14.29% w/w CR polymer for the remainder of the study in PBS.

4.7 RESULTS AND DISCUSSION

4.7.1 Mean dissolution time

Table 4-2: Dissolution profiles of 0.1 M HCl time 0 - 60 minutes.

0.1 M				
	MDT1	**MDT2**	**MDT3**	**Ave MDT**
CB	21.10	22.38	22.86	22.11 ± 0.91
A1B	23.78	24.33	22.78	23.63 ± 0.79
A2.5B	23.51	24.87	20.97	23.12 ± 1.98
A5B	20.68	21.53	21.68	21.30 ± 0.54
E1B	23.35	25.02	23.98	24.12 ± 0.84
E2.5B	23.44	27.82	26.20	25.82 ± 2.21
E5B	19.14	17.97	19.76	18.96 ± 0.91
K1B	20.97	19.54	21.44	20.65 ± 0.99
K2.5B	19.89	19.81	20.82	20.17 ± 0.56
K5B	19.68	18.06	18.95	18.90 ± 0.81
CG	23.30	23.74	19.78	22.27 ± 2.17
A1G	24.66	25.33	26.20	25.40 ± 0.77
A2.5G	23.32	23.36	25.05	23.91 ± 0.99
A5G	20.61	19.68	19.84	20.04 ± 0.50
E1G	25.30	23.68	24.27	24.42 ± 0.82
E2.5G	28.00	26.52	25.36	26.63 ± 1.32
E5G	18.80	18.63	18.09	18.51 ± 0.37
K1G	21.75	23.18	21.28	22.07 ± 0.99
K2.5G	20.01	18.62	20.16	19.60 ± 0.85
K5G	13.64	8.91	13.36	11.97 ± 2.65

Table 4-3: Dissolution profiles of PBS pH 7.2 time 60 - ∞ minutes.

PBS pH7.2						
	MDT	**MDT**	**MDT**	**Ave MDT**		
CB	82.97	89.04	83.75	85.25	±	3.30
A1B	80.34	80.32	80.32	80.33	±	0.01
A2.5B	82.54	83.24	83.37	83.05	±	0.45
A5B	80.15	79.12	83.24	80.84	±	2.15
E1B	78.32	77.96	78.14	78.14	±	0.18
E2.5B	82.71	81.53	82.12	82.12	±	0.59
E5B	89.35	87.30	99.76	92.14	±	6.68
K1B	98.28	99.00	97.75	98.34	±	0.63
K2.5B	88.55	89.20	87.39	88.38	±	0.92
K5B	105.59	109.05	107.09	107.24	±	1.73
CG	76.91	82.96	82.79	80.88	±	3.44
A1G	65.03	64.94	64.98	64.98	±	0.05
A2.5G	67.28	66.87	66.59	66.91	±	0.35
A5G	69.04	68.47	68.42	68.64	±	0.35
E1G	76.91	82.96	82.79	80.88	±	3.44
E2.5G	69.79	69.94	69.42	69.72	±	0.26
E5G	67.48	67.47	66.92	67.29	±	0.32
K1G	93.32	91.65	89.69	91.55	±	1.82
K2.5G	65.61	65.58	65.59	65.59	±	0.01
K5G	-	-	-	-	±	-

4.7.2 Similarity factor (f_2)

Table 4-4: Similarity factor for all samples in 0.1 M and PBS pH 7.2 vs. polymer free beads and granules.

Similarity Factor (f2)				
	0.1 M		PBS pH 7.2	
	(f1) vs CB	(f1) vs CG	(f1) vs CB	(f1) vs CG
A1B	98.87 ± 1.33	79.76 ± 2.15	62.98 ± 2.27	71.82 ± 3.46
A2.5B	98.96 ± 0.21	81.02 ± 1.79	55.98 ± 1.14	83.42 ± 3.55
A5B	78.52 ± 5.97	91.94 ± 2.64	54.11 ± 8.79	77.76 ± 8.52
E1B	97.64 ± 0.86	77.94 ± 2.58	69.76 ± 1.42	63.39 ± 1.54
E2.5B	97.30 ± 2.13	76.59 ± 3.72	58.25 ± 1.02	71.34 ± 1.35
E5B	95.60 ± 2.99	84.08 ± 4.36	62.03 ± 1.19	67.69 ± 2.78
K1B	99.09 ± 0.37	78.19 ± 2.94	94.72 ± 0.98	51.77 ± 0.70
K2.5B	99.29 ± 0.55	80.07 ± 2.48	90.37 ± 2.83	56.12 ± 0.95
K5B	80.98 ± 2.72	70.44 ± 2.46	34.32 ± 0.96	44.85 ± 1.89
CB	100.00 ± 0.00	80.03 ± 2.86	100.00 ± 0.00	53.07 ± 1.17
A1G	85.66 ± 2.91	93.08 ± 5.25	45.52 ± 0.29	70.69 ± 2.25
A2.5G	83.72 ± 0.36	96.33 ± 1.45	55.43 ± 0.98	85.96 ± 2.67
A5G	75.35 ± 3.99	90.42 ± 6.36	59.55 ± 1.22	69.53 ± 1.98
E1G	84.84 ± 7.97	87.93 ± 2.08	38.16 ± 0.50	52.68 ± 0.25
E2.5G	83.73 ± 3.14	94.16 ± 3.91	50.50 ± 0.58	86.36 ± 2.67
E5G	63.21 ± 5.01	75.10 ± 10.51	41.01 ± 0.42	59.11 ± 0.64
K1G	76.43 ± 2.50	93.21 ± 2.56	56.33 ± 0.82	87.84 ± 1.32
K2.5G	79.06 ± 6.53	92.29 ± 2.35	73.55 ± 1.51	46.60 ± 0.59
K5G	65.60 ± 2.01	78.73 ± 5.55	67.05 ± 1.13	44.28 ± 0.56
CG	80.03 ± 2.86	100.00 ± 0.00	53.07 ± 1.17	100.00 ± 0.00

4.7.3 Formulations of 1% w/v and 14.29% w/w CR polymer

4.7.3.1 Results

The comparative results for bead formulations containing 1% w/v controlled release polymer, and for granule formulations containing 14.29% w/w polymer are depicted in Figure 4-4 and Figure 4-5.

4.7.3.2 Discussion

4.7.3.2.1 Dissolution in 0.1 M HCl

The effect of modern controlled release polymers on the release of ketoprofen from the formulations appeared to be in contradiction to what was expected. It was thought that the addition of these polymers most of which only dissolve at high pH levels would led to little, if any, release of ketoprofen during the first 60 minutes in 0.1 M HCl. Observation of the graph indicates that there are several differences between the release of drug from beads and granules. As expected, granules exhibited a much more prominent burst effect during the first 5 minutes. This difference could be attributed to the difference in surface structure between the beads and the granules. Granules had a much less smooth and more cracked or flaky appearance. This type of surface morphology increased the total surface area of the granules exposed to the dissolution medium and more drug dissolved during the first 5 minutes. Only a very slight initial burst effect was noticed for the bead formulations. Chitosan was cross-linked giving a slow release matrix whereas no cross-liking occurred in the granule formulations.

The results also show that the deviation between baskets was much lower for beads than for granules. Once again it was speculated that this correlated to the structural differences between the formulations. Where beads consisted mainly of solid spherical structures, granules were asymmetrical and random in shape. Although granulate formulations were well sieved, size differences were greater in granulate formulations than in bead formulations.

The inclusion of controlled release polymers into both of the formulation types led to a noticeable increase in amount of drug released and in the case of the granules, rate of drug release (See Annexure B). The mean dissolution time was increased Eudragit® S100 and Aqoat® AS-HF, but was decreased by Kollidon® SR (See Table 4-2). Release profiles of Kollidon® S100 beads did not differ statistically from those obtained for polymer free chitosan beads ($f_2 = 99.09 \pm 0.37$). Only a slight increase of

0.79% in total drug released was seen for Kollidon® SR 1% w/v beads. Eudragit® S100 and Aqoat® AS-HF also statistically gave very similar release profiles to each other, with Eudragit® S100 beads releasing a total amount of 20.42 ± 0.20% (6.53% more than chitosan beads) and Aqoat® AS-HF 22.53 ± 1.23% drug after 60 minutes (f_2 = 97.99 ± 1.81). Although the release rate appeared higher for these two formulations ($A1B_{MDT}$ = 23.63 ± 0.79 & $E1B_{MDT}$ = 24.12 ± 0.84 *vs.* $K1B_{MDT}$ = 20.65 ± 0.99) the slope still retained the character of the slope of the chitosan beads (See Table 4-4).

For granulate formulations there were a statistical correlation between the Eudragit® S100 and Kollidon® SR formulations, with Eudragit® S100 granules releasing only 1.51% more drug in total after 60 minutes (f_2 = 85.69 ± 3.98). Aqoat® AS-HF granules had a very high release rate and released a total of 61.18 ± 5.60% of its total drug content. The generally high amount of drug released during the first 60 minutes in 0.1 M HCl by the granules ($CG_{(60min)}$ = 31.76 ± 3.02%, $A1G_{(60min)}$ = 61.18 ± 5.60%, $E1G_{(60min)}$ = 45.89 ± 1.45% and $K1G_{(60min)}$ = 44.38 ± 1.75%) is unacceptable in the study, as this can be seen as a contradiction to the objectives. Aqoat® AS-HF has been omitted in Figure 4-5 due to the high total amount of drug released after only 60 minutes

Furthermore it must be motioned that when the burst effect is not taken into consideration and some of the formulations do seem to show zero-order kinetics. Straight lines for concentration against time could be plotted, with correlation efficiencies of at least $r \geq 0.99$ for formulations A1G, E1G and CG, for 10 minutes onwards, and $r \geq 0.99$ for CB after 5 minutes. This is an interesting fact and might indicate the ability of these formulations to act as perfect controlled release formulations in and acidic medium. Further study is recommended.

4.7.3.2.2 Dissolution in PBS pH 7.2

It was obvious that the release of active from the formulations was pH dependant, as the release profiles of the formulations differed from the profiles obtained during the first 60 minutes in 0.1 M HCl. As can be seen from Figure 4-4 and Figure 4-5, the amount of active released in the second medium (PBS pH 7.2) was largely dependant on the amount of drug left in the formulations after the first 60 minutes. In PBS pH 7.2 the differences between release profiles from bead and granulate formulations, was not as well defined as in 0.1 M HCl. The burst effect from granulate formulations during the first 5 minutes was found to be more pronounced than for bead formulations. Eudragit® S100 granules released 17.45% more drug than its correlating bead formulations ($E1G_{(65min)}$ = 37.91 ± 0.29%, $E1B_{(65min)}$ = 20.46 ± 0.31%). Kollidon® SR released 8.98% more drug than its correlating bead formulations ($K1G_{(65min)}$ = 19.02 ± 0.07%, $K1B_{(65min)}$ = 10.04 ± 0.12%). The same was true for the chitosan granule formulations vs. chitosan bead formulations, with chitosan granules releasing 10.67% more active for the first 5 minutes ($CG_{(65min)}$ = 19.83 ± 0.69%, $CB_{(65min)}$ = 9.16 ± 0.15%). Furthermore it was interesting to see that the standard deviation between baskets was much lower for granules in PBS pH 7.2 than in 0.1 M HCl. Although the burst effect was most prominent during the first 5 minutes, a sharp increase in amount of active dissolved was still observed for some formulations up to 100 minutes.

The standard deviation between baskets for Aqoat® AS-HF and chitosan bead formulations actually increased when compared to the values during the first 60 minutes. The standard deviation between points of the Eudragit® S100 formulation remained small. These differences in values can be interpreted as the difference in stability of the formulations in mediums of different pH. In an acidic medium the binder in the granules, namely chitosan, is soluble and therefore the integrity of the granules comes at risk, while in an alkaline medium the binder is not soluble. The difference in standard deviation of bead formulations can not be explained as easily.

On observation of Figure 4-5 in its entirety and Table 4-3, it becomes clear that some formulations faired better at controlled release than others. While some formulations such as Eudragit® S100 1% w/v beads could only sustain release for up to 180 minutes before reaching a plateau, others such as Kollidon ® SR, continued to release drug for up two 360 minutes ($E1B_{MDT}$ = 78.14 ± 0.18, $K1B_{MDT}$ = 98.34 ± 0.63). Polymer free chitosan beads appeared to be the best formulation, but the MDT-values seem to point at Kollidon® SR beads as being the best, with a low initial MDT-value of $K1B_{MDT\,(0.1M)}$ = 20.65 ± 0.99 and the highest MDT-value ($K1B_{MDT}$ = 98.34 ± 0.63) in PBS pH 7.2. In general it seemed that all formulations gave a initial high release of drug for the first 60 minutes in pH 7.2 followed by a steady increase in drug concentration until all drug was released or the study was ended (Also see Table 4-2 and 4-3) . This initial high release indicated a success in achieving enteric release, and might be useful *in vivo* to reach optimal blood levels. The consecutive sustained increase in drug concentration proved that controlled release was present, and this could be useful in vivo to retain the desired blood levels of a drug for some time. All formulations gave good total drug release with an approximate average of 103.71% ± 11.78% for all the formulations. This high a value might be as a result of ketoprofen's high solubility in PBS pH 7.2.

From Figure 4-4 and Figure 4-5 the ranking order of CR polymer and type of formulation, based on total amount of drug released in 0.1 M HCL, total time of drug release in PBS pH 7.2 and mean dissolution time, is thus K1B > CB > A1B > E1B > CG > (E1G & K1G) > A1G.

4.7.4 Formulations of 2.5% w/v and 29.41% w/w CR polymer

4.7.4.1 Results

The comparative results for bead formulations containing 2.5% w/v controlled release polymer, and for granule formulations containing 29.41% w/w polymer are depicted in Figure 4-6 and Figure 4-7.

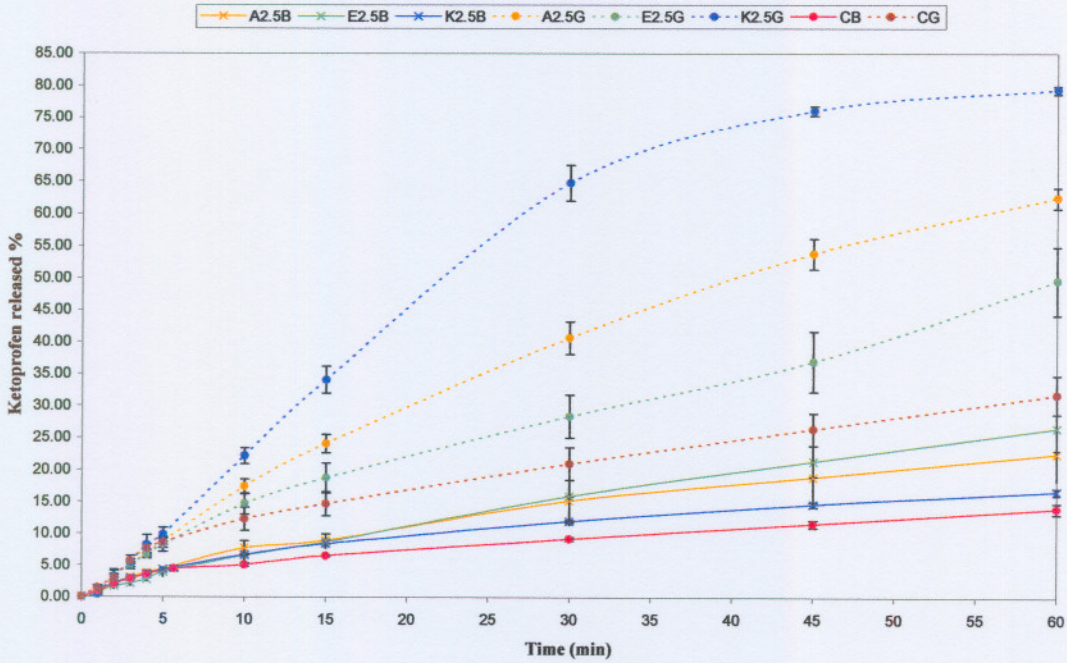


Figure 4-6: Ketoprofen release from beads containing 2.5% w/v CR polymer and granules containing 29.41% w/w CR polymer during the first 60 minutes in 0.1 M HCl.

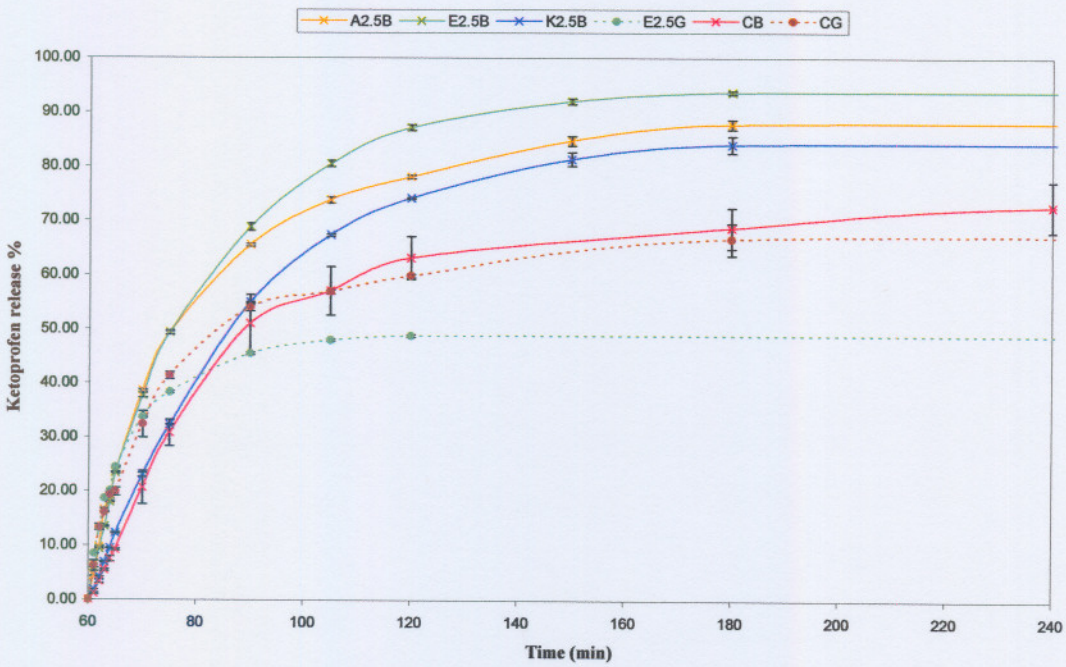


Figure 4-7: Ketoprofen release from beads containing 2.5% w/v CR polymer and granules containing 29.41% w/w CR polymer for the remainder of the study in PBS pH 7.2.

4.7.4.2 Discussion

4.7.4.2.1 Dissolution in 0.1 M HCl

At first glance of Figure 4-6 it is clear that almost all the CR polymer containing granules formulations did not succeed in limiting of drug release in 0.1 M HCl. Kollidon® SR granules released a total amount of drug of 79.30 ± 0.59% and Aqoat® AS-HF granules released 62.37 ± 1.64% of their drug content. Eudragit® S100 granules showed the best release profile for the 29.41% CR polymer containing granules and released 49.46 ± 5.30% of its total drug content ($A2.5G_{MDT}$ =23.91 ± 0.99, $E2.5G_{MDT}$ = 26.63 ± 1.32 $K2.5G_{MDT}$ = 19.60 ± 0.85). No significant burst effect was visible; as the concentration increased steadily for granulate formulations until the dissolution was ended. Although the granulate formulations released a lot of drug during the first hour, controlled release is still visible in the release profiles. It is speculated that tablets prepared from these granules may produce viable controlled release tablets. Further study is recommended. The amount of drug released during one hour at 0.1 M HCl was not acceptable, as this high a concentration is bound to cause gastric irritation. Thus all formulations excessive drug release during the first 60 min was omitted in Figure 4-7.

Bead formulations appeared more successful than granulate formulations. With the exception of Eudragit® S100 beads, the standard deviation between baskets of the same formulations was once again much smaller than for the granulate formulations. A small burst effect was visible for up to 5 minutes, much the same as with the 1% w/v bead formulations. All bead formulations released less than 30% of the total drug content, with Eudragit® S100 2.5% w/v beads releasing the most active, but proved to have the highest MDT-value ($E2.5B_{(60min)}$ = 26.50 ±8.26%, $E2.5B_{MDT}$ = 25.82 ± 2.21). Aqoat® AS-HF 2.5% w/v beads released 22.45 ± 0.58% active ($A2.5B_{MDT}$ = 23.12 ± 0.56). Once again the release profiles obtained for chitosan beads and Kollidon® SR 2.5% w/v beads was very similar, with Kollidon® SR beads releasing 1.19% more drug than chitosan beads ($CB_{(60min)}$ = 13.89 ± 0.85 % & $K2.5B_{(60min)}$ = 16.58 ± 0.54%) (f_2 = 99.29 ± 0.55).

Closer observation of the graph shown in Figure 4-6 showed that three profiles followed zero–order kinetics. Not taking the burst effect of the first 5 minutes into consideration and fitting a straight line through the remaining points gave a correlation efficiency of at least $r \geq 0.99$ for CB, E1B, CG and E1G. This is an interesting fact and might indicate the ability of these formulations to act as perfect controlled release formulations in and acidic medium.

4.7.4.2.2 Dissolution in PBS pH 7.2

Close observation of Figure 4-7 shows that an important burst effect occurs during the first 5 minutes in PBS pH 7.2. The release rate during the first 5 minutes is almost identical for Aqoat® AS-HF and Eudragit® S100 beads, with both formulations releasing a total amount of approximately 23.30% active after 5 minutes ($E2.5B_{(65min)}$ = 23.36 ± 0.12% & $A2.5B_{(65min)}$ = 23.32 ± 0.13%) (f_2 = 86.97 ± 0.79). Chitosan granules released only 3.47% less drug than the above mentioned formulations with a total of 19.83 ± 0.69% active being released after 5 minutes by chitosan beads. Although the release profile of Kollidon® SR was very similar to that of chitosan beads, Kollidon® SR 2.5% w/v beads released 3.08% less active during the first 5 minutes ($CB_{(65min)}$ = 9.16 ± 0.15% & $K2.5B_{(65min)}$ = 12.24 ± 0.11%). Although the burst effect was most prominent during the first 5 minutes, all formulations showed a steady increase in drug release during the first hour in pH 7.2. As mentioned earlier chitosan bead and chitosan granules appeared much the same (f_2 = 80.03 ± 2.86). All the CR polymer loaded bead profiles had a good correlation in release profiles, with the increase in drug release slowing down after 60 minutes in pH 7.2 (See Table 4-4).

It should be noted that the standard deviation between baskets of the same bead formulations appeared to be much lower for 2.5% w/v CR polymer containing bead formulations than for 1 and 5% w/v formulations. This effect could not be explained.

Formulations of 2.5 % w/v was not very successful in sustaining release of the active for a long time, within two hours in pH 7.2 all the remaining drug was released from the CR polymer formulations (Also see Table 4-2 and 4-3). The time that the 2.5% w/v formulations could sustain release was much lower than the time pure chitosan beads could sustain release. (CB = 3 hours in pH 7.2). As can be seen from Table 4-3 the MDT-value of most formulations decreased with the addition of 2.5% w/v or 29.41% w/w CR polymer, with the exception of 2.5% w/v Kollidon® SR beads ($K2.5B_{MDT}$ = 88.38 ± 0.92% vs CG_{MDT} = 85.25 ± 3.30) All formulations, except chitosan beads, released the entire amount of drug entrapped within the formulations.

From Figure 4-6 and Figure 4-7 the ranking order of 2.5% w/v or 29.41% w/w CR polymer and type of formulation, based on total amount of drug released in 0.1 M HCL, total time of drug release in PBS pH 7.2 and mean dissolution time, thus is CB > K2.5B ≥ A2.5B > E2.5B > CG > E2.5G ≥ A2.5G > K2.5G.

4.7.5 Formulations of 5% w/v and 45.45% w/w CR polymer

4.7.5.1 Results

The comparative results for bead formulations containing 5% w/v controlled release polymer, and for granule formulations containing 45.45% polymer are depicted in Figure 4-8 and Figure 4-9.

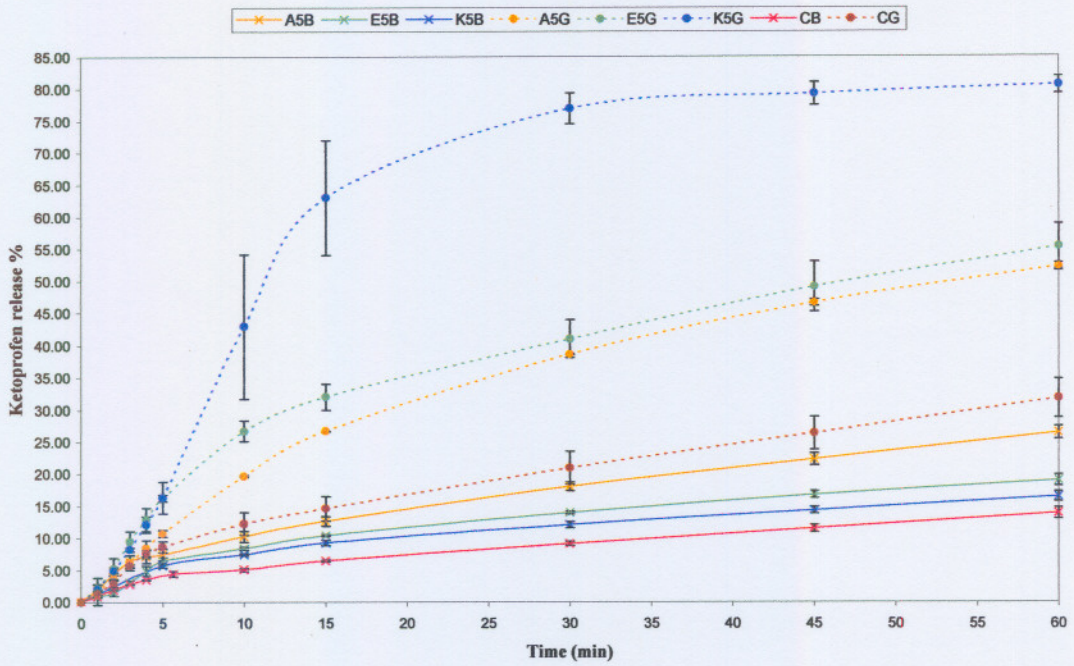


Figure 4-8: Ketoprofen release from beads containing 5% w/v CR polymer and granules containing 45.45% w/w CR polymer during the first 60 minutes in 0.1 M HCl.

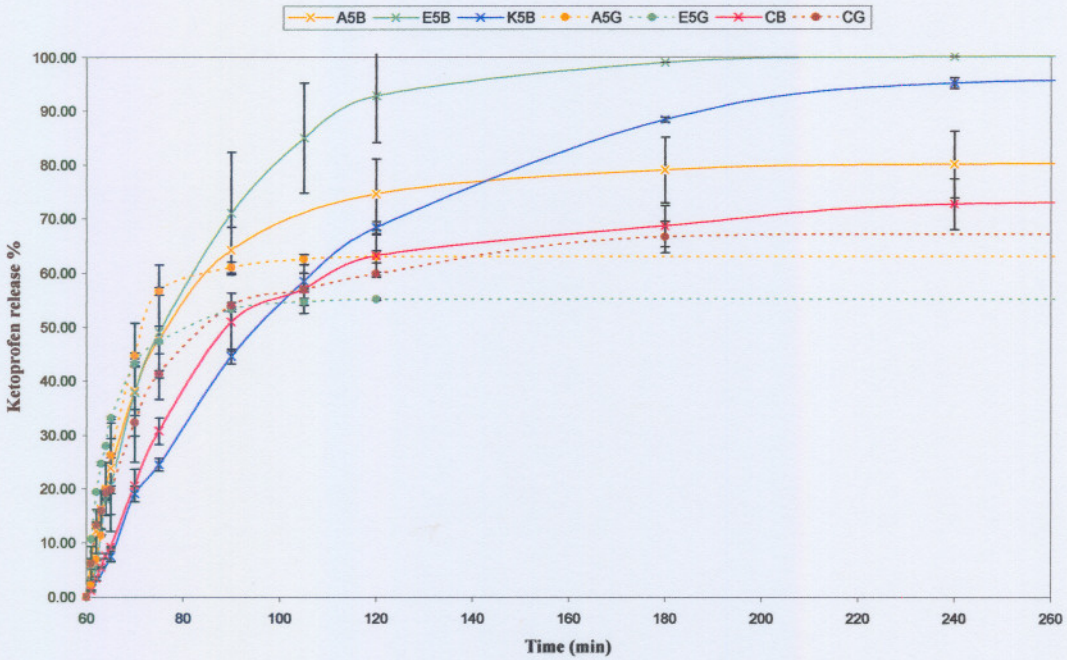


Figure 4-9: Ketoprofen release from beads containing 5% w/v CR polymer and granules containing 45.45% CR polymer for the remainder of the study in PBS pH 7.2.

4.7.5.2 Discussion

4.7.5.2.1 Dissolution in 0.1 M HCl

From the release profiles in Figure 4-8 it is clear that as for 29.41% w/w CR polymer granules, 45.45% w/w CR polymer granules proofed somewhat unsuccessful. Kollidon® SR 45.45% w/w granules once again showed the worst release profile in 0.1 M HCl, with the lowest MDT-value of $K5G_{MDT}$ 11.97 ± 2.65. All granulate formulations showed a sharp initial increase in drug concentration during the first 15 minutes, with Eudragit® S100 and Aqoat® AS-HF releasing equally high amounts of active ($E5G_{(15min)}$ = 32.02 ± 2.02% & $A5G_{(15min)}$ = 26.66 ± 0.05%, f_2 78.50 ± 7.82) Kollidon® SR released the highest concentration of drug after 15 minutes ($K5G_{(15min)}$ = 63.02 ± 8.94%), and disintegrated after 45 minutes. Almost empty baskets of Kollidon® SR granules were removed from the dissolution after 60 minutes, with some granulate matter remained at the bottom of the dissolution beakers. The high initial rate of drug release did however appear to decrease after 15 minutes for the Aqoat® and Eudragit® S100 formulations, but Aqoat® granules still released more drug after 60 minutes than Eudragit® formulations ($A5G_{(60min)}$ = 62.37 ± 0.64% & $E5G_{(60min)}$ = 49.46 ± 3.51%, although MDT-values pointed to Aqoat® granules as being more successful at controlling drug release ($A5G_{MDT}$ = 20.04 ± 0.50, $E5G_{MDT}$ = 18.51 ± 0.37. Once again this high concentration of drug released indicates a failure to produce formulations with enteric release and all granulate formulations, with the exception of chitosan granules, were omitted in Figure 4-9.

The release profiles obtained for bead formulations differed significantly from granulate formulations. The bead formulations appeared to be more successful in achieving enteric release, with slightly higher MDT-values obtained (See Table 4-2). There was still a burst effect visible for the first 5 minutes in 0.1 M HCl and this was attributed to the fact that drug particles might have been on the surface of the beads, or due to the high initial swell rate of beads in 0.1 M HCl. Statistically there was little difference in the concentration of drug released after 5 minutes from the bead formulations, ($A5B_{(5min)}$ = 7.44 ± 0.94%, $E5B_{(5min)}$ = 6.45 ± 0.60%, $K5B_{(5min)}$ = 5.73 ± 0.28% and $CB_{(5min)}$ = 5.66 ± 0.43%) (See Annexure B). Thereafter the release rate appeared to remain relatively constant, but still very low, until completion of the

dissolution. In total 5% w/v Aqoat® AS-HF beads released the highest concentration of active and with the highest MDT-value in 0.1 M HCl ($A5B_{(60min)}$ = 26.39 ± 0.99%, $A5B_{MDT}$ = 21.30 ± 0.54) and Eudragit® S100 the second highest concentration of active, with a MDT-value similar to that of Kollidon® beads ($E5B_{(60min)}$ = 19.01 ± 0.87%, $E5B_{MDT}$ = 18.96 ± 0.91, $K5B_{MDT}$ = 18.90 ± 0.81). As with the 2.5% w/v bead formulations the release profiles for 5% w/v Kollidon® SR beads and chitosan beads were comparable, with Kollidon® SR beads releasing only 2.51% more active ($K5B_{(60min)}$ = 16.49 ± 0.77% & $CB_{(60min)}$ = 13.89 ± 0.85%, f_2 = 80.98 ± 2.72).

Furthermore it was interesting to see that if the initial burst effect of 15 minutes is left out of calculation 45.45% Aqoat® AS-HF granules appeared to follow zero-order kinetics, with r = 0.9917. The correlation efficiency was also higher than r ≥ 0.99 for 5 % Kollidon® SR beads (r = 0.9961). No other samples containing 45.45% w/w or 5% w/v controlled release polymer had significant correlation efficiencies.

4.7.5.2.2 Dissolution in PBS pH 7.2

The most noticeable feature in the graph obtained for 5% w/v or 45.45% CR polymer containing formulations is the high deviation between baskets of the same formulations, especially for the bead formulations. This was ascribed to the low level of chitosan in these bead formulations and the possible poor cross-linking. There was also a great initial increase in concentration visible during the first 15 to 20 minutes in pH 7.2. The initial increase in concentration of drug released by Aqoat® AS-HF and Eudragit® S100 statistically did not differ from that of chitosan granules ($E5B_{(65min)}$ = 20.79 ± 8.64% & f_2 = 67.69 ± 2.78, $A5B_{(65min)}$ = 23.72 ± 8.53% & f_2 = 77.76 ± 8.52, $CG_{(65min)}$ = 19.83 ± 0.69%), while the release from chitosan beads and Kollidon® beads was much lower and statistically similar to each other ($K5B_{(65min)}$ = 19.03 ± 0.99%, $CB_{(65min)}$ = 20.61 ± 0.15%, f_2 = 97.31 ± 2.69).

The rate of release of active from 5% w/v Eudragit® S100 beads appeared to decline with passing time, but remained higher than that of the other formulations and all drug

was released after only 150 minutes (90 minutes in pH 7.2) ($E5B_{MDT}$ = 92.14 ± 6.68). As with chitosan beads and granules, the high initial release rate of 5% w/v Aqoat® AS-HF beads seemed to decline after 100 minutes and reached a plateau after 180 minutes ($A5B_{MDT}$ = 80.84 ± 2.15). Therefore 5% w/v Aqoat® AS-HF beads could only sustain drug delivery as long as untreated chitosan granules could ($A5B_{MDT}$ = 80.84 ± 2.15 vs. CG_{MDT} =80.88 ± 3.44)). The release of active from 5% w/v Kollidon® SR beads continued for up to 240 minutes, as was the case with chitosan beads, although the high MDT-value of 107.24 ± 1.73 for Kollidon® beads indicates that this formulation well outperformed polymer free beads (CB_{MDT} = 85.25 ± 3.30). Kollidon® SR beads released all of the drug entrapped in the beads compared to chitosan beads which could only release approximately 86% the total amount of drug.

From Figure 4-8 and Figure 4-9 the ranking order of 5% w/v or 45.45% CR polymer and type of formulation, , based on total amount of drug released in 0.1 M HCL, total time of drug release in PBS pH 7.2 and mean dissolution time,K5B > CB > A5B > E5B > CG > A5G > E5G > K5G.

4.8 SUMMARY AND CONCLUSION

When the release profiles of Figure 4-4 is compared to that of Figure 4-6 and 4-8, and the data in Table 4-4 is considered, it is clear that there is a strong correlation between the profiles of the bead formulations, but less of a correlation between the granulate formulations. Although there was a strong correlation polymer free beads and CR polymer containing beads ($f_2 \geq 50$) the correlation seemed to decrease with an increase in CR polymer concentration. This was also found to be the case with the MDT-values. Initially Eudragit® and Aqoat® led to an increase in the MDT-values, but decreased the MDT-values with an increase in the CR polymer concentration. The effects on the MDT-values by CR polymer concentration correlated to the MDT-values of granulate samples in 0.1 M HCl (See Table 4-2). It is however important to mention the difference concentration and medium had on the MDT-values. While an initial decrease in the MDT-values was noted for higher concentrations of CR

polymer in chitosan beads, it seemed to increase the MDT-values in PBS pH 7.2 (See Table 4-2 and 4-3). For granulate samples however it was noted that the initial decrease in the MDT-values of 0.1M HCl was also followed by a substantial decrease in the MDT-values of PBS pH 7.2. This could be attributed to the fact that granules of lower concentration released less total drug after 60 minutes granules of higher concentration CR polymer. This phenomenon could have been due to the lower total concentration of the binder chitosan in the granulate formulations and the high solubility of uncrossed linked chitosan in 0.1 M HCl. Therefore low concentrations of chitosan dissolved more rapidly and could not sustain the formulations integrity.

When the release profiles for 1% w/v and 14.29% CR polymer formulations (See Figure 4-5) is compared to the release achieved for 2.5% w/v or 29.41% and 5% w/v or 45.45% as seen in Figures 4-7 and 4-9, it was noted that the best control of release was achieved with low concentrations of CR polymers added, with the exception of 5% w/v Kollidon® SR beads ($K5B_{MDT}$ = 107.24 ± 1.73). This might have been related to the decrease of the total chitosan concentration in the granules and consequent lower binding or cross-linkage.

It should be noted that the standard deviation between baskets of Kollidon® SR containing bead formulations appeared to be much lower than the values obtained for the Aqoat® and Eudragit® formulations. This was consistent with the more consistent degree of swelling and even the lower friability and solubility of the Kollidon® SR bead formulations.

Since ketoprofen has a short half-life of about 1.8 ± 0.4 hours, any formulation delaying release for more than 2 hours should be an improvement. As seen in Figure 4-10, several formulations succeeded in doing so. The best results were for formulations with low concentrations of Kollidon® SR. This effect of Kollidon® SR is not well documented and further study is recommended.

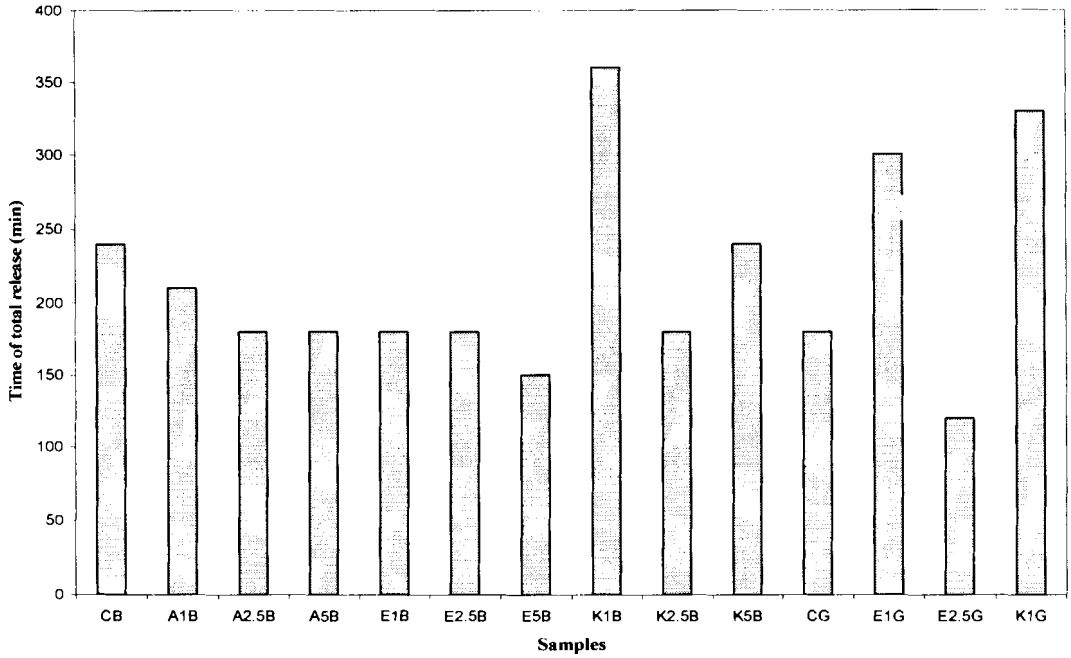


Figure 4-10: Total time of release for formulations with optimum enteric release characteristics.

When all the dissolutions profiles are taken into account, as well as the MDT-values, the ranking order for type and concentration of CR polymer formulations vs. chitosan only formulations is thus K1B ≥ K5B > CB > A1B > K2.5B > E1B > A2.5B > A5B > E5B> E2.5B > CG > (E1G & K1G) > E2.5G > A5G > E5G > A1G > A2.5G > K2.5G > K5G.

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, commonly used in the pharmaceutical industry. Chitosan has been, compared to traditional excipients, shown to have superior characteristics and is especially flexible in its use. It is a biocompatible, biodegradable, and muco-adhesive polymer. Chitosan also has anti-ulcer and antacid activities (Açikoz *et al.*, 1995). Chitosan was found to be an excellent candidate for the production of beads via the inotropic gelation technique. This technique was chosen above others as being simple, and gives reproducible results. Chitosan is soluble in acetic conditions. Not only does this pose a problem with possible high release of drug in the acidic regions of the stomach, but also with the ability of the formulation to control the release in the alkaline environments, of the gastrointestinal tract. To enhance the effect of the chitosan bead matrix, modern controlled release polymers was incorporated into the chitosan bead formulation. The effect of these polymers on drug release as well as on the morphology and strength of the chitosan beads was investigated and compared to granules with similar composition.

Ketoprofen was chosen as the model drug because of its physical and chemical properties. Ketoprofen is a non-steroidal anti-inflammatory drug with analgesic and antipyretic properties, commonly utilized in the treatment of seve e rheumatoid arthritis. The short half-life of ketoprofen makes it an excellent candidate for formulation into the chitosan matrix bead formulation. By entrapping ketoprofen in chitosan beads, the production of a controlled release formulation with less gastric irritation could be achieved.

Beads were produced by dispersing ketoprofen in an aqueous acetic acid solution of chitosan. Varying concentrations of different types of controlled release polymers was added to the dispersion. The dispersion was dropped though a needle into a gently agitated solution of tripolyphosphate (TPP) and beads formed on contact with the TPP-solution. The beads were then separated, washed with deionised water, and freeze-dried. Matrix chitosan granules were produced by mixing of the dry ingredients, and wetting with a weak aqueous solution of acetic acid. The resulting mass was granulated through a 2 mm sieve and dried. These granules were further reduced in size by means of milling. Varying concentrations of controlled release polymers was also added to the granulate formulations prior to granulation. The concentrations of the polymers were added to render granules with the same polymer concentration as the beads.

On close investigation of the spherical beads by means of scanning electron microscopy the beads were found to have a porous surface structure with possible poor cross-linking. The inclusion of low concentrations 1% w/v of controlled release polymer appeared to enhance the surface structure but seemed to enhance the porosity of the internal structure. Granulate formulations appeared to have an irregular surface structure, which could have been a result of the milling process. The addition of controlled release polymers to the granules appeared to weaken the structure of the granules, as more surface cracks, and a flakier appearance was visible.

Studies on the drug loading capacity showed that the addition of controlled release polymers to the bead formulations improved the drug loading capacity of the chitosan beads. The increase in drug loading was dependant on the type and concentration of polymer added, with Eudragit® S100 5% w/v having the most significant effect. The drug loading capacity of the granules remained constant throughout the study.

Friability testing revealed that beads produced by the inotropic gelation technique offered poor resistance to mechanical force. Once again the inclusion of certain controlled release polymers enhanced the resistance to mechanical force. This effect was also dependant on the type and concentration of polymer used, with Aqoat® AS-HF weakening the beads, and Kollidon® SR strengthening the beads resistance to friability.

Solubility and swelling tests revealed that bead formulations would be a much better option for obtaining controlled release, as granules showed a high degree of solubility and degradation. This is due to the fact that the cross-linking of chitosan with TPP significantly reduces the solubility of the beads.

Dissolution testing on formulations showed that some formulations succeeded in the initial objectives of the study. Kollidon® SR formulations delayed drug release with almost the same effectiveness as chitosan beads in acidic environments, and also proved to act as controlled release formulations in the alkaline testing medium. Furthermore bead formulations all fared better at delaying and controlling the release of the model drug in both dissolution mediums. Although some formulations proved to be successful, it is speculated that the amounts of controlled release polymer used in the study might have been too high. Future studies conducted with less than 1% controlled release polymer, especially Kollidon® SR may prove to be very rewarding.

5.2 FUTURE PROSPECTS

In this study several factors that may prove rewarding in future studies have been identified. The difference in release profiles obtained for the bead and granulate formulations suggests that bead formulations may be more effective in the achieving the objectives of the study. The zero-order type release obtained from some of the granulate formulations may point to the fact that these formulations could be useful in obtaining perfect sustained release in acetic mediums. It is therefore recommended that a future study into variables that might affect the release of drug from these granules be considered. Such variable include:

- Size of the granules
- Shape of the granules
- The possible compression into tablets or mini-tablets
- Enhancing of the binder efficiency
- Use a more effective binder.

By adapting the above mentioned variables an even more economical method for the production of sustained release particles might be discovered.

With regard to the beads, several factors affecting the characteristics were identified, emphasizing the importance of several factors in the development of beads for controlled drug delivery. These include

- The viscosity of the polymers used should be taken into consideration. The release rate of drug from the polymer matrix is dependant on the diffusion of the drug though the gelled polymer matrix. An increase in the polymer viscosity, especially chitosan, might improve control over the drug release rate (Säkkinen 2003:38).

- The time allowed for cross-linking appeared not to be sufficient. With an increase in cross-linking time allowed, the TPP could diffuse deeper into the bead formulations, thereby decreasing the porosity of the bead internal structure increasing the mechanical strength of the beads and decreasing the solubility.

- The choice of drug used in the formulation may have affected the release profiles and drug loading efficiency. Ketoprofen has a relatively high pKa resulting in a loss of drug to the external TPP phase during inotropic gelation. It is therefore recommended that a drug be used with a low pKa that would not dissolve in the alkaline TPP-solution. The drug loading may be improved by this alteration, and the method could be redesigned to allow for optimal cross-linking of the bead formulations during production. It may also be of interest to study the effect of other cross-linking agents such as sodium sulfate or sodium citrate.

- The method of drying might affect the release of drug from the bead formulations. Beads produced by freeze-drying appeared to be very porous. Oven dried beads utilized in other studies appeared more solid in structure (Lubbe, 2002:53). A more solid matrix formulation might prove to delay drug release even better than freeze-dried bead.

A deeper investigation into the swelling behavior of bead formulations may prove useful. With a better understanding of the correlation between the degree of swelling of formulations and the dissolution profiles obtained, it is speculated that a mathematical formulation could be devised predicting the release profile of drugs from bead formulations. In future it might even be possible to avoid tedious dissolution testing simply by a better understanding of swelling behavior.

ANNEXURE A
CERTIFICATES OF APPROVAL

CERTIFICATE OF ANALYSIS FOR AQOAT® AS-HF

Shin-Etsu

2003/10/23 (1/1)

Certificate of Analysis

Shin-Etsu Chemical Co., Ltd.
Naoetsu Plant Quality Assurance Department
28-1 Nishifukushima, Kubiki-mura,
Nakakubiki-gun, Niigata, Japan

Product Name	Shin-Etsu AQOAT (Hydroxypropyl Methylcellulose Acetate Succinate)
Grade	AS-HF
Lot Number	203001
Quantity	3kg
Manufacture Date	2002/03/16
Expiration Date	2005/03/15
Analysis Date	2003/03/25
Issue No.	D5120031002136002-1-01
Remark	

This product complies with the specifications described in the current JPE.
This product is manufactured in accordance with GMP.

Test Item	Unit	Test Result	Specification
Description		Conforms	Conforms
Identification (1)		Conforms	Conforms
Identification (2)		Conforms	Conforms
Identification (3)		Conforms	Conforms
Identification (4)		Conforms	Conforms
Identification (5)		Conforms	Conforms
Viscosity	mm2/s	2.59	2.4 - 3.6
Purity:(1) Heavy Metals	ppm	Not more than 10	Not more than 10
Purity:(2) Arsenic	ppm	Not more than 2	Not more than 2
Purity:(3) Free Succinic Acid	%	0.13	1.0 Max.
Loss on Drying	%	1.2	5.0 Max.
Residue on Ignition	%	0.02	0.20 Max.
Particle size Average	μm	4.8	10 Max.
Particle size 90% Cumulation	μm	10.5	20 Max.
Methoxyl Content	%	24.0	22.0 - 26.0
Hydroxypropoxyl Content	%	7.7	6.0 - 10.0
Acetyl Content	%	11.8	10.0 - 14.0
Succinoyl Content	%	5.9	4.0 - 8.0

K.Araume

KIYOSHI ARAUME
General Manager, Q. A. Dept.

Shin-Etsu No. : 13062350-04-01

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Shin-Etsu Chemical Co., Ltd.
Cellulose Division
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TEL 81-3-3246-5261 FAX 81-3-3246-5372

Judgment:
Shin-Etsu Chemical Co., Ltd.
Naoetsu Plant Quality Assurance Department
28-1 Nishifukushima, Kubiki-mura,
Nakakubiki-gun, Niigata, Japan

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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

9

CERTIFICATE OF ANALYSIS FOR CHITOSAN

Original

Report No.: 021009

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

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

XIAMEN JIANGYUAN IMPORT AND EXPORT 厦门江源 经济发展公司 进出口单证章

WCI 7109

CERTIFICATE OF ANALYSIS FOR EUDRAGIT® S100

degussa.

Röhm Pharma Polymers

RÖHM GmbH & Co. KG, D-64275 Darmstadt

> CERTIFICATE OF ANALYSIS <

Pharma Polymers

Date 2005-06-29

Customer order item/date

Delivery item/date

Order item/date

Invoice number/date

Contact person/phone/fax

EUDRAGIT S 100 PE FL. 400 G

Lot No. B030505062
Quantity

Dry substance/Residue on evaporation (%)	97,4
Loss on drying (%)	2,6
Methacrylic acid units, based on DS (%)	28,8
Acid value (mg KOH per g dry substance)	188
Viscosity/Apparent viscosity (mPa s)	129
Viscosity (mm2/s)	33
Monomers (ppm)	96

The product conforms to:
Specification (INFO 7.3/E, 2004/09)
Ph. Eur.: "Methacrylic Acid - Methyl Methacrylate Copolymer (1:2)"
USP/NF: "Methacrylic Acid Copolymer, Type B"
Ph. Mex.: "Polimetacrilatos Tipo B"
JPE: "Methacrylic Acid Copolymer S"

Stability: at least until 2006-07

The product is manufactured by Röhm GmbH & Co. KG, Kirschenallee, D-64293 Darmstadt, Germany. The product is manufactured according to current Good Manufacturing Practices for pharmaceutical excipients as published in USP < 1078 >. The results of analysis were obtained using the methods given in the named specification. Tests not listed above are performed on selected lots. Since transport and storage can cause changes in the material that are beyond our control, the material should be further examined for its suitability before use.

Roehm GmbH & Co. KG

Weber (Head of Sales) Dr. Brendel (Head of Quality Assurance.
(Written by computer and therefore not signed)

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Sitz der Gesellschaft: Darmstadt
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Gregor Hetzke
Bernd Pfeiffer

Vorsitzender des Aufsichtsrats:
Dr. Manfred Spindler

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CERTIFICATE OF ANALYSIS FOR KOLLIDON® SR

Technical Information

June 200
Supersedes issue dated June 2004

MEMP 030728e-04/Page 1 of 12

Kollidon® SR

BASF

® = Registered trademark of
BASF Aktiengesellschaft

PBG-Number: 10 235 112
Date of Manufacture: 11/04/03
Date of expiration: 12/04/05
Lot Number: N15-C1285-1
Bin Number: 65842-19

Trivial name:	Polyvinyl acetate / polyvinylpyrrolidone
Compositions:	Kollidon® SR consists of 80% polyvinyl acetate and 19% povidone Ph.Eur./USP (Kollidon® 30) in a physical mixture.
Description:	White or slightly yellowish, free-flowing powder.
Solubility:	Insoluble in water (The povidone part is soluble but the polyvinyl acetate part is insoluble). It is very soluble in N-methylpyrrolidone.
Molecular weight:	The average molecular weights Mw of the polyvinyl acetate part is about 450,000. That one of the povidone K 30 part it is about 50,000. The average molecular weight of Kollidon® SR as mixture is expressed as K-value according to the method described in the monographs "Povidone" and measured in a 1% solution in tetrahydrofurane. The typical K-value is 60 to 65.
Particle size distribution:	The average particle size is about 100 µm.
Glas transition temperature:	The glas transition temperature Tg of the anhydrous material is 35°C.
Bulk density:	0.45 g/ml.
Flowability:	Kollidon® SR has outstanding flow properties with an angle of repose well below 30°. It can enhance the flow ability of other components added for a tablet formulation.
Hygroscopicity:	The water uptake is much less than that of povidone or copovidone.
Storage:	Store below 30°C

BASF Aktiengesellschaft
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CERTIFICATE OF ANALYSIS FOR KETOPROFEN

Boehringer Ingelheim
Bidachem

Certificate of analysis — Nr° F229-04

Product : KETOPROFEN

Batch n°: 04040675

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

Warren Chem Specialities (PTY) LTD
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34 BRUNTON CIRCLE
FOUNDERS HILL
LETHABONG
FAX (011) 452-2810

WC 1 . 8743

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

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

Release date: 20 May 2004

BIDACHEM s.p.a
Quality Control
Manager

Dr. T. De Quarti

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

ANNEXURE B
EXPERIMENTAL DATA

Tables B-1: Mean dissolution data of all formulations.

Chitosan Bead formulations					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	0.84	0.06	61	1.14	0.14
2	1.92	0.04	62	3.39	0.36
3	2.82	0.05	63	5.45	0.15
4	3.51	0.05	64	7.52	0.47
5	4.43	0.43	65	9.16	0.15
10	5.07	0.27	70	20.61	3.06
15	6.48	0.13	75	30.78	2.47
30	9.11	0.30	90	50.97	5.37
45	11.50	0.58	105	57.06	4.45
60	13.89	0.85	120	63.15	3.91
			180	68.66	3.79
			240	72.65	4.67

Chitosan Granule formulations					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	1.38	0.24	61	6.20	0.94
2	2.96	0.59	62	13.27	0.60
3	5.69	0.19	63	15.98	0.14
4	7.62	0.68	64	19.28	0.32
5	8.63	0.85	65	19.83	0.69
10	12.25	1.80	70	32.36	2.42
15	14.63	1.90	75	41.33	0.67
30	20.90	2.56	90	53.99	0.64
45	26.33	2.57	105	56.92	0.37
60	31.76	3.02	120	59.85	0.30
			180	66.61	2.91

Aqoat AS-HF® 1% w/v beads					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	1.24	0.50	61	4.48	0.22
2	1.95	0.46	62	7.71	0.26
3	2.85	0.30	63	11.36	0.45
4	3.37	0.07	64	16.60	0.69
5	3.93	0.16	65	22.20	0.92
10	6.79	0.22	70	39.07	1.53
15	8.99	0.43	75	50.79	2.12
30	14.94	0.87	90	70.09	3.19
45	18.74	1.00	105	79.05	3.63
60	22.53	1.23	120	83.69	3.62
			150	88.08	3.88
			180	89.43	3.94
			210	90.20	4.00

Aqoat AS-HF® 14.29% granules					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	1.24	0.86	61	10.54	0.07
2	2.73	0.67	62	17.04	0.09
3	4.76	0.72	63	20.37	0.13
4	6.39	0.55	64	23.44	0.13
5	8.30	0.60	65	26.07	0.14
10	15.86	1.10	70	32.51	0.18
15	21.68	2.01	75	35.32	0.22
30	38.09	4.09	90	37.24	0.27
45	50.03	4.67	105	37.64	0.26
60	61.18	5.60			

Aqoat® AS-HF 2.5% w/v beads					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	0.50	0.25	61	4.36	0.02
2	1.92	0.46	62	9.65	0.12
3	3.05	0.13	63	16.46	0.31
4	3.78	0.36	64	18.14	0.29
5	4.33	0.13	65	23.32	0.13
10	7.69	1.17	70	38.56	0.18
15	8.88	1.05	75	49.31	0.28
30	15.12	0.74	90	65.48	0.24
45	18.77	0.10	105	73.78	0.57
60	22.42	0.58	120	78.09	0.32
			150	84.77	0.88
			180	87.78	0.85

Aqoat AS-HF® 29.41% granules					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	1.14	0.66	61	5.57	0.12
2	3.20	0.21	62	10.01	0.13
3	5.25	0.14	63	14.74	0.12
4	6.58	0.20	64	19.73	0.16
5	8.69	0.51	65	23.23	0.18
10	17.32	1.15	70	32.96	0.27
15	24.06	1.42	75	37.73	0.28
30	40.57	2.54	90	41.27	0.34
45	53.69	2.39	105	41.88	0.41
60	62.37	1.64			

Aqoat® AS-HF 5% w/v beads					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	1.90	0.10	61	6.19	3.11
2	4.35	0.96	62	12.09	4.08
3	6.35	0.96	63	16.19	3.50
4	7.12	0.99	64	19.97	4.94
5	7.44	0.94	65	23.79	8.53
10	10.23	0.86	70	38.14	4.47
15	12.63	0.76	75	47.65	2.56
30	18.03	0.66	90	64.15	4.32
45	22.26	0.92	120	74.54	6.62
60	26.39	0.99	180	79.05	6.09
			240	80.04	6.19

Aqoat AS-HF® 45.45% granules					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	1.13	1.61	61	2.25	0.03
2	4.40	0.16	62	6.92	0.20
3	6.23	1.17	63	11.36	0.15
4	8.44	1.25	64	19.94	0.25
5	10.72	0.54	65	26.23	0.35
10	19.64	0.13	70	44.72	0.56
15	26.66	0.05	75	56.64	0.75
30	38.58	0.08	90	61.01	1.00
45	46.58	0.51	105	62.52	0.92
60	52.21	0.64	120	62.98	1.14

Eudragit® S100 1% w/v beads					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	1.17	0.62	61	0.96	0.04
2	1.95	0.36	62	6.26	0.13
3	2.38	0.54	63	9.34	0.01
4	3.06	0.33	64	14.79	0.09
5	3.40	0.39	65	20.46	0.31
10	6.03	0.09	70	34.58	0.28
15	7.85	1.26	75	44.91	0.44
30	13.30	0.31	90	63.89	0.45
45	16.86	0.25	105	72.98	0.69
60	20.42	0.20	120	76.17	0.38
			150	77.55	0.35
			180	78.38	0.33

Eudragit® S100 14.29% granules					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	2.00	0.34	61	7.39	0.14
2	3.62	1.89	62	20.22	0.22
3	4.99	0.87	63	28.91	0.30
4	6.11	1.04	64	32.24	0.28
5	7.95	1.18	65	37.91	0.29
10	15.12	0.66	70	51.16	0.57
15	19.49	0.72	75	57.96	0.56
30	28.30	0.36	90	66.23	0.46
45	36.83	0.50	105	69.79	0.73
60	45.89	1.45	120	71.37	1.74
			150	72.67	1.39
			180	74.87	1.11
			210	76.05	0.81
			240	77.65	0.38
			270	78.90	0.37
			300	80.24	0.52

Eudragit® S100 2.5% w/v beads					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	0.82	0.28	61	5.86	0.02
2	1.67	0.48	62	9.34	0.10
3	2.14	0.44	63	13.43	0.13
4	2.75	0.36	64	17.91	0.13
5	3.91	0.73	65	23.36	0.12
10	6.56	1.66	70	37.70	0.44
15	8.67	1.00	75	49.34	0.36
30	15.84	4.53	90	68.80	0.65
45	21.29	6.55	105	80.51	0.55
60	26.50	8.26	120	87.16	0.54
			150	92.03	0.53
			180	93.63	0.35

Eudragit® S100 29.41% granules					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	0.37	0.09	61	8.43	0.04
2	3.21	0.92	62	13.01	0.15
3	5.02	0.31	63	18.56	0.15
4	6.74	0.67	64	19.97	0.08
5	8.19	1.05	65	24.40	0.15
10	14.67	1.63	70	33.69	0.19
15	18.68	2.34	75	38.31	0.21
30	28.37	3.37	90	45.41	0.22
45	36.92	4.72	105	47.96	0.21
60	49.46	5.30	120	48.74	0.28

Eudragit® S100 5% w/v beads					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	0.82	0.07	61	2.65	0.49
2	1.44	0.49	62	5.29	0.16
3	3.11	0.27	63	12.01	0.49
4	4.85	0.81	64	18.06	0.18
5	6.45	0.60	65	20.79	8.64
10	8.43	0.23	70	37.86	12.89
15	10.42	0.15	75	49.06	12.40
30	13.96	0.23	90	71.03	11.33
45	16.78	0.56	105	85.00	10.21
60	19.01	0.87	120	92.82	8.62
			180	105.72	4.80
			240	109.69	3.56

Eudragit® S100 45.45% granules					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	2.24	1.58	61	10.68	0.10
2	5.27	1.67	62	19.43	0.20
3	9.49	1.55	63	24.73	0.32
4	12.90	1.82	64	28.00	0.19
5	16.25	0.53	65	33.19	0.23
10	26.67	1.65	70	43.21	0.35
15	32.02	2.02	75	47.37	0.30
30	40.98	2.95	90	53.30	0.39
45	49.10	3.92	105	54.71	0.62
60	55.38	3.51	120	55.13	0.20

Kollidon® SR 1% w/v beads					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	0.75	0.27	61	1.10	0.03
2	1.93	0.22	62	2.62	0.06
3	2.41	0.09	63	3.94	0.06
4	3.15	0.20	64	7.63	0.10
5	3.99	0.83	65	10.04	0.12
10	5.82	0.63	70	21.69	0.43
15	7.28	0.37	75	34.33	0.49
30	10.43	0.50	90	56.26	0.90
45	12.61	0.23	105	70.93	0.77
60	14.68	0.26	120	78.43	0.79
			150	83.90	0.84
			180	86.17	0.69
			210	87.79	0.41
			240	89.21	0.35
			270	90.20	0.14
			300	90.92	0.13
			330	91.60	0.06
			360	92.29	0.08

Kollidon® SR 14.29% granules					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	1.15	1.38	61	6.25	0.17
2	4.31	0.69	62	12.56	0.03
3	5.90	0.39	63	13.62	0.08
4	8.27	0.56	64	17.39	0.04
5	9.40	0.50	65	19.02	0.07
10	15.99	0.79	70	27.71	0.17
15	21.11	1.53	75	31.88	0.22
30	29.96	0.66	90	39.51	0.25
45	37.40	0.15	105	43.43	0.23
60	44.38	1.75	120	45.61	0.26
			150	49.68	1.02
			180	51.70	0.62
			210	52.65	0.47
			240	53.62	0.37
			270	55.14	0.70
			300	55.91	0.25
			330	56.97	0.50

Kollidon® SR 2.5% w/v beads					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	0.79	0.57	61	1.91	0.00
2	2.09	0.26	62	4.19	0.01
3	2.93	0.13	63	6.89	0.10
4	3.50	0.14	64	9.47	0.06
5	4.31	0.01	65	12.24	0.11
10	6.67	0.12	70	22.94	0.32
15	8.35	0.09	75	32.27	0.05
30	11.89	0.18	90	54.95	0.04
45	14.55	0.38	105	67.31	0.19
60	16.58	0.54	120	74.12	0.16
			150	81.38	1.25
			180	84.06	1.55

Kollidon® SR 29.41% granules					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	0.40	0.30	61	1.17	0.04
2	3.23	1.09	62	1.56	0.02
3	5.41	1.05	63	1.57	0.02
4	8.18	1.58	64	1.71	0.01
5	9.79	1.14	65	2.23	0.03
10	22.13	1.21	70	2.43	0.10
15	34.05	2.10	75	3.59	0.08
30	64.72	2.80			
45	76.05	0.73			
60	79.30	0.59			

Kollidon® SR 5% w/v beads					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	0.68	0.19	61	1.81	0.89
2	2.07	0.28	62	3.61	0.75
3	2.96	0.16	63	4.82	0.18
4	3.61	0.24	64	6.91	0.64
5	5.73	0.28	65	8.23	0.99
10	7.43	0.19	70	19.12	1.46
15	9.26	0.38	75	25.51	1.18
30	12.06	0.46	90	45.15	1.36
45	14.36	0.53	105	59.16	1.49
60	16.49	0.77	120	68.59	1.07
			180	88.30	0.45
			240	95.64	1.02

Kollidon® SR 45.45% granules					
0.1 M HCl			PBS pH 7.2		
Time	% Release	Stdev	Time	% Release	Stdev
1	1.99	0.82	61	0.00	0.00
2	4.85	0.62	62	0.00	0.00
3	8.23	0.31	63	0.00	0.00
4	12.13	1.25	64	0.00	0.00
5	16.31	2.48	65	0.00	0.00
10	42.94	11.25			
15	63.02	8.94			
30	76.95	2.40			
45	79.24	1.78			
60	80.53	1.32			

Tables B-2: Statistical interpretation of Drug loading.

Effect	Multivariate Tests of Significance (DLC Friablity Solubility) Sigma-restricted parameterization Effective hypothesis decomposition					
	Test	Value	F	Effect df	Error df	p
Intercept	Wilks	0.000047	271962.3	3	38.0000	0.00
Type	Wilks	0.000000	677.8	57	114.1298	0.00

Effect	Univariate Tests of Significance for %DLC (DLC Friablity Solubility) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	390884.5	1	390884.5	316633.1	0.00
Type	7302.1	19	384.3	311.3	0.00
Error	49.4	40	1.2		

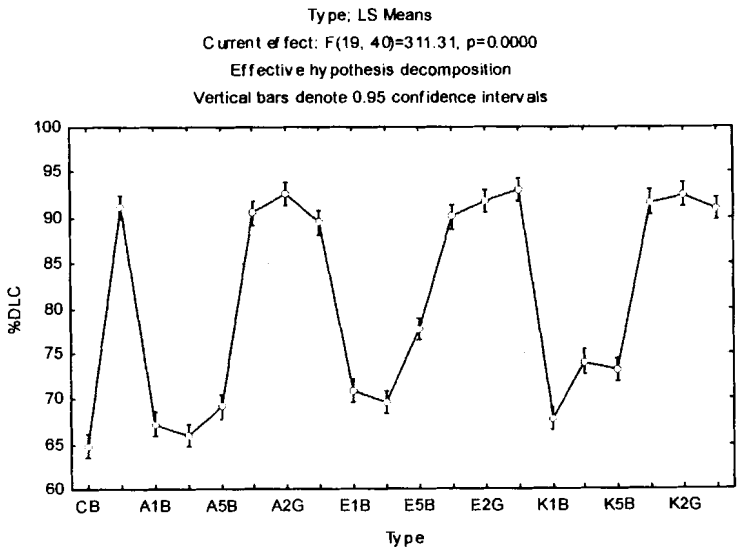

Table B-3: Tukey HSD test; variable %DLC
Approximate Probaboloties for Post Hoc Tests
Error: Between MS= 1.2345, df= 40.000

Type	CB	CG	A1B	A2.5B	A5B	A1G	A2.5G	A5G	E1B	E2.5B	E5B	E1G	E2.5G	E5G	K1B	K2.5B	K5B	K1G	K2.5G	K5G
CB		0.000	0.424	0.999	0.003	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.156	0.000	0.000	0.000	0.000	0.000
CG	0.000		0.000	0.000	0.000	1.000	0.987	0.935	0.000	0.000	0.000	0.999	1.000	0.866	0.000	0.000	0.000	1.000	0.994	1.000
A1B	0.424	0.000		0.992	0.835	0.000	0.000	0.000	0.033	0.507	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
A2B	0.999	0.000	0.992		0.081	0.000	0.000	0.000	0.001	0.022	0.000	0.000	0.000	0.000	0.869	0.000	0.000	0.000	0.000	0.000
A5B	0.003	0.000	0.835	0.081		0.000	0.000	0.000	0.938	1.000	0.000	0.000	0.000	0.000	0.987	0.001	0.012	0.000	0.000	0.000
A1G	0.000	1.000	0.000	0.000	0.000		0.727	1.000	0.000	0.000	0.000	1.000	0.996	0.410	0.000	0.000	0.000	0.999	0.797	1.000
A2G	0.000	0.987	0.000	0.000	0.000	0.727		0.120	0.000	0.000	0.000	0.413	1.000	1.000	0.000	0.000	0.000	1.000	1.000	0.959
A5G	0.000	0.935	0.000	0.000	0.000	1.000	0.120		0.000	0.000	0.000	1.000	0.582	0.038	0.000	0.000	0.000	0.684	0.155	0.975
E1B	0.000	0.000	0.033	0.001	0.938	0.000	0.000	0.000		0.998	0.000	0.000	0.000	0.000	0.126	0.107	0.600	0.000	0.000	0.000
E2B	0.001	0.000	0.507	0.022	1.000	0.000	0.000	0.000	0.998		0.000	0.000	0.000	0.000	0.850	0.003	0.047	0.000	0.000	0.000
E5B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.028	0.002	0.000	0.000	0.000
E1G	0.000	0.999	0.000	0.000	0.000	1.000	0.413	1.000	0.000	0.000	0.000		0.933	0.175	0.000	0.000	0.000	0.968	0.489	1.000
E2G	0.000	1.000	0.000	0.000	0.000	0.996	1.000	0.582	0.000	0.000	0.000	0.933		0.997	0.000	0.000	0.000	1.000	1.000	1.000
E5G	0.000	0.866	0.000	0.000	0.000	0.410	1.000	0.038	0.000	0.000	0.000	0.175	0.997		0.000	0.000	0.000	0.989	1.000	0.765
K1B	0.156	0.000	1.000	0.869	0.987	0.000	0.000	0.000	0.126	0.850	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000
K2B	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.107	0.003	0.028	0.000	0.000	0.000	0.000		1.000	0.000	0.000	0.000
K5B	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.600	0.047	0.002	0.000	0.000	0.000	0.000	1.000		0.000	0.000	0.000
K1G	0.000	1.000	0.000	0.000	0.000	0.999	1.000	0.684	0.000	0.000	0.000	0.968	1.000	0.989	0.000	0.000	0.000		1.000	1.000
K2G	0.000	0.994	0.000	0.000	0.000	0.797	1.000	0.155	0.000	0.000	0.000	0.489	1.000	1.000	0.000	0.000	0.000	1.000		0.978
K5G	0.000	1.000	0.000	0.000	0.000	1.000	0.959	0.975	0.000	0.000	0.000	1.000	1.000	0.765	0.000	0.000	0.000	1.000	0.978	

Tables B-4: Statistical interpretation of Friability.

Effect	Multivariate Tests of Significance (DLC Friablity Solubility) Sigma-restricted parameterization Effective hypothesis decomposition					
	Test	Value	F	Effect df	Error df	p
Intercept	Wilks	0.000047	271962.3	3	38.0000	0.00
Type	Wilks	0.000000	677.8	57	114.1298	0.00

Effect	Univariate Tests of Significance for %Friability (DLC Friablity Solubility) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	165.5518	1	165.5518	32637.84	0.00
Type	160.1570	19	8.4293	1661.80	0.00
Error	0.2029	40	0.0051		

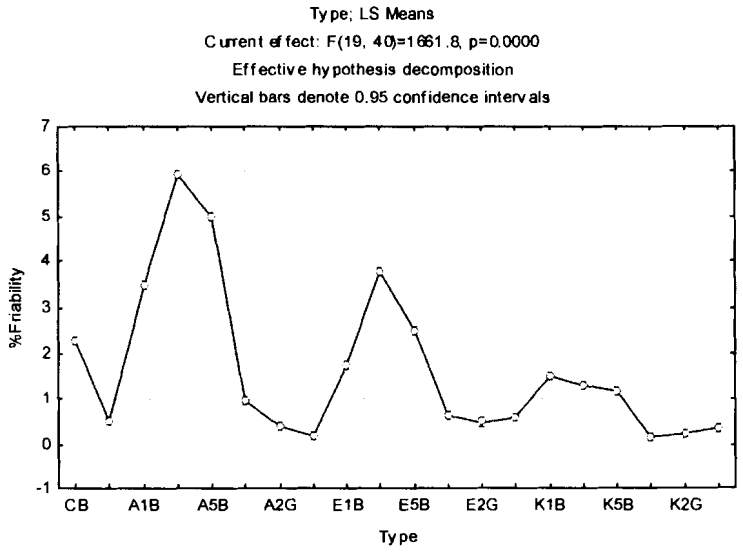

Table B-5: Tukey HSD test; variable %Friability
Approximate Probaboloties for Post Hoc Tests
Error: Between MS= 0.00507, df= 40.000

Type	CB	CG	A1B	A2.5B	A5B	A1G	A2.5G	A5G	E1B	E2.5B	E5B	E1G	E2.5G	E5G	K1B	K2.5B	K5B	K1G	K2.5G	K5G
CB		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.087	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CG	0.000		0.000	0.000	0.000	0.000	0.522	0.000	0.000	0.000	0.000	0.949	1.000	1.000	0.000	0.000	0.000	0.000	0.002	0.364
A1B	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A2B	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A5B	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A1G	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.047	0.000	0.000	0.000
A2G	0.000	0.522	0.000	0.000	0.000	0.000		0.147	0.000	0.000	0.000	0.010	0.891	0.060	0.000	0.000	0.000	0.029	0.662	1.000
A5G	0.000	0.000	0.000	0.000	0.000	0.000	0.147		0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	1.000	1.000	0.242
E1B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000
E2B	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E5B	0.087	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E1G	0.000	0.949	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000		0.646	1.000	0.000	0.000	0.000	0.000	0.000	0.005
E2G	0.000	1.000	0.000	0.000	0.000	0.000	0.891	0.001	0.000	0.000	0.000	0.646		0.963	0.000	0.000	0.000	0.000	0.010	0.767
E5G	0.000	1.000	0.000	0.000	0.000	0.000	0.060	0.000	0.000	0.000	0.000	1.000	0.963		0.000	0.000	0.000	0.000	0.000	0.032
K1B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000		0.205	0.001	0.000	0.000	0.000
K2B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.205		0.879	0.000	0.000	0.000
K5B	0.000	0.000	0.000	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.879		0.000	0.000	0.000
K1G	0.000	0.000	0.000	0.000	0.000	0.000	0.029	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.982	0.054
K2G	0.000	0.002	0.000	0.000	0.000	0.000	0.662	1.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.982		0.813
K5G	0.000	0.364	0.000	0.000	0.000	0.000	1.000	0.242	0.000	0.000	0.000	0.005	0.767	0.032	0.000	0.000	0.000	0.054	0.813	

Tables B-6: Statistical interpretation of Solubility.

Effect	Multivariate Tests of Significance (DLC Friablity Solubility) Sigma-restricted parameterization Effective hypothesis decomposition					
	Test	Value	F	Effect df	Error df	p
Intercept	Wilks	0.000047	271962.3	3	38.0000	0.00
Type	Wilks	0.000000	677.8	57	114.1298	0.00

Effect	Univariate Tests of Significance for %Solubility (DLC Friablity Solubility) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	84359.03	1	84359.03	560200.4	0.00
Type	26292.01	19	1383.79	9189.3	0.00
Error	6.02	40	0.15		

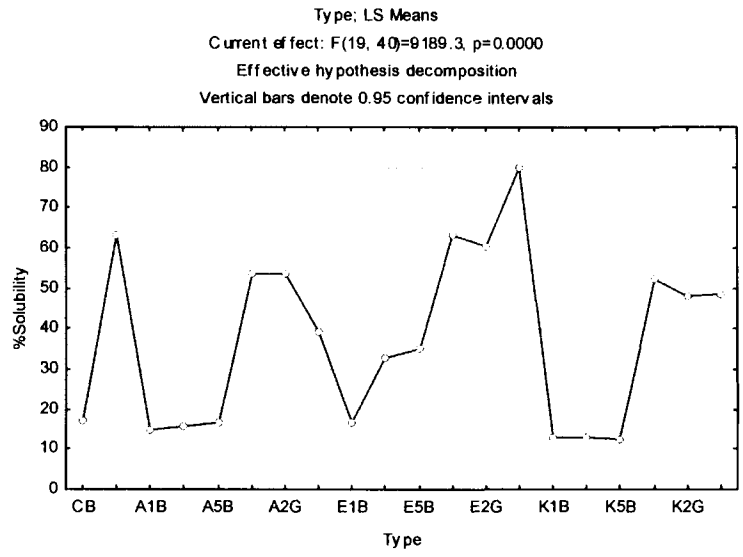

Table B-6: Tukey HSD test; variable %Solubility
Approximate Probaboloties for Post Hoc Tests
Error: Between MS= 0.15059, df= 40.000

Type	CB	CG	A1B	A2.5B	A5B	A1G	A2.5G	A5G	E1B	E2.5B	E5B	E1G	E2.5G	E5G	K1B	K2.5B	K5B	K1G	K2.5G	K5G
CB		0.000	0.000	0.199	1.000	0.000	0.000	0.000	0.999	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CG	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A1B	0.000	0.000		0.167	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A2B	0.199	0.000	0.167		0.551	0.000	0.000	0.000	0.904	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A5B	1.000	0.000	0.000	0.551		0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A1G	0.000	0.000	0.000	0.000	0.000		1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000
A2G	0.000	0.000	0.000	0.000	0.000	1.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000
A5G	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E1B	0.999	0.000	0.001	0.904	1.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E2B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E5B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E1G	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E2G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000
E5G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000
K1B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		1.000	0.774	0.000	0.000	0.000
K2B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000		0.868	0.000	0.000	0.000
K5B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.774	0.868		0.000	0.000	0.000
K1G	0.000	0.000	0.000	0.000	0.000	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000
K2G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		1.000
K5G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	

ANNEXURE C
REASERCH ARTICLE

The influence of polyvinylpyrrolidone on the release profile of ketoprofen from chitosan matrix particles.

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Abstract

Matrix beads were prepared by inotropic gelation in tripolyphosphate and granules were prepared by wetting chitosan with acetic acid. Ketoprofen was chosen as the drug model due to the relatively high pKa of 5.94. This caused the drug to leak out of the beads during gelation in tripolyphosphate. Common methods to overcome this problem lead to weaker beads. It was found that polyvinylpyrrolidone increased the drug loading of the chitosan matrix beads to as high as 74.02%. Scanning electron microscopy reviled the beads containing polyvinylpyrrolidone appeared to have smoother surface layers, with polyvinylpyrrolidone particles clearly visible on the surface, while granules appeared more cracked. Friability tests were preformed to evaluate the resistance to mechanical force. Inclusion of polyvinylpyrrolidone lowered the friability of beads to an almost acceptable value. Polyvinylpyrrolidone also managed to lower the solubility, swelling and lessen the degradation, thereby increasing the integrity of the matrix. Polyvinylpyrrolidone caused more drug to be released from the granules, shorter MDT-values. In beads the results obtained during one hour in 0.1 M HCl was very similar to chitosan only beads, with drug release of up to 6 hours in pH 7.2 for 2.5% w/v polyvinylpyrrolidone and high MDT-values.

Keywords: Chitosan; Polyvinylpyrrolidone; ketoprofen; controlled release; particles

1. Introduction

In recent years there has been a phenomenal increase in interest in controlled release dosage forms [1-3]. These dosage forms have a significant potential to provide economic value by enhancing efficacy and decreasing treatment costs [4-5]. Chitosan, a natural polymer obtained from the alkaline deacetylation of chitin, which is widely distributed in nature as the principal component of exoskeletons of crustaceans and insects, has several properties of interest to the pharmaceutical industry [6]. The simplest way of achieving slow release of drugs by means of chitosan is to employ it as a gel-forming excipient in matrix-type formulations [7-8].

Kawashima *et al.* [9] found that chitosan retarded release of aspirin from tablets during dissolution testing in an acidic environment. Chitosan has been shown to be a viable excipient for controlled release bead formulation [10-14]. Win *et al.* [15] suggested that chitosan gel beads may be useful as a controlled drug delivery system in oral administration and may avoid drug release in the highly acidic gastric fluid region of the stomach. Chitosan matrix granules also proved to sufficiently retard drug release and were considered as a controlled release dosage from [10; 8; 16-17]. Säkkinen found that matrix beads and granules prepared with the addition of modified release excipients could be valuable in the development of prolonged-release formulation. The effect polyvinylpyrrolidone (PVP) was however not investigated.

Kollidon® SR (KSR) also known as polyvinyl acetate or polyvinylpyrrolidone is derived from a polyvinyl acetate-dispersion and appears as a spray dried, non hygroscopic powder consisting of polyvinyl acetate (80% w/w) and PVP (20% w/w). KSR, an excipient for drug delivery matrices based on polyvinyl acetate, possesses good controlled release properties [18]. Reza *et al.* [19] were able to produce sustained release formulations containing high levels of KSR. Higher polymeric content (75%) in the matrix decreased the release rate of drug because of increased tortuosity and decreased porosity. KSR outperformed hydroxypropyl methylcellulose while Cumba wax retarded drug release even more than KSR.

Since Bodmeier *et al.* introduced a method whereby chitosan beads can be prepared by inotropic gelation using oppositely charged tripolyphosphate (TPP) much work has been done on the subject [8; 10-16]. Bodmeier suggested methods by which drug loading of drug soluble in alkaline solutions could be improved. These included adjusting the pH of the TPP-solution, shortening the time of contact with TPP-solution, and keeping the TPP-solution to chitosan solution concentration to a minimum [11]. According to Shu and Zhu [20] the mechanical strength of these chitosan beads was very poor and its usage in the pharmaceutical industry is still very limited and thus a novel method based on gelatin coagulated in sesame seed oil was proposed.

Herein we report a new method to prepare TPP/chitosan matrix beads containing KSR, with increased resistance to mechanical force and improved dissolution profiles. These bead formulations have also been compared to better known granulate formulations.

2. Materials and methods

2.1. Materials

The following materials were used during the preparation of chitosan matrix drug loaded particles. Chitosan (83% deacetylated). Glacial acetic acid 100% (Merck, South Africa). Tripolyphosphate (Sigma, South Africa). Kollidon® SR (BASF, South Africa). Ketoprofen (Boehringer Ingelheim Bidachem, Italy). All chemicals used were analytical grade and were used as received.

2.2. Preparation of chitosan matrix beads

Chitosan drug loaded beads were produced by the method of Bodmeier *et al.* with adjustments to improve drug loading by dispersing 3% w/v ketoprofen in a solution of 3% w/v chitosan in 2% v/v aqueous solution of acetic acid [11]. The solution was

placed in an ultra sonic bath (Julabo Labortechnik, Swarzwald, Germany) until it was bubble free. Drug loaded chitosan-polymer beads were produced much in the same way as polymer free beads, but amounts of 1, 2.5 or 5% w/v KSR was additionally dispersed in the chitosan acetic acid solution.

The beads were formed by dropping the chitosan drug dispersion or chitosan, drug, polymer dispersion through a gauge 21 needle into a bubble free gently agitated solution of TPP with a peristaltic pump (Watson-Marlow 205S, Watson-Marlow Ltd., England). The pH (WTW pH 320 SET, Wissenschaftlich-technische werkstätten, Germany) of the TPP-solution was adjusted to pH 5.0 and an amount of 150mg ketoprofen was added to 100ml of the TPP-solution. The ration chitosan drug dispersion to TPP-solution was kept steady at 1:5 to ensure optimal drug loading. A stirring time of 30 minutes was allowed for cross-linking to occur, before the beads were removed from the TPP-solution. The beads were washed twice with 50ml of deionised water and freeze-dried in a Secfroid freeze-dryer (Lausanne, Switzerland), for 24 hours.

2.3. Preparation of chitosan matrix granules

Granules were prepared by means of wet granulation. Weighed amounts of ketoprofen chitosan, and polymer, similar to the composition of the beads, were added to a glass jar. To minimize the difference between the bead and granules formulations, the composition of the granules had to be based on the amount of polymer contained in the beads theoretically, therefore 1% w/v polymer beads correlates to 14.25% w/w polymer granules, and so 2.5% and 5% w/v beads correlates to 29.41% and 45.45% w/w granules respectively. The formulation powder was then mixed in a Turbulator mixing apparatus (Model T2C, Wily A. Bachofen Maschinefabrik, Basel, Switzerland) for 5 minutes. After mixing the powder mass was wetted with a aqueous solution of 2.5% v/v glacial acetic acid in a mortar, and granulated trough a 2 mm sieve.

The resulting granulate was dried over night at 35°C in an oven (Term-O-Mat oven, Labotec, South Africa), and dried an additional 8h at 35°C under vacuum. The granulate was then broken up by force, and sieved. Only granules of sieve fraction 710 - 850 μm were used in the study (Fritsch analysette, Germany). This method of preparing chitosan granules was used to eliminate the any additional effects that excipients such as binders might have on the release of drug from the granules.

2.4. Model drug loading determination

Beads can only incorporate a certain amount of drug. For pharmaceutical particles to be considered as a viable dosage form, an adequate amount of drug must be incorporated into such particles. 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 *et al.* [13] and Bodmeier *et al.* [11] described a method whereby a formulation of drug loaded beads or micro-granules was accurately weighed and kept in a known amount of a aqueous 2% v/v acetic acid solution at 30°C for 48 hours, or 0.1 M NaOH at 22°C for 48 hours, respectively. The formulations were filtered and solubility was obtained by measuring the concentration of the drugs spectrophotometrically after appropriate dilution in 0.1 M NaOH. The drug loading capacity (*DLC*) of the beads was calculated from the following equation:

$$DLC = \frac{\text{Total amount of drug} - \text{Free amount of drug}}{\text{Weight of the Beads}}$$

2.5. Morphology: Scanning electron microscopy

Scanning electron microscopy (SEM) was used to characterize the surface and cross sections of the formulations. Cross sections were obtained by cutting the formulations with a razor blade, much in the same way as Bodmeier *et al.* [11]. Formulations were mounted on aluminum formulation mounts and coated with gold palladium alloy to minimize surface charging. A Philips XL30DX4i scanning electron microscope

(Philips, Netherlands) was used. Lubbe [21] 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 exactly using the above described method.

2.6. Formulation resistance to mechanical force determination

A weighed amount of formulations were randomly selected from each batch and dusted with compressed air and accurately weighed. The formulation was 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 formulations were dusted and weighed again. The percentage friability was determined using the following equation:

$$\% \text{ Friability} = [(W_0\text{-}Wt)/W_0] \times 100$$

where W_0 is the weight of the formulation before testing, Wt is the weight of the formulation after testing.

2.7. Formulations solubility study

Gupta *et al.* [13] described a method by which solubility of chitosan beads can be determined. The formulation 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 by the following equation:

$$\%S = [(W_0 - W_t)/W_0] \times 100$$

were 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.

2.8. Formulations swelling and degradation

The swelling and degradation studies were conducted in much the same manner as Gupta *et al.* [13] and Mi *et al.* [14] A known amount of formulation (100 mg) was weighed and placed in 10 ml of the media (0.1 M HCl and Phosphate buffered solution pH 7.2 at 37°) for the required time. Swollen beads were collected at time intervals of 10, 60 and 360 minutes. The net weight of the formulation was determined by first blotting the formulation with filter paper to remove additional water on the surface of the formulation, and then weighing immediately on an electronic balance (Precisa 240A, Precisa balances, Zürich, Switzerland). The percentage swelling of the formulation in the media was calculated using the formula:

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

where E_{sw} is the percentage swelling of the sample at a specific time, W_e 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.

2.9. Model drug release studies

The release properties of all the formulations were studied in 0.1 M HCl or simulated intestinal fluid (USP XXI) with a pH of 1.2 and phosphate buffered solution (PBS) pH 7.2 (USP XXI) respectively. The dissolution method used to determine the drug release from the formulations consists of *apparatus 1* in the USP [19]. The basket stirring element was used. An Erweka DT6R (Erweka® Apparatebau GmbH, Germany) dissolution apparatus was used. An amount of 0.140g formulation was weighed and placed in the basket. The basket was lowered into 1000 ml dissolution medium and rotation was started after the basket was submerged into the medium. The rotation was kept constant at 50 revolutions per minute. Formulations of 5 ml were drawn through 3 μm (Millipore) membranes to eliminate any suspended

particles. The formulation containing baskets were first submerged in 0.1 M HCl for 60 minutes and dissolution formulations were extracted at time intervals of 1, 2, 3, 4, 5, 10, 15, 30, 45 and 60 minutes, and replaced with fresh preheated medium.

After 60 minutes in 0.1 M HCl the baskets was removed from the dissolution medium, patted dry, and immediately placed in PBS pH 7.2 for the remainder of the dissolution. Dissolution formulations of 5 ml were withdrawn through 3 μm membrane at 61, 62, 63, 64, 65, 70, 75, 90, 105, 120, 150, 180, 210, 240, 270, 300, 330, 360 minutes, and replaced with fresh preheated medium. All formulations were run in triplicate and assayed spectrophotometrically, using a Unicam spectrophotometer at 258 nm wavelength. The following equation was used to correct for dilution of the dissolution media after each formulation extraction and addition of pure medium:

$$Y_n^* = Y_n + \frac{V_s}{V_m} \bullet \sum^{n-1} Y^*$$

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

To characterize the drug release rate further the mean dissolution time (MDT) was calculated from dissolution data according to Mockel and Lippold using the following equation [21]:

$$MDT = \frac{ABC}{X_{d,\max}}$$

where MDT is the mean dissolution time and $X_{d,\ max}$ is the actual maximum cumulative mass dissolved. ABC is the area calculated from the graph.

Additionally the similarity factor (f_2) is used to compare the dissolution profile of each formulation with that of marketed formulation. In this approach, recommended by the FDA Guidance for Industry, a value between 50 and 100 indicates similarity between two dissolution profiles. If the f_2 value is close to 100, the two profiles are nearly identical [22, 23]. The similarity factor can be determined by the following equation (4-3):

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

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 pints, and w_t is an optional weight factor. The equation is a logarithmic transformation of the sum of squared error. It takes the average sums of squares of the difference between test and reference profiles and fits the result between 0 and 100. The similarity factor (f_2) is 100 when the test and reference profiles are identical and approaches zero as the dissimilarity increases.

3. Results and Discussion

3.1. Model drug loading determination

As can be seen clearly from the Figure 3.1, the addition of KSR to granules had no significant effect on the drug loading of granules, while the addition of KSR proved to substantially increase the drug loading efficiency of the beads, with higher amounts of KSR leading to the most pronounced improvement. With 2.5% w/v KSR added to the chitosan drug dispersion lead to an increase of 9.4%. According to Fussnegger [19] the release profiles of his tablet formulations with KSR were not influenced by different dissolution media. This increased in drug loading experienced, may be as a result of the pH independent sustaining of drug release from KSR already noticeable during the wet inotropic gelation phase of production.

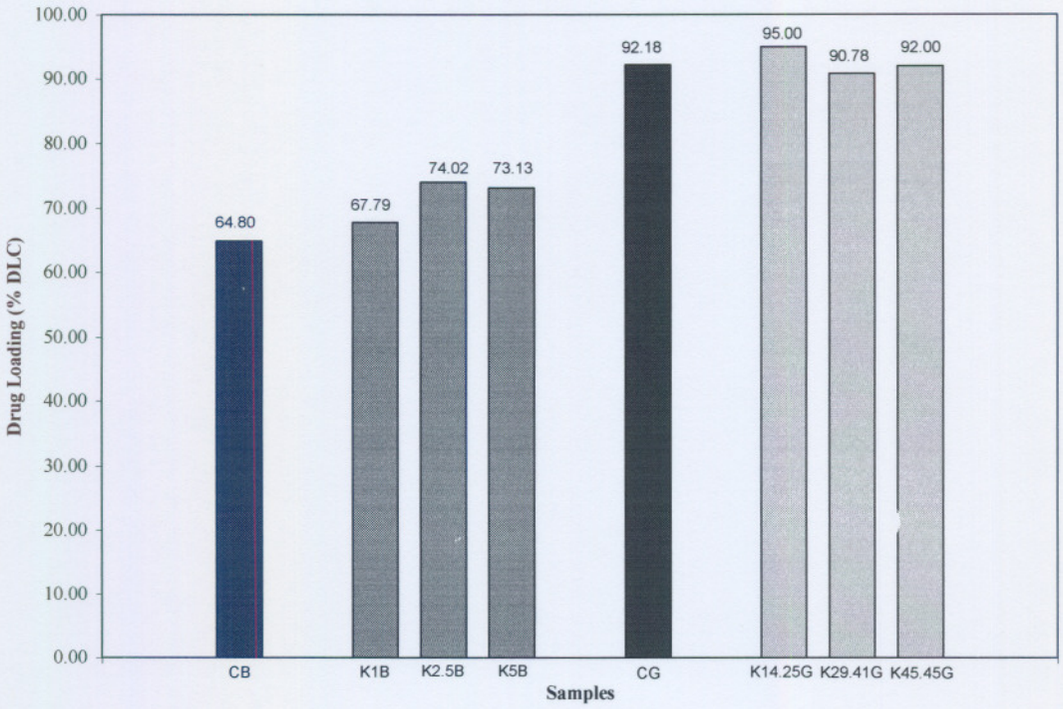

Figure 3:1: Results of drug loading efficiency study on chitosan bead and granules formulations containing increasing amounts of KSR.

3.2. Morphology: Scanning electron microscopy

The results obtained from the SEM analysis are represented in Figure 3.2. Beads produced by the recommended adjustments to the method of Bodmeier, namely an adjustment in pH, shortened stirring time, and lower TPP to chitosan dispersion ratio, appeared to weaker and more porous (Fig 3.2 a) than beads produced without the adjustments. According to Mi *et al.* [26] these adjustments interferes with the diffusion of TPP into the chitosan bead matrix, thereby reducing the effectiveness of TPP to cross-link chitosan and produce beads with a finely porous internal structure and smooth surface layer. By adding KSR to the bead matrix a significant improvement in the surface structure could be seen (Fig 3.2 b-d) with lower concentrations producing the most pronounced improvement in surface morphology. Beads produced from higher concentrations once again appeared grainier, due to the appearance of KSR particles on the bead surface. Also important to mention was the significant layering effect noticed upon examining the internal structure of the beads. In 1% w/v KSR beads there is a clear indication of two distinct layers, a thin outer

layer and an internal thick layer. Although this effect was noticeable also in beads produced with higher concentrations of KSR the phenomenon was less pronounced. It is not well understood why these layers formed. All beads produced appeared to have a spherical shape, with diameters varying from 1.8 to 2.2mm.

Granules (Fig 3:2 i-l) had a relatively simple structure. The granules prepared during the study were well mixed and very hard. Since granules had to be milled in a mortar to achieve the required size no need was seen to cut the granules, as the surface views and internal views were identical. Surface views of granules were very much alike for ketoprofen loaded chitosan granules and for granules with added polymer. Due to the fact that the required size of granules was collected by means of a sieve fraction, the size of the granules was uniform. Polymer free granules appeared as a tightly packed clump with little or no definition between the drug particles and chitosan (Fig 3:2 i). This could be attributed to the method used to produce the granules. Chitosan dissolves in acetic acid, to from a gel. When acetic acid was used to granulate the drug-chitosan mixture, chitosan formed a gel which coated all the particles to from the tightly packed granule. Spherical particles of KSR were mostly visible on the surface of granules made with a concentration of 45.45% w/w polymer. There was however a more significant decrease in the denseness of the bead with every increase in polymer concentration, and high concentrations of polymer did not seem to from good tightly packed matrixes. A large hole was visible for granules produce with 45.45% w/w polymer added (Fig 3:2 l). This was found to be due to the lower total concentration of the binder chitosan in the granule, with higher concentrations of KSR

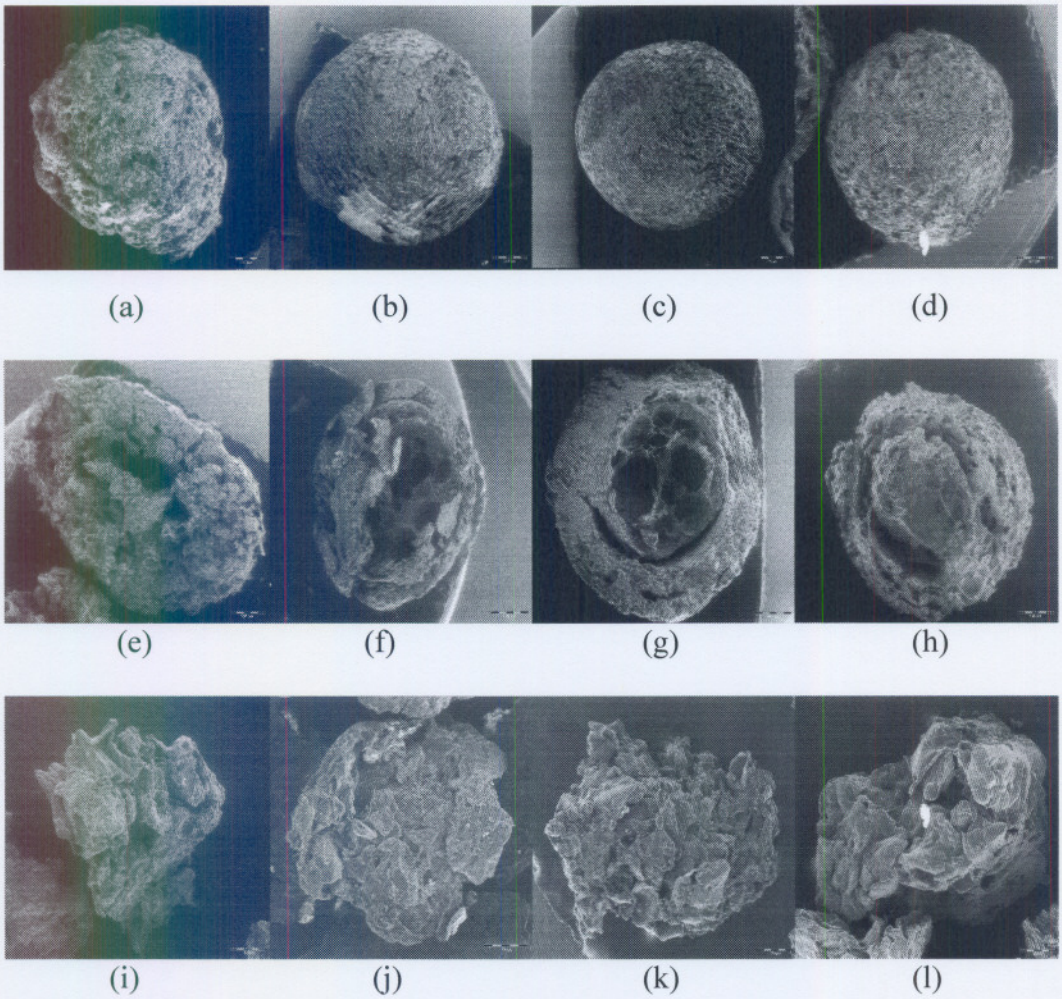

Figure 3.2: SEM micrographs of (a) the surface layer of a chitosan/ketoprofen bead (b) 1% w/v KSR bead, (c) 2.5% w/v KSR and (d) 5% w/v KSR bead. The internal structure of (e) a chitosan/ketoprofen bead, (f) a 1% w/v KSR bead, (g) a 2.5% w/v bead, and (h) 5% w/v KSR bead. The surface morphology of granules is depicted in (i) a chitosan/ketoprofen granule, (j) a 14.25% w/w KSR granule, (k) a 29.41% w/w KSR granules and (l) a 45.45 w/w granule.

3.3. Formulation resistance to mechanical force determination

The results of the evaluation of friability are given as a percentage in Figure 3:3. The results of the friability of the granules were all lower than 1%. The results of the friability of the granules were found to be 0.25 ± 0.09%. From this value it can be concluded that the granules prepared in the study are relatively hard. This means that not much granulate would be lost during packaging and transport of the granules to the patient and that the granules would remain intact until the usage. Higher amounts of KSR lead to a higher degree of friability, as the amount of binder in the granules decreased with an increase in KSR concentration.

The friability of the beads is quite high with 2.27 ± 0.14% for ketoprofen loaded chitosan beads prepared by the method with adjustments recommended by Bodmeier *et al* [11]. The effect of KSR was a noticeable decrease in friability with an optimal value of 1.19 ± 0.08% for 5% w/v KSR. The increase in resistance to mechanical force depended on the concentration of KSR in the beads, with higher concentrations yielding the best results (Fig 3:3). This was clear even before the tests were conducted as KSR beads were much easier to handle and less prone to breakage during normal processes.

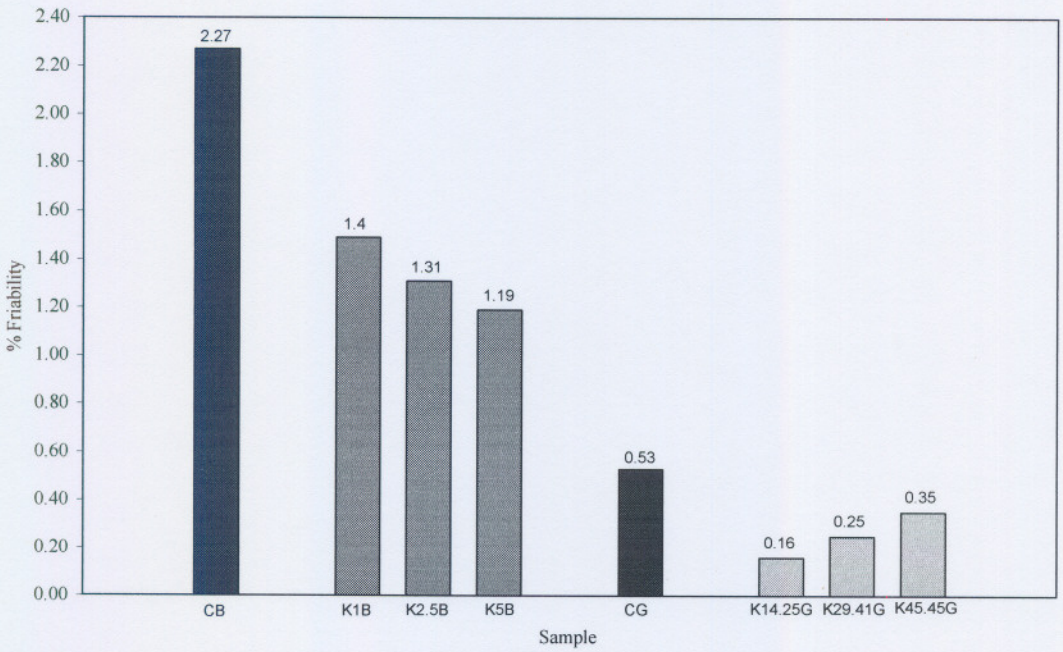

Figure 3:3: Results of friability study on chitosan bead and granules formulations containing increasing amounts of KSR.

3.4. Formulations solubility study

Polymer free beads showed a rather high degree of solubility, with an average solubility of 16.93 ± 0.17%. According to Gupta *et al.* [27] 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. The high solubility of ketoprofen loaded chitosan beads can be attributed to the poor cross-linking due to the changes

made to the method described in section 2.2. By adding KSR to the bead formulation the degree of solubility could noticeably be decreased, with higher concentrations of KSR yielding beads with even lower solubility. The effect of was most pronounced for 5% w/v KSR, with an average solubility of 12.36 ± 0.03%, much lower than that of chitosan.

Since granules produce by the method described in section 2.3 does not involve cross-linking of chitosan it was expected that the solubility of granules would be much higher than that of cross-linked chitosan beads. This was found to be the case. It is clear from Figure 3:4 that the solubility of ketoprofen loaded chitosan granules, with a solubility of 63.00 ± 1.01% was much higher than that of the similar beads (%S = 16.93 ± 0.17%). It was noticed that KSR managed to lower the solubility of the granules. The effect of KSR on the solubility of the granules seemed to be influenced by concentration, with higher concentrations being more pronounced as with beads. But the effect seemed to reach a plateau with higher concentrations than 29.41% w/w. This may also be caused by the lowering level of the binder chitosan in the granules.

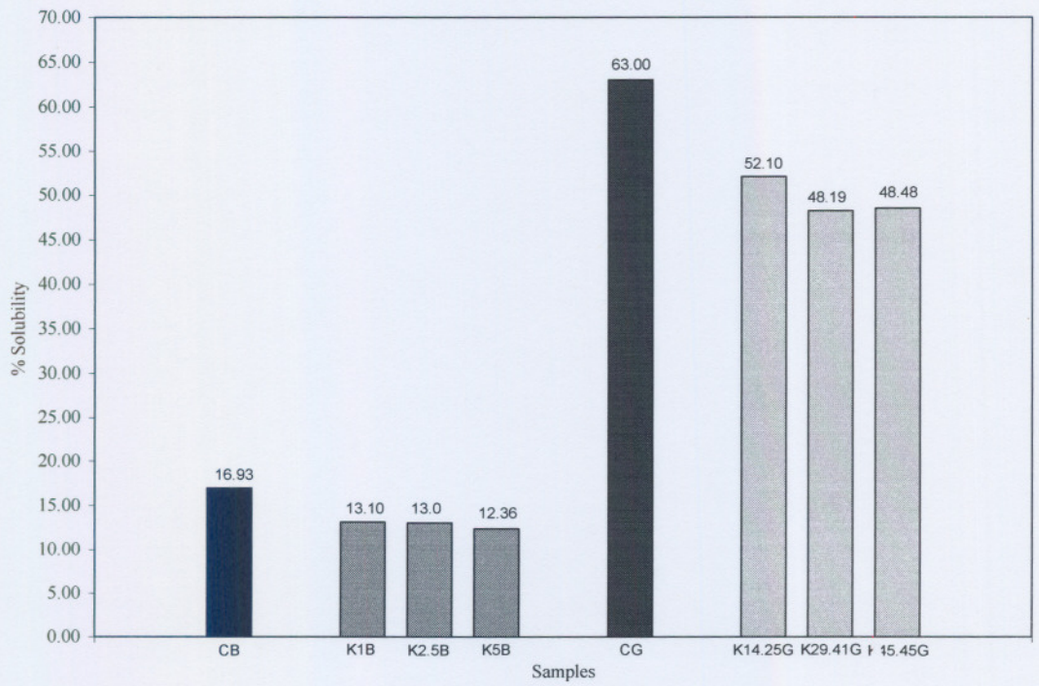

Figure 3.4: Results of solubility study on chitosan bead and granules formulations containing increasing amounts of KSR.

3.5. Formulations swelling and degradation

Due to the high solubility of the granule formulations used the study utilized to determine the swelling of formulations was not appropriate for granules and test were not preformed on granules. Beads made from only chitosan and drug showed good swelling during the firs 10 minutes submerged in 0.1 M HCl but started losing shape and dissolving after 60 minutes reaching a maximum Esw of 9.42 ± 0.72%. After 6 hours the beads almost completely dissolved forming a gel. The swelling behavior of ketoprofen loaded chitosan beads was much weaker in PBS pH 7.2 than in 0.1 M HCl. Beads swelled only a maximum of 4.10 ± 0.87% after 6 hours. The rate of swelling seemed to increase only slightly after each time interval.

KSR formulations showed a decrease in swelling after 6 hours in 0.1 M HCl, possibly due to degradation of the matrix beads. The effect was however not noticed in PBS pH 7.2. Beads degree of swelling continued to increase up to 6 hours for 1 and 5% w/v formulations but seemed to reach a constant after 60 minutes for 2.5% w/v KSR beads. Swelling was mostly inhibited by 5% KSR with maximum values for degree of swelling of 2.10 ± 0.02 and 1.89 ± 0.07% for 0.1 M HCl and PBS pH 7.2 respectively (Fig 3.5).

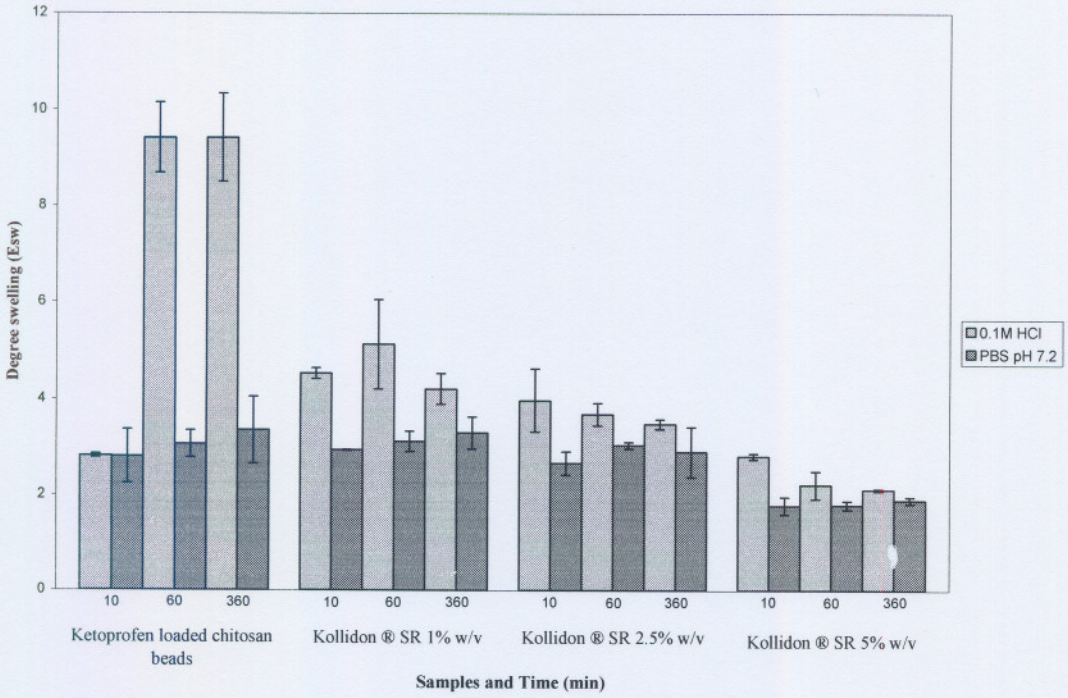

Figure 3.5: Results of swelling and degradation study on chitosan bead formulations containing increasing amounts of KSR.

3.6. Model drug release studies

The results of dissolution testing are shown in Figure 3.6 and Figure 3.7. The effect of modern KSR on the release of ketoprofen from the formulations appeared to be in contradiction to what was expected. It was thought that the addition of KSR would lead to little, if any, release of ketoprofen during the first 60 minutes in 0.1 M HCl. Keen observation of the graph indicates that there are several differences in between the release of drug from beads and granules. As expected, granules showed a much more prominent burst effect during the first 5 minutes. This difference could be attributed to the difference in surface structure between the beads and the granules. Granules had a much less smooth and more cracked or flaky appearance. This type of surface morphology increased the total surface area of the granules exposed to the dissolution medium and more drug dissolved during the first 5 minutes. Only a very slight initial burst effect was noticed for the bead formulations. Further observation also reviled that the deviation between baskets was much lower for beads than for granules. Once again it was speculated that this correlated to the structural differences between the formulations. Where beads consist mainly of solid spherical

structures, granules were asymmetrical and random in shape. Although granulate formulations were well sieved, size differences were greater in granulate formulations than in bead formulations.

Table 3.6.1: Mean dissolution time values for both dissolution mediums.

0.1 M HCl						
	MDT1	**MDT2**	**MDT3**	**Ave MDT**		
CB	21.10	22.38	22.86	22.11	±	0.91
K2.5B	19.89	19.81	20.82	20.17	±	0.56
K5B	19.68	18.06	18.95	18.90	±	0.81
CG	23.30	23.74	19.78	22.27	±	2.17
K1G	21.75	23.18	21.28	22.07	±	0.99
K2.5G	20.01	18.62	20.16	19.60	±	0.85
K5G	13.64	8.91	13.36	11.97	±	2.65
PBS pH7.2						
	MDT	**MDT**	**MDT**	**Ave MDT**		
CB	82.97	89.04	83.75	85.25	±	3.30
K1B	98.28	99.00	97.75	98.34	±	0.63
K2.5B	88.55	89.20	87.39	88.38	±	0.92
K5B	105.59	109.05	107.09	107.24	±	1.73
CG	76.91	82.96	82.79	80.88	±	3.44
K1G	93.32	91.65	89.69	91.55	±	1.82
K2.5G	65.61	65.58	65.59	65.59	±	0.01
K5G	-	-	-	-	±	-

Granules released much more drug during the first hour in 0.1 M HCl as can also be seen from the short MDT values in Table 3.6.1. Although the release profile of 14.25% w/w KSR granules appeared to follow the same curve as chitosan granules (See Table 3.6.2), a higher amount of drug was released ($CG_{(60min)}$ = 31.76 ± 3.02%, $K14.25G_{(60min)}$ = 44.38 ± 1.75%, f_2 = 87.84 ± 1.31). Granules of 29.41 and 45.45% w/v KSR quickly released the drug into the dissolution medium with virtually all of the drug released after 45 minutes ($K29.41G_{(45min)}$ = 76.05 ± 0.59% $K45.45G_{(45min)}$ = 79.24 ± 1.32%). The granules quickly disintegrated and only empty baskets were removed after 60 minutes. Due to the high amount of drug released by granules during the first hour in 0.1 M HCl not much drug was released in PBS pH 7.2. Both 29.41 and 45.45% w/w released virtually no drug in PBS pH 7.2, while 14.25% w/w

KSR granules sustained release for up to 330 minutes and released a further 56.97 ± 0.50% drug ($MDT_{K14.25G}$ = 91.55 ± 1.82).

Table 3.6.2: Similartity factor values for all samples compared with chitosan beads and granules.

Similarity Factor (f2)				
	0.1 M		PBS pH 7.2	
	(f1) vs CB	(f1) vs CG	(f1) vs CB	(f1) vs CG
K1B	99.09 ± 0.37	78.19 ± 2.94	94.72 ± 0.98	51.77 ± 0.70
K2.5B	99.29 ± 0.55	80.07 ± 2.48	90.37 ± 2.83	56.12 ± 0.95
K5B	80.98 ± 2.72	70.44 ± 2.46	34.32 ± 0.96	44.85 ± 1.89
CB	100.00 ± 0.00	80.03 ± 2.86	100.00 ± 0.00	53.07 ± 1.17
K1G	76.43 ± 2.50	93.21 ± 2.56	56.33 ± 0.82	87.84 ± 1.32
K2.5G	79.06 ± 6.53	92.29 ± 2.35	73.55 ± 1.51	46.60 ± 0.59
K5G	65.60 ± 2.01	78.73 ± 5.55	67.05 ± 1.13	44.28 ± 0.56
CG	80.03 ± 2.86	100.00 ± 0.00	53.07 ± 1.17	100.00 ± 0.00

As expected the beads fared far better at sustained release of ketoprofen (See Table 3.6.1). There was only a slight difference between the release profiles obtained for different concentrations of KSR beads compared to chitosan beads (See Table 3.6.2). The release profiles of 1% w/v KSR beads correlated well to that of chitosan, with 1% KSR beads releasing only 0.79% more drug after 60 minutes ($K1B_{(60min)}$ = 14.68 ± 0.26%, $CB_{(60min)}$ = 13.89 ± 0.85%, f_2 = 99.09 ± 0.37). Beads of 2.5 and 5% w/v KSR beads released almost the same amount of drug as each other and only slightly more than chitosan beads ($CB_{(60min)}$ = 13.89 ± 0.85%, $K2.5B_{(60min)}$ = 16.58 ± 0.58% & $K5B_{(60min)}$ = 16.49 ± 0.77%).

Differences between the KSR bead formulations were only visible after dissolution testing in PBS pH 7.2. Chitosan beads and granules showed a similar dissolution profile with release for up to 180 minutes for chitosan granules, and 240 minutes for beads. Beads of 2.5% w/v KSR appeared to be the weakest formulation with only releasing drug up to 180 minutes ($K2.5B_{(180min)}$ = 84.06 1.15% $MDT_{K2.5B}$ = 88.38 ± 0.59), where as beads of 5% w/v KSR appeared to control drug release better than

chitosan, with a smoother curve obtained from the study and more drug being released in total, and also sustained drug release to 240 minutes ($CB_{(240min)}$ = 72.65 ± 4.67%, $K5B_{(240min)}$ = 95.64 ± 1.02%, MDT_{K5B} = 107.24 ± 1.73). It was noted that 1% w/v KSR beads showed the most favorable results, with drug release being sustained up to 360 minutes, and a high concentration of drug released from the beads ($K1B_{(360min)}$ = 92.29 ± 0.08%, MDT_{K1B} = 98.34 ± 0.63).

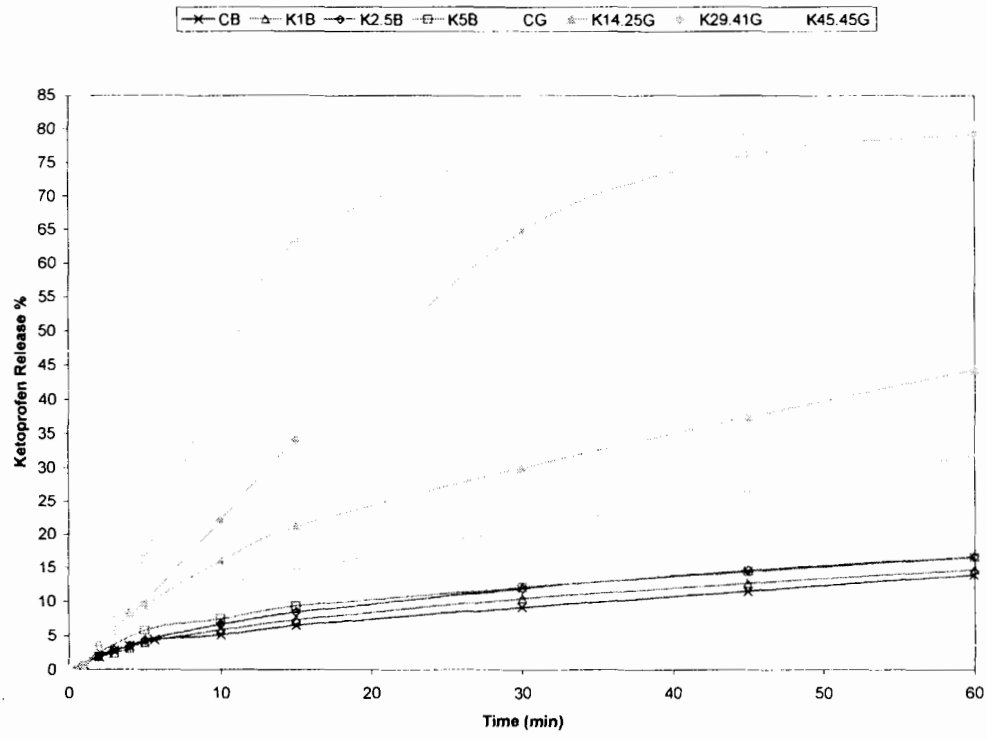

Figure 3.6: Results of dissolution study on chitosan bead and granules formulations containing increasing amounts of KSR after 60 minutes in 0.1 M HCl.

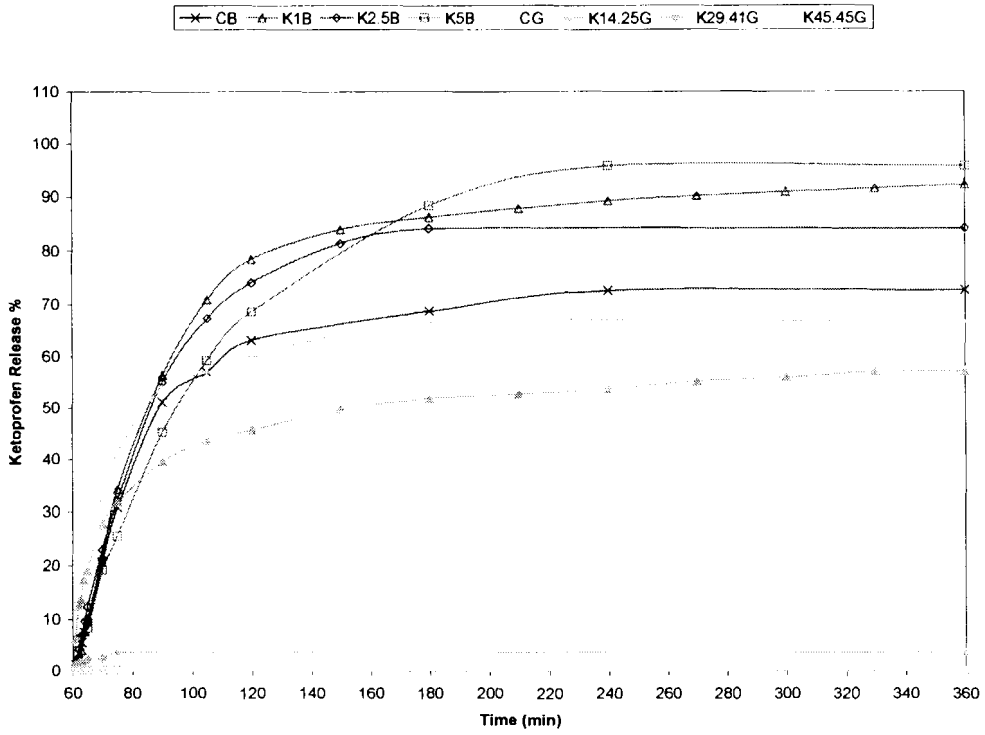

Figure 3.7: Results of dissolution study on chitosan bead and granules formulations containing increasing amounts of KSR after 360 minutes in PBS pH 7.2.

4. Conclusion

It was possible to prepare chitosan beads and granules with high concentrations of KSR. KSR weakened the characteristics of the granules by increasing friability, and drug release. This was found to be as a result of the decreasing concentration of the binder chitosan with increasing amounts of KSR. Chitosan beads prepared by the method of Bodmeier *et al.* with adjustments to pH, stirring time, and a lower chitosan dispersion to TPP ratio, offered poor resistance to mechanical force, solubility, degradation and only moderately controlled drug release. Inclusion of KSR into the bead formulations produced statistically similar release profiles for low concentrations of KSR added to the beads, and significantly increased the MDT –value of the dissolutions with higher concentrations. Additional KSR enhanced the characteristics of the beads, lowered solubility and friability, regulated swelling and controlled degradation. With low levels of KSR there was also no statistical increase in the MDT-values of controlled drug release samples.

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LIST OF FIGURES

Figure 1-1: Representation of a reservoir diffusion controlled release system (Sigma Aldrich, 2004). 7

Figure 1-2: Representation of a monolithic diffusion controlled release system (Sigma Aldrich, 2004). 7

Figure 1-3: Representation of a biodegradable controlled release system (Sigma Aldrich, 2004). 8

Figure 1-4: Chitosan production flow chart (Paul & Sharma, 2000). 15

Figure 1-5: The chemical structure of chitosan (Adapted from Egawa *et al.*,1999:238). 15

Figure 2-1: Structure of Ketoprofen (British Pharmacopoeia: 2002)..... 28

Figure 2-2: Effect TPP-solution pH on percentage drug loading (%DLC)..... 32

Figure 2-3: Effect of chitosan-drug dispersion to external TPP-phase ratio on the percentage drug loading (%DLC)..... 33

Figure 2-4: Example of a standard curve plotted for ketoprofen in 0.1M HCl. 41

Figure 3-1: Full view of a ketoprofen loaded bead, prepared in TPP, pH 9.0 (a), and a ketoprofen loaded chitosan bead, prepared in TPP pH 5.0 (b) (CB)..... 47

Figure 3-2: Cross sectional view of a ketoprofen loaded chitosan bead prepared in TPP, pH 9.0 (a), and a ketoprofen loaded chitosan bead, prepared in TPP, pH 5.0 (b) (CB)..... 47

Figure 3-3: Full view of 1 (a), 2.5 (b) and 5% w/v (c) Aqoat® AS-HF beads (A1B, A2.5B, and A5B)..... 47

Figure 3-4: Full view of 1 (a), 2.5 (b) and 5% w/v (c) Eudragit® S100 beads (E1B, E2.5B, and E5B)..... 48

Figure 3-5: Full view of 1 (a), 2.5 (b) and 5% w/v (c) Kollidon® SR beads (K1B, K2.5B, and K5B)..... 48

Figure 3-6: Cross-sectional view of 1 (a), 2.5 (b) and 5% w/v (c) Aqoat® AS-HF beads (A1B, A2.5B, and A5B)..... 48

Figure 3-7: Cross-sectional view of 1 (a), 2.5 (b) and 5% w/v (c) Eudragit® S100 beads (E1B, E2.5B, and E5B)..... 49

Figure 3-8: Cross-sectional view of 1 (a), 2.5 (b) and 5% w/v (c) Kollidon® SR beads (K1B, K2.5B, and K5B)..... 49

Figure 3-9: Full view of ketoprofen loaded chitosan granule (CG)..... 49

Figure 3-10: Full view of 1 (a), 2.5 (b) and 5% w/v (c) Aqoat® AS-HF granules (A1G, A2.5G, and A5G)..... 50

Figure 3-11: Full view of 1 (a), 2.5 (b) and 5% w/v (c) Eudragit® S100 granules (E1G, E2.5G, and E5G)..... 50

Figure 3-12: Full view of 1 (a), 2.5 (b) and 5% w/v (c) Kollidon® SR granules (K1G, K2.5G, and K5G)..... 50

Figure 3-13: Graphic representation of percentage drug loading (%DLC) of bead formulations..... 55

Figure 3-14: Percentage friability of bead samples..... 57

Figure 3-15: Percentage solubility of ketoprofen loaded beads..... 59

Figure 3-16: Percentage solubility of ketoprofen loaded granules..... 60

Figure 3-17: Degree of swelling for Aqoat ® AS-HF formulations. 64

Figure 3-18: Degree of swelling for Eudragit ® S100 formulations. 64

Figure 3-19: Degree of swelling for Kollidon® SR formulations..... 65

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 (Ritger and Peppas 1987:31)..... 73

Figure 4-2: Example of a standard curve plotted for ketoprofen in 0.1 M HCl..... 76

Figure 4-3: Example of a standard curve plotted for ketoprofen in PBS pH 7.2..... 76

Figure 4-4: Ketoprofen release from beads containing 1% w/v CR polymer and granules containing 14.29% w/w CR polymer during the first 60 minutes in 0.1 M HCl..... 80

Figure 4-5: Ketoprofen release from beads containing 1% w/v CR polymer and granules containing 14.29% w/w CR polymer for the remainder of the study in PBS minutes in 0.1 M HCl..... 80

Figure 4-6: Ketoprofen release from beads containing 2.5% w/v CR polymer and granules containing 29.41% w/w CR polymer during the first 60 minutes in 0.1 M HCl..... 85

Figure 4-7: Ketoprofen release from beads containing 2.5% w/v CR polymer and granules containing 29.41% w/w CR polymer for the remainder of the study in PBS pH 7.2..... 85

Figure 4-8: Ketoprofen release from beads containing 5% w/v CR polymer and granules containing 45.45% w/w CR polymer during the first 60 minutes in 0.1 M HCl..... 89

Figure 4-9: Ketoprofen release from beads containing 5% w/v CR polymer and granules containing 45.45% CR polymer for the remainder of the study in PBS pH 7.2..... 89

Figure 4-10: Total time of release for formulations with optimum enteric release characteristics. 94

LIST OF TABLES

Table 1-1:	Features unique to controlled release dosage forms (Adapted from Thrombre, 1991:156).	4
Table 1-2:	Characteristics of available Aqoat® coatings.	21
Table 1-3:	Characteristics of available Eudragit® coatings (Degusa.2004)...	23
Table 2-1:	Composition of the granules used in the study.	38
Table 3-1:	Degree of swelling (E_{sw}) of bead samples prepared in the study in 0.1 M HCl and PBS pH 7.2.....	63
Table 3-2:	Degree of swelling (E_{sw}) of bead formulations prepared in the study in 0.1 M HCl and PBS pH 7.2.	59
Table 4-1:	Amount of ketoprofen (in μg) per 0.140g of dissolution formulation as calculated from the drug loading capacity (DLC).....	75
Table 4-2:	Dissolution profiles of 0.1 M HCl time 0 - 60 minutes.....	77
Table 4-3:	Dissolution profiles of PBS pH 7.2 time 60 - ∞ minutes.....	78
Table 4-4:	Similarity factor for all samples in 0.1 M and PBS pH 7.2 vs. polymer free beads and granules.....	79

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