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A chitosan-polymer hydrogel bead system for a metformin HCI controlled release oral dosage form

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A Thesis

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A Chitosan–Polymer Hydrogel Bead System For A Metformin HCl

Controlled Release Oral Dosage Form

by

Sanjeev Dogra

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

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An Abstract of

A Chitosan–Polymer Hydrogel Bead System For A Metformin HCl Controlled Release Oral Dosage Form

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The hydrogels or polyelectrolyte systems are based on ionic cross linking. They have been employed to prepare a controlled release dosage forms. These systems are based upon the fact that their structure can entrap the drug within them. They normally employ a hydrophilic matrix system.

Metformin hydrochloride (MH) a Class III drug was used as the model drug. Metformin hydrochloride (MH) is a highly water soluble and low permeability drug. Thus, a controllable dosage form system for the controlled release of metformin hydrochloride (MH) was attempted. Metformin hydrochloride (MH) loaded chitosan polyelectrolyte complex (PEC) hydrogel beads were prepared via ionotropic crosslinking with sodium tripolyphosphate (TPP). A combination of various polymers were studied with chitosan having metformin hydrochloride (MH) dispersed within them. Thus, metformin hydrochloride (MH) dispersed in 1.5% glacial acetic acid and having chitosan and polymers dispersed within it was cross linked with 1% sodium tripolyphosphate solution adjusted to a pH of 6.0-6.4. The beads prepared were examined for the optimal stirring conditions and curing time in order to obtain spherical beads. The prepared beads were filtered and kept at 35^{o}C overnight to dry. The ratio of polymer: drug combination was

kept around 1:1. The physical and chemical characterization of the resultant beads was done to determine their particle shape, particle diameter, weight, percent swelling, DSC studies, drug entrapment efficiency (DEE) as well as in vitro release. Fourier Transform Infrared (FTIR) and Powder X-ray Diffraction (PXRD) studies were performed to determine any possible drug polymer interaction. The effect of incorporating polymers such as sodium alginate, xanthan gum, hydroxy propyl methyl cellulose, and hydroxy ethyl cellulose on the physico-chemical properties of the beads were also studied. Spherical to oval beads with varying particle size, weight, DEE, and sustained release profile were obtained depending on the polymer combination used. These beads were able to sustain the release of the metformin HCl from the beads. The in vitro dissolution studies were done to assess the release pattern of the drug from the beads over a five to six hour period. A sample volume of 5 mL was taken from the dissolution apparatus and the same amount was replaced with an equal volume of phosphate buffer to maintain the sink conditions. The in vitro dissolution rate profile showed a sustained release of the drug from the beads. The dissolution profile was observed to be similar in nature for all the polymers studied. The chitosan beads were subjected to stability studies for one month. The stability results for the chitosan beads after one month were shown to be favorable with respect to providing a possible controlled release dosage form from the beads.

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Chapter One

Introduction

For many disease states, the ideal dosage regimen is one in which an acceptable therapeutic concentration of the drug is immediately attained at the site of action and is maintained constant for the desired duration of treatment. Conventional dosage systems release the complete drug immediately after its administration. These systems have limitations including plasma concentration fluctuations over successive dosing intervals. For drugs having short biological half lives, frequent doses are required to maintain the steady state plasma concentrations within the therapeutic range.

Thus, for such drugs, maintaining a therapeutic plasma concentration becomes a problem especially in the case of missing doses and the overnight no dose period.

Thus, modified dosage forms have been found to maintain the steady state and provide a concentration over the specified period. A number of terms have been used to describe these systems. (1)

Conventional dosage form systems aim for an effective concentration. However, these dosage systems have the disadvantages of frequent dosage administration and varying half life.The dosing interval may not be appropriate for the biological half life of the drug. This will result in large peaks in the blood level. For example, drugs with short half lives require frequent dosing to sustain constant therapeutic levels.

1. The drug levels may not be within the therapeutic range at sufficiently early times. This is an important consideration for certain disease states.
2. Patient non compliance with a multiple dosing regimen can result in the failure of this therapeutic approach.

Thus, sustained release dosage forms have been proven to be a better choice in overcoming these problems.

A conventional dosage form releases its active ingredient(s) into an absorption pool immediately. Their kinetic pattern is as follows in Figure 1.1.

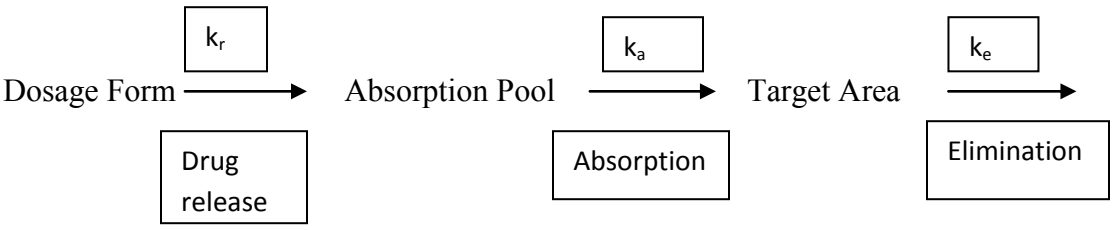

Figure 1-1: Conventional dosage form release pattern

The absorption pool has drug available already in solution and ready for absorption at the site of absorption. The k_r, k_a, and k_e are first order rate constants for drug release,

absorption, and overall elimination, respectively. Immediate release from a conventional dosage forms has $k_r >>> k_a$. For non immediate release dosage forms $k_r << k_a$ (i.e., the release of the drug from the dosage form is the rate limiting step). The kinetic pattern is seen in Figure 1.2. (2)

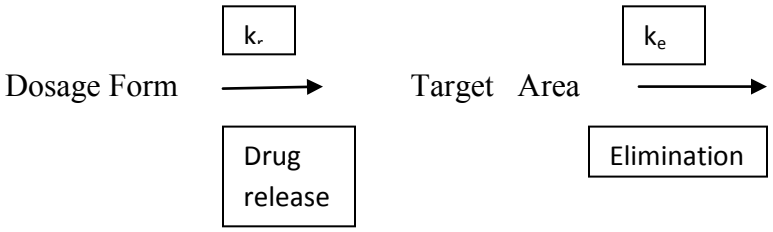

Figure 1-2: Non immediate release dosage pattern

1.1 Types of Non Immediate Release Dosage Forms

A non immediate release dosage form alters the release rate by affecting the value of k_r. A non immediate release dosage form may be conveniently divided into four categories:

1. Delayed release
2. Sustained release
    a. controlled release
    b. prolonged release
3. Site specific release
4. Receptor release

Delayed release employs a repetitive, intermittent dosing of drugs from one or more immediate release units incorporated into a single dosage form. These types of dosage forms include repeat action tablets and capsules, and enteric coated tablets where timed release is achieved by barrier coating.

Sustained release dosages are drug delivery systems that achieve slow release of the drug over an extended period of time.

If the system is successful in sustaining a constant drug level in the body or target tissue, it is considered a controlled release system.

If it is unsuccessful in providing a constant level, but extends the duration of action over that which is achieved by conventional delivery, it is known as prolonged release system.

Site specific and receptor release refers to the targeting of a drug directly to a certain biological location.

For site specific release, the target should be a certain organ or tissue. For receptor release, the target should be the particular receptor for a drug within an organ or tissue.

1.2 Drug Release from Controlled Oral Dosage Forms

The purpose of a sustained release system is to deliver a drug at a rate necessary to achieve and maintain a constant drug blood level.

This means that the rate of delivery should be independent of the amount of drug remaining in the dosage form and constant over a certain time. It implies that the rate

should follow zero order kinetics. Zero order release may be theoretically desirable. Non zero order release rates may be clinically equivalent to constant release in many cases. (3)

In order to achieve a therapeutic level promptly and maintain that level for a given period of time, the dosage form generally should consist of two parts.

With controlled oral dosage forms, the total drug in the dosage form should consist of two portions, a loading dose and a maintenance dose. The initial loading dose is released immediately on its administration. The release of the drug is characterized by a first order kinetic process. The loading dose immediately obtains the acceptable therapeutic plasma levels. The remaining dosage is released at a slow and a controlled rate in order to maintain the constant plasma concentration of the drug.

The release pattern has to follow zero order kinetics. Therefore, the rate of release of the drug is independent of the remaining fraction of the dose. The controlled oral dosage form involves releasing the maintenance dosage at a rate which is equivalent to the elimination rate of the drug. (4)

1.3 Methods of Preparing Controlled Oral Dosage Forms

A number of formulation methods have been developed to overcome the barrier seen with immediate release oral dosage forms. These processes include: inert insoluble matrices; use of coatings; hydrophilic matrices; as well as the combination of hydrophilic and hydrophobic polymers (5); embedding of the drug in a wax or plastic matrix; ion exchange resins; osmatic pumps; and microencapsulation. The physiology of the gastrointestinal tract, the physico-chemical property of the drug, the drug release pattern, the pharmacological action of the drug, are parameters that must be considered. The

physico-chemical properties of the drug involve such parameters as: aqueous solubility; stability; pK_a and permeability values.

The Biopharmaceutical Classification System (BCS) involves placing a drug into four classifications (6):

1. High solubility and high permeability
2. Low solubility and high permeability
3. High solubility and low permeability
4. Low solubility and low permeability

Class 1 is considered the preferred category while Class 4 is the worst category.

A drug having high solubility in the intestine is a good drug for a controlled oral dosage form. The drug permeability value must also be considered and should be more then the prescribed value.

The drug having a biological half life of between two and six hours is the preferred drug because we want to avoid accumulation of the drug in the body. (7)

1.4 Drug Release Patterns

The two main patterns involving drug release are dissolution of the active component and diffusion of the dissolved or solubilized species. These processes have been shown to involve:

1. Hydrating of the device (swelling of the hydrocolloid or dissolution of matrix).
2. Diffusion of water into the system.
3. Dissolution of the drug.
4. Diffusion of the dissolved drug out of the matrix.

Drug release can be constant, declining or bimodal.

1. **Declining release**. The release of the drug from such models is a function of the square root of time or follows first order kinetics. These systems do not provide constant plasma concentration but can give a sustained release dosing pattern.
2. **Constant release**. These systems involve the release of drug at a constant rate. They depend on the diffusion of the drug upon osmosis'absorption.
3. **Bimodal release**. These systems provide a rapid initial delivery of the drug followed by a slower rate and an increased rate at a later time. (8)

Matrix Devices

The rate of release of a drug dispersed as a solid in an inert matrix has been described by Highuchi. The drug release rate is described as follows:

$$M = kt^{1/2} \qquad \text{Equation 1.1}$$

Where K is a constant. A plot of the amount of drug released (M) versus the square root of time should be linear if the release of the drug from the matrix is diffusion controlled. (9)

1.5 Controlled Oral Dosage Form Systems

These systems can be placed under the following headings:

Monolithic Matrix Delivery Systems

These dosage systems have:

1. Drug particles dispersed in a soluble matrix, with the drug becoming available as the matrix dissolves or swells and dissolves.
2. Drug particles dispersed in an insoluble matrix, with drug becoming available as the solvent enters the matrix and dissolves the particles.

Swellable polymers can be used to make monolithic matrix systems for controlled release dosage forms. The swellable matrix systems are also known as diffusion controlled systems. These systems have several rates balancing each other: polymer swelling rate, drug dissolution and diffusion rate, and the polymer erosion rate. (10)

Matrix Tablets

These are a type of controlled drug delivery system. They release the drug in a continuous manner. They release the drug by both dissolution controlled as well as diffusion controlled mechanisms. For controlling the release of the drugs having different solubility properties, the drug is dispersed in a swellable hydrophilic substance. This can become an insoluble matrix of rigid nonswellable hydrophobic materials or plastic materials. (11)

Chapter Two

Polyelectrolyte Complexes or Hydrogels

2.1 Introduction

Metformin hydrochloride is an oral antidiabetic drug used for the treatment of Type 2 diabetes. It belongs to BCS Class III (i.e. high solubility and low permeability). The present study involves preparing a controlled release dosage form of metformin hydrochloride using hydrocolloid polymers and chitosan in a 1% sodium tripolyphosphate solution.

The polymers used have been found to be more of a thickening and gelling agent. Chitosan is a natural polysaccharide and has been widely used as a polymeric drug carrier excipient in the dosage form since it has good biocompatibility, biodegradability and low toxicity and is naturally abundant.

Chitosan is positively charged. It associates with negatively charged polyions in solution to form polyelectrolyte complexes or beads. These chitosan beads show favorable physicochemical properties. They are excipients for the design of various types of dosage forms. This study describes the complexation of chitosan with selected natural and

synthetic polyanions and demonstrates some of the factors that influence the formation and stability of these hydrogel beads.Hydrogels can respond to their surrounding conditions such as pH, ionic strength, temperature, and electric current. The pH-sensitivity of a hydrogel is an important factor in designing polymers for controlled drug release in the gastrointestinal tract. (1) The GIT has a variety of pH's including the stomach (pH 1.1 at rest and 2-3 when at work) to the intestine (pH 5-7). In recent years, chitosan has been used as an excipient to prepare a sustained release dosage form for water soluble drugs and to enhance the bioavailability of poorly water-soluble compounds.

Various factors potentially influence the drug release (chitosan concentration as well as the type and amount of cross-linking agent) were studied. The controlled release dosage form of the drug utilizing this formulation was found to deliver the drug within 2 hours while the whole study lasted for 5~6 hours. The difference in the drug release behavior can be attributed to the differences in the ionic interaction between the oppositely charged ions and the concentration of the drug within the beads.

The purpose of this study was to prepare and evaluate the chitosan gel beads as a possible controlled drug release system for metformin hydrochloride. The study also investigated the conditions under which the polymer bead is formed, and hence the dependence of the drug releases on bead formation. We also reported the effects of the agents forming the matrix on in vitro dissolution profile of the metformin hydrochloride controlled-release beads as well as to characterize the chitosan beads using DSC, PXRD, FTIR, and dissolution studies.

The chitosan beads have a homogeneous crosslinked structure. The polyanionic polymers interact with cationic chitosan on the surface of these sodium tripolyphosphate (TPP) solutions to form a polyelectrolyte complex film. This polyelectrolyte complex film provides the improvement of the drug's sustained release and metformin loading. The loading efficiency was very high. Thus TPP/ Chitosan beads prepared were found to have greater metformin drug loading in the beads. Chitosan beads have been used due to their ability to deliver drugs. Their advantage is that chitosan beads focus on making beads with polymers for drug entrapment.

2.2 Chitosan Based Polyelectrolyte Complexes as Potential Carrier Materials in Drug Delivery Systems (1)

Upon mixing oppositely charged polyelectrolyte in solution, spontaneous association takes place in the form of a strong and reversible electrostatic link.

Basically, these direct interactions between the polymeric chains lead towards the formation of a polyelectrolyte complex network with non- permanent structures. These hydogels or polyelectrolyte complexes are well tolerated, biocompatible and more sensitive to changes in their environmental conditions. (2, 3) The formation and stability of the polyelectrolyte complexes depend on many factors such as: the degree of ionization of each of the oppositely charged polyelectrolyte; the charge density of the polyelectrolyte; the charge distribution over the polymeric chains; the concentrations of the polyelectrolyte; its mixing ratio; the mixing order; the duration of the interaction; the nature of the ionic groups; the position of the ionic group on the polymeric chains; the

molecular weights of the polyelectrolyte; the polymer chain flexibility and temperature; as well as ionic strength and pH of the reaction medium. (4-6)

Depending on the ratio in which the polyelectrolyte is mixed, there may be an excess of one charge (either positive or negative), and a non-stoichiometric complex is formed which is usually soluble.

On the other hand, if a stoichiometric polyelectrolyte complex contains the same amount of each charge with a zero net charge in the resultant complex, then the stoichiometric complex will be normally insoluble and precipitate out of the solution upon formation. (8)

Chitosan belongs to a series of polymers that are deacetylated derivatives of the natural polysaccharide, chitin, having different degrees of deacetylation and molecular weights. It is comprised of β -1,4 –linked glucosamine (deacetylated units) and N–Acetyl–D-glucosamine (acetylated units) with degrees of deacetylation between 70% and 95% and molecular weights between 10 and 1,000 KDa. (9, 10)

The cationic amino groups on the C2 position of the repeating glucopyranose units of chitosan can interact electrostatically with anionic groups, normally carboxylic acid groups of other polyions, to form polyelectrolyte complexes. A number of different polyanions of natural origin including pectin, alginate, xanthan gum, carboxymethyl cellulose or from synthetic origin including polyacrylic acid and polyphosphoric acid. These materials are used with chitosan to make the polyelectrolyte complexes and to provide the required physicochemical properties for the design of specific drug delivery systems. (3)

2.3 Polyelectrolyte Complexes Between Chitosan-Alginate Polyelectrolyte Complex

Alginate is a natural, linear, unbranched, biodegradable polysaccharide consisting of 1, 4 linked β-d-mannuronic acid and alguluronic acid monomers in various proportions. The negatively charged carboxylic acid groups of manuronic and guluronic acid units of alginate interact electrostatically with the positively charged amino groups of chitosan to form polyelectrolyte complexes. Many studies have been done using alginate as an anionic polyelectrolyte in the complexation with chitosan or as a polyelectrolyte complex formed by electrostatic linkages between these two polymers. They are biodegradable and biocompatible and also strong enough at lower pH values where chitosan dissolves. (11)

The charge ratio, ionic strength, molecular weight, pH, mixing order, diameter and speed of the dispersing elements have been shown to effect the particle size, particle surface charge (zeta potential) and the stability of the alginate chitosan polyelectrolyte complexes. (12)

Chitosan alginate polyelectrolyte complexes have demonstrated good results for controlling the release of charged molecules and exhibit high encapsulation efficiencies for these molecules. (13) Compared with the matrix type tablets made by using polyelectrolyte complexes between chitosan and alginate and the others formed between chitosan and carrageenan, they showed better results for the formed complex in prolonging diltiazem release at lower concentrations of the polymers. (14)

2.4 Chitosan–Pectin Polyelectrolyte Complex

Pectin is a linear polysaccharide which consists of α 1, 4 linked D galacturonic acid units, interrupted with a highly branched region in the polymer chain. (1)

The acidic chitosan and pectin solutions on mixing make a homogenous solution without any ionic interaction between the polymers.

A polyelectrolyte complex can be obtained on adjusting the pH of the mixture to around pH 5.5. The electrostatic interaction occurred between the negatively charged carboxylic groups of pectin and the positively charged amino groups of the chitosan. The extent of the interaction depends on the pH of the surrounding medium that determines the extent of ionization of the polymers. (15)

2.5 Chitosan-Xanthan Gum Polyelectrolyte Complexes

Xanthan gum is an exopolysaccharide secreted from the plant Xanthomonas campestris. It is comprised of a cellulose backbone β–(1, 4)–D- glucopyranose glucan, with a trisaccharide side chain, namely (3, 1) α-D-mannopyranose- (2, 1) β-D-glucuronic acid – (4, 1) β- D-mannopyranose, on every glucose residue. (16)

Results from modulated differential scanning calorimetry analysis and the swelling degree of the microcapsules prepared from chitosan–xanthan-gum polyelectrolyte complexes showed the cross linking density was interdependent of xanthan concentrations, chitosan concentration and the chitosan solution pH. (17)

The kinetic analysis using rheological measurements of chitosan–xanthan polyelectrolyte complex formation indicated that the coacervate is formed in two distinctive steps.

The kinetic curve indicated a classic sol-gel transition and the hydrogel is organized in a quasi-ordered network during coacervation. (18)

After a certain mixing period, the hydrogel was obtained which showed a structural modification and a stable storage modulus with a solid like behavior. The mechanical grinding of the freeze dried polyelectrolyte complex made a powder having particles with a suitable diameter for pharmaceutical applications. (18)

2.6 Chitosan-Polymethacrylate Copolymer (Eudragit^{R}) Polyelectrolyte Complexes

Polymethacrylate copolymers or Eudragit^{R} are commonly used as coating agents for oral dosage forms such as capsules or tablets. (1)

There are different types of Eudragit^{R} polymers which are synthetic methacrylic acid copolymers comprising of various ratios of dimetylaminoethylmethacrylates, methacrylic acid and methacrylic acid esters. Some are polycations (Eudragit E, RL, RS and NE) and are formed due to the presence of dimethylamino groups or quaternary amino groups. Others are polyanions (Eudragit L and S) and are formed due to the presence of carboxylate groups. (19, 20)

Polyelectrolyte complexes formed between chitosan having different molecular weights and Eudragit L100 or Eudragit L100-55 were compressed into matrix type tablets having diclofenac sodium as the model drug. (21) The polyelectrolyte complexes were studied for their molar ratio, structure, swelling and drug release profiles. The results showed the potential of the polyelectrolyte complex for producing a controlled release drug delivery system. The polymer ratio, (Eudragit type) and molecular weight of the chitosan effects the drug release rate from the matrix type tablets. (21)

2.7 Guar Gum Hydrogel

Guar Gum is used as a binder and disintegrating agent in solid dosage forms and also as a thickening and stabilizing agent in liquid products. (22) Baveja et al. (23) explored guar gum for sustained release dosage form applications. It was found to be a release retardant for phenylpropanolamine (24), theophylline (25), diltiazem hydrochloride (26, 27) and ionized sustained release tablets. (28) Recently it was used to prepare a three-layer matrix tablet for the controlled release of trimetazidine dihydrochloride as demonstrated by Krishnaiah et al. (29)

Guar gum is also used as a hydrophilic polymer to sustain the drug delivery of water soluble drugs. (30) It was also previously reported that guar gum is a potential hydrophilic matrix carrier for oral controlled delivery of drugs with varying solubility. (31)

2.8 Tripolyphosphate (TPP)

The ionic crosslinking interaction has been found to be more advantageous when compared to chemical cross linking. Chitosan beads, micro or nano particles, can be produced by ionic cross linking with tripolyphosphate (TPP). (32, 33, 34) Shu and Zhu (35, 36) reported that chitosan beads, prepared with TPP not only increased the drug loading efficiency, but also prolonged the drug release period. They also showed that citrate cross linked chitosan possessed a pH sensitive swelling and drug controlled release properties.

TPP is a non toxic and multivalent anion. (37) It can form a gel by ionic interaction between the positively charged amino groups of chitosan and the negatively charged

counter ion of TPP. (35, 36, 38, 39) The charge density of TPP and chitosan controls the interaction. The charge density is also dependent on the pH of the solution. The chitosan matrix depends on the molecular weight of chitosan. Puttipipakhachorn et al. (40) reported that the higher the molecular weight and degree of deacetylation of chitosan, the lower the release rate from the chitosan film.

2.9 Hydrogels

Hydrogels have been in existence for a long time. (41) The use of hydrogels has been extended to a number of biomedical (42) and pharmaceutical applications. (43) They closely look like tissue in their physical properties due to the high water content and rubbery consistency. The ability of materials of different molecular size to diffuse into the hydrogels and come out from the hydrogels makes these dry or swollen polymeric networks a good drug delivery system for oral administration. The hydogels can also be used as controlled drug delivery systems. (44)

They are a cross linked networks comprised of hydrophilic polymers. They can absorb a lot of water, but still maintain its three dimensional structure. (45) They swell in aqueous media as it dissolves in this same aqueous media to make a polymer solution. Basically, the hydrophilicity of the polymer makes them water attractive models. They have a physical and chemical cross linking network, giving them water insoluble behavior. They are just like a polymer which combines with water to form a solid with water-like properties which demonstrates permeabilities for many water soluble substances.

2.10 Preparation of Hydrogel Based Drug Delivery Systems

The preparation of a hydrogel based drug formulation system is done either by its crosslinking of polymers or polymerization of monomers and cross linking with poly functional monomers. (46, 47) Synthetic, semi synthetic or natural source based polymers can be used to make hydrogels. Normally polymers having hydroxyl, amine, amide, ether, carboxylate and sulfonate as their functional groups in its side chain are employed in this method. (45)

2.11 Drug Release Mechanism

The hydrogels are primarily glassy in nature in its dehydrated state. The release of drug involves a simultaneous absorption of water and desorption of drug via a swelling controlled mechanism. (48) The rate determining factor is how the polymer resists an increase in volume and a change in shape. (46) A glassy hydrogel on its interaction with an aqueous medium permits the aqueous medium to penetrate into the free spaces on the surface between the macromolecular networks. When a lot of aqueous medium enters the hydrogel, its glassy transition temperature drops to the experimental temperature. The presence of an aqueous medium in a glassy polymer causes a stress which leads to an increase in gyration radius and the end to end distance of the polymer molecules is seen as swelling. The penetration of the aqueous medium taken into the systemic velocity pattern causes an increase in the thickness as a swollen area which simultaneously takes place in the opposite place**.** The pH responsive drug release pattern is styled in a monophasic or pulsatile pattern. The peroral controlled dosage form is designed to have

uniform drug release with an increase in pH gradient in different parts of the gastrointestinal lumen.

2.12 Hydrogels as Stimuli Responsive Drug Delivery Systems

The drug release profile for these dosage forms should meet the physiological conditions and variations of the site of the release. Basically, a required dosage form should react to physiological requirements, sense the changes and accordingly adjust the drug release profile. A disease state is associated with rhythmic pattern alterations and hence the required dosage form should follow the same pattern. The drug release pattern should be optimized, in order to avoid side effects, which is associated with the extra drug release. Hydrogels can show changes in their swelling pattern, network structure, and permeability with respect to different stimuli, both internally and externally to the body. (49)

2.13 Hydrogels as pH Responsive Drugs Delivery Systems

Changes in pH occur as the drug passes through the GIT (50) and therefore a pH responsive drug is required. The local pH changes can be used to release the drug profile for controlling the drug release. Peroral controlled drug delivery requires that the pH be responsive to the drug delivery system. (51, 52)

A pH responsive hydrogel comprises the polymeric backbone with an associated ionic dependant group. On contact with an aqueous medium of appropriate pH and ionic strength, the dependant groups ionize and evolve into charges on the polymer network. This, in turn, develops an electrostatic repulsive force responsible for the pH dependent swelling or deswelling of the hydrogels (44) and thus controls the drug release pattern.

pH changes cause a change in the mesh size of the polymer chain. Pendant groups of the anionic hydrogels are unionized below and ionized above the pK_a of the polymer matrix. This leads to a swelling at a pH above the polymer pK_a due to the presence of ions which causes a large osmotic swelling force. The cationic hydrogels swell at a lower pH. (41)

2.14 Hydrogels Factors Influencing the pH Responsive Swelling

The factors that commonly affect the swelling of hydrogels include the properties of the polymer such as charge, concentration and pK_a of the ionizable group. It is also effected by the degree of the ionization, density of the cross linking as well as hydrophiliticity or hydrophobicity. The swelling medium properties include pH, ionic strength, counterion and its valency. (53)

Apart from the polymer and swelling medium properties, the physical structure of the network also contributes towards the hydrogen swelling. During this study the pH sensitive semi IPN (an interpenetrating network) of chitosan and polyvinylpyrolidone were used for the controlled release of amoxicillin. (54)

2.15 Sodium Alginate Complex

Sodium alginate is employed to prepare gel beads for the delivery of biomolecules such as drugs, peptides and proteins. (55, 56) The gel beads were prepared by sol-gel transformation of the alginate. They were produced by the crosslinking of alginate with different cations such as Ca^{2+} and Zn^{2+}. (57) The alginate matrix, which has an open lattice structure, forms porous beads. (58) Chitosan is a polycationic polysaccharide derived from chitin, whereas alginate is polyanionic in nature. (59)

Because of the gel forming property, it is commonly used in designing drug delivery systems. (60, 61, 62, 63) Chitosan is commonly reacted with tripolyphosphate in the preparation of the beads. In order to improve the limitations faced by pure alginate and chitosan bead systems, an alginate–chitosan polyelectrolyte complex was introduced. (64, 65, 66) Alginate-chitosan beads were prepared by the ionic interaction between carboxyl residues of alginate and the amino terminals of chitosan. Alginate forms a complex with chitosan, and decreases the porosity in the beads which decreases the leakage of the encapsulated drugs. (67, 68) Apart from this, chitosan gains a higher level of mechanical strength with the alginate gel mass. (59)

Chapter 3

Optical Microscopy

3.1 Introduction

There are many methods available for particle size measurements. One of these is microscopy which gives the actual size of particles. Microscopy is a technique which uses microscopes to view samples and objects that cannot be observed with the naked eye. Magnification is the principal aim of microscopy. (1) The microscope is used to observe shape, size and physical morphology of the specimen. It can be done with a small quantity of the sample.

Microscopic methods can be divided into three main instrumentation categories.

1. Optical microscopy
2. Scanning electron microscope
3. Transmission electron microscope.

3.1.1. **Optical Microscope** Optical microscopy, using an optical microscope, is a very old technique. It involves the use of visible light lenses. The microscope used should have the following parts:

1. Stage with a slide holder
2. Objective lenses
3. Illuminator
4. Eye pieces of 10X, 15X or larger magnification
5. Condenser lens
6. Diaphragm

Figure 3-1: Optical microscope (2)

3.1.2 The Eye Clops Bionic Eye Magnifier

A digital magnifying glass can also be used to capture optical images. The Eye Clops Bionic Eye Magnifier and Multizoom, manufactured by Jakks Pacific was used to capture images of the product prepared. It is the size and shape of a softball, with a short pistol

grip. The ball shape holds a fat camera, surrounded by LED bulbs. It has great magnification potential with an in- focus and out- of- focus (depth of field) being a matter of a tenth of a millimeter. The Eye Clops magnifier is equipped with a white translucent shell, which can be minutely adjusted for quick focus-adjustment. The Eye Clops magnifies objects 200-400 times their actual size. (3)

The Eye Clops BioniCam has a full tone LCD shade as well as a battery pack. The LCD shade can be used as a viewfinder for specimens. It has the capability of choosing one of three absolute magnifications namely 100X, 200X as well as 400X.

Operation

Once the 5 AA batteries are inserted, a USB Memory card within the BioniCam is capable of taking and storing single pictures as well as recording video clips to be shown on a computer, LCD screen or in a power point presentation. (3) The magnified images crop up in genuine time as well as full tone upon a LCD screen.

A multiclick dial offers the possibly of 100X, 200X or 400X magnification. In order to concentrate on an image, one simply turns an I.R.I.S. lens connection until we obtain the desired image. Obtaining a clear picture takes time and manipulation of the biocam appertinal assembly, especially at the top magnification. However, with the use of an appropriate base with the assistance of an easy-to-read focusing gauge, focusing of the lens can be done very quickly. The Eye Clops is a handheld bionic microscope meant to be used for magnifying small objects and capturing video clips of experiments in progress.

Figure 3-2: Accessories of Eye Clops[R]

Figure 3-3: A whole assembly of Eye Clops[R]

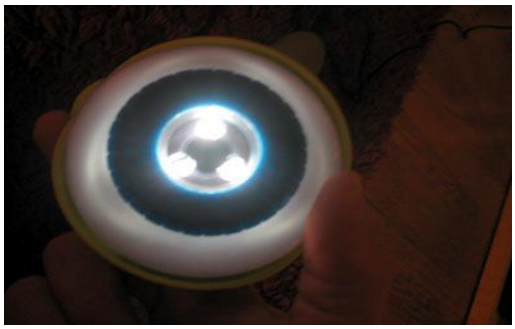

Figure 3-4: Focusing the lens of the Eye Clops

3.1.3 Scanning Electron Microscope (SEM)

Introduction

SEM is used for surface microstructure studies. (4) It takes the image of a solid specimen by using a beam of high energy electrons. The image has details such as surface morphology, and crystallographic information. (5)

The Basic Principles. It involves the interactions of electrons with the solid specimen. When electrons are bombarded onto a specimen, the electrons penetrate into the sample surface. They also lose energy by repeated, random scattering, which is known as elastic scattering. (4) It produces backscattered electrons. Any inelastic scattering transfers energy to the secondary emitted electrons. The signal is amplified by the amplifier and it is scanned by the cathode ray tube.

Instrumentation

The SEM has an electron gun, sample stage, detectors, data output devices and a vacuum system. (4) The gun generates an electron beam which accelerates at the 0.1-0.3 KeV energy level. The electron guns commonly use tungsten and lanthium hexaboride. (6) The

lenses are used to focus the electron beams. The two types of lenses used are condenser lens and objective lenses. A stigmator consists of a series of coils surrounding the electron beams.

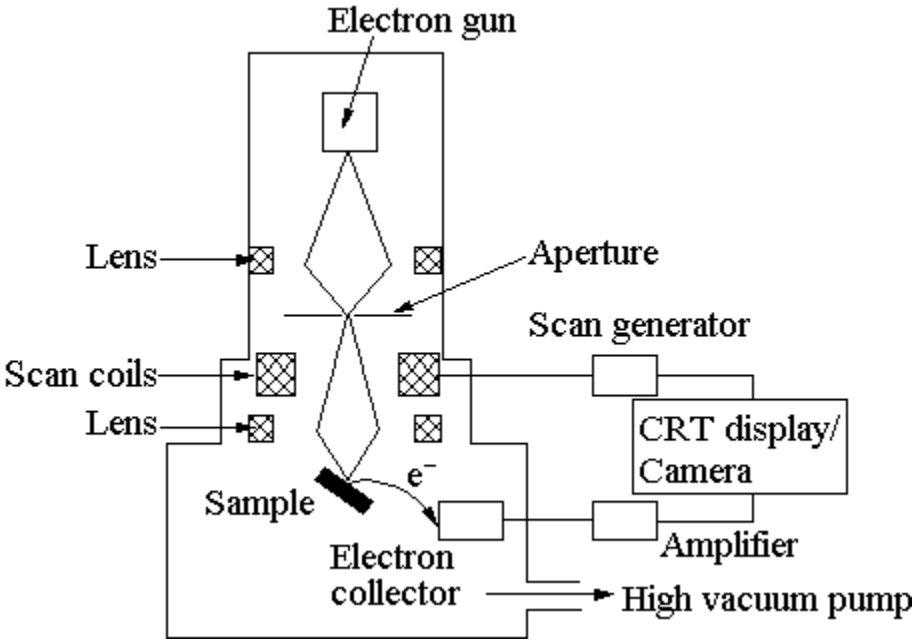

Figure 3-5: Schematic diagram of a Scanning Electron Microscope (7)

Sample Preparation

Sample preparation depends upon the sample to be focused. If it consists of conductive materials, its surface can be visualized directly. For non conducting surface samples, the most common techniques of obtaining SEM images involve coating the surface with a thin (~10 nm) metallic film produced by sputtering or by vacuum evaporation. (8)

The application of this technique is to visualize the surface morphology. SEM also finds its application in the identification of the micro fabric and crystalline orientations of materials. (4)

3.1.4 Transmission Electron Microscopy (TEM)

This process of taking images in the TEM involves the use of electrons. The image is obtained when the electrons interact with the specimen and the electrons are transmitted through it. The small details of the sample can be visualized by the TEM down to atomic levels. This makes TEM an important tool in the medical, biological and material sciences as well as in environmental geochemistry research. (9)

Basic Principle

TEM produces an image upon the interaction of the specimen with the electron beam. Visualization is due to the elastic scattering of electrons, illumination conditions, objective lens and arranged apertures. Various modes of operation can improve the image quality including bright and dark field, diffraction contrast and phase contrast. (10)

Instrumentation

The electron emission source is either tungsten or lanthanum hexaboride. (11) Electromagnets are used to accelerate and focus the electrons into a very thin beam by varying the magnetic field of the electromagnetic lenses. The microscope is interiorly evacuated to a low pressure of 10^{-4} Pa. This minimizes scattering of the electrons and

increases the mean free path. The detector normally used is a CCD camera. The TEM is seen in Figure 3.6 below:

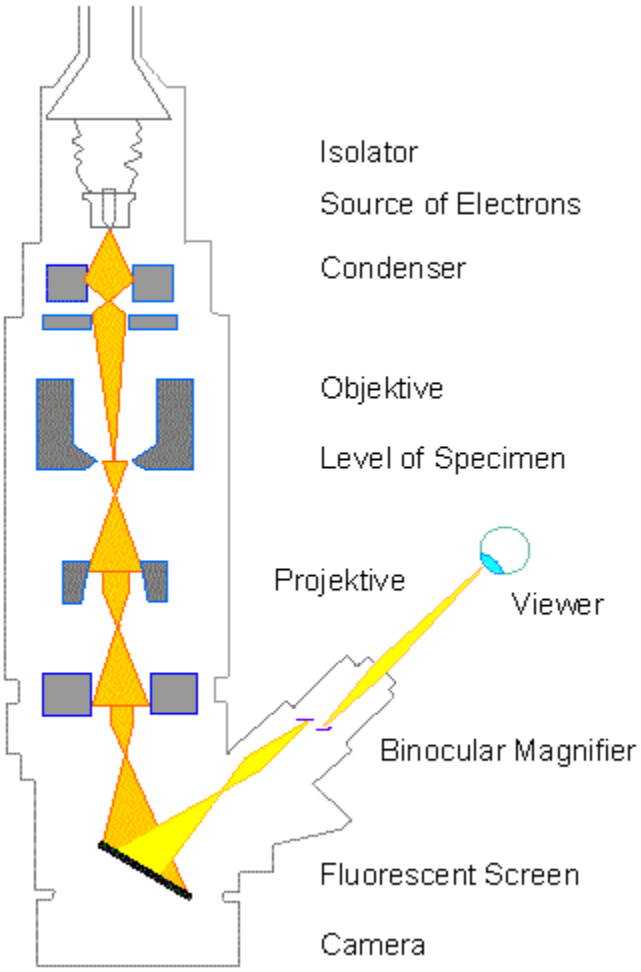

Figure 3-6: Schematic picture of a Transmission Electron Microscope (12)

Preparation of the Sample (10)

The pharmaceutical samples to be studied are powders in the solid state which are either dissolved or dispersed in a solvent and deposited onto a support mesh known as a grid. The grid is composed of copper, molybdenum, gold or platinum and is 2.5 to 3.0 mm in

diameter and 50-400 mesh. For biological materials, the samples are fixed onto the grid using a negative staining material such as uranium acetate.

Application

TEM is used to determine structural and compositional information for various materials.

Chapter 4

Powder X-Ray Diffraction Analysis (PXRD)

4.1 Introduction

X-ray diffraction is an important method used for structural studies including crystal imperfections, preferred orientations in polycrystalline sheets and the network of atoms in solids. (1) X-rays are electromagnetic waves, but have smaller wavelengths (10^{-10}m) and of the same magnitude as the interatomic spacing in crystals. (1) X-ray Diffraction has been used extensively for the structure determination of crystalline materials. An X-ray diffractometer has two main applications which include X-ray crystallography and X-ray powder diffraction. X-ray crystallography involves structural determination of the crystalline phases. X-ray powder diffractometery uses a sample in powder form. It is used for the identification of crystalline phases. (2)

4.2 Basic Principles

The powder diffraction method has a monochromatic beam of X-rays made to direct at a powdered sample spread on a support and the diffraction intensity is measured as the detector is moved through different angles. The pattern obtained is characteristic of the

sample material and thus can be used for the identification on comparing with a pattern database. Powder X-ray diffraction takes a fingerprint of the sample. By measuring the spacing of the lines in the diffraction pattern, it helps in the identification of the size and shape of the unit cell. The Bragg equation is the central equation in analyzing the results for a powder diffraction experiment. (3)

Bragg's law relates the wavelength of the X-rays (λ), the angle of incidence (θ) and the interatomic distance (d). (4) Bragg's law states that when a monochromatic X-ray beam having wavelength (λ) is incident upon a crystalline compound at an angle (θ), diffraction occurs as follows:

$$n\lambda = 2d \sin\theta \quad (4) \qquad \text{Equation 4.1}$$

where (d) is the distance between the successive planes in the crystal lattice, and (n) is order of the diffracted beams.

The d-spacing characteristics of the X-ray scan give a unique fingerprint of the sample. On comparing the diffraction pattern with a standard reference pattern, the material can be identified.

4.3 Instrumentation for PXRD

An X-ray diffractometer has three main components, namely an X-ray tube generator, sample holder and detector. This is seen in Figure 4.1.

An X-ray tube has an evacuated tube containing a heated cathode and an anode (target). A high voltage is imposed across the electrodes which are then used to accelerate the emitted electrons to the target. Usual X-ray methods for getting a characteristics spectrum

of a substance are performed by using the sample as an anode or affixing the specimen on the target. The detector commonly used is an energy dispersive spectrometer. (4)

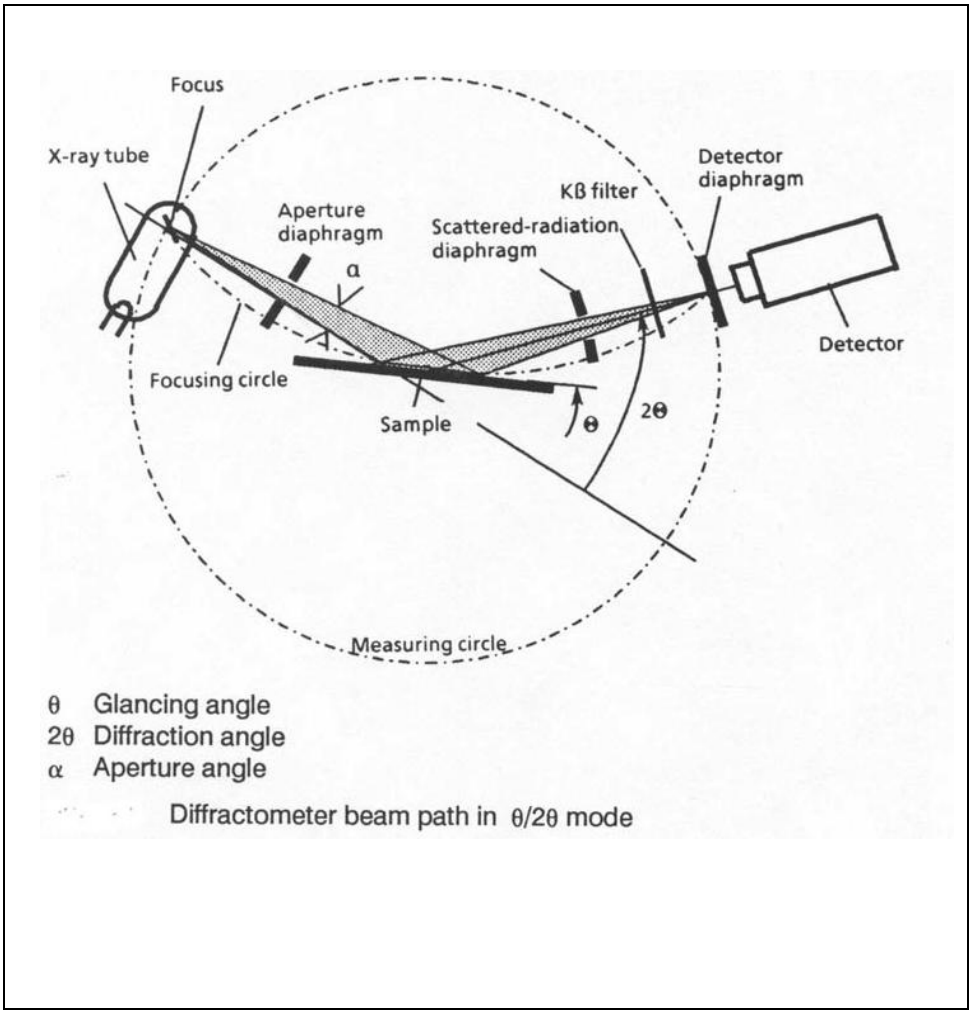

Figure 4-1: Powder Diffractometer Diagram (5)

4.4 Preparation of the Sample.

A few grams of the sample are required. Either powdered samples obtained using a 325 mesh (<44 μm) sieve or a flat surface sample are used. (6) The sample is uniformly

placed into the sample assembly. The upper surface of the sample should be flat in order to have uniform distribution.

4.5 Application

The role of PXRD as used in pharmaceutical analysis is that each crystal form of a drug will have a diffraction pattern. This can be used as a signature or fingerprint which is representative for that particular form of the drug. Thus, it can be used to test for polymorphs and solvates during drug discovery, formulation development and manufacturing. (7) X-ray powder diffraction can also be implemented as a means of rapid characterization of organic solids. (8) Powder X-ray diffraction analysis is used for the characterization of crystalline structures. (4)

CHAPTER 5

Differential Scanning Calorimetry (DSC)

5.1 Introduction

Differential Scanning Calorimetry is a thermal analytical technique. Apart from the measurement of heat, DSC can also be used to measure heat flow rate (power) and to identify characteristic temperatures of a reaction as well as its transition. The heat measurements not only have integral (total) heats of reaction or transition, but also may include the determination of partial heats developed within a specified temperature interval. These values are useful for kinetic evaluations, determinations of crystallinity and purity. (1)

Thus, DSC measures the difference in heat flow between a sample and a reference material when they are subjected to the same heating rate in a controlled atmosphere. (2)

5.2 Basic Principle of DSC

In DSC, the measurement signal (ΔT) is obtained as an electric voltage. The heat flow rate (φm) where (m) is the measured quantity, is internally assigned to this signal by an installed calibration (1):

$$\varphi m = - k'. \Delta T \qquad \text{Equation 5.2 A}$$

DSC analyzer measures the energy changes that occur when the sample is heated, cooled or held isothermally, with the temperature at which the changes occur. These energy changes measure the transitions that happen in the sample quantitatively and also measure the material characteristics and properties such as melting point, measurement of glass transitions and many others. DSC measures the heat flow, the flow of energy into and out of the sample, as a function of temperature or time and is normally shown as milliwatts (mW) on the Y axis. (3)

Platinum resistance thermometers are used to monitor the temperature of the sample and reference holders. They electronically keep track of the temperature of the two holders to assure they are constant. Once a thermodynamic event occurs, either exothermic or endothermic, there is a difference in the power requirements for the coils used to maintain a constant temperature. The difference in power is plotted with respect to temperature as recorded by the device. (4)

5.3 Instrumentation

DSC measures the differential signal between a sample and a reference. This signal can be amplified into a basic signal. There are two types of DSC calorimeters used, heat flux and power compensated DSC.

5.3.1 The Heat Flux DSC - These systems have a constantan disc which provides the main means of transferring heat to the sample and reference positions. It also acts as one element of the temperature measuring thermo electric junction. The sample and reference are kept in pans during the scan that are positioned on raised platforms on the constantan

disc. The disc transfers the heat from the sample pan to the contained sample and reference. The chromel–constantan area thermocouples, made by the junction of constantan disc and the chromel wafer, monitors the differential heat flow. The temperature of the sample is monitored directly by the chromel– alumel thermocouples, formed from chromel and alumel wires, which are connected to the chromel wafers's underside. (5)

5.3.2 The Power Compensated DSC -These systems have separate heaters for the sample and reference. The sample and reference are maintained closely at the same temperature by the system operated using platinum resistance thermometers which causes different amounts of heat to be supplied to each specimen as necessary. (5)

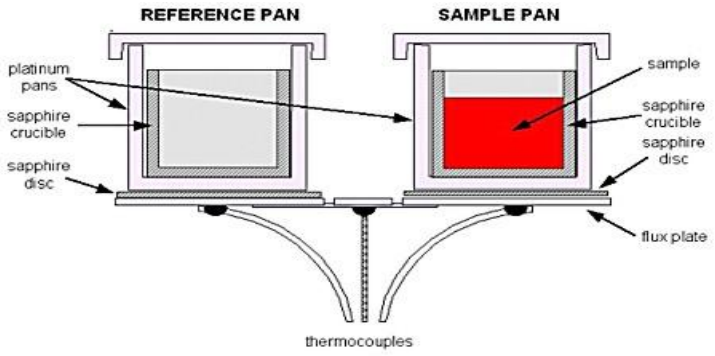

Figure 5-1: Heat Flux DSC (6)

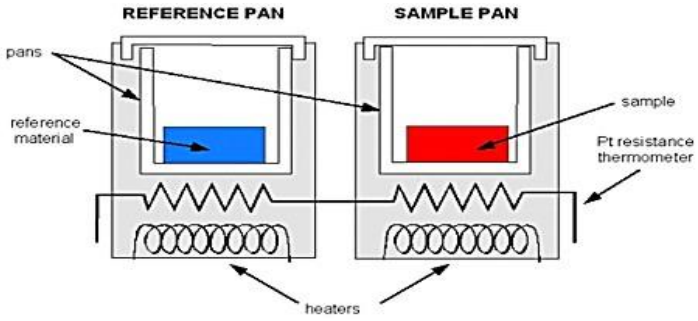

Figure 5-2: Power Compensated DSC (6)

5.4 DSC Thermograms demonstrate heat flows vs. temperature or time. It has a baseline, the peaks of which are due to transitions or reactions. Thermal transitions of materials are measured as glass transitions, melting, crystallization, oxidation or decomposition.

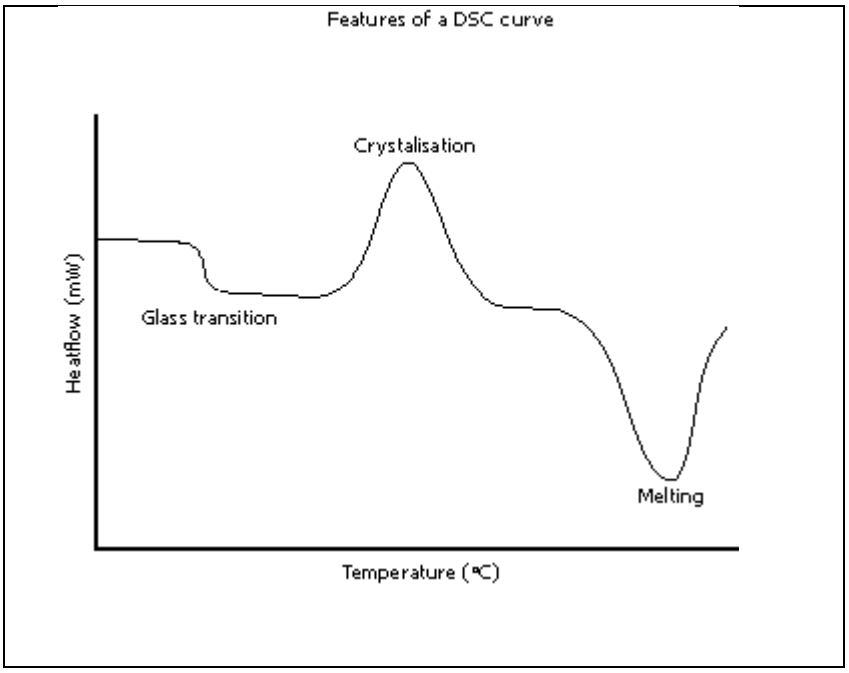

Figure 5-3: DSC Thermogram and its features (7)

5.5Application

DSC can also be used to detect the glass transition temperature in a protein solution as well as in lyophilized products. (8) DSC is an important tool in the drug development process. (9)

CHAPTER 6

UV-Visible Spectroscopy

6.1 Introduction

Spectrophotometery deals with the measurement of the tendency of a dissolved substance to absorb electromagnetic radiation within a definite and narrow wavelength range. These absorptions are measured at wavelengths that are generally characteristic for the chemical composition of the dissolved absorbing substances. UV and visible bands occur due to electronic transitions in the region of 200 nm – 780nm. (1) UV-Visible spectroscopy is a powerful analytical technique which can identify the presence of functional groups. It can also be used to determine the concentration of the analyte in solution.

6.2 Basic Principle

When UV or visible light is absorbed by molecules, it causes valence electrons in their orbits to gain energy and jump to a higher energy state. In the case of organic molecules, the electronic transitions could be due to a σ, π or n electron transition from the ground state to an excited state (σ^*, π^* or n*). (2) The molecule thus undergoes an

electronic transition. Only (π to π*) and (n to n *) transitions, which occur in the UV visible region, are observed. UV-Visible spectroscopy involves chromophores. They cause electronic transitions within a molecule, promoting bonding and non-bonding electrons into higher and less stable anti-bonding orbitals. The absorption of UV- Visible light leads to a broad band since it involves vibrational and rotational energy levels of the electronic transitions. Normally the absorptions are due to n to π* and π to π * transitions and thus molecules having conjugated double bonds as aromatic compounds absorb strongly. (2)

In UV-Visible Spectroscopy a large number of chemicals in solution obey the Beer Lambert Law. The Beer Lambert Law states that the absorbance of the solution is directly proportional to the concentration of the absorbing species and the associated path length of the light. When the path length in UV-Visible Spectroscopy is fixed, the concentration of the analyte in solution can be determined by the following equation:

$$A = -\log (I/I_o) = Є.c.l \qquad \text{Equation 6.1}$$

Where (A) is absorbance; (I_o) is the intensity of the incident light at a given wavelength; (I) is the transmitted intensity; (l) is the thickness of the absorber (in cm) and (c) is the concentration of the absorbing species, and (Є) is the molar absorptivity or (molar extinction coefficient) for the concentrations in moles /L. (1) The absorbance and absorptivity are both wavelength dependent. (2)

UV-Visible Spectroscopy has a few terms associated with it but includes terminologies such as bathochromatic shift (5), hyperchromic and hypochromic shift, chromophore (5) and auxochrome.

6.3 Instrumentation: The main components of an UV-Visible spectrometry are the light source, monochrometer, the beam splitting system (if required) and the detector. The deuterium arc and the tungsten filament lamps are the most commonly used light sources for lower and higher wavelength regions, respectively. The most modern instruments have grating monochromaters in reflection mode as dispersing elements. Several types of beam splitters are used in double beam spectrometers. (3)

They have detectors as photocells or photomultiplier tubes that produce voltage proportional to the radiation energy that strikes them. (4)

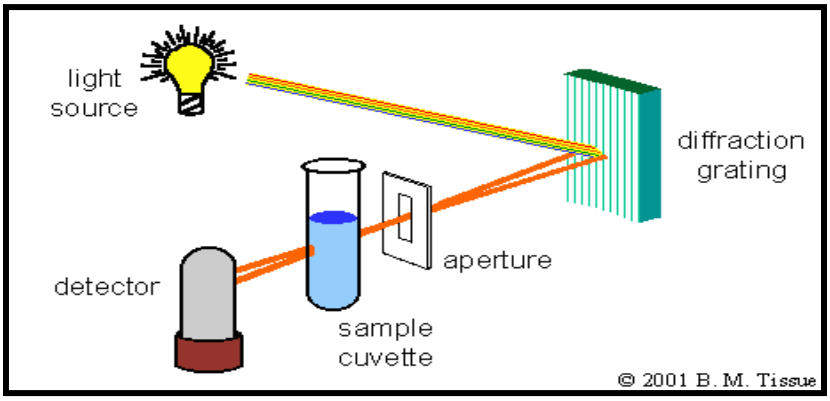

Figure 6-1: Single beam UV-Visible spectrophotometer (6)

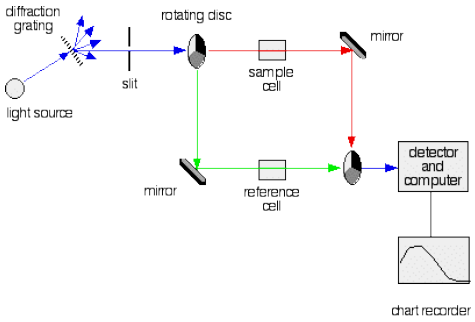

Figure 6.2: Double beam UV-Visible spectrophotometer (7)

The double beam spectrophotometer has a beam chopping device or a beam switching assembly which allows the beam to pass alternatively through the sample and reference cells.

6.4 Application

UV spectroscopy is an easy and sensitive method used for the analysis of nucleic acids and other chemical moieties. (8) Due to its sensitivity and non destructive nature, UV spectroscopy is frequently utilized as a detection method for analytical procedures such as HPLC. (8) Studies have been conducted where UV-Visible spectroscopy has been used to study the reaction pathways of various solid- state reactions. (9)

CHAPTER 7

Fourier Transform Infrared Spectroscopy (FTIR)

7.1 Introduction

Infra-red spectroscopy involves the study of the interaction of the infrared light with matter. Upon interaction of infrared radiation with matter, absorption takes place, which then causes the chemical bonds in the material to vibrate. The functional groups present in the molecules tend to absorb infrared radiation in the same wavenumber. Thus, the structure of the unknown molecule can be determined by the Infra red spectrum. (1) The infra-red spectroscopy provides molecular structural information by using the frequency of the vibrational modes of a compound. Infra-red spectroscopy uses the infrared region of the electromagnetic spectrum.

Fourier transform infrared (FT-IR) spectrometers are most commonly used today. In an FT-IR spectrometer, the spectra are collected by using a time domain measurement. The FT-IR spectrometer has a number of advantages over the conventional dispersive IR spectrometer with respect to superior sensitivity, resolution of bands, absolute wavelength accuracy, and higher precision of measurements. (2) The FT-IR

spectrometer has an interferometer which measures the signal and performs a Fourier transformation of the data.

7.2 Principle When IR radiation interacts with a compound at the proper frequency, it results in the transfer of energy to the molecule. The rate of energy transfer depends upon the frequency of radiation, after which, the vibration and /or rotation of the molecule is altered. If the frequency of the electromagnetic radiation matches a natural vibrational frequency of the molecule, a net transfer of energy occurs. (3) When the frequency of the IR radiation matches the vibrational frequency of a functional group of the compound, absorption occurs. The absorption intensity depends upon the dipole moment. Atoms in a functional group vibrate in a number of ways such as symmetrical and anti- symmetrical stretching, scissoring and rocking.

A sample upon absorbing energy causes the molecule to transition between two vibrational energy levels.

IR Spectroscopy has three typical spectral regions based on wavelength. The infrared region is subdivided into the near infra- red, infrared and far- infrared spectrum. (4)

Near IR: 10000 to 4000 cm^{-1}	It has overtones of fundamental vibrations.
IR: 4000 to 20 cm^{-1}	It excites the fundamental vibration. This is the most widely used region, as it has spectral fingerprints and most organic molecules are active in this region.

Far IR : 200 to 10 c It excites low energy vibrations and higher energy rotations.

The IR Spectrum records transmitted light which tells how energy was absorbed at each wavelength when the IR radiation passes through the sample. Thus, a transmittance spectrum is produced. The fingerprint region is 1450 to 600 cm^{-1} and is unique to a given molecule. Absorption bands in the region 4000 to 1450 cm^{-1} are known as the group frequency region since it represents the stretching vibrations of diatomic units.

7.3 **Instrumentation.**

An FTIR spectrophotometer has an IR radiation source, interferometer and a detector. The main four instrument components include: a radiation source, analyser, detector and electronics for signal processing which can change accordingly to the instrument type and application. (5)

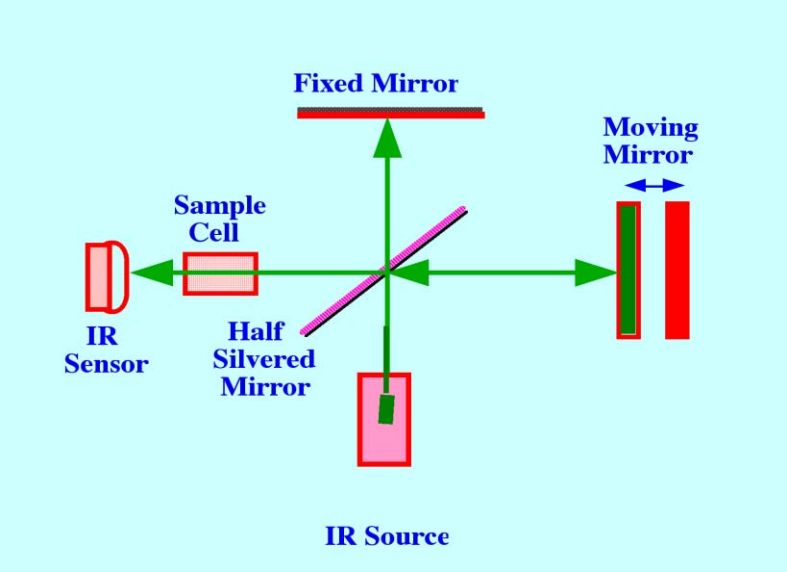

Figure 7-1: A diagram for an FTIR spectrophotometer (6)

An IR radiation source is comprised of a Nernst glower or a Glowbar unit (silicon carbide rod). (2) The interferometer combines separate waves together. A Michelson interferometer is most commonly used.

The monochromatic light, after splitting into two beams, is then recombined to form a visible pattern of areas of constructive and destructive interference of bright and dark fingers.

The IR detectors commonly used are the thermocouple and Bolometer. (2)

The signal is fed to a computer where the data is fourier transformed and a spectra having a transmission versus wavenumber is obtained. Various vibrational modes of the functional groups show characteristic IR absorption peaks within a specified wave number range.

7.4 Sample Preparation

For gas cells, the samples are retained in a short path length cell between 1 and 10 cm. The most basic cells are glass or metal tubes. (5)

For liquid samples, the liquids are sandwiched between two transparent IR plates made of sodium chloride or potassium bromide.

For solid samples, the solid is mixed with a mulling agent, Nujol (mineral oil) and is applied onto salt plates.

A solid sample can be made into a KBr disk. The sample can be prepared by grinding it with potassium bromide powder (3) using a mortar and a pestle. The mixture is then

placed between a punch and die and compacted into a translucent film using a mechanical die press.

7.5 Application

FTIR is commonly used to identify compounds. It can be used for qualitatively and quantitative purposes. FTIR is used to identify drugs, excipients and polymorphism. Fourier Transform Infrared spectrometry has been used for the assay of Polyhedral Boron compounds in plasma and pharmaceutical formulations. (7) FTIR data has been utilized for the confirmation of cross linking in the polymeric network. (8)

CHAPTER 8

Dissolution

8.1 Introduction

Dissolution is the process in which a solid, because of its solubility characteristics, enters into and becomes a solution within a given solvent. (1) Drug release /dissolution testing is routinely done to demonstrate drug release and bioavailability within a biological host. The bioavailability of a drug is studied vigorously. Dissolution testing can be used to determine the bioequivalence of drugs. The dissolution test must meet the legal requirements for that drug. Dissolution is a regular quality control procedure utilized in industry. The standard dissolution test is a measure of the dosage consistency in drug release. Dissolution studies are important during pre-formulation studies which correlates the data with the biological activity.

The dissolution test measures the amount of active drug released from a solid oral dosage form, such as a tablet or capsule, by using a known volume of dissolution medium collected within a predetermined length of time. (2)

The in-vitro dissolution testing indicates that only the process of dissolution is studied. This is somewhat misleading because the standard in-vitro dissolution testing models not only measure the dissolution of a drug substance in the GI Fluids, but also includes the drug release from a formulation. (3)

A tablet or other solid dosage form on interaction with water disintegrates and the drug starts to pass into solution from the intact solid dosage form. Thus, the tablet first disintegrates into granules and these granules further de-aggregate into fine particles. The disintegration, de-aggregation and dissolution process can occur simultaneously with the release of drug from its delivery form at each individual step.

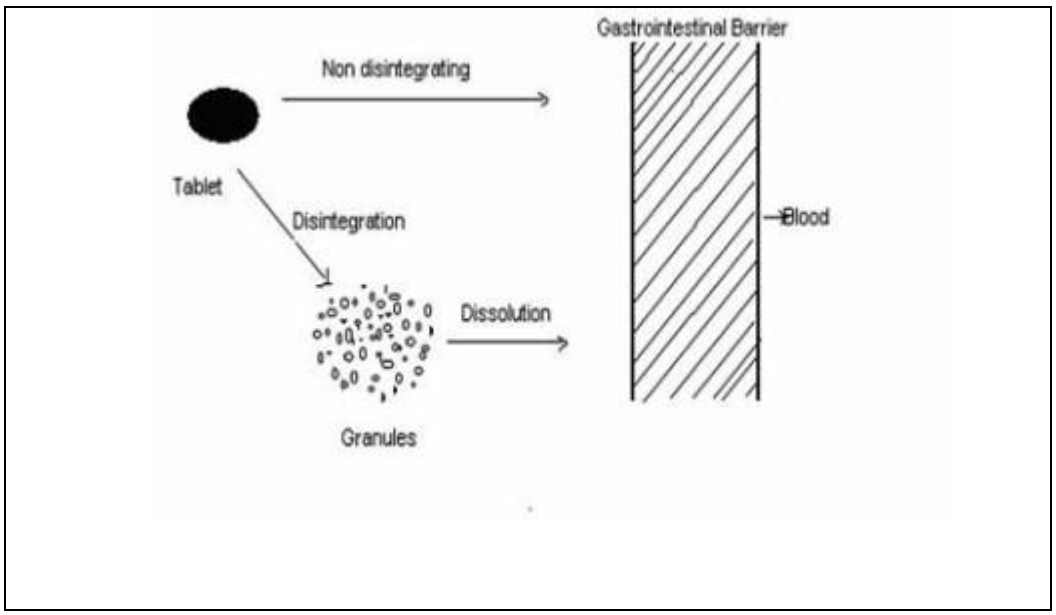

Figure 8-1: Tablet showing various stages during the dissolution process (4)

Drug dissolution has been found to be the rate limiting or controlling step in the bio-availability of drugs having low solubility. This is due to the fact that it takes a lot of time for the drug to get into and appear in the systemic circulation.

The mathematical expression for the dissolution rate is given by the Noyes Whitney equation (1) as:

$$dC/dt = K(C_s - C_t) \qquad \text{Equation 8.1}$$

where (dC/dt) is the dissolution rate of the drug, (k) is the dissolution constant, (C_s) is the concentration of the saturated solution and (C_t) = Concentration after some time.

This equation was modified by Nernst and Brunner as:

$$dM/Dt = D.A /H(C_s - C_t) \qquad \text{Equation 8.2}$$

where (k) is the dissolution constant; (C_s) is the concentration of saturated solution; (C_t) = Concentration after some time; (D) is the diffusion coefficient; (h) is the thickness of the diffusion layer; (M) is the mass of the solid material at time (t) and (A) is the area available for mass transfer.

In order to simulate the in-vivo sink condition, an in-vitro dissolution testing process can involve using a large volume of dissolution medium or a mechanism in which the dissolution medium is constantly replenished with fresh solvent at a specified rate so that the solute concentration never reaches more then 10–15 % of its maximum solubility. (1) Sink conditions must be maintained by utilizing a large volume of solvent with respect to the saturation point.

Figure 8-2: Digital Dissolution Test Apparatus (Six Stage) (5)

8.2 Method

There are seven different types of compendia apparatii described in the USP. The most commonly used apparatii in dissolution testing includes Apparatus 1 (Basket) and Apparatus 2 (Paddle). (6) The others include Apparatus 3 (reciprocating cylinder); Apparatus 4 (Flow cell); Apparatus 5 & 6 (Paddle over disk & Cylinder); and Apparatus 7 (Reciprocating disk) (7)

8.2.1 The Rotating Basket Apparatus 1 (1)

This apparatus consists of a 1000 mL vessel made of glass or other inert transparent material, a variable speed drive, and a cylindrical basket. The vessels are immersed in a water bath which is kept at a temperature of 37 $\pm$ 0.5 ^{o}C during the test. The bath fluid should also be kept in constant smooth circulating operation.

The basket consists of two parts, one of which is the top attached to the shaft. The detachable part of the basket is fabricated from a welded seam, stainless steel cloth,

formed into a cylinder 3.66 cm high and 2.5 cm in diameter, with a narrow rim of sheet metal around the top.

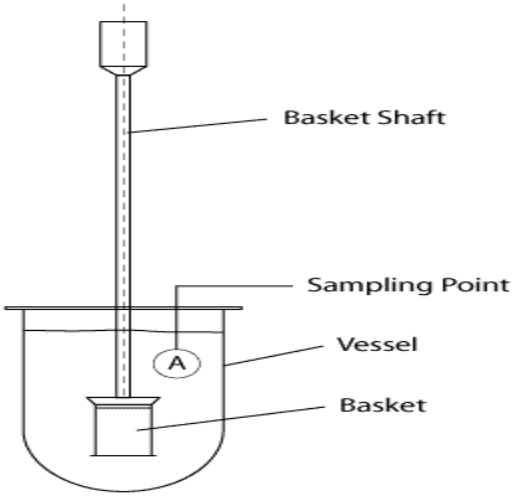

Figure 8-3: USP Dissolution Apparatus I (Basket method) (8)

8.2.2 The Paddle Method Apparatus 2 (1)

It has the same assembly as Apparatus 1, except that a paddle formed into a blade and a shaft is used as the stirring element.

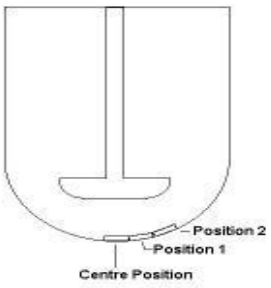

Figure 8-4: USP Dissolution Apparatus II (Paddle method) (9)

Chapter 9

Materials and Methods Used

9.1 Materials

9.1.1 **Metformin Hydrochloride**: It is 1, 1-Dimethyl biguanide hydrochloride. It has the chemical formula of $C_4H_{11}N_5$.HCl and a molecular weight of 165.6. It is a white, almost odorless, hygroscopic, crystalline powder with a bitter taste. It has a melting point of about 225°C. It is soluble as 1 gm in 2 gm of water and 1 gm in 100 mL of alcohol. It is insoluble in chloroform and ether. It should be stored in an airtight container. (1)

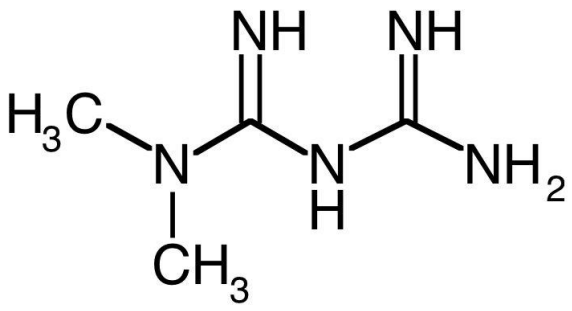

Figure 9-1: Metformin Structure (2)

Pharmacological Action:

Metformin increases the peripheral utilization of glucose, by increasing uptake, and decreasing gluconeogenesis. To work, metformin requires the presence of endogenuous insulin; therefore, patient must have some β-cells. (3)

Dose: Initially given as 1 to 1.5 g daily in divided doses, while subsequent doses of up to 3 gm daily in divided doses are possible. The initial dose is 500 mg twice or thrice daily with meals. This gradually is increased over 10 to 14 days to a maximum of 3 g daily. The usual maintenance dose is 1 to 1.5 gm daily, although up to 3 g daily may be necessary in some cases. When metformin is given to patients who are already receiving insulin, the full dose of insulin should be maintained for 2 days and then gradually reduced every few days. (1)

Metformin is absorbed over 6 hours and the bioavailability is 50 to 60% under fasting conditions. Food delays absorption. Metformin hydrochloride is readily absorbed from the gastro-intestinal tract and is excreted unchanged in the urine. More then 60% of a dose of metformin was reported to be absorbed from the gastro-intestinal tract. It is excreted unchanged in the urine within 36 hours. The biological half–life was about 2.8 hours. (1)

Toxic Effects. Metformin hydrochloride may cause anorexia, nausea, vomiting and diarrhea. A metallic taste and urticaria may occasionally occur in some patients. (1)

Source: Metformin hydrochloride (MH) was obtained from Spectrum Chemical Mfg Corp, New Jersey, Lot No: YO3098.

9.1.2 Chitosan

Chitosan is a polysaccharide consisting mainly of unbranched chains of β – (1, 4) –2– acetoamido–2–deoxy–D–glucose. The amino group in chitosan has a pK_a value of ~ 6.5. It is positively charged and thus soluble in acidic to neutral solution. It is an amorphous solid which is practically insoluble in water, dilute acids, dilute and concentrated alkalies, alcohol and other organic solvents. (4)

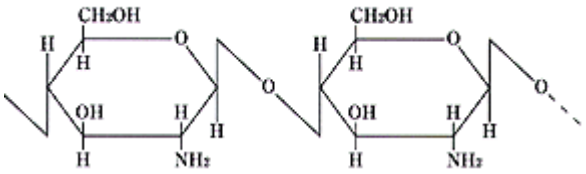

Figure 9-2: Chitosan Structure (5)

The Chitosan used was [Deacetylated Chitin; Poly (D-glucosamine) β–(1, 4) -2 –Amino -2-deoxy –D-glucose] powder has a degree of deacetylation of 75-92%.

Use: Deacetylated chitin, chitosan is used in water treatment and in photographic emulsions.

Source: Chitosan was obtained from Spectrum Chemical Mfg Corp, New Jersey, Lot No: YE0240.

9.1.3 Sodium Tripolyphosphate

Sodium tripolyphosphate written as STPP or sodium tripolyphosphate (TPP). It has a chemical formula of $Na_5O_{10}P_3$ and molecular weight of 367.91. (6)

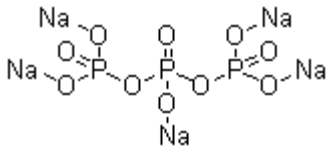

Figure 9-3: Sodium Tripolyphosphate structure (7)

Therapeutic Category: In water softening, as a peptizing agent, emulsifier and dispersing agent.

Source: Sodium Tripolyphosphate (TPP) was obtained from Spectrum Chemical Mfg Corp, New Jersey, Lot No: YR3092.

9.1.4 Sodium Alginate

Sodium alginate, alginic acid sodium salt is also called algin or alginate. It is an anionic polysaccharide. Alginic acid sodium salt is a gelling polysaccharide extracted from giant brown seaweed. (8)

Sodium alginate is a cream colored powder. It is soluble in water, forming a viscous, colloidal dispersion. It is insoluble in alcohol and hydro-alcoholic solutions in which the alcohol content is greater then 30% w/w. It is insoluble in chloroform, ether and in aqueous acid solution in which the pH is below 3.

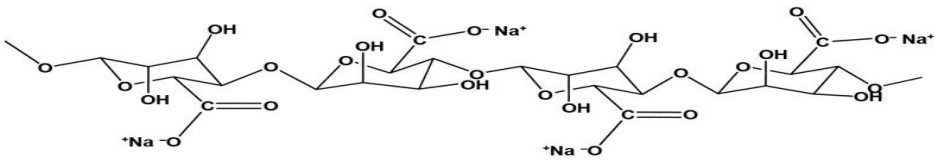

Figure 9-4: Sodium Alginate structure (9)

Therapeutic Category: Pharmaceutical aid (suspending agent). (8)

Source: Sodium alginate was obtained from Sigma Chemical Co,St Louis, MO, Lot no: 61K0119

9.1.5 Pectin

Pectin is a polysaccharide present in the cell walls of all plant tissues which function as intracellular cementing agents. One of the richest sources of pectin is lemon or orange rind which has 30% of this polysaccharide. The molecular weight of pectin is 20,000-400,000. (10)

It occurs as a coarse or fine yellowish-white colored powder which is practically odorless and with a mucilaginous taste. It is almost completely soluble in 20 parts of water, forming a viscous solution containing negatively charged, very much hydrated particles. It is acid to litmus. It is insoluble in alcohol or in diluted alcohol, and other organic solvents. It dissolves more readily in water, if first moistened with alcohol, glycerol or sugar syrup, or if first mixed with 3 or more parts of sucrose. It is stable under mildly acidic conditions. Under more strongly acidic or basic conditions, the pectin depolymerizes. (10)

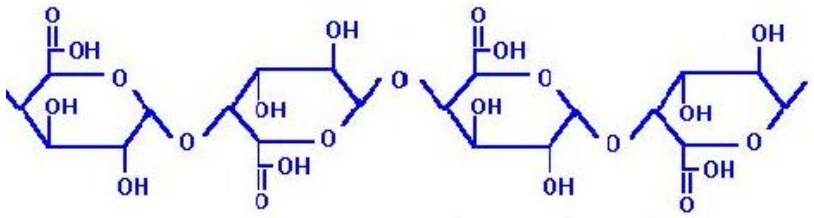

Figure 9-5: Pectin structure (11)

Therapeutic Category: Protectant.

Source: Pectin was obtained as a sample from the University of Toledo, College of Pharmacy, Pharmaceutics Stock Room with no lot number

9.1.6 Guar gum

Guar gum has a molecular weight of about 220, 000. The water soluble fraction (85 %) of guarflour is called guaran which consists of linear chains of (1, 4)–β-D-mannopyranosyl units with α–D-galactopyranosyl units attached by (1, 6) linkages. (12)

Guar gum is a free flowing powder. It is completely soluble in cold and hot water. It is practically insoluble in oils, grease hydrocarbons, ketones and esters. Water solutions are tasteless, odorless, non toxic, of a pale, translucent gray color and neutral. It is stable to heat. It has five to eight times the thickening power of starch. Aqueous dispersions are neutral. (12)

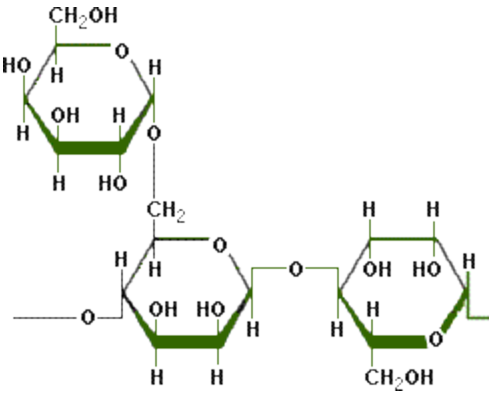

Figure 9-6: Guar gum structures (13)

Therapeutic Category: It is a protective colloid, stabilizer and thickening agent, a film forming agent for cheese and as a binding and disintegrating agent in tablet formulations. (12)

Source: Guar gum was obtained from Spectrum Chemical Mfg Corp, New Jersey, Lot No: YV3076.

9.1.7 Xanthan Gum

Xanthan gum is a polysaccharide. Its molecular weight is more then 10^6.

Xanthan gum is a cream colored, odorless, free flowing powder. It dissolves readily in water with stirring to give highly viscous solutions at low concentrations. It forms strong films on evaporation of its aqueous solutions. It is resistant to heat degradation. Aqueous solutions are highly pseudoplastic. (14)

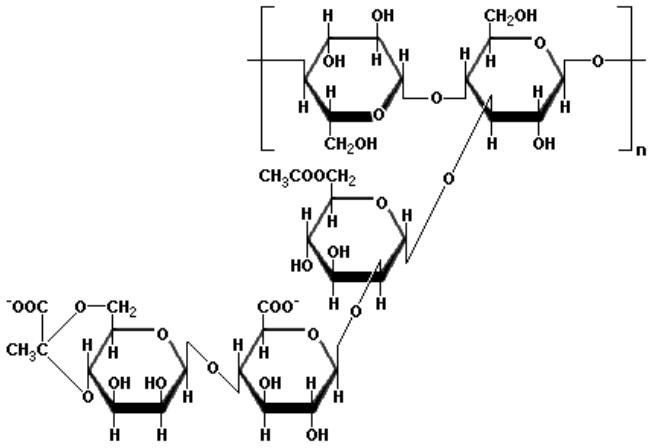

Figure 9-7: Xanthan gum structure (15)

Therapeutic Category: It is used as a stabilizer and emulsifying agent.

Source: Xanthum gum was obtained from Spectrum Chemical Mfg Corp, New Jersey, Lot No: XV0506

9.1.8 Hydroxy Ethyl Cellulose

Hydroxyethyl cellulose is ether of cellulose in which the hydroxyl-ethyl groups are attached to the anhydroglucose rings of the cellulose by ether linkages. (16)

A white or off-white colored powder which is odourless and tasteless. It is soluble in cold or hot water. It is insoluble in most organic solvents. Its 2% solution in water has a pH of 6.5 to 8.5.

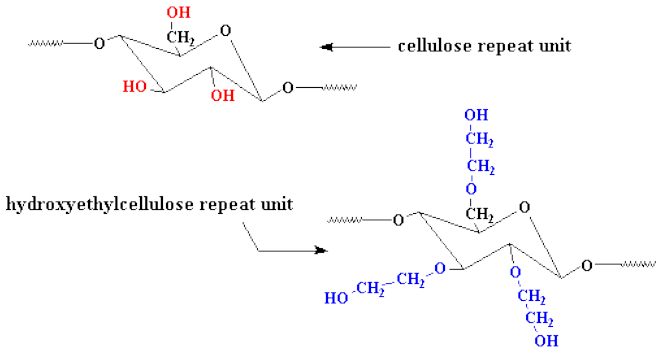

Figure 9-8: Hydroxy Ethyl Cellulose Structure (17)

Use: Since it does not gel on heating, it may be useful in preparations where gel formation is undesirable. (16)

Source: Hydroxyethyl Cellulose QP 4400 was obtained from Union Carbide Corporation, Chemicals and Plastics, New York, Lot No. W – 1485-L

9.1.8 Potassium Dibasic Phosphate, Sodium Hydroxide and Glacial Acetic Acid

Potassium dibasic phosphate and sodium hydroxide were used to prepare the pH 7.4 buffer employed as the dissolution medium to measure the drug release studies. Glacial Acetic acid was also used.

Source: Potassium Dibasic phosphate was obtained from Amend Drug & Chemical CO., Irvington, N.J. LOT X 1730H29

Source: Sodium hydroxide was obtained from Fisher Scientific, New Jersey, LOT 066852

Source**:** Glacial Acetic acid was obtained from Fisher Scientific, New Jersey, LOT 095402

9.2 Preparation of Chitosan / TPP Poly Electrolyte Hydrogel Beads

The beads were prepared by the "Ionotropic cross linking method ''. Thus, the complexation mechanism is an ionotropic crosslinking or interpolymer complex. (18) The drug, metformin hydrochloride, (400 mg) was dissolved in and dispersed in 10 ml of 1.5 % glacial acetic acid. Chitosan (200 mg or 400 mg) with/without another polymer such as sodium alginate (200 mg) was dispersed in the above dispersion of metformin hydrochloride with low heat. (19) A microtip attached to a syringe was used to deliver the dispersion drop wise into 50 ml of 1% TPP solution (adjusted at a pH of approximate 6-6.5) with magnetic stirring at room temperature. The beads were allowed to cure for 20 minutes in the solution. They were then, separated by filtration and allowed to dry at 35 ^{o}C temperature overnight. The ratio of metformin hydrochloride (400 mg): chitosan: polymer was 2:1:1. The effect of the types of polymers such as HEC, guar gum (20), pectin (21) and, and the amounts of cross-linking agents with chitosan were also studied as given in Table 1.1. The scheme for preparing the metformin hydrochloride beads is shown in Figure 9.9.

Initially only the drug and chitosan beads were prepared. The second study involved using the drug, chitosan and the polymer. The detailed compositions of each formulation are seen in Table 9.1 and Table 9.2.

The remaining compositions were made using 200 mg of polymers to increase the crosslinking and electrostatic attraction. The polymers used include: sodium alginate, hydroxyethyl cellulose, pectin, guar gum, cholesterol, hydroxy propyl methyl cellulose, Eudragit and xanthan gum. The compositions and observations are seen in Table. 9.2.

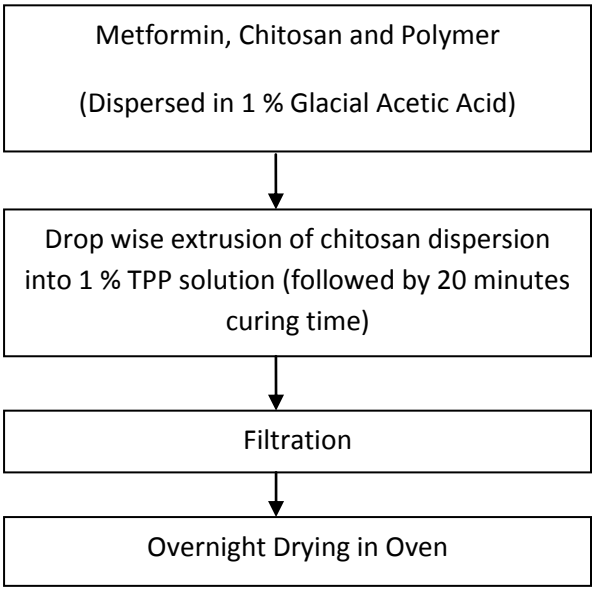

Figure 9-9: Scheme for the formation of metformin, chitosan and polymers beads

Table 9.1: Composition, Curing time and Observations for the Chitosan-TPP Metformin HCl (MH) beads

Drug MH)	**Chitosan**	**Volume of polymer solution**	**Volume of 1% TPP**	**Curing time**	**Observation**
400 mg	200 mg	10 mL	50 mL	20 min	beads not strong
400 mg	400 mg	10 mL	50 mL	20 min	diameter of beads improved, but not strong enough to hold integrity

Table 9.2: Composition, Curing time and Observations for the Chitosan and different Polymers-TPP beads for Metformin HCl (MH)

Drug (MH)	Chitosan (A)	Polymer (B)	Polymer (B) amount	Vol. of polymer solution	Vol. of 1% TPP	Curing time	Observation
400 mg	200 mg	HPMC	200 mg	10 mL	50 mL	20 min	highly dispersed, beads not strong
400 mg	200 mg	HEC	200 mg	10 mL	50 mL	20 min	Spherical beads
400 mg	200 mg	Eudragit^{R} RSPO	200 mg	10 mL	50 mL	20 min	Spherical beads
400 mg	200 mg	Xanthan gum	200 mg	10 mL	50 mL	20 min	aggregates layer
400 mg	200 mg	Sod alginate	200 mg	10 mL	50 mL	20 min	Spherical beads
400 mg	300 mg	Sod alginate	100 mg	10 mL	50 mL	20 min	Spherical beads
400 mg	100 mg	Sod alginate	300 mg	10 mL	50 mL	20 min	aggregates layer
400 mg	200 mg	Guar gum	200 mg	10 mL	50 mL	20 min	Spherical beads
400 mg	200 mg	Pectin	200 mg	10 mL	50 mL	20 min	bigger beads, viscous
400 mg	200 mg	Cholesterol	200 mg	10 mL	50 mL	20 min	aggregates layer

9.3 Morphological Characterization

The surface morphology of the beads was observed using an Eye Clops [R] magnifying camera at 200X magnification, which was then connected to the computer. The dried beads were placed on a glass slide and its eye piece focused by the magnifying camera to obtain the bead's image. (22, 23) The particle size of the beads was measured using standard sieves.

9.4 Measurement of the Bead Size, Weight, Yield and Swelling

The average diameter for both wet and dry beads (10 beads) was measured using a slide caliper scale. The weight of 10 beads from each batch was taken and the average was calculated. The total yield (%) and percent swelling were calculated as follows:

$$\text{Yield (\%)} = \frac{\text{(weight of the dry beads)}}{\text{Total weight of raw material}} \times 100 \qquad \text{Equation 9.1}$$

$$\text{Swelling (\%)} = \frac{\text{(weight of wet beads – weight of dry beads)}}{\text{Weight of weight beads}} \times 100 \qquad \text{Equation 9.2}$$

The swelling kinetics for the hydrogels can be classified as diffusion controlled and relaxation controlled. (24)

9.5 Drug Entrapment

The drug entrapment efficiency was studied by taking the filtrate solution of the beads in a pH 7.4 phosphate buffer. The amount of metformin hydrochloride loaded into the beads was determined by a UV Spectrophotometer at 251 nm. The absorbance value for the drug in the filtrate gives the concentration of the drug in the filtrate. This concentration is the amount of the drug that was not trapped into the chitosan beads. This amount of the drug in the filtrate solution was subtracted from the total amount of the drug added into the beads. This quantity was then divided by the total drug, and then multiplies with 100 to obtain the value of Drug entrapment efficiency (DEE). All the experiments were carried out in triplicate.

$$\text{DEE \%} = \frac{(\text{Total drug} - \text{Drug in solution}) \times 100}{\text{Total drug}} \qquad \text{Equation 9.3}$$

DEE = Drug entrapment efficiency

9.6 DSC Studies

Differential Scanning Calorimetry studies were done using a DSC 822e with a TS0801RO Sample Robot, TS0800GCI Gas Controller from Mettler-Toledo Inc., and Star ^{R} Software. The DSC studies were used to characterize the phase equilibria, polymorphic changes, melting, and crystallization and decomposition behavior of the chitosan beads. (25) The weight of the dried beads for the DSC experiment was between 3 and 8 mg. The experiments were conducted using closed aluminum pans with a pin

hole cover. The sample was examined under a nitrogen flow at a scan rate of 10°C /min with the range from 0°C to 450°C. The DSC is used for the determination of melting point, changes in crystallinity and to detect any possible interaction between the drug, carrier and adsorbent. The DSC runs for pure polymer, metformin hydrochloride and beads were done in triplicate. Thus, the DSC was used for the characterization of the complex. (26)

9.7 In-vitro Release Studies.

The dried beads after weighing were suspended in 900 mL of Phosphate Buffer (pH 7.4) in a glass flask and maintained at 37°C, and at 50 rpm. A 5 ml sample of the solution was removed from the flask after 15, 30, 45 and 60 minutes and then 2, 3, 4, 5 and 6 hours. The volume of each sample was replaced with the same volume of phosphate buffer (pH 7.4) to maintain the sink conditions. (22) The amount of metformin hydrochloride released from the beads was analyzed using a UV spectrophotometer at 251 nm. The in vitro release studies were performed in triplicate for each of the samples.

9.8 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum was performed using a Perkin Elmer 1600 spectrophotometer with a resolution of 2 cm^{-1} and eight scans in the spectral region between 4000 and 600 cm^{-1} were done. Solid powder samples were finely crushed, mixed with potassium bromide (1:10 ratio by weight) and pressed at 15,000 psig (using a Carver Laboratory Press Model C, Fred S. Carver Inc., WIS 53051) to make the disc. The detector was purged carefully using clean dry nitrogen gas to increase the signal level and reduce moisture.

An FTIR spectrum is used to see the presence of interactions and identification of the changes in functional groups. (26)

9.9 Powder X-ray Diffraction Analysis The drug's crystalline state in the polymer was valuated by Powder X- Ray Diffraction (PXRD) Analysis.

X-ray spectra were recorded using X' Pert – PRO multipurpose X-Ray diffractometer manufactured by Panalytical, Tokya, Japan using a Ni-filtered, Cu K radiation at a voltage of 45 kv, and a current of 40 mA with a scintillation counter. The instrument was operated in the continuous scanning speed of 4 ^{o} /min over a 2θ range of 5^{o} to 40 ^{o}.

Chapter Ten

Results and Discussion

10.1 Preparation of the Beads

The formation of gel beads by the ionotropic method using polymers was found to be simple, rapid and reproducible. The use of the polymer matrix for preparing a modified release dosage system is common. (1) The extrusion of a chitosan dispersion having both the drug and polymer into a 1% TPP solution causes gel formation to occur due to the action of sodium ions with the negatively charged groups of an anionic polymer. The incorporation of polymer with chitosan was found to be effective in giving rigidity to the beads. (2) The 20 minutes curing time was found to be the standard time for the bead's stabilization.

The bead picture was taken using magnifying lenses. The beads prepared were slightly grayish white in color. The mean diameter for the beads was found to be 2.2 mm. The results showed that the amount and type of polymer used was found to change the morphology of the beads. The polymer gave sphericity to the beads. (3)

10.2 Physical Morphology

The physical morphology including shape and surface observation was performed using the Eye Clops^{R} magnifying biogenic camera. A sponge like internal structure was seen. The pores of the beads containing drug were observed in the beads. Figure 10.1 to Figure 10.6 show the presence of the gel structure during the process. (4) The beads were observed to be spherical to oval and the surface was found to be smooth. (5) From these images, we can see that the drug is dispersed and entrapped within the pores of the beads. Magnification at 200X sharply and distinctly demonstrated the pore shape structure of the beads.

10.3 Measurement of the Bead Size, Weight, Yield and Swelling

The beads shape, diameter, size, weight and percentage swelling are the important characteristics which can be evaluated and demonstrated the properties performed by them. (2)

The weight of the chitosan beads was evaluated. The pK_a value for chitosan was 6.3. Thus, at pH 6.0-6.3, a higher amount of precipitation of the complexes took place. Chitosan acted as a polymeric drug carrier because of its biocompatibility, biodegradability, low toxicity and natural source of origin. (5)

The dry diameters for most of the various beads were observed to be in the range of 1.5 to 2.8 mm. The swelling rate was found to be around (9 % to 12 %) which describes the amount of hydrophilicity, the beads can sustain on interaction with water. The yield was found to be less due to the small batch size and thus can be improved by making bigger batches and improving the crosslinking methods. (6) The drug, polymer type and

composition shaped the formation of the beads in terms of rigidity and spherical shape. The drug to total polymer used ratio was kept the same for every formulation.

Table 10.1: Composition and Curing time for the Chitosan and different polymers-TPP beads for Metformin HCl (MH)

Drug Quantity (MH)	Chitosan (A) Quantity	Polymer(B) Type	Polymer (B) Amount	Volume of polymer solution	Volume of 1% TPP	Curing time
400 mg	200 mg	HPMC	200 mg	10 mL	50 mL	20 min
400 mg	200 mg	HEC	200 mg	10 mL	50 mL	20 min
400 mg	200 mg	Eudragit	200 mg	10 mL	50 mL	20 min
400 mg	200 mg	Xanthan gum	200 mg	10 mL	50 mL	20 min
400 mg	200 mg	Sod alginate	200 mg	10 mL	50 mL	20 min
400 mg	300 mg	Sod alginate	100 mg	10 mL	50 mL	20 min
400 mg	100 mg	Sod alginate	300 mg	10 mL	50 mL	20 min
400 mg	200 mg	Guar gum	200 mg	10 mL	50 mL	20 min
400 mg	200 mg	Pectin	200 mg	10 mL	50 mL	20 min
400 mg	200 mg	Cholesterol	200 mg	10 mL	50 mL	20 min
400 mg	200 mg	X	X	10 mL	50 mL	20 min
400 mg	400 mg	X	X	10 mL	50 mL	20 min

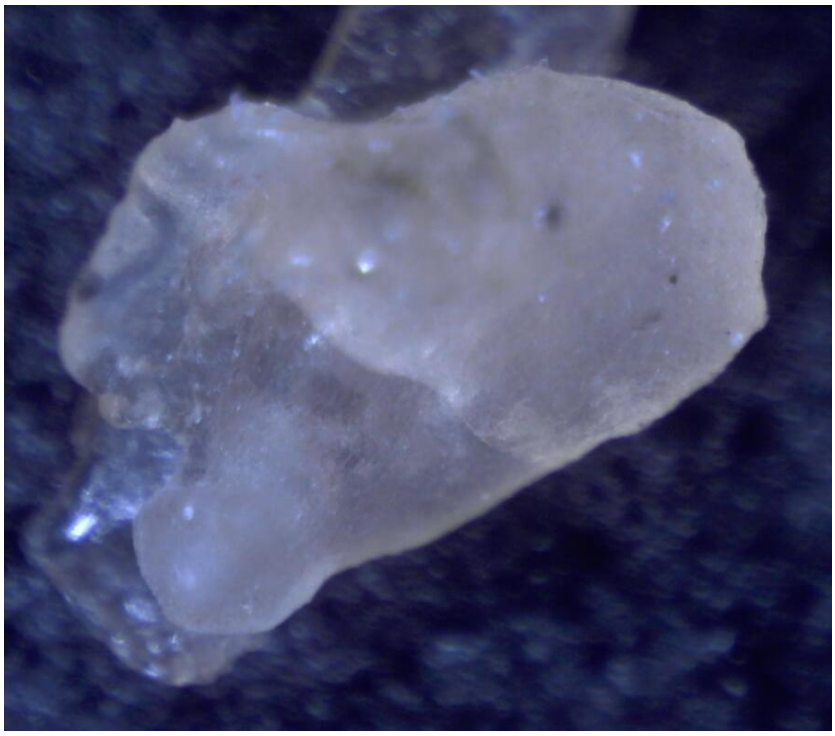

Figure 10-1: Eyeclops image of Chitosan-TPP beads taken at a magnification of 200X

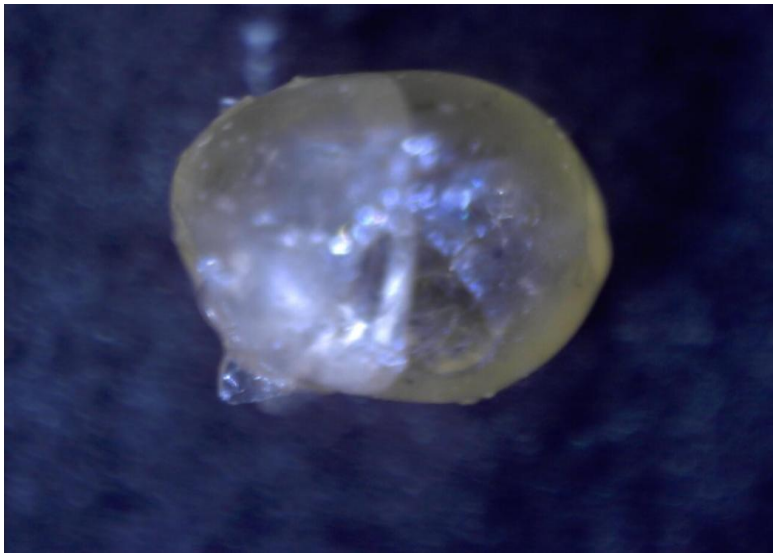

Figure 10-2: Eyeclops image of Pectin-Chitosan TPP beads taken at a magnification of 200X

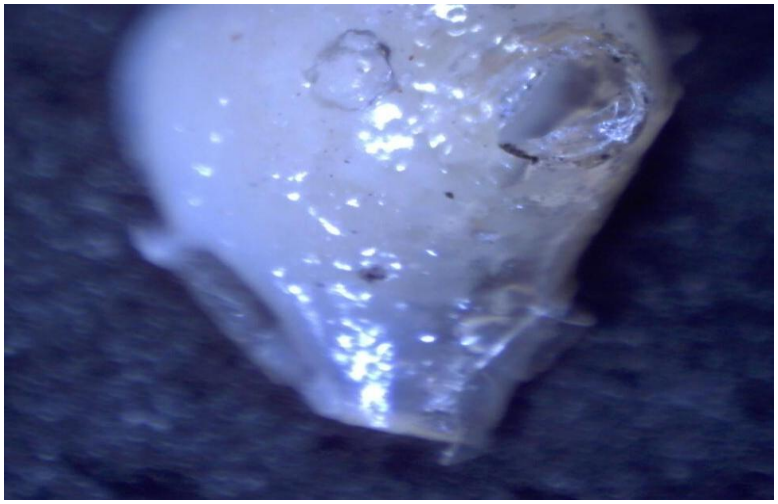

Figure 10-3: Eyeclops image of HEC- Chitosan TPP beads taken at a magnification of 200X

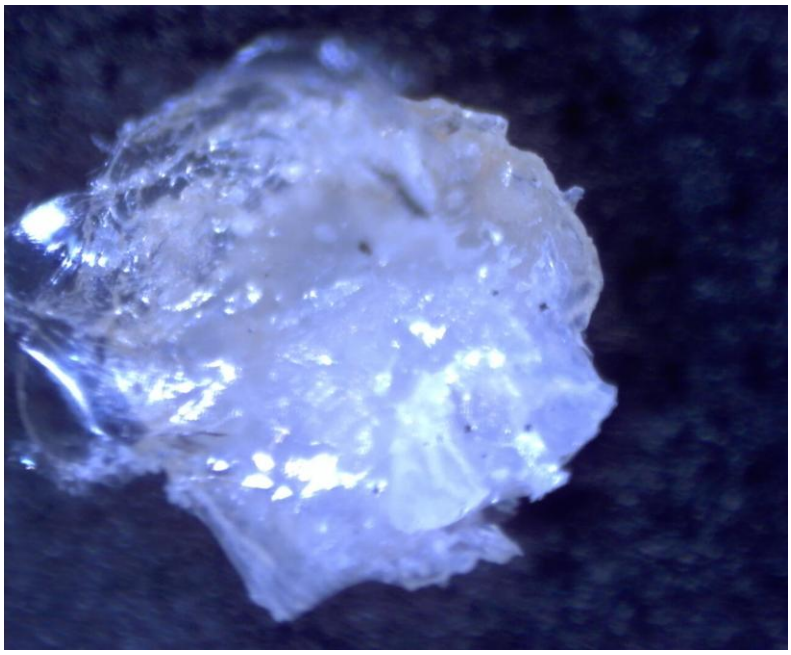

Figure 10-4: Eyeclops image of Sodium Alginate-Chitosan TPP beads taken at a magnification of 200X

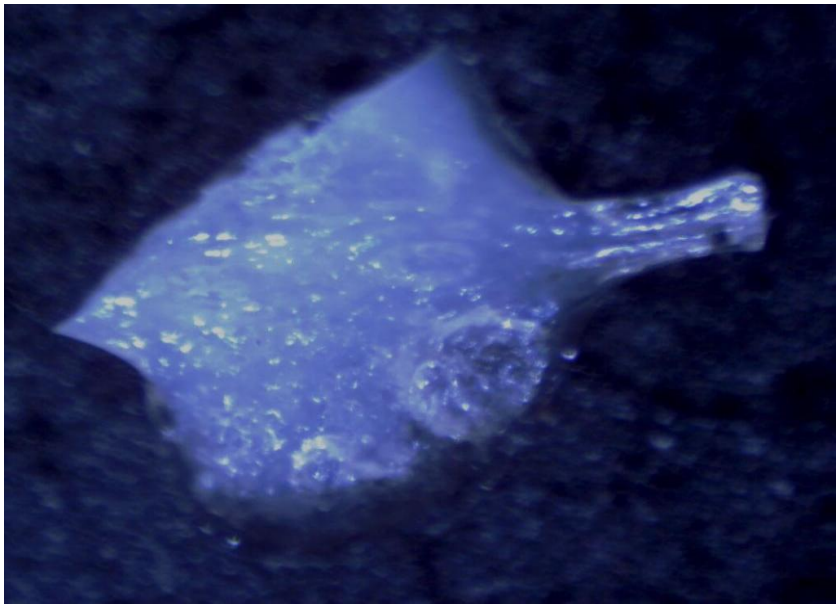

Figure 10-5: Eyeclops image of Guar gum-Chitosan TPP beads taken at a magnification of 200X

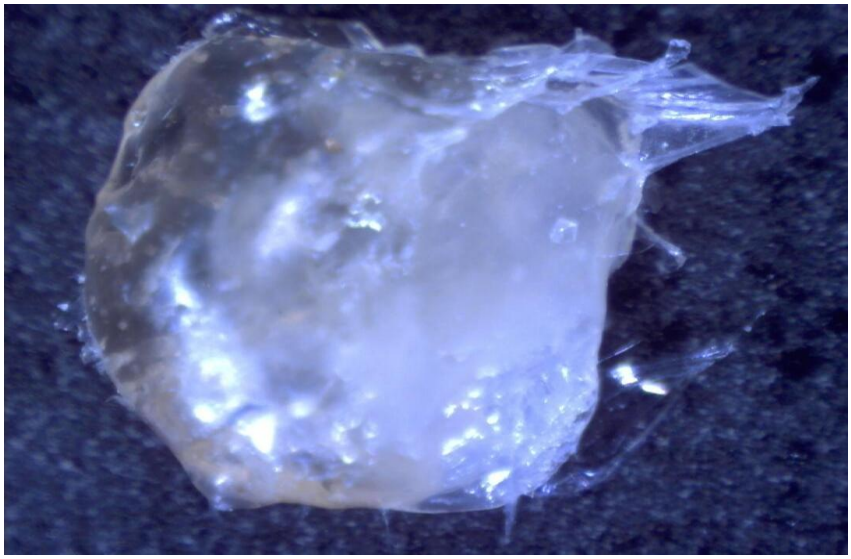

Figure 10-6: Eyeclops image of Xanthan-Chitosan TPP beads taken at a magnification of 200X

Table10.2: Observations reported for Chitosan and different polymers-TPP beads for Metformin HCl (MH) beads

Drug (MH)	Chitosan (A)	Polymer (B)	Observation
400 mg	Chitosan	HPMC	beads not strong
400 mg	Chitosan	HEC	Spherical beads
400 mg	Chitosan	Eudragit	Spherical beads
400 mg	Chitosan	Xanthan gum	aggregates layer
400 mg	Chitosan	Sod alginate	Spherical beads
400 mg	Chitosan	Sod alginate	Spherical beads
400 mg	Chitosan	Sod alginate	aggregates layer
400 mg	Chitosan	Guar gum	Spherical beads
400 mg	Chitosan	Pectin	bigger beads, viscous
400 mg	Chitosan	Cholesterol	aggregates layer
400 mg	Chitosan	No polymers	beads not strong

Table 10.3: Diameter, Weight and Swelling Studies for Chitosan and Metformin beads

Bead Characteristics	Chitosan and MH
Yield (%)	60
Wet diameter (mm)	2.5 ± 0.25
Dry diameter (mm)	1.6 ± 0.18
Weight(10 wet beads) gms	0.342 ± 0.031
Weight(10 dry beads) gms	0.312 ± 0.031
Wt % swelling	9.61

Table 10.4: Diameter, Weight and Swelling Studies for Sodium Alginate, Chitosan and Metformin HCl (MH)

Bead Characteristics	**Sod Alginate, Chitosan and MH**
Yield (%)	58.8
Wet diameter (mm)	2.8 ± 0. 30
Dry diameter (mm)	2.50 ± 0.09
Weight(10 wet beads) gms	0.317 ± 0.041
Weight(10 dry beads) gms	0.285 ± 0.034
Wt % swelling	11.22

Table 10.5: Diameter, Weight and Swelling Studies for Hydroxyethyl Cellulose (HEC), Chitosan and Metformin HCl (MH)

Bead Characteristics	**HEC, Chitosan and MH**
Yield (%)	57.50
Wet diameter (mm)	2.5 ± 0.25
Dry diameter (mm)	2.30 ± 0.20
Weight(10 wet beads) gms	0.312 ± 0.031
Weight(10 dry beads) gms	0.280 ± 0.020
Wt % swelling	11.42

Table 10.6: Diameter, Weight and Swelling Studies for Xanthan, Chitosan and Metformin HCl (MH)

Bead Characteristics	**Xanthan, Chitosan and MH**
Yield (%)	59.20
Wet diameter (mm)	1.9 ± 0 .25
Dry diameter (mm)	1.5 ± 0.20
Weight(10 wet beads) gms	0.280 ± 0.025
Weight(10 dry beads) gms	0.250 ± 0.018
Wt % swelling	12.00

Table 10.7: Diameter, Weight and Swelling Studies for Guargum, Chitosan and Metformin HCl (MH)

Bead Characteristics	**Guargum, Chitosan and MH**
Yield (%)	57.50
Wet diameter (mm)	2.5 ± 0.25
Dry diameter (mm)	1.90 ± 0.30
Weight(10 wet beads) gms	0.399 ± 0.031
Weight(10 dry beads) gms	0.35919 ± 0.020
Wt % swelling	11.14

Table 10.8: Diameter, Weight and Swelling Studies for Pectin, Chitosan and Metformin HCl (MH)

Bead Characteristics	Pectin, Chitosan and MH
Yield (%)	45.80
Wet diameter (mm)	2.7 $\pm$ 0.25
Dry diameter (mm)	2.2 $\pm$ 0.20
Weight(10 wet beads) gms	0.278 $\pm$ 0.029
Weight(10 dry beads) gms	0.250 $\pm$ 0.020
Wt % swelling	11.20

10.4 Drug Entrapment Efficiency

The drug entrapment efficiency (7) from the hydogel beads was obtained using UV spectrophotometry. The filtrate obtained after bead collection on the filter medium and diluting with phosphate buffer 7.4 was analyzed using a UV-Visible spectrophotometer and the absorbance value of the solution was noted from the UV spectrophotometer. This value was then compared using the calibration curve. The calibration curve was first obtained by scanning the wavelength at maximum absorbance. This occurred at about 251 nm. The concentration of the drug was known at this wavelength. Five different drug concentrations were used to construct the calibration curve as seen in Figure 10.7.

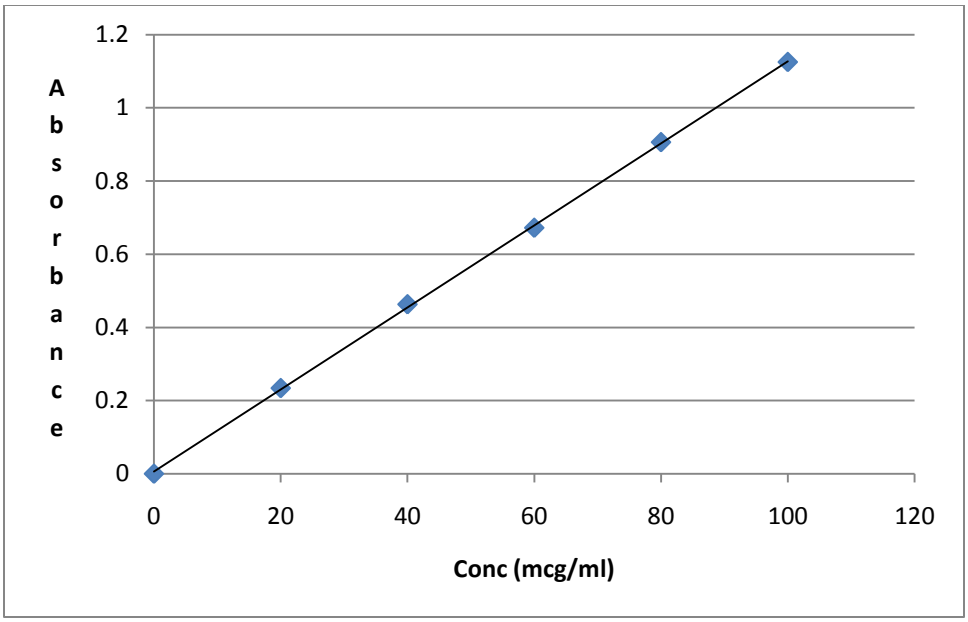

Figure 10-7: Calibration curve for Metformin HCl in Phosphate Buffer pH 7.4 at 251 nm wavelength , where slope is 0.0112,the Intercept is 0.006 and $R^2 = 0.9998$.

The percentage of entrapment efficiency (%EE) for the chitosan beads prepared using various compositions were calculated using the formula. (8)

$$\text{DEE \%} = \frac{(\text{Total drug} - \text{Drug in solution}) \times 100}{\text{Total drug}} \qquad \text{Equation 10.1}$$

DEE = Drug entrapment efficiency

The % EE was found to be in the range of 43.17% to 82.70% for the polymers studied. Sodium alginate gave the highest % EE of 82.70% and cholesterol gave the lowest % EE of 43.17%. The results might indicate that sodium alginate exhibited electrostatic interactions with the $-NH_3^+$ group of chitosan in the polyelectrolyte complex form. This might result in the drug being entrapped in the PEC forming a better bead than that with the pure chitosan bead. (9)

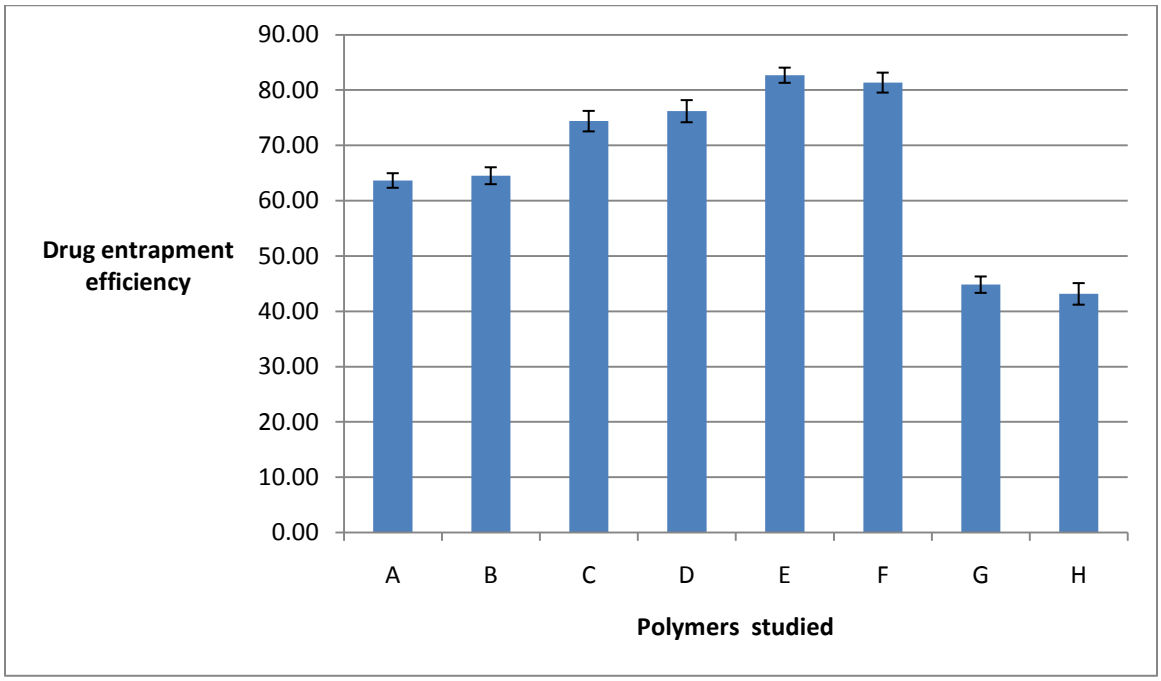

Figure 10-8: Drug entrapment efficiency values for different polymers used with chitosa and metformin HCl where A = HPMC; B = HEC; C = Eudragit; D = Xanthan Gum; E = Sodium Alginate; F = Guar Gum; G = Pectin; and H = Cholesterol

10.5 Differential Scanning Calorimeter studies

The DSC thermograms for the chitosan, polymers, metformin, chitosan-polymer-TPP drug loaded beads are shown in Figures 10.10 and 10.14. The pure alginate polymer showed an endothermic peak at 240^{o}C into the cycle. (9) This peak represents the degradation of alginate. The chitosan-polymer drug loaded beads showed a lower endothermic peak than that of the pure chitosan and pure metformin. This phenomenon

indicates that the polyelectrolyte complex between chitosan, polymer and metformin had formed.

The DSC thermograms for metformin has an endothermic peak at 235^{o} C, a peak temperature of 236.4^{o} C and two exothermic crystallization peaks at 310^{o} C and 330^{o} C, respectively. (10)

The endothermic peaks were also observed in the bead. The changes in the transition temperature results from the formation of beads, since we observe a slight lowering in the melting endotherm. This indicates the formation of a polyelectrolyte complex.

The thermal behavior of the beads showed that the ionic interaction decreases the endothermic temperature. The HEC beads showed a sharp endothermic transition and a decrease in the endothermic peak at 178^{o} C which confirmed the presence of an electrostatic interaction. The DSC spectra also showed the presence of an exothermic crystallization peaks at 251^{o}C. Thus, the shift in endothermic and exothermic peaks suggests the presence of a polyelectrolyte complex. (10) Similar observations were noted for the other batches of beads.

10.6 Fourier Transform Infrared Spectroscopy (FTIR)

The chitosan beads were analyzed using FT-IR and the spectra were compared. The FTIR spectrum for chitosan has an intense and broad band at 1584cm^{-1} which is the –NH group as seen Figure 10.9.

The spectra for metformin loaded chitosan / alginate / TPP beads showed a new prominent absorption band at 1547.94 due to the ($–NH_3^+$) groups and suggested the

formation of a strong polyelectrolyte complex. The intensity of the peaks seen at 1600-1400 cm $^{-1}$ were found to be reduced in intensity in the beads as seen in Figures 10.9. (11) The broad stretch band at 3400-3000 cm $^{-1}$ suggests the presence of a secondary amine as seen in Figure 10.9. The stretching at 2800-3000 cm $^{-1}$ is due to the presence of CH_3 groups as shown in Figure 10.9.

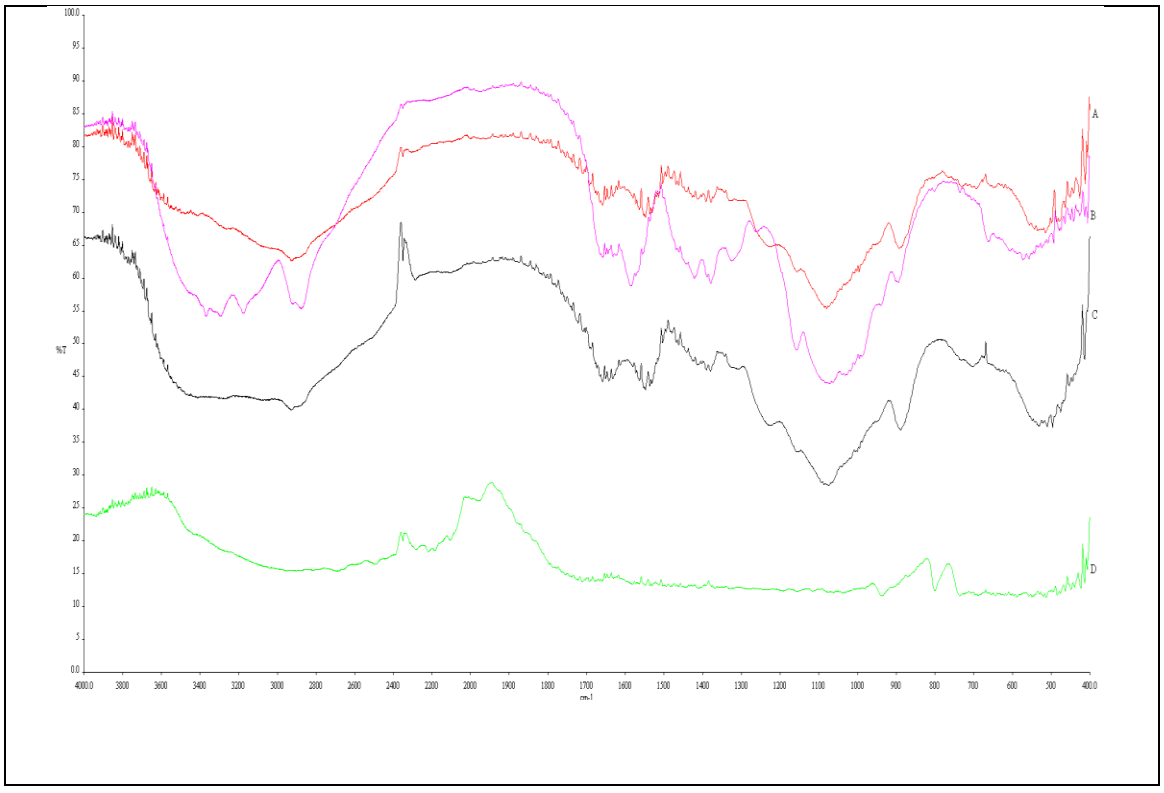

Figure 10-9: FTIR spectra of Drug loaded Sodium Alginate Chitosan TPP beads (A); Chitosan polymer (B); Drug loaded Chitosan TPP beads (C); and Metformin HCl (D)

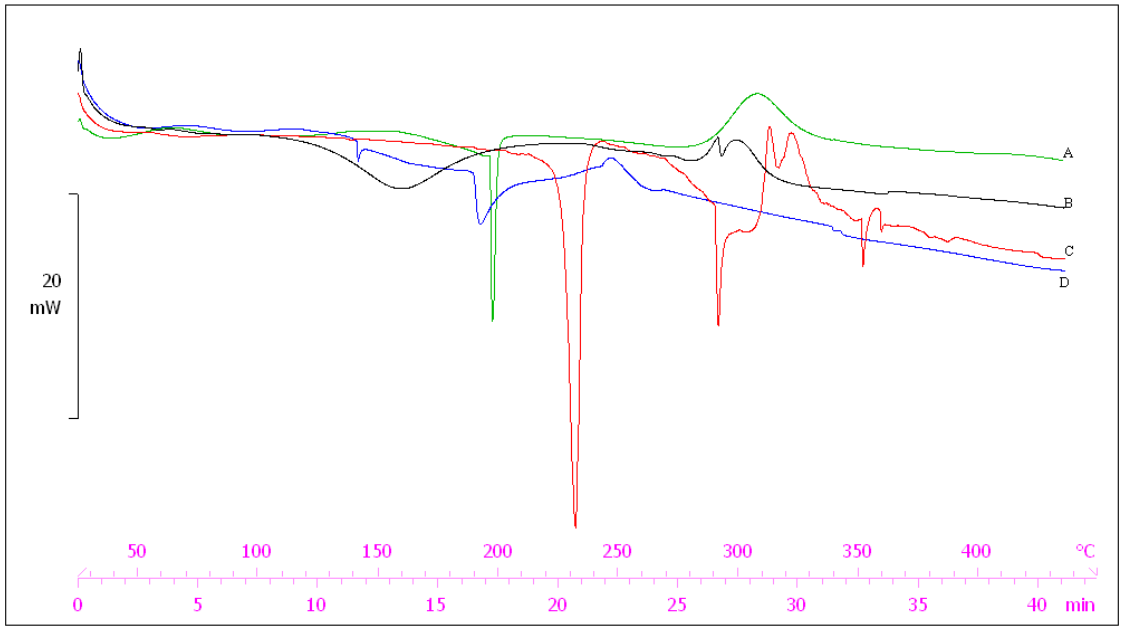

Figure10-10: DSC thermograms of chitosan polymer (A); Guar gum polymer (B); metformin HCl (C); and Guargum chitosan drug loaded beads (D)

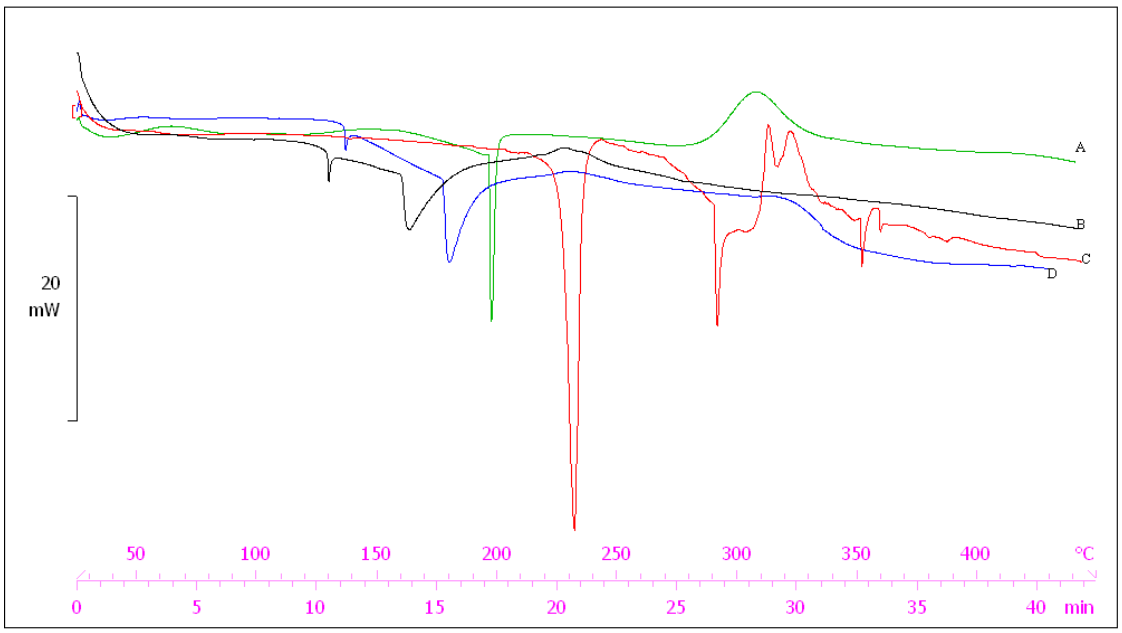

Figure10-11: DSC thermograms of chitosan polymer (A); Pectin polymer (B); metformin HCl (C); and Pectin chitosan drug loaded beads (D)

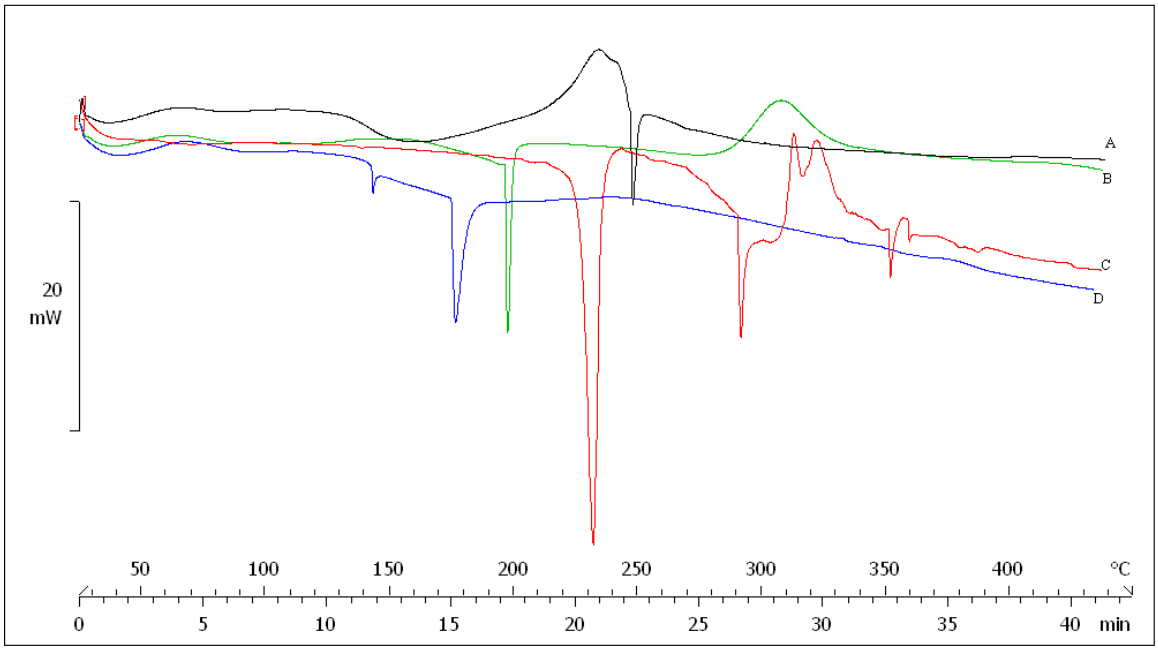

Figure10-12: DSC thermograms of sod alginate polymer (A); chitosan polymer (B); metformin HCl (C); and sod alginate chitosan drug loaded beads (D)

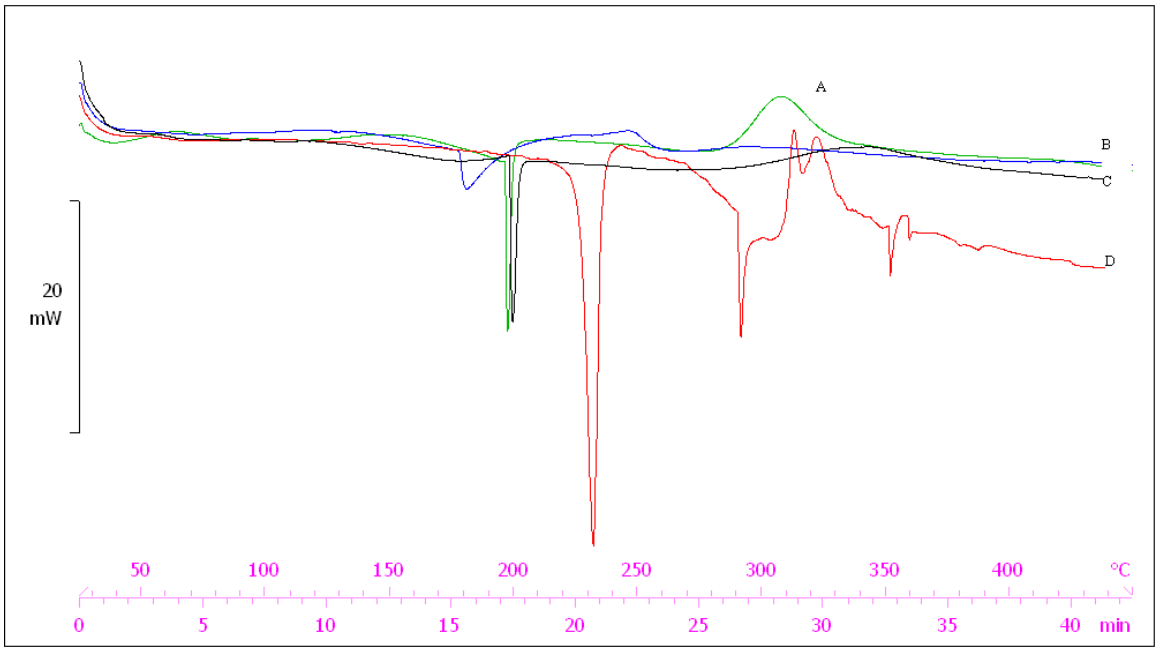

Figure10-13: DSC thermograms of chitosan polymer (A); HEC chitosan drug loaded beads (B); HEC polymer (C); and metformin HCl (D)

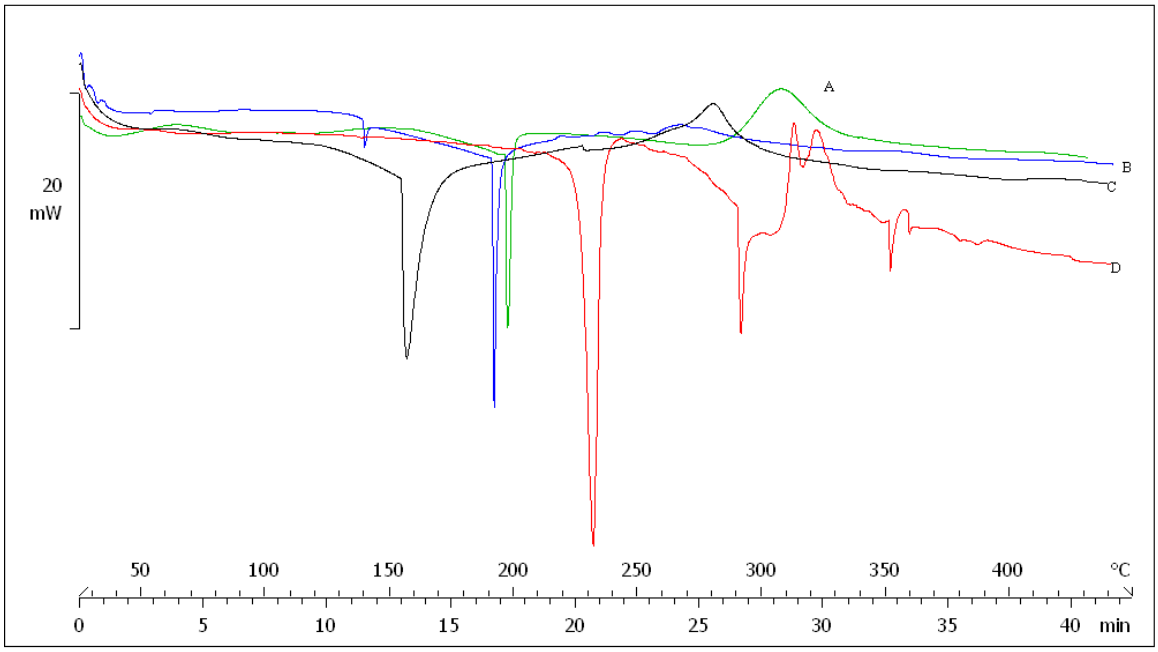

Figure10-14: DSC thermograms of chitosan polymer (A); Xanthan chitosan drug loaded beads (B); Xanthan polymer(C); and metformin HCl (D)

10.7 Powder X-Ray Diffraction Analysis

The powder X-ray diffraction technique (12) is commonly employed to determine the physical nature of the polyelectrolyte, drug and polymer. The physical morphology can explain that the drug is molecularly dispersed in the polymer matrix or occur as an ionic interaction complex. The metformin has crystalline peaks which are indicated by the presence of sharp intense peaks. The polymers used also have sharp peaks, indicating the presence of their semi crystalline nature. The binding of metformin in the polyelectrolyte complex still shows the presence of sharp peaks as seen in the PXRD for the chitosan-alginate-TPP metformin beads. We can thus conclude that the metformin is present in its crystalline state in the polyelectrolyte. Powder X-ray diffraction studies (13) were

performed to detect any changes in the crystallinity of the drug. Metformin showed numerous peaks which demonstrate the crystalline nature of the drug. The chitosan beads also showed peaks which indicative of some crystalline characteristics.

10.8 In-Vitro Release Study

The in-vitro drug release from the beads in phosphate buffer pH 7.4 was performed using the USP II Dissolution test apparatus. (14) The calibration curve was first obtained scanning the wavelength at which the maximum absorbance took place, which was 251 nm. The concentration of the drug in solution at a wavelength of 251 nm was determined. Five different concentrations of drug in solution were used to construct the calibration curve as previously seen in Figure 10.7. The in-vitro release of plain metformin hydrochloride dispersed in pH 7.4 phosphate buffer as seen in Figure 10.16 was done to determine the release pattern of the drug. The in-vitro drug release study was done for the chitosan –polymer-TPP drug loaded beads for a 5-6 hour study. The in-vitro dissolution results show that beads were sustaining the drug release. (11) The dissolution characteristics are used to compare the release rates of drugs prepared as chitosan beads. (14, 15)

From Figures 10.17 and 10.18, , it can be seen that the beads released the drug within 2 hours to its maximum over the entire 5-6 hour study period. The beads were able to achieve the maximum solubility due to encapsulated drug. This may be due to the crosslinking. The reason for the sustained release might be due to the polymer chains in the beads were entangled together with the strong ionic interactions. Thus, the penetration of the dissolution medium into the hydrogel beads was difficult.

We were not able to ascertain a sustained release effect due to the fact that an in-vivo study was not performed.

We can only make the conclusion of a sustained release dosage form if an in vivo study was performed and blood levels obtained. It should be noted that in an in-vitro study, the drug has no where to go and therefore it may reach maximum concentrations within 2-3 hours.

We can only compare the maximum percent cumulative release as an indication of how well the beads were able to maintain or release the drug over a specific time period.

10.9 Short Term Stability Study

The short term stability study (16) for the hydrogels prepared was done by keeping the hydogels or beads at room temperature and at 4 different Relative Humidity (RH) storage conditions, namely 22.51%, 57.57%, 75.30% and 97.30% (17) for one month. A saturated salt solution of the following salts was used for making the specified relative humidity values as seen in Table 10.9. (17, 18)

The physical morphology of the chitosan beads remains almost the same after a storage period of one month. There was no color change and the beads were observed to be intact and rigid.

The dissolution studies for the beads showed that the chitosan beads were able to control the release of the metformin hydrochloride.

Thus, it can be concluded that the chitosan beads were stable after one month of storage at room temperature and at various relative humidity storage conditions.

Table 10.9: Salt used, Concentration, and Associated Relative Humidity

Salt used	**Concentration used (%)**	**Published RH at 25^{o}C**
Potassium Acetate	78	22.51%
Sodium Bromide	75	57.57 %
Sodium Chloride	40	75.30%
Potassium Sulfate	14	97.30%

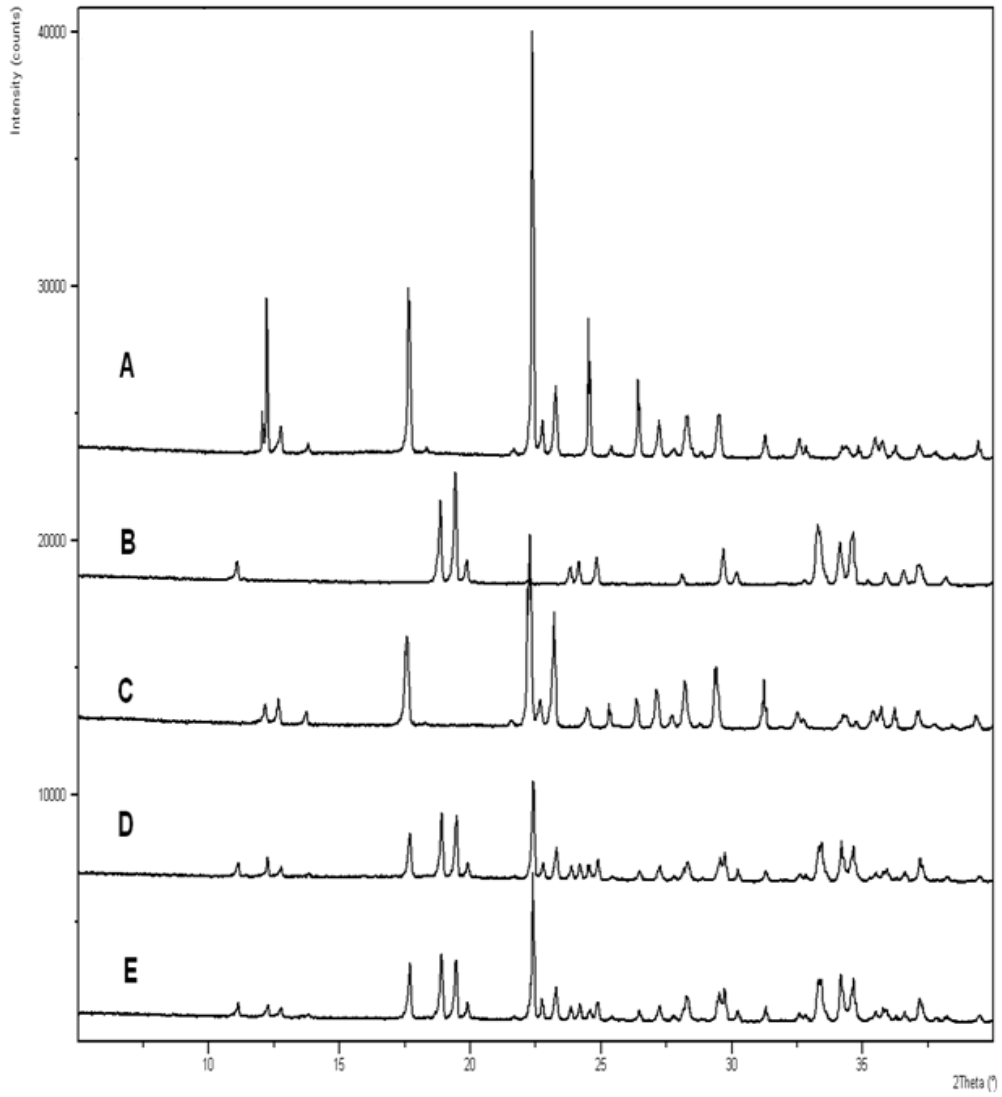

Figure 10-15: PXRD of Chitosan Metformin HCL mix (A); Chitosan Sod Alginate Metformin HCL TPP beads (B); Metformin HCL(C); Chitosan Metformin HCL TPP mix (D); and TPP polymer (E)

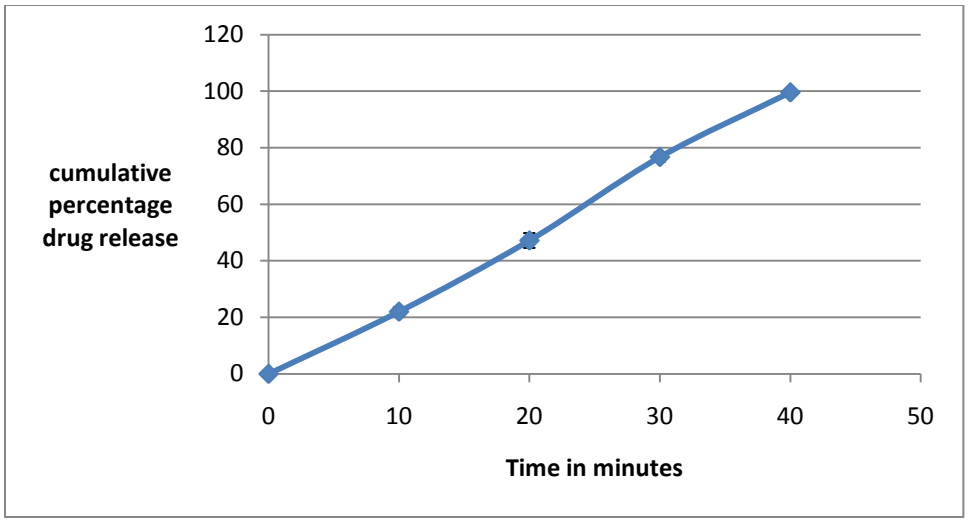

Figure 10-16: Cumulative percentage drug release of plain Metformin HCl in the pH 7.4 Phosphate Buffer

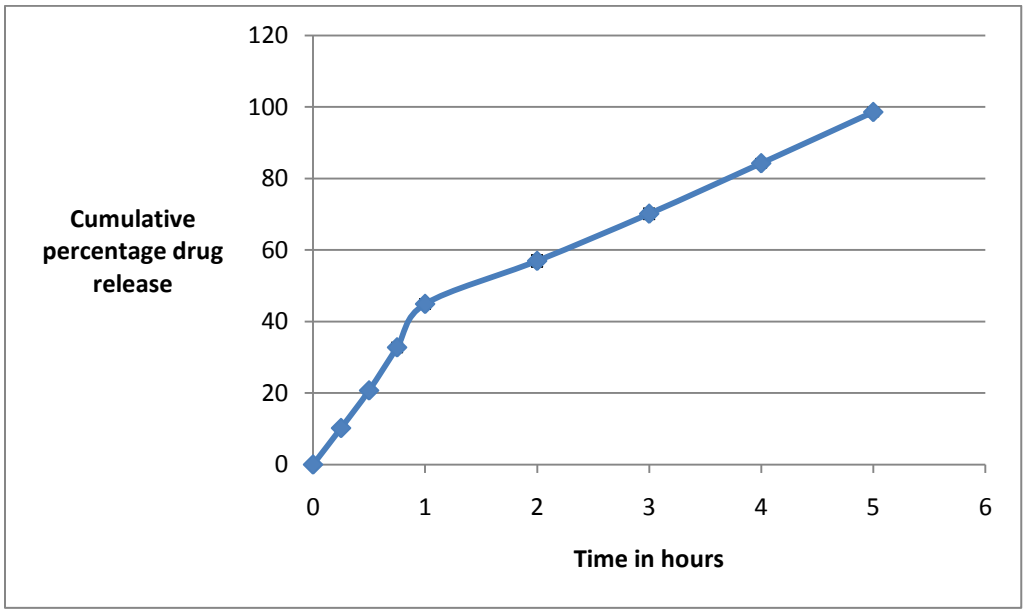

Figure 10-17: Cumulative percentage drug release of Metformin HCl from Sod Alginate-Chitosan beads in the pH 7.4 Phosphate Buffer

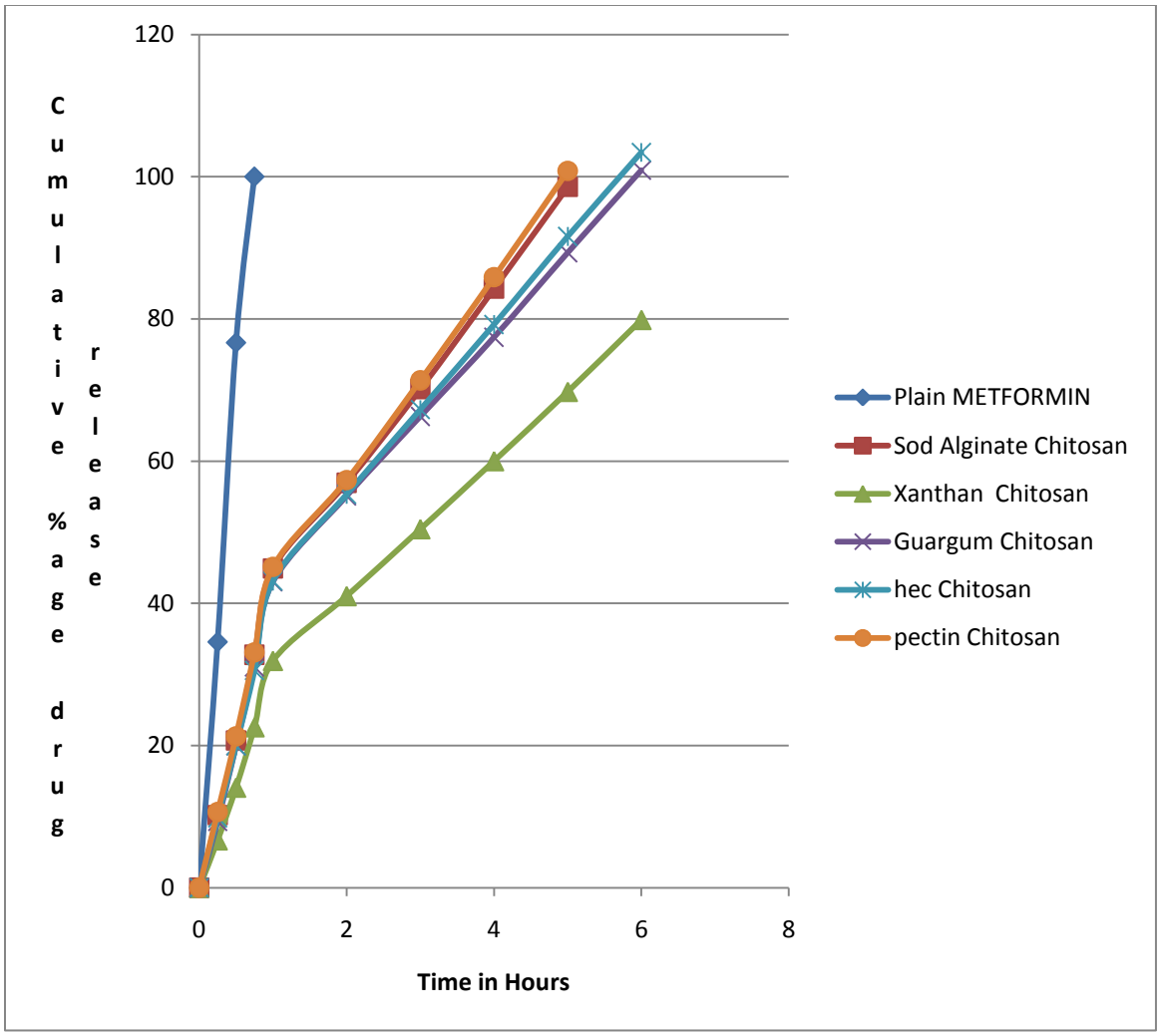

Figure 10-18: Cumulative percentage drug release of Metformin HCl from the various polymers noted above in the pH 7.4 Phosphate Buffer

Table 10.10: Cumulative Percent and Standard Deviations Release for the Metformin HCl from various Chitosan-Polymers Combination versus time

Polymers / **Time**	**Sod Alginate & Chitosan**	**Xanthan gum & Chitosan**	**Guagum & Chitosan**	**Hydroxyethyl Cellulose & Chitosan**	**Pectin & Chitosan**
15 min	10.21 ± 0.51	6.67 ± 0.48	9.31 ± 0.26	9.83 ± 0.25	10.65 ± 0.20
30 min	20.72 ± 0.68	14.08 ± 0.33	19.96 ± 0.19	19.98 ± 0.27	21.31 ± 0.23
45 min	32.77 ± 1.34	22.57 ± 0.25	31.06 ± 0.28	30.56 ± 0.10	33.08 ± 0.20
1 hr	44.91 ± 1.37	31.90 ± 0.31	43.04 ± 0.16	43.13 ± 0.24	45.11 ± 0.25
2 hr	56.95 ± 1.64	41.01 ± 0.32	55.11 ± 0.31	55.34 ± 0.34	57.34 ± 0.22
3 hr	70.14 ± 1.49	50.43 ± 0.30	66.29 ± 0.36	67.26 ± 0.38	71.36 ± 0.18
4 hr	84.26 ± 1.22	59.99 ± 0.31	77.42 ± 0.28	79.21 ± 0.18	85.87 ± 0.37
5 hr	98.58 ± 1.20	69.73 ± 0.44	89.30 ± 0.23	91.60 ± 0.33	100.81 ± 0.23
6 hr	---	79.84 ± 0.31	100.91 ± 0.21	103.43 ± 0.18	-----

CHAPTER 11

Conclusion

Chitosan / TPP beads were successfully prepared by the ionic cross–linking of cationic chitosan and polyanionic TPP. The polyanion interacts with the cationic chitosan to form a polyelectrolyte complex hydrogel in which drug molecules are trapped. This type of system shows a controlled drug release behavior due to a diffusion and swelling process.

The influence of various combinations of chitosan and other polymers showed improvement in the drug entrapment efficiency (DEE). The replacement of half of the chitosan with another polymer improved drug entrapment efficiency due to the hydrogel forming nature of the polymer.

Metformin hydrochloride loaded chitosan / sodium alginate / TPP beads were selected as the model since it had the highest Drug Entrapment Efficiency percentage. The beads were also found to have a uniform spherical shape. Their wet and dry diameters were found to be 2.8 $\pm$ 0. 30 mm and 2.50 $\pm$ 0.09 mm, respectively. Swelling percentage was around 11.22 for these beads.

FTIR studies showed the presence of functional groups. Characteristic IR absorption peaks were found to be superimposed in the beads. No significant chemical interaction was observed. The drug crystalline state in the beads was evaluated using powder X-Ray Diffraction (PXRD) analysis. Metformin hydrochloride (MH) was found to exist in its crystalline form with all sharp peaks matching with pure metformin inside the beads. There was no chemical interaction observed. The in vitro drug release study showed a slow release profile for chitosan / polymer/ TPP beads. The prepared beads may provide for a possible sustained drug release of a highly water soluble molecule metformin hydrochloride. This should be confirmed using an in-vivo study which was not part of this research project.

Finally this study demonstrated the observations of a uniform spherical to oval particles, high drug entrapment efficiency and a possible sustained drug release profile from the Chitosan / TPP polyelectrolyte complexes or beads. Thus, the prepared drug delivery system could be utilized for a possible sustained release product of highly water soluble drugs such as metformin hydrochloride.

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