PREPARATION OF THE CONTROLLED RELEASE CHITOSAN MICROSPHERES AND OPTIMISATION OF THE FORMULATION PARAMETERS

A thesis submitted in partial fulfillment of the requirement
for the award of the degree of

Master of Science
in
Biotechnology

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DECLARATION

I hereby declare that the work presented in the thesis entitled **"Preparation of the controlled release chitosan microspheres and optimisation of the formulation parameters"** in partial fulfillment of the requirement for the award of the degree of Masters of Science in Biotechnology, is an authentic record of my own work during the academic year 2010-2011, under the guidance of Mrs. M. Vasundhara, Assistant Professor, Department of Biotechnology and Environmental Sciences, Thapar University, Patiala. The report has not been submitted for the award of any other degree or certificate in this or any other University.

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This is to certify that the above statements made by the student are correct and true to the best of our knowledge and belief.

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CERTIFICATE

This is to certify that the thesis entitled **"Preparation of the controlled release chitosan microspheres and optimisation of the formulation parameters"** submitted by Amit Kumar Singal in partial fulfillment of the requirement for the award of the Degree of Masters of Science in Biotechnology to Thapar University, Patiala, is a record of student's own work carried out by him under my supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other University.

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ABSTRACT

The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. In most cases (conventional dosage forms), only a small amount of administered dose reaches the target site, while the majority of the drug distributes throughout the rest of the body in accordance with its physicochemical and biochemical properties. In recent years, controlled drug delivery formulations and the polymers used in these systems have become much more sophisticated, with the ability to do more than simply extend the effective release period for a particular drug.

In the present study, an attempt has been made to prepare microspheres by ionotropic gelation method using chitosan as a polymer and sodium tripolyphosphate (TPP) as a cross linking agent. Chitosan is a biodegradable natural polymer with a great potential for pharmaceutical applications due to its good biocompatibility, nontoxicity and mucoadhesion. TPP is an extensively researched well established, charged, non toxic, multivalent, anionic crosslinking agent with five bonding sites on the molecules.

Metformin hydrochloride loaded chitosan - TPP microspheres were prepared by dropping the drug containing solution of chitosan into TPP solution. The droplets instantaneously formed gelled spheres by the ionotropic gelation. The influence of drug concentration, drug-chitosan concentration, and pH of TPP solution was studied. The microspheres were characterized by their percentage yield, particle size, surface morphology, encapsulated amount of drug and in vitro drug release rate. Release studies were done in buffer (pH 1.2) and subsequently in buffer (pH 6.8). The release of drug from microspheres was greatly affected by pH of TPP solution, chitosan concentration, and drug concentration. After studying various parameters it was examined that highest encapsulation efficiency and highest percentage release was achieved at pH 9 having TPP conc. 2% (w/v), chitosan conc. 1% (w/v) and drug conc. of 1.5% (w/v). Morphology and stability of microspheres was observed to be good as compared to others at these optimized values.

Chitosan microspheres cross-linked by a combination of tripolyphosphate, not only had a good shape, but also had good pH-responsive drug release properties. High drug incorporation in chitosan – TPP microspheres could be achieved by ionotropic gelation method without using any toxic chemicals which causes undesirable effects. Hence this method is of particular interest in

the pharmaceutical field. Also the ionotropic gelation can be carried out under very mild conditions using simple equipments. The control of various manufacturing parameters plays a very important role in obtaining microspheres of good sphericity, high yield and high drug encapsulation.

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CHAPTER I

INTRODUCTION

Drugs are rarely administered as pure chemical substances alone and are almost always given as formulated preparations or medicines (i.e. drug delivery systems or dosage forms). These can vary from relatively simple solutions to complex drug delivery systems through the use of appropriate additives or excipients in the formulations. The method by which a drug is delivered can have significant effect on its efficacy.

The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. In most cases (conventional dosage forms), only a small amount of administered dose reaches the target site, while the majority of the drug distributes throughout the rest of the body in accordance with its physicochemical and biochemical properties. Therefore, developing a drug delivery system that optimizes the pharmaceutical action of a drug while reducing its toxic side effects *in vivo* is a challenging task (Waree Tiyaboonchai, 2003).

In the last 100 years, drug delivery systems have enormously increased their performances, moving from simple pills to sustained/controlled release and sophisticated programmable delivery systems. Meanwhile, drug delivery has also become more specific from systemic to organ and cellular targeting (Grassi M *et al., 2005).*

Traditional delivery systems are characterized by immediate and uncontrolled drug release kinetics. This is not economical and sometimes results in damaging side effects. Accordingly, drug absorption is essentially controlled by the body's ability to assimilate the therapeutic molecule and thus, drug concentration in different body tissues such as the blood, typically undergoes an abrupt increase followed by a similar decrease. As a consequence, it may happen that drug concentration dangerously approaches the toxic threshold to subsequently fall down below the effective therapeutic level (Grassi M *et al., 2005).*

So, increasing attention has been focused on methods of giving drugs continually for prolonged time periods and in a controlled fashion. Here, it is important to answer a fundamental question: What is controlled released technology? Controlled release products provide prolonged delivery of a drug while maintaining its blood concentration within therapeutic limits. The justification for a controlled release dosage form over a conventional tablet is either to circumvent problems in drug absorption or metabolism, or

to optimize therapy itself. An ideal drug delivery system should release the drug in the right body compartment at a rate required for a specific treatment (Srinath A *et al., 2008)*.

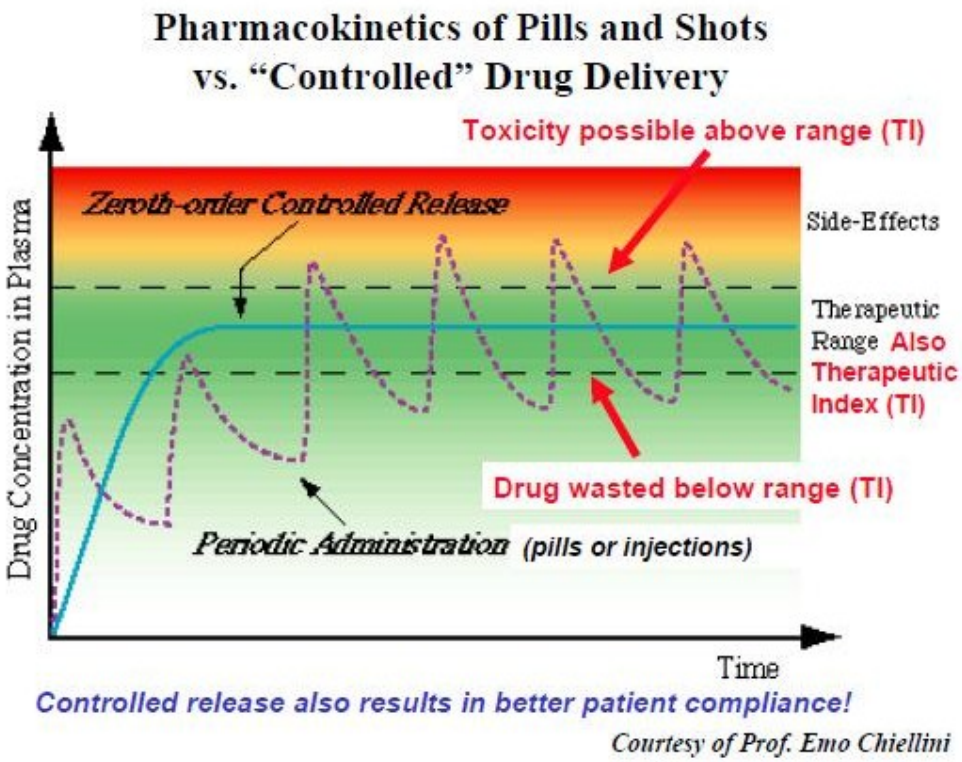

Figure 1: Drug levels in the blood with traditional drug dosing and controlled-delivery dosing.

The primary method of accomplishing the controlled release has been through incorporating the chemicals within polymers. Most available drug delivery systems use biodegradable biopolymers to act as carriers for controlled drug release (Li-Chun *et al., 2006)*. The release of the active agent may be constant over a long period, it may be cyclic over a long period, or it may be triggered by the environment or other external events. Among the various ways of achieving long-term drug delivery, polymeric microspheres have been effectively used for many years because of their biocompatibility, high bioavailability, and ability to encapsulate small molecules that can diffuse out of the barrier with precise kinetics modeling, and sustained drug release characteristics to the body fluid (Li-Chun *et al., 2006)*. The goal of many of the original controlled release systems was to achieve a delivery profile that would yield a high blood level of the drug over a long period of time. According to the release behavior controlled release systems can be subdivided into three different categories: passive pre-programmed, active pre-

programmed and active self-programmed. While in the first category (passive preprogrammed) release rate is predetermined and is irresponsive to external biological stimuli, in the second category (active preprogrammed), release rate can be controlled by a source external to the body as in the case of insulin delivery. The last category, representing the future of controlled release systems, is characterized by delivery systems whose release rate is controlled by biological stimuli such sugar concentration in blood. While until 15 years ago the majority of controlled release systems fell into the first category, nowadays the importance of the last two categories has become recognized. (Grassi M *et al., 2005).*

Rationale for controlled release of drugs includes:

- Increased patient compliance i.e. less frequent dosing.
- Safety: can control pharmacokinetics to remain within therapeutic index "window".
- Decreased cost: lower doses leads to more efficient use of drug.
- Greater profits: patent extension for drug, controlled and therapeutic release feature of drug are more profitable than traditional drug delivery system (Prof. Allan S Hoffman, ScD. University of Washington Seattle WA, 98195, USA).

Modified-oral controlled release dosage forms have been developed over the past three decades due to their considerable therapeutic advantages over conventional forms:

- reduced side-effects, drug concentration
- decreased dosing frequencies
- Improvement of patient compliance
- kept at effective levels in plasma, and improved utilization of drug
- Improvement in bioavailability of some drugs (Sevgi A Y F , 2010).

However these advantages can be significant, the potential disadvantages cannot be ignored (Brannon- Peppas *et al., 1995*):

a. the possible toxicity
b. non biocompatibility of the materials used
c. undesirable by-products of degradation
d. any surgery required to implant or remove the systems

e. the chance of patient discomfort from the delivery device
f. the higher cost of controlled-release systems compared with traditional pharmaceutical formulations.

In recent years, controlled drug delivery formulations and the polymers used in these systems have become much more sophisticated, with the ability to do more than simply extend the effective release period for a particular drug. In addition, materials have been developed that should lead to targeted delivery systems, in which a particular formulation can be directed to the specific cell, tissue, or site where the drug it contains is to be delivered. While much of this work is still in its early stages, emerging technologies offer possibilities that scientists have only begun to explore.

Objectives of Study:

The objectives of the present study were to optimize the variables influencing the preparation of the chitosan-TPP microspheres. The study involved:

- Preparation of chitosan-TPP microspheres by altering the process variables.
- Characterization of cross linked chitosan-TPP microspheres such as weight variation, percentage yield, average particle size and encapsulated amount of drug.
- Evaluation of the drug release behavior of the prepared chitosan - TPP microspheres.

CHAPTER II

REVIEW OF LITERATURE

Dubey R *et al., (2003)* studied the effect of formulation parameters such as concentration of chitosan solution, stirring speed, and concentration of drugs on preparation of microspheres using chemical denaturation method. They also analyzed morphology, mean particle size, particle size distribution, percentage drug entrapment (PDE), drug loading capacity, and in vitro drug release from the microspheres.

Balau L *et al., (2004)* performed structural investigations such as X-ray diffraction and Fourier transform IR analysis on chitosan films. Thermal investigations were also performed which revealed that the decomposition of the chitosan films proceeded in two stages, starting from 180°C and 540°C.

Anal *et al., (2005)* developed multilayer beads with improved properties for controlled delivery of the antibiotic ampicillin. They applied ionotropic gelation to prepare single and multilayer beads using various combinations of chitosan and Ca^{2+} as cationic components and alginate and polyphosphate as anions. Beads prepared with higher concentration of chitosan entrapped more ampicillin. During incubation in simulated gastric fluid, the beads swelled and started to float but did not show any sign of erosion.

Marques K L *et al., (2005)* described a multi-pumping flow system for the chemiluminometric determination of the hypoglycemic drug metformin. The developed methodology was based on the metformin-induced inhibition (metformin acts as a Cu (II) scavenger) of the catalytic effect of Cu (II) ions on the chemiluminescent reaction between luminol and hydrogen peroxide.

Oliveira B F *et al., (2005)* prepared chitosan microspheres by spray-drying method followed by treatment with a cross-linking agent, d,l-glyceraldehyde. They analyzed cross-linked spray-dried chitosan microspheres for their morphological aspects, particle size, zeta potential and water uptake capacity and reported that an increase either in d,l-glyceraldehyde concentration or in duration of cross-linking causes a decrease in both the swelling capacity and the zeta potential of the chitosan microspheres. They also reported d,l-glyceraldehyde to be a better cross-linking agent as compared to glutaraldehyde, for chitosan microspheres with the advantage that it is non toxic.

Anal *et al.,* (2006) prepared pentasodium tripolyphosphate cross-linked chitosan microspheres with higher acid resistance for controlled release of ampicillin. The microspheres were prepared by two different microencapsulation procedures (by emulsification and by spray drying) and characterized by their particle size, surface morphology, stability, entrapment efficiency and drug release.

Devika R Bhumkar *et al., (2006)* studied the effect of pH on cross linking of chitosan with sodium tripolyphosphate. The ionotropic gelation method for formation of cross-linked chitosan particles can be easily modified from ionic cross-linking to deprotonation by adjusting the pH of TPP. Chitosan was cross-linked ionically with TPP at lower pH and by deprotonation mechanism at higher pH.

Li-Chun Lin *et al., (2006)* attempted to expand the versatilities and applications in chitosan microspheres. They converted chitosan into micro-droplets by using a high voltage electrostatic field system, and then treated with TPP/NaOH solution of varying volume ratio to fabricate chitosan microspheres. By varying the pH values of these reacting agents, distinct morphological structure and properties of chitosan microspheres were further changed.

Patel A *et al., (2006)* prepared chitosan microspheres by non-aqueous solvent extraction method. They reported major advantage of this preparation technique as short processing time, the lack of exposure of the ingredients to high temperature, and high encapsulation efficiencies. They prepared different microspheres by mixing different ratio of components in the organic phase and analyzed drug polymer compatibility by FTIR spectroscopy. They concluded that drug loaded floating microspheres are a suitable delivery system for metformin hydrochloride, and may be used for effective management of NIDDM type II.

Basak S C *et al., (2008)* explained an ultraviolet spectrophotometric method based on absorption at 232nm in water for the estimation of metformin hydrochloride. The method showed very good linearity in the concentration range of 0-20 µg/mL. When standard drug solution was assayed for number of times (n = 6) the relative error (accuracy) and the relative standard deviation were found to be 0.8% and 0.47% respectively.

R Garg and G D Gupta (2008) analysed that controlled release dosage forms have been extensively used to improve therapy with several important drugs. However, the development processes are faced with several physiological difficulties such as the inability to restrain and localize the system within the desired region of the gastrointestinal tract and the highly variable nature of the gastric emptying process. This variability may lead to unpredictable bioavailability and times to achieve peak plasma levels. On the other hand, incorporation of the drug in a controlled release gastro retentive dosage forms (CR-GRDF) which can remain in the gastric region for several hours would significantly prolong the gastric residence time of drugs and improve bioavailability, reduce drug waste, and enhance the solubility of drugs that are less soluble in high pH environment.

Wanzari M M *et al., (2008)* described a simple reverse phase high-performance liquid chromatographic method for determining concentration of metformin in rat plasma. The method employed C18 column, ammonium acetate and acetonitrile were taken as mobile phase and ultraviolet detection was done at 236nm. The assay was linear in the concentration range of 0.33 - 16.6 µg/ml with co-efficient of correlation as 0.994. The retention time for metformin was found to be 4.7 minute.

Jain S K *et al.*, (2009) determined metformin hydrochloride content in gelucire (a lipid) microspheres. They dispersed 100 mg of drug in 100 ml of distilled water followed by heating at 65°C and agitation at 50 rpm with a magnetic stirrer and allowed to cool at room temperature. The lipid was solidified and the drug solution was filtered through a Whatman filter paper no 41. The sample was analyzed for drug content by UV spectrophotometry at 233 nm using UV–visible spectrophotometer after suitable dilutions.

Nair R *et al.*, (2009) reviewed various pharmaceutical applications of chitosan in microspheres based drug delivery. Drug release behavior and encapsulation in chitosan microspheres prepared by different methods like spray-drying, solvent evaporation, multiple emulsion, and complex coacervation, thermal cross-linking was also analyzed.

AppaRao B *et al., (2010)* prepared prolonged release microspheres of diclofenac sodium by employing ethyl cellulose as a polymer. The release kinetics study revealed that the drug follows first order kinetics and the mechanism of drug release was diffusion

controlled type. From the in vitro release profile, it was also observed that the drug released from microspheres decreased with an increase in coating material in the microspheres.

Dhakar R C *et al., (2010)* envisaged to reduce the dosing frequency and improve patient compliance by designing and evaluating sustained release mucoadhesive (SRM) microspheres of metformin hydrochloride (MH) for effective control of diabetes type-II. The microspheres exhibited good mucoadhesive properties and showed high drug entrapment efficiency. MH release from these microspheres was slow and extended and dependent on the type of polymer used. The mean particle size decreased and the drug release rate increased at higher stirring speed of emulsion content.

Sahu M, (2010) described work to improve oral therapeutic efficacy of metformin HCl and glimepiride by formulating a dosage form having one active ingredient in modified release (needs high dose) form and the other active ingredient as immediate release form (needs low dose). They performed and analyzed different FTIR spectra of metformin hydrochloride and studied the drug release rates from the microspheres.

CHAPTER III

RESEARCH ENVISAGED

The basic rationale for controlled drug delivery is to alter the pharmacokinetics and pharmacodynamics of pharmacologically active moieties by using novel drug delivery systems or by modifying the molecular structure and/or physiological parameters inherent in a selected route of administration. It is desirable that the duration of drug action becomes more a design property of a rate-controlled dosage form, and less, or not at all, a property of the drug molecules inherent kinetic properties. Thus, optimal design of controlled release systems necessitates a thorough understanding of the pharmacokinetics and pharmcodynamics of drug. The successful designing of a drug delivery system also involves a basic understanding of the properties and characteristics of polymer and a thorough knowledge of the nature of polymer.

3.1 Drug selection

The bioavailability of drugs with an absorption window in the upper small intestine is generally limited with conventional pharmaceutical dosage forms. The residence time of such systems and, thus, of their drug release into the stomach and upper intestine is often short. To overcome this restriction and to increase the bioavailability of these drugs, controlled drug delivery systems with a prolonged residence time in the stomach can be used (Alexander S *et al, 2006)*.

3.1.1 Physico-chemical properties of drug

Dose size: The therapeutic dose of the drug should be low.

Aqueous solubility: Extremes in aqueous solubility of a drug are undesirable.

Partition coefficient: Extremes in partition coefficient of a drug are also undesirable.

Drug stability: Drug used for sustained drug delivery should be stable over the entire length of the gastro-intestinal tract.

Molecular size: Large molecules will show small diffusion coefficients and may be unsuitable for a sustained release system.

3.1.2 Biological properties

Biological half-life: A drug with a short half life requires frequent dosing and this makes it a desirable candidate for sustained release formulation.

Absorption: Drugs that are slowly absorbed or absorbed with variable absorption rate are poor candidates for sustained release formulation.

Distribution: Drugs with high apparent volumes of distribution are poor candidates.

Therapeutic index: Drugs with a narrow therapeutic index require precise control over the blood levels of drug placing a constraint on controlled release dosage form. Metformin hydrochloride was chosen as a model drug for the preparation of crosslinked microspheres.

3.2 Metformin Hydrochloride

It is an oral antihyperglycemic biguanide agent used in the treatment of non-insulin-dependent diabetes Mellitus (type II diabetes) not responding to dietary modification. Metformin improves glycemic control by improving insulin sensitivity and decreasing intestinal absorption of glucose.

3.2.1 Drug description

Metformin hydrochloride (N, N-dimethylimidodicarbonimidic diamide hydrochloride) is a white crystalline substance with a molecular weight of 129.16 g/mol (free) and 165.63 g/mol (HCl), and is not chemically or pharmacologically related to any other classes of oral antihyperglycemic agents. Particle size does not influence dissolution of metformin hydrochloride because it is freely soluble in water but is practically insoluble in acetone, ether and chloroform. It is odourless, hygroscopic and has a slightly bitter taste. It should be stored in light resistant container at a temperature 15 -30 ºC. Its empirical formula is $C_4H_{11}N_5HCl$ and its chemical structure is as follows *(Indian pharmacopoeia, 1996):*

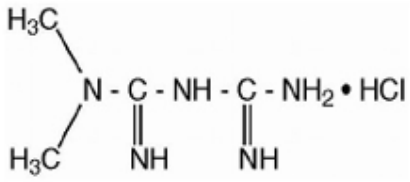

Figure 2: Chemical structure of Metformin Hydrochloride

Metformin is sold under several trade names, including Glucophage XR, Riomet, Fortamet, Glumetza, Obimet, Dianben, Diabex, and Diaformin. It is usually available in

500 mg, 850 mg, and 1000 mg dosage tablets. Metformin is sometimes prescribed to type II diabetes patients in combination with rosiglitazone.

3.2.2 Clinical uses of metformin hydrochloride

The main use for metformin is in the treatment of diabetes mellitus type II, especially in over weight people and those with normal kidney function. Unlike the other most-commonly prescribed class of oral diabetes drugs, the sulfonylureas, metformin does not induce hypoglycemia when taken alone and used at recommended dosages. Metformin is also being used increasingly in polycystic ovary syndrome (PCOS), non-alcoholic fatty liver disease (NAFLD) and premature puberty, three other diseases that feature insulin resistance but the use is not widespread and lack extensive experimental evidence.

3.2.3 Adverse Effects

Common side effects of metformin include gastrointestinal upset, including diarrhoea, cramps, nausea, vomiting and increased flatulence. The most serious potential side effect of metformin use is lactic acidosis which has fewer occurrences in usual people but risk is high in those with impaired liver or kidney function (Khurana *et al 2010)*. Feeling very weak, tired, unusual muscle pain and stomach discomfort, trouble breathing, feeling cold and feeling dizzyare some common signs of lactic acidosis. Metformin has also been reported to reduce the blood levels of thyroid-stimulating hormone in patients with hypothyroidism, in men, lutenizing hormone and testosterone. The clinical significance of these changes is still unknown (Vigersky R A, 2006; Shegem N S *et al., 2002)*.

3.2.4 Precautions

Metformin is avoided in those people who

- Have kidney problems
- Have liver problems
- Drink a lot of alcohol
- Have heart problems that is treated with medicines, such as Lanoxin (digoxin) or Lasix (furosemide)

- Are going to have an x-ray procedure with injection of dyes (contrast agents)
- Are going to have surgery
- Are 80 years or older and have not had kidney function tested (Thomsen H S *et al., 2003*; Jones G *et al., 2003)*.

3.2.5 Analysis of Metformin hydrochloride

There are various methods found for simultaneous determination of metformin and combined drugs. For rapid screening of metformin molecularly imprinted solid phase extraction is done. To determine metformin in humans, dried blood spot liquid chromatography for therapeutic drug monitoring of metformin is done. For formulation and optimization of sustained release matrix tablet of metformin HCl surface methodology is done. There is no method found for stability determination of metformin HCl having degradation studies.

3.2.6 Pharmacology

3.2.6.1 Mechanism of action

Metformin is an antihyperglycemic agent which improves glucose tolerance in patients with type II diabetes, lowering both basal and postprandial plasma glucose. Its pharmacologic mechanisms of action are different from other classes of oral antihyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Unlike sulfonylureas, metformin does not produce hypoglycemia in either patients with type 2 diabetes or normal subjects and does not cause hyperinsulinemia. With metformin therapy, insulin secretion remains unchanged while fasting insulin levels and day-long plasma insulin response may actually decrease. The glucose-lowering effects of metformin are mainly a consequence of reduced hepatic glucose output (primarily through inhibition of gluconeogenesis and, to a lesser extent, glycogenolysis) and increased insulin-stimulated glucose uptake in skeletal muscle and adipocytes (Bailey C J *et al., 1996*; Hundal R S *et al., 2000)*.

3.2.7 Pharmacokinetics

3.2.7.1 Absorption and Bioavaiblaility

The absolute bioavailability of a metformin hydrochloride 500 mg tablet given under fasting conditions is approximately 50-60% (Sweetman, 2002). Metformin doses of 0.5 to 1.5 g have an absolute oral bioavailability of 40 to 60%. The discrepancy between the amount of drug absorbed and the amount available may result from presystemic clearance or binding to the intestinal wall. Studies using single oral doses of metformin tablets of 500 mg and 1500 mg, and 850 mg to 2550 mg, indicate that there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alteration in elimination. Food decreases the extent of and slightly delays the absorption of metformin. The clinical relevance of these decreases is unknown.

3.2.7.2 Distribution

Metformin reached steady state plasma concentrations within 24 hours at therapeutic doses and are usually in the range of 1-2 mg/ml. It is rapidly distributed and negligibly bound to plasma proteins in contrast to sulfonylureas which are more than 90% protein bound, metformin partitions into erythrocytes, most likely as a function of time. At usual clinical doses and dosing schedules of metformin hydrochloride tablets, steady state plasma concentrations of metformin are reached within 24-48 hours and are generally < 1 μg/ml. During controlled clinical trials, maximum metformin plasma levels did not exceed 5 μg/ml, even at maximum doses. No metabolites or conjugates have been identified. As metformin is primarily excreted unchanged in the urine, significant accumulation may occur in renal impairment, resulting in increased risk of lactic acidosis. Therefore, Competact is contraindicated in patients with moderate renal impairment, with a creatinine clearance <60ml/min.

3.2.7.3 Metabolism and Elimination

Intravenous single-dose studies in normal subjects demonstrate that metformin is excreted unchanged in the urine and does not undergo hepatic metabolism (no metabolites have been identified in humans) or biliary excretion. Renal clearance is approximately 3.5 times greater than creatinine clearance which indicates that tubular secretion is the major route of metformin elimination. Following oral administration, approximately 90% of the absorbed drug is eliminated via the renal route within the first 24 hours, with a plasma elimination

half-life of approximately 6.2 hours. In blood, the elimination half-life is approximately 17.6 hours, suggesting that the erythrocyte mass may be a compartment of distribution.

3.3 Selection of polymer

If an application requires rapid development and commercialization, then the polymer selection will most likely be made from those polyesters that already received regulatory approval. Another factor to be taken into account is choice, whether to use homopolymers or copolymers containing multiple monomer species. If copolymers are employed than the relative ratio of different monomers may be manipulated to change polymeric properties i.e. morphology, structure and extent of drug polymer interactions. Ultimately all these properties will influence the performance of the drug delivery systems via changes to relative rates of mass transport and degradation rate of both, the polymer and the device (Kotwal and Saifee, *2007).*

Range of materials has been employed to control the release of drugs and other active agents. The earliest of these polymers were originally intended for non-biological uses and were selected because of their desirable physical properties, which include: poly (urethanes) for elasticity, poly (siloxanes) or silicones for insulating ability, poly (methyl methacrylate) for physical strength and transparency, poly (vinyl alcohol) for hydrophilicity and strength, poly (ethylene) for toughness and lack of swelling and poly (vinyl pyrrolidone) for suspension capabilities.

3.3.1 Criteria for ideal polymer

- Easy to synthesize and characterize
- Non-toxic
- Non-immunogenic
- Biocompatible and biodegradable
- Inexpensive (W Tiyaboonchai, *Naresuan Uni. J., 2003).*

3.3.2 Cross-linking of polymers

Forming cross-links between polymer functional groups decreases its hydrophilicity, thus slowing down the rate of permeation of biological fluids throughout the hydrated matrix and subsequently the rate of drug diffusion from the matrix. Formation of physical bonds

between polymer molecules can be achieved by ionotropic gelation, a process that involves interaction of one polymer with other polymers, electrolytes or polyelectrolytes. Cross-linking by adding agents to form chemical bonds has the major drawback of the toxicity of the chemicals used. These substances (e.g. formaldehyde) are highly toxic on inhalation and ingestion, and act as irritants to the skin and the respiratory tract. Ionic cross-linking has the advantage of avoiding addition of toxic chemicals; hence this method is of particular interest in the pharmaceutical field (Genta *et al*., 2002).

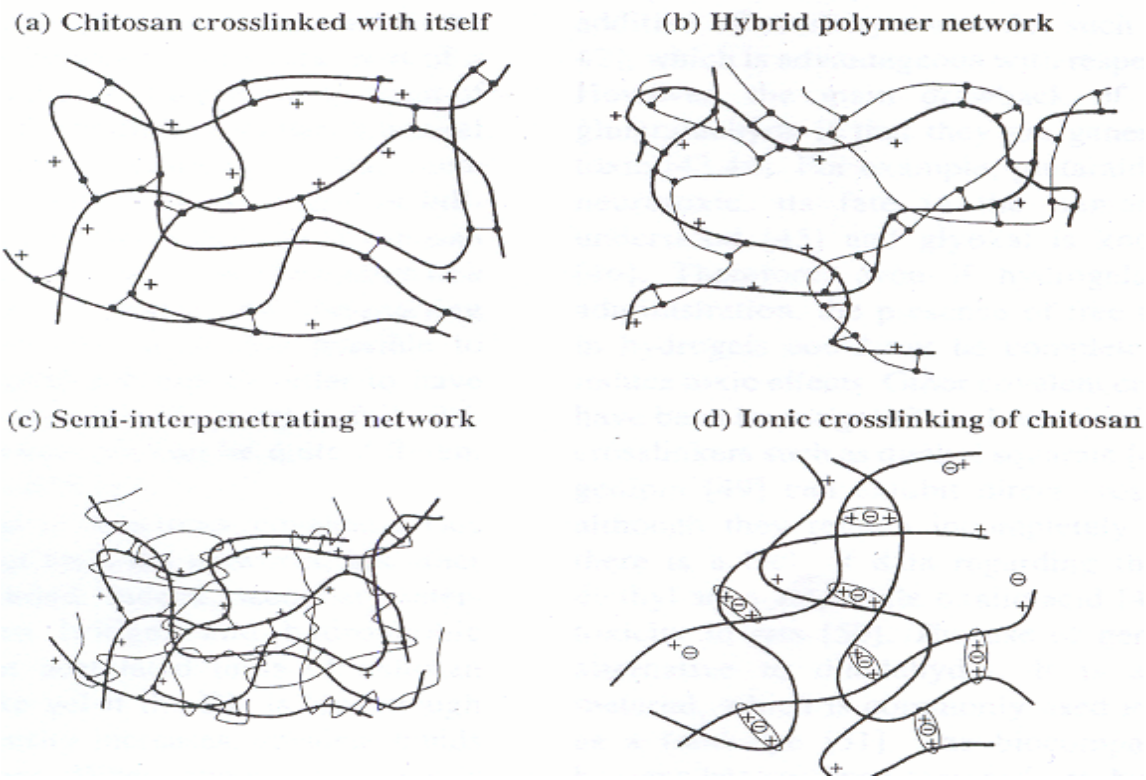

Figure 3: Formation of cross-linked gels by use of polyelectrolyte complexation (Sinha *et al.*, 2004).

For this application, chitosan has come to be a particularly interesting polymer for the association and delivery of labile macromolecular compounds (M Prabaharan, *2005).* Among water-soluble polymers available, chitosan is one of the most extensively studied.

3.4 Chitosan

Chitosan or beta (1,4)-2-amino-2-deoxy-D-glucose is a modified natural carbohydrate polymer prepared by the partial N-deacetylation of chitin, a natural biopolymer derived from crustacean shells such as crabs, shrimps and lobsters. Chitosan is also found in some

microorganisms, yeast and fungi (Illum, *1998)*. The primary unit in the chitin polymer is 2-deoxy-2-(acetylamino) glucose.

Chitosan (pronounced ky-toe-san) is derived from a material called chitin (second most abundant polysaccarides in nature next to cellulose), which is an amino polysaccharide, extracted from the powdered shells of crustaceans like shrimps and crabs. To prepare chitin, crab and shrimp shells are demineralised in dilute hydrochloric acid (HCl), deproteinated in dilute sodium hydroxide (NaOH), and then decolourised in potassium permanganate ($KMnO_4$). The chitin is then deacetylated to become chitosan by boiling it in a concentrated sodium hydroxide solution. Biochemical grade/purified chitosan is prepared by repeating the deacetylation process. Pharmaceutical grade chitosan is deacetylated between 90 and 95% and food grade between 75 and 80% (Paul and Sharma, *2000*).

Chitosan is structurally similar to glycosaminoglycans with a chemical formula of $(C_6H_{11}O_4N)_n$ (Hejazi and Amiji, *2003*), as represented in Figure 4:

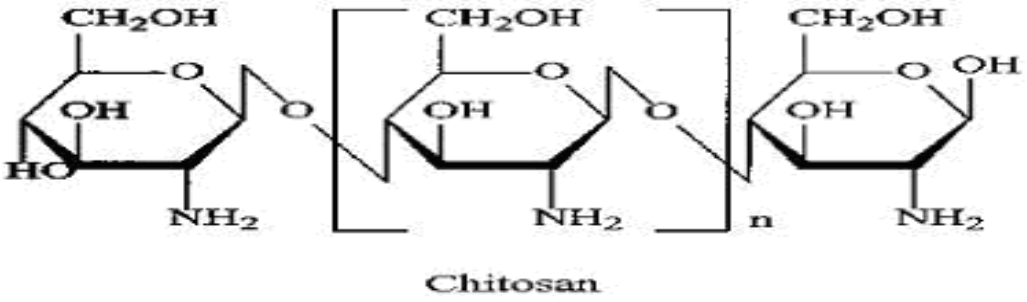

Figure 4: Chemical structure of chitosan

Chitosan is a weak base with a pKa of about 6.2-7.0, and it requires a certain amount of acid to become soluble and has shown promising characteristics as a modified drug delivery polymer (Paul and Sharma, *2000*). The word 'chitosan' refers to a large number of polymers, which differ in their degree of N-deacetylation (40-98%) and molecular weight (50000 - 20000000 Daltons). These two characteristics are very important to its physicochemical properties and may have a major effect on the biological properties. Chitosan salts are soluble in water; the solubility depends on the degree of deacetylation and the pH of the solution. The pharmaceutical requirements of chitosan are: particle size <30 µm, density between 1.35 and 1.40 g/cm^3, pH 6.5-7.5, insoluble in water, and partially soluble in acids .

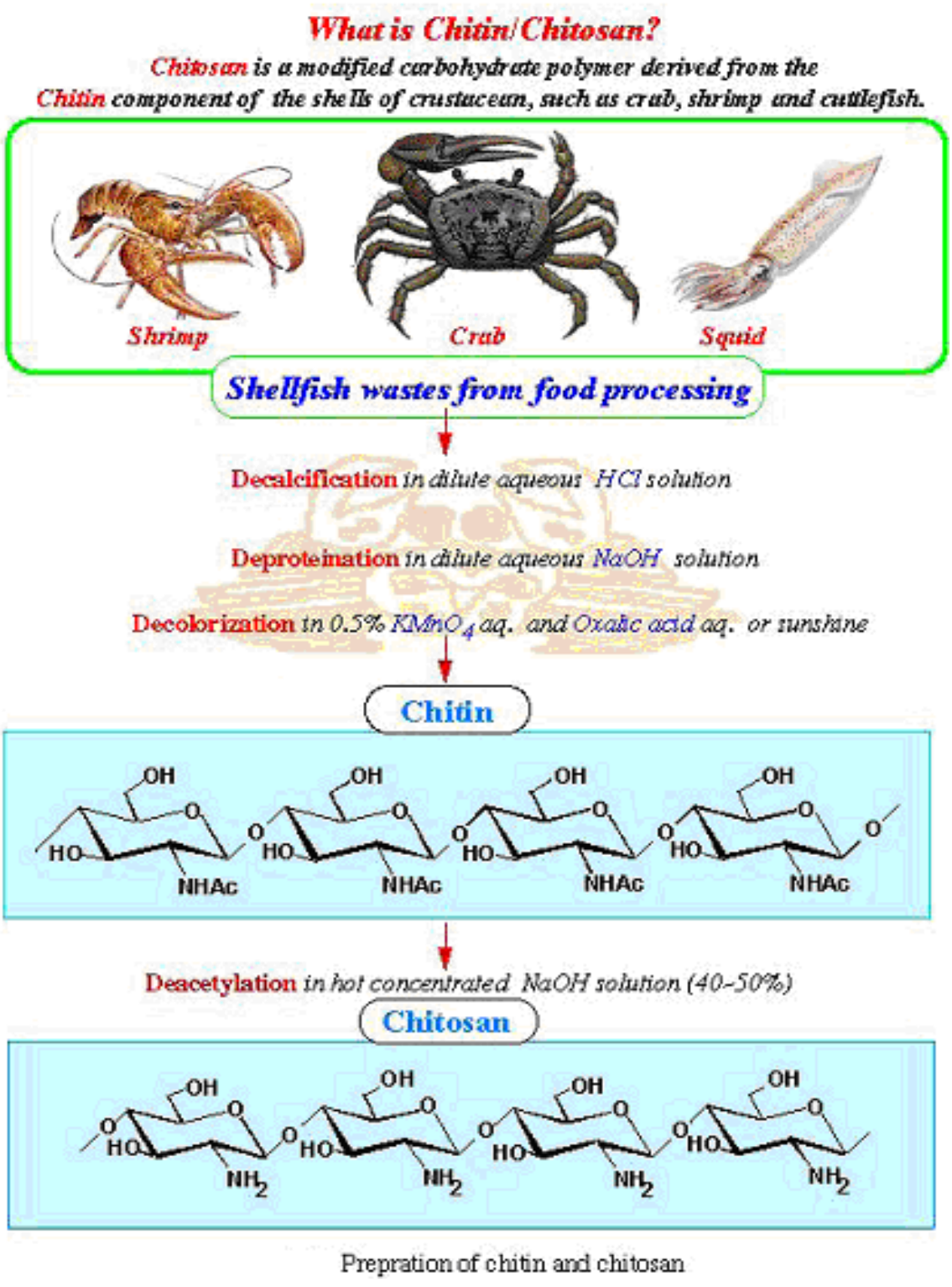

Figure 5: Synthesis of chitosan from chitin (Nair R *et al., 2009*)

Table1: Chemical and biological properties of chitosan (Hejazi and Amiji, *2003*, Sinha V R *et al., 2004*).

Chemical properties of chitosan	Biological properties of chitosan
• Cationic polyamine. • High charge density at pH <6.5 • Adheres to negatively charged surfaces • Forms gels with polyanions • High molecular weight, linear polyelectrolyte • Viscosity, high to low • Chelates certain transitional metals • Ease of chemical modification • Reactive amino/hydroxyl groups • Insoluble in water and organic solvents	• Biocompatible and Biodegradable • Safe and non-toxic • Mucoadhesive properties • Spermicidal • Anti-cancerogen • Anti-cholesteremic • Suitably permeable • Wound healing acceleration • Immune system stimulant • Hemostatic, bacteriostatic and fungistatic

3.4.1 Application of chitosan polymer

- Chitosan by itself is haemostatic (stops bleeding), some derivatives such as sulfated chitosan are anticoagulants. By utilizing the haemostatic effect, chitosan bandages and sponges were prepared for surgical treatment and wound protection.
- Chitosan has a capacity of forming film and has been suggested as a biopolymer of choice for the development of contact lenses (soft and hard contact lenses). Chitosan has been used for the manufacturing of ocular bandage lenses used as protective devices for acutely or chronically traumatized eyes. Chitosan membranes have also been found useful as artificial kidney membranes because of their suitable permeability and high tensile strength.
- Used for hypobilirubinaemic and hypercholesterolemia effects, antacids and antiulcer activities wound and burn healing properties, immobilization of enzymes and living cells and in ophthalmology.
- Among pharmaceutical applications it has been used as a vehicle for directly compressed tablets, as a disintegrant, binder, granulating agent in ground mixtures, as a drug carrier for sustained release preparations as well as co-grinding diluents

for the enhancement of dissolution rate and bioavailability of water insoluble drugs.

- Chitosan is known to possess mucoadhesive properties due to molecular attractive forces of electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces.
- The special affinity of chitosan for biomolecules has been utilized to reduce side effects of drugs. Membranes prepared from chitosan have shown greater permeability for ascidic drugs than basic drugs. Chitosan polymer is also used as a carrier for microsphere drug delivery. Chitosan microspheres are most widely studied delivery systems for the controlled release of drugs i.e. antibiotics, antihypertensive agents, anticancer agents, proteins, peptide drugs and vaccines. (Nair R *et al., 2009*).

3.5 Crosslinking agent

3.5.1 Sodium tripolyphosphate (STPP)

Tripolyphosphate (TPP) has the following synonyms: Sodium triphosphate; Triphosphoric acid, pentasodium salt; Sodium Tripolyphosphate (STPP); pentasodium triphosphate; Pentasodium Tripolyphosphate. TPP is non toxic and multivalent anion that forms crosslinks by ionic interaction between positively charged amino groups of chitosan and multivalent negatively charged TPP molecules (Bodmeir *et al., 1989*).

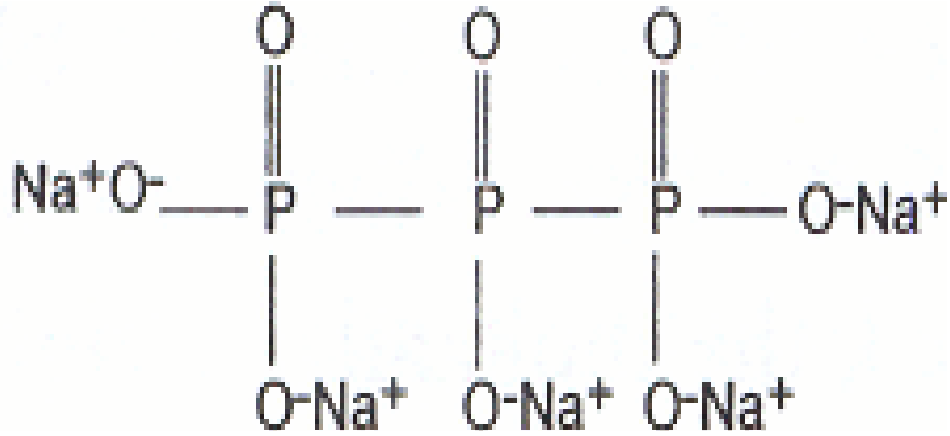

Figure 6: Chemical structure of sodium tripolyphosphate

3.5.2 Preparation

Industrially sodium tripolyphosphate is prepared by heating a stoichiometric mixture of disodium phosphate, Na_2HPO_4 and monosodium phosphate, NaH_2PO_4 in carefully controlled conditions.

$$\mathbf{2Na_2HPO_4 + NaH_2PO_4 \rightarrow Na_5P_3O_{10} + 2H_2O}$$

3.5.3 Uses

1. The United States Food and Drug Administration lists STPP as "generally recognized as safe", along with salt, vinegar, and baking powder.

2. STPP is a solid inorganic compound used in a large variety of household cleaning products, mainly as a builder, but also in human foodstuffs, animal feeds, industrial cleaning processes and ceramics manufacture.

3. Chemical functions of STPP includes: sequestration of "water hardness", enabling surfactants to function effectively; pH buffering; dirt emulsification and prevention of deposition; hydrolysis of grease; and dissolving-dispersing dirt particles.

TPP is an extensively researched, well-established cross-linking agent with five bonding sites on the molecule. Chitosan beads cross-linked by a combination of tripolyphosphate, not only had a good shape, but also had good pH-responsive drug release properties. The TPP treatment of chitosan microspheres is expected to improve their stability and their applicability in controlled drug delivery.

CHAPTER IV

EXPERIMENTAL WORK

4.1 Experimental Material and Equipments

Various chemicals and instruments used for the preparation and evaluation of crosslinked microspheres are listed as given below in table 2.

Table 2: Experimental materials and equipments

Experimental Material	**Equipments**
• Chitosan (85% deacetylated, Sigma Aldrich) • Metformin hydrochoride • Pentasodium tripolyphosphate (Practical grade 90-95%, Sigma-Aldrich) • Acetic acid • Buffer pH 1.2 • Buffer pH 6.8 • Conc. hydrochloric acid • Potassium chloride • Sodium hydroxide • Potassium dihydrogen orthophosphate • Distilled water	• Analytical balance • Magnetic stirrer • Magnetic bead • Optical microscope • Ulrta violet -visible spectrophotometer • Incubator • Shaking incubator • Disposable syringes • Glass beakers (100ml, 150ml, 200ml, 250ml) • Measuring cylinders (100ml, 500ml,1000ml) • Micropipettes (200µl, 1ml) • Glass pipettes (1ml, 2ml)

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4.2 Preparation of standard curves of metformin hydrochloride

Concentration of metformin hydrochloride in the solution was estimated by stable beam spectrophotometer by reading the instrument at 233nm. The standard curves were prepared in distilled water, pH 1.2 buffer, pH 6.8 buffer solutions and 0.1N HCl.

4.2.1 Preparation of buffer pH 1.2

50ml of the potassium chloride solution (0.2M) was placed in a 200ml volumetric flask and to it was added 85ml of hydrochloric acid solution (0.2M) and then distilled water was added to make the volume to 200ml.

4.2.2 Preparation of buffer pH 6.8

50ml of the potassium dihydrogen phosphate (0.2M) was placed in 200ml volumetric flask and to it 22.4ml of sodium hydroxide solution (0.2M) was added and the volume was made upto 200ml with distilled water.

4.2.3 Preparation of standard stock and working solution of drug in distilled water

100mg of accurately weighed drug was dissolved in distilled water and the volume was made upto 100ml with distilled water. 1ml of standard stock solution was diluted to 10ml with distilled water in a 25ml test tube to get a stock solution of 100µg/ml. Aliquots of 0.06ml to 0.3 ml were pipetted out from the standard working solution and volume was made up to 3ml to obtain solution containing the desired concentration of metformin hydrochloride ranging from 2µg/ml to 10µg/ml. The absorbance of the different dilutions was measured at 233 nm against distilled water as blank using a stable beam spectrophotometer.

Similarly, standard stock solution and standard working solutions were prepared in buffer pH 1.2 and buffer pH 6.8 and standard curves were obtained by plotting the data.

Table 3: Standard curve data of metformin hydrochloride in distilled water

S.No.	Concentration (µg/ml)	Absorbance (at 233nm)
1.	2	0.169
2.	4	0.392
3.	6	0.588
4.	8	0.780
5.	10	0.996

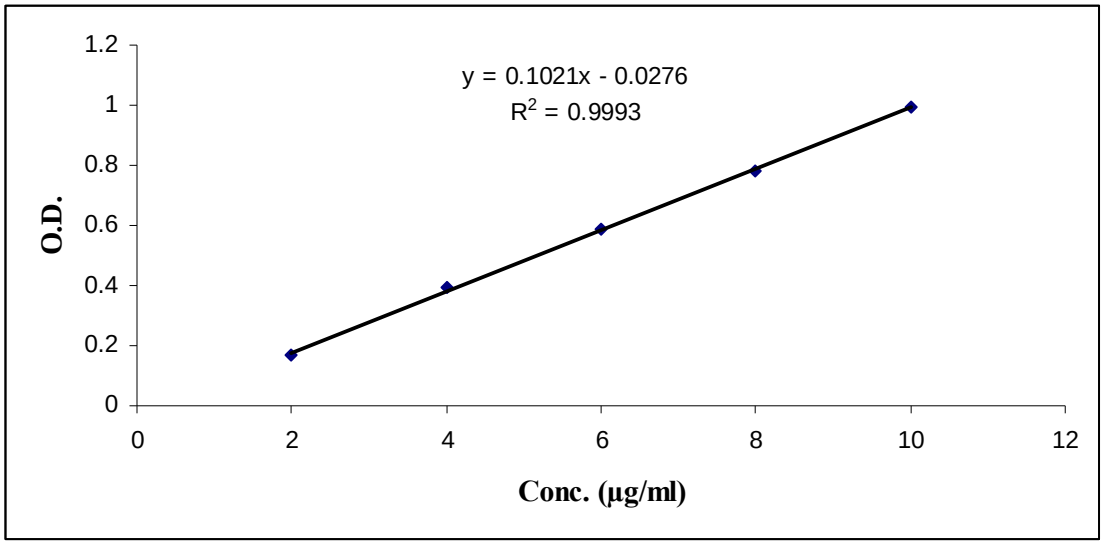


Figure 7: Standard curve of metformin in distilled water

Table 4: Standard curve data of metformin hydrochloride in buffer pH 1.2

S.No.	Concentration (µg/ml)	Absorbance (at 233nm)
1.	2	0.035
2.	4	0.073
3.	6	0.109
4.	8	0.146
5.	10	0.180

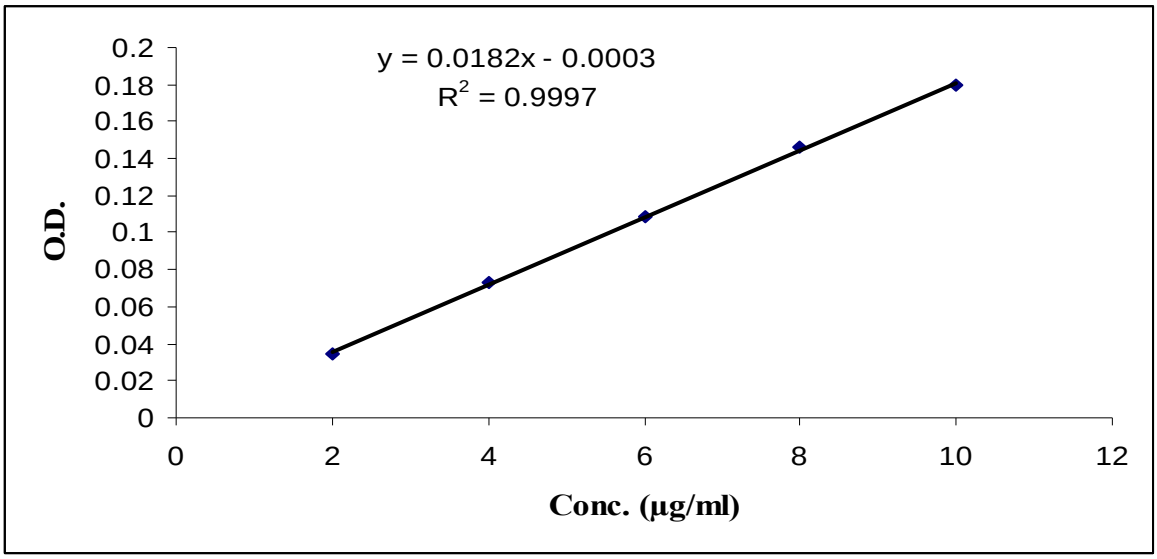


Figure 8: Standard curve of metformin in buffer of pH 1.2

Table 5: Standard curve data of metformin hydrochloride in buffer (pH 6.8)

S. No.	Concentration (µg/ml)	Absorbance (at 233nm)
1.	2	0.150
2.	4	0.319
3.	6	0.465
4.	8	0.608
5.	10	0.753

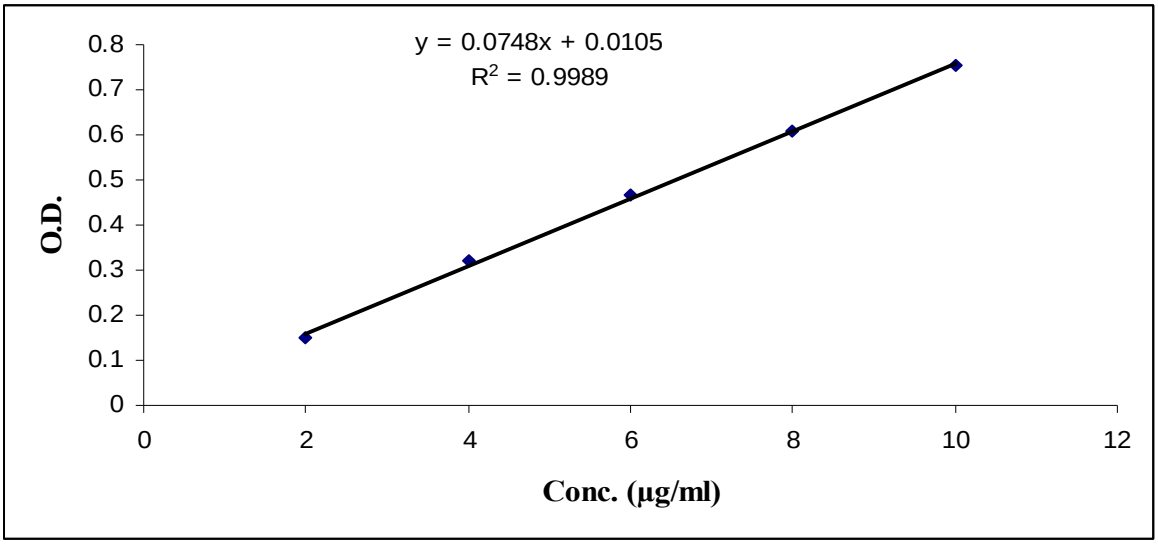


Figure 9: Standard curve of metformin hydrochloride in buffer pH 6.8

Table 6: Standard curve data of metformin hydrochloride in 0.1N HCl.

S. No.	Concentration (µg/ml)	Absorbance (at 233nm)
1.	10	0.171
2.	20	0.353
3.	30	0.526
4.	40	0.736
5.	50	0.889

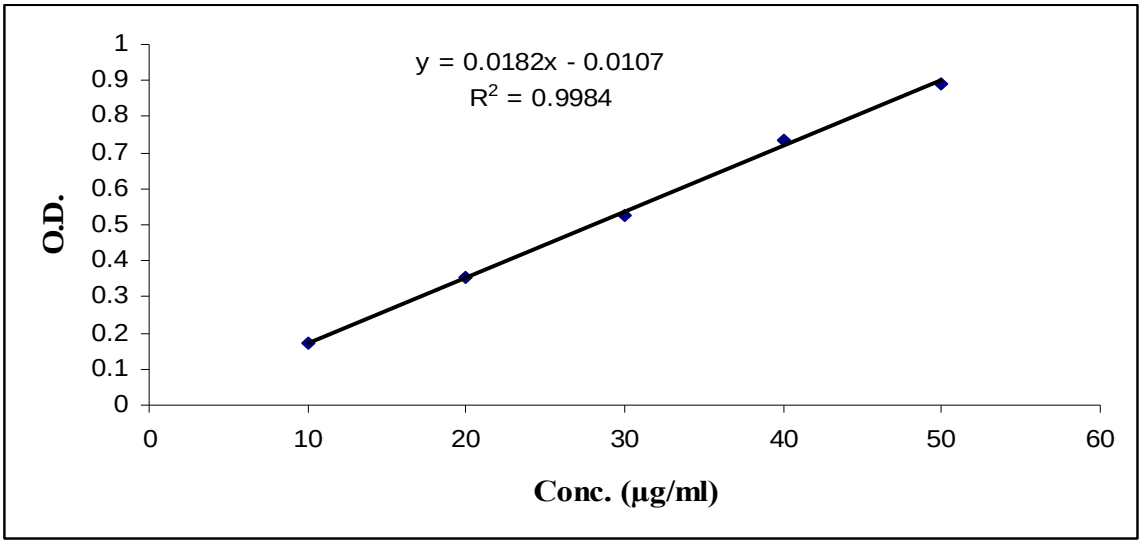


Figure 10: Standard curve of metformin hydrochloride in 0.1N HCl.

4.3 Preparation of chitosan microspheres

Microspheres are matrix systems that contains drug throughout their structure and are potential candidates for oral controlled release. Microsphere can be defined as solid spherical particles ranging from one to 1000 µm in size. These particles consist of the drug which is the core material, and a coating material. (Tamizharasi S *et al., 2009*). The choice of methods for the preparation of microspheres depends on many factors such as the drug solubility, partition co-efficient, polymer composition, molecular weight etc. For instance, ionotropic gelation method may be a method of choice for the preparation of microspheres of water soluble drugs. Chitosan microspheres were prepared by ionotropic gelation method.

In this method chitosan stock solution (1% w/v) was prepared by dissolving chitosan in acetic acid (1% v/v) at room temperature. The drug metformin hydrochloride (1% w/v) was dissolved directly into the above prepared chitosan solution. 10 ml of this bubble free solution was dropped through a disposable syringe needle into a gently agitating 100ml of 2% (w/v) sodium tripolyphosphate solution. The dropping rate and falling distance were kept constant. The solution was magnetically stirred for half an hour followed by filtration and rinsing with distilled water. Gel like beads were obtained which were air dried for twenty four hours followed by oven drying for six hours at 40˚C.

Different batches of cross linked chitosan microspheres were prepared by altering the following formulation variables:

Table 7: variables for preparation of microspheres

Drug concentration	(0.5-2% w/v)
Drug: polymer concentration	(0.5-2% w/v)
pH of TPP solution	(pH3,5,7 and 9)

Table 8: Formulation of cross linked chitosan microspheres prepared by varying drug conc.

Batch No.	pH of TPP solution	Conc. of TPP (%w/v)	Conc. of chitosan (%w/v)	Conc. of drug (%w/v)	Stirring time (min)	Volume of chitosan-drug solution (ml)
B0	9	2	1	-	30	10
B1	9	2	1	0.5	30	10
B2	9	2	1	1	30	10
B3	9	2	1	1.5	30	10
B4	9	2	1	2	30	10

Table 9: Formulation of cross linked chitosan microspheres prepared by varying drug-polymer concentration.

Batch No.	pH of TPP solution	Conc. of TPP (%w/v)	Conc. of chitosan (%w/v)	Conc. of drug (%w/v)	Stirring time (min)	Volume of chitosan-drug solution (ml)
BN	9	2	0.5	0.5	30	10
B5	9	2	1	1	30	10
B6	9	2	1.5	1.5	30	10
B7	9	2	2	2	30	10

Table 10: Formulation of cross linked chitosan microspheres prepared by varying pH at various chitosan: drug ratio.

Batch No.	pH of TPP solution	Conc. of TPP (%w/v)	Conc. of chitosan (%w/v)	Conc. of drug (%w/v)	Stirring time (min)	Volume of chitosan-drug solution (ml)
B8	3	2	1.5	1.5	30	10
B9	5	2	1.5	1.5	30	10
B10	7	2	1.5	1.5	30	10
B11	9	2	1.5	1.5	30	10
B12	5	2	1	1.5	30	10
B13	7	2	1	1.5	30	10
B14(3)	9	2	1	1.5	30	10
B15	5	2	1	1	30	10
B16	7	2	1	1	30	10
B17(2)	9	2	1	1	30	10

4.4 Evaluation of cross linked chitosan-TPP microspheres

All batches of chitosan-TPP microspheres were evaluated based upon the following characteristics.

(1) Percentage Yield

(2) Average particle size

(3) Encapsulated amount of drug

(4) In vitro release properties.

4.4.1 Percentage Yield

The prepared microspheres were collected, dried and weighed. The percentage yield was calculated by the formula:

$$\text{Percentage yield(w/w)} = \frac{\text{Weight of dried microspheres recovered}}{\text{Weight of chitosan + Weight of TPP}} \times 100$$

Table 11: Percentage yield of different batches of chitosan-TPP microspheres

Batch no.	Yield (gm)	Percentage yield (%)
B0	0.0889	4.24
B1	0.1125	5.48
B2	0.1102	5.25
B3	0.1412	6.7
B4	0.0998	4.75
B5	0.1100	5.24
B6	0.2541	8.75
B7	0.1881	11.55
B8	0.1823	8.48
B9	0.1901	7.87
B10	0.1931	8.98
B11	0.2072	9.64
B12	0.2645	6.3
B13	0.3134	7.4
B14(3)	0.1412	6.7
B15	0.1207	5.74
B16	0.1198	5.7
B17(2)	0.1102	5.25

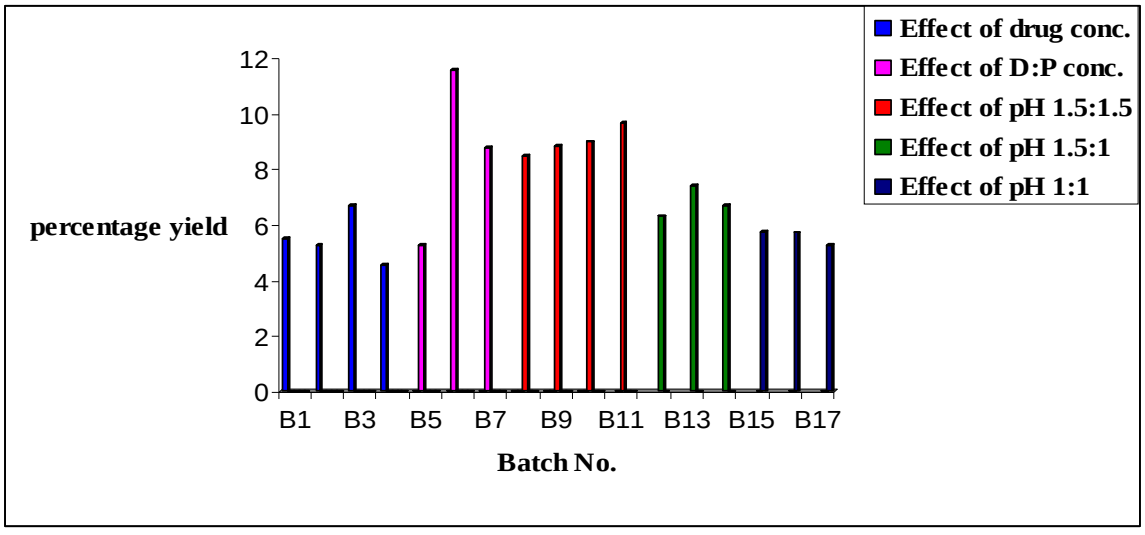


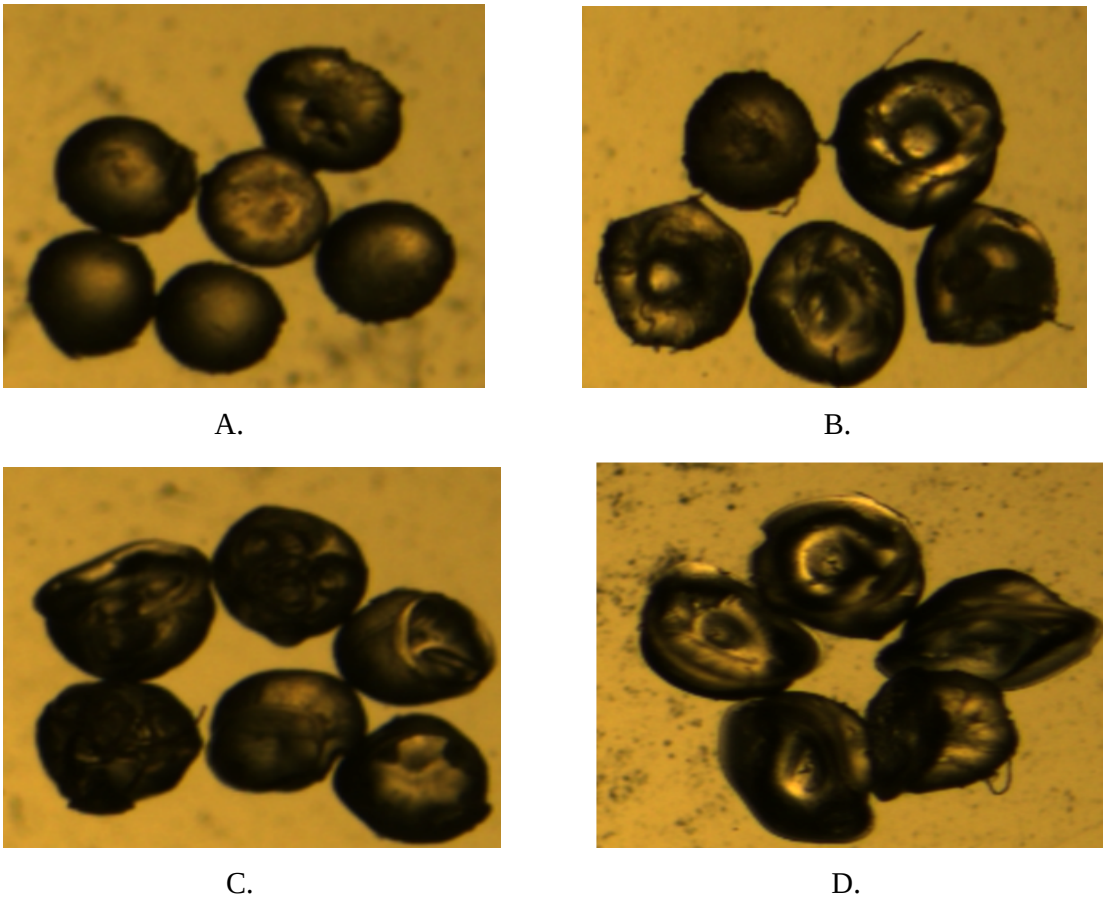
Figure 11: Histogram showing effect of various formulation parameters on percentage yield.

4.4.3 Particle Size Determination

The various batches of cross linked microspheres prepared were studied for the following micrometric properties.

4.4.3.1 Average particle size

The particle size of the microspheres was measured directly by using stereomicroscope. The slide containing chitosan microspheres was mounted on the stage of microscope. Then the microspheres were focused to measure the average particle size. Stereomicrographs of the cross linked chitosan-TPP microspheres were taken. Morphological characteristics of the microspheres were also observed.



A. B. C. D.

Figure12: Stereomicrographs of microspheres (A, B, C and D) at pH 9, 7, 5 and 3 respectively at 1X.

Table 12: Average diameter of different batches of chitosan-TPP microspheres

Batch no.	Average size (µm)
B1	697
B2	595
B3	586
B4	618
B5	636
B6	759
B7	803
B8	972
B9	788
B10	817
B11	937
B12	624
B13	603
B14(3)	586
B15	646
B16	623
B17(2)	595

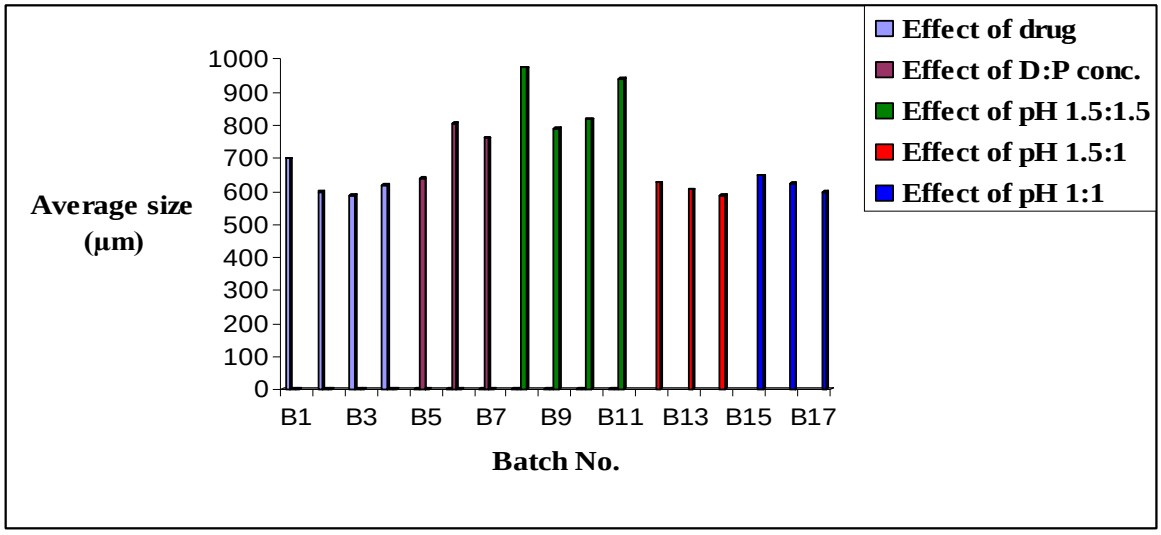


Figure 13: Histogram showing effect of various formulation parameters on average particle size of microspheres

4.4.4 Determination of Encapsulated Amount of Drug (EAD)

100 mg of microspheres were crushed in a pestle and mortar and digested in 100ml of 0.1N HCl for 24 hrs in a beaker. The solution was filtered and aliquot was used to measure the drug content (EAD) spectrophotometrically at 233 nm against a suitable blank.

Table 13: EAD of different batches of chitosan-TPP microspheres

Batch No.	EAD (µg/ml)
B1	13
B2	16.38
B3	20.06
B4	21.4
B5	18.28
B6	16.89
B7	22.95
B8	19.45
B9	23.51
B10	26.84
B11	22.89
B12	25.73
B13	22.89
B14(3)	20.06
B15	19.12
B16	18.73
B17(2)	16.38

4.4.5 Evaluation of *in vitro* drug release rate

100 mg of drug loaded chitosan-TPP microspheres were incubated in 100ml buffer (pH1.2) in a 250ml conical flask kept in a shaking incubator at 37^{o}C at 60 rpm. After 4 hours microspheres were filtered and transferred in to 100ml buffer (pH6.8) incubated at 37^{o}C in a shaking incubator at 60rpm. Starting from time 0 hour and at desired intervals of time, 5ml sample was withdrawn and replaced with same amount of fresh medium. Drug in the release medium was measured spectrophotometrically. Release studies and release profiles of cross linked chitosan microspheres of various batches are given below.

CAD= Cumulative amount of drug (µg/ml)

Table 14: CAD data of cross linked chitosan-TPP microspheres prepared by varying drug conc.

S.No.	TIME(hr.)	CAD 1	CAD 2	CAD 3	CAD 4
1.	1	3.67	3.94	8.94	7.17
2.	2	4	4.72	11.5	7.39
3.	3	4.5	6.33	11.61	7.44
4.	4	4.78	7.22	14.94	7.64
5.	5	5.12	7.9	17.30	7.79
6.	6	5.15	8.16	17.12	7.79
7.	7	5.36	8.3	17.65	7.77
8.	8	5.44	8.36	17.60	7.86
9.	24	5.16	8.46	17.21	8.17

Table 15: Cumulative percentage drug release data of cross linked chitosan-TPP microspheres prepared by varying drug conc.

S.No.	TIME(hr.)	B1	B2	B3	B4
1.	1	28.23	24.05	44.7	33.5
2.	2	30.77	28.82	57.5	34.53
3.	3	34.62	38.64	64.05	34.77
4.	4	36.77	44.08	74.7	35.84
5.	5	39.4	48.23	85.6	36.31
6.	6	40	49.82	86.5	36.4
7.	7	41.23	50.67	87.85	36.4
8.	8	41.85	51.04	88	36.73
9.	24	39.7	51.65	86.05	38.2

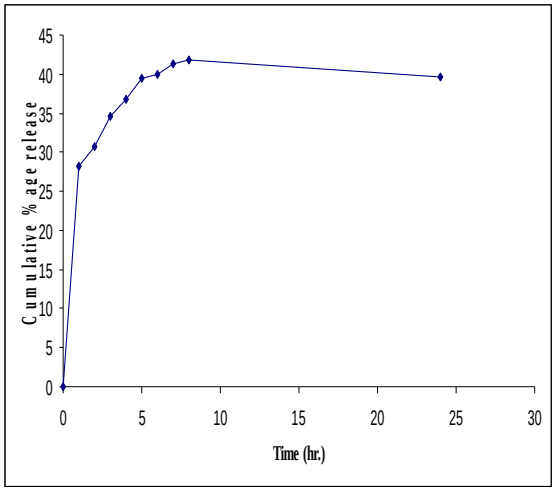


Figure 14: The drug release curve of B1

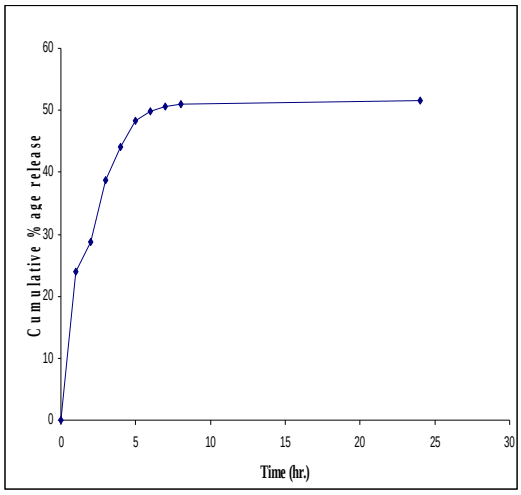


Figure 15: The drug release curve of B2

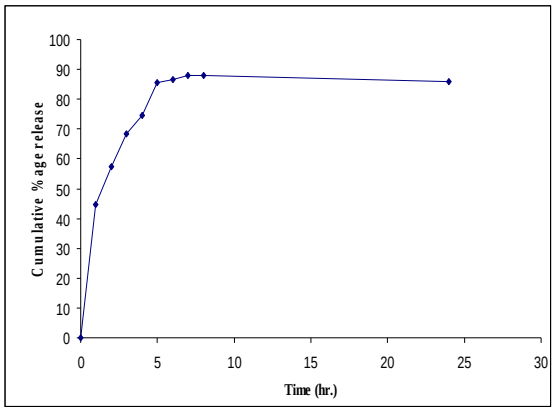


Figure 16: The drug release curve of B3

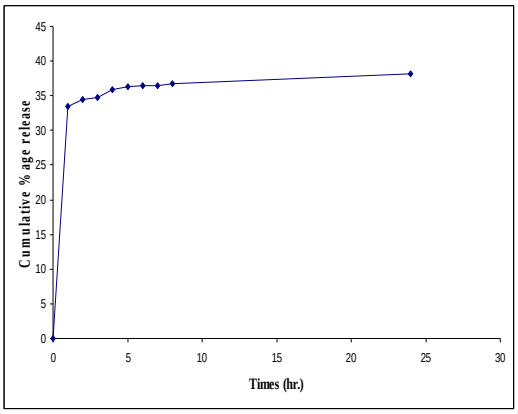


Figure 17: The drug release curve B4

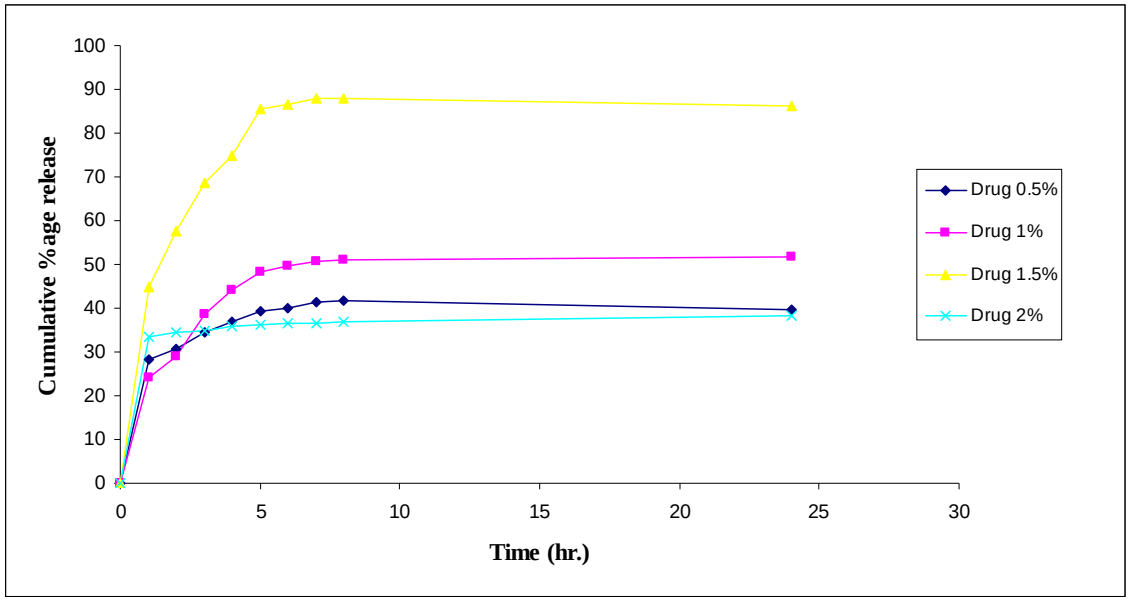


Figure 18: The comparative drug release curves of B1-4

Table 16: CAD data of cross linked chitosan-TPP microspheres prepared by varying drug-polymer concentration.

S.No.	Time (hr.)	CAD5	CAD6	CAD7
1.	1	5.56	6.83	10.97
2.	2	5.89	7.05	11.05
3.	3	7	7.17	11.67
4.	4	7.39	7.44	11.78
5.	5	8.25	8.26	12.6
6.	6	8.47	8.17	12.83
7.	7	8.47	8.18	12.73
8.	8	8.45	8.19	12.7
9.	24	8.04	7.94	12.46

Table 17: Cumulative percentage drug release data of cross linked chitosan-TPP microspheres prepared by varying drug-polymer concentration.

S.No.	Time (hr.)	B5	B6	B7
1.	1	30.42	40.44	47.8
2.	2	32.22	41.74	48.15
3.	3	38.3	42.45	50.85
4.	4	40.43	44.05	51.33
5.	5	45.13	48.9	54.9
6.	6	46.33	48.4	55.9
7.	7	46.33	48.43	55.47
8.	8	46.23	48.5	55.34
9.	24	43.98	47	54.3

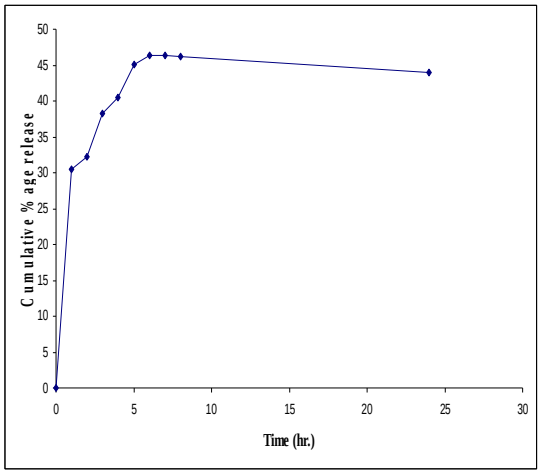


Figure 19: The drug release curve of B5

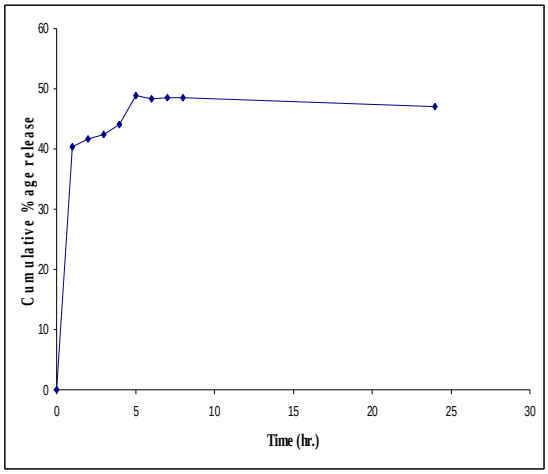


Figure 20: The drug release curve of B6

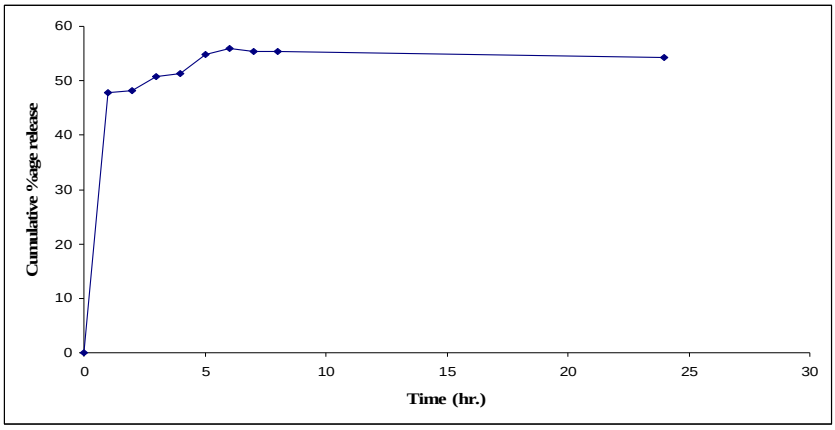


Figure 21: The drug release curve of B7

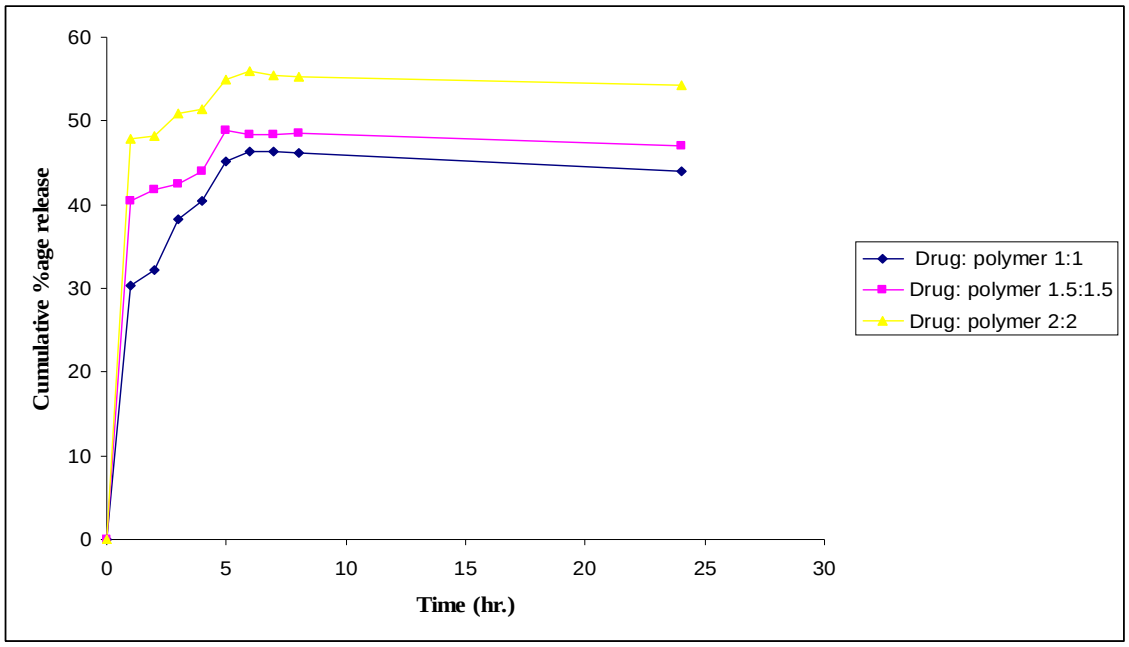


Figure 22: The comparative drug release curves of B5-7

Table 18: CAD data of cross linked chitosan-TPP microspheres prepared by varying pH at various chitosan: drug ratio.

S.No.	TIME(hr.)	CAD8	CAD9	CAD10	CAD11
1.	1	6.28	5.22	4.5	5.06
2.	2	5.61	5.5	4.11	5.33
3.	3	5.33	5.44	3.89	5.56
4.	4	4.89	5.17	4	5.56
5.	5	5.27	5.38	4.46	5.13
6.	6	5.18	5.4	4.58	5.17
7.	7	5.09	5.5	4.51	5.17
8.	8	4.96	5.27	4.33	5.08
9.	24	5.04	5.34	4.39	5.12

Table19: Cumulative percentage drug release data of cross linked chitosan-TPP microspheres prepared by varying pH at various chitosan: drug ratio.

S.No.	TIME(hr.)	B8	B9	B10	B11
1.	1	18.05	13.11	16.77	22.11
2.	2	16.11	13.81	15.31	23.3
3.	3	15.3	13.66	14.49	24.3
4.	4	14.04	12.98	14.9	24.3
5.	5	15.13	13.51	16.62	22.41
6.	6	14.87	13.56	17.06	22.6
7.	7	14.61	13.81	16.8	22.6
8.	8	14.24	13.23	16.13	22.2
9.	24	14.47	13.41	16.36	22.4

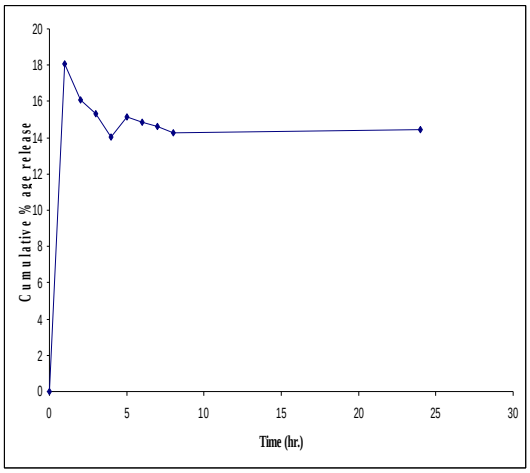


Figure 23: The drug release curve of B8

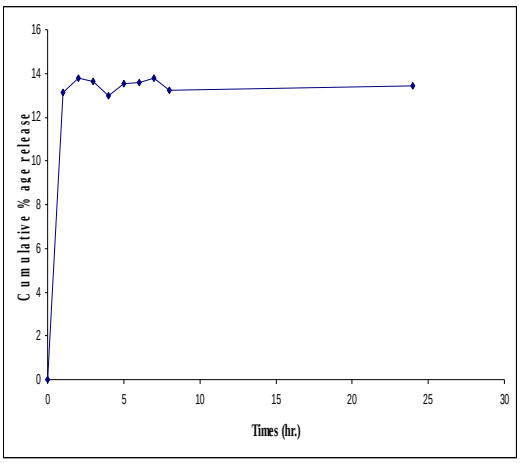


Figure 24: The drug release curve of B9

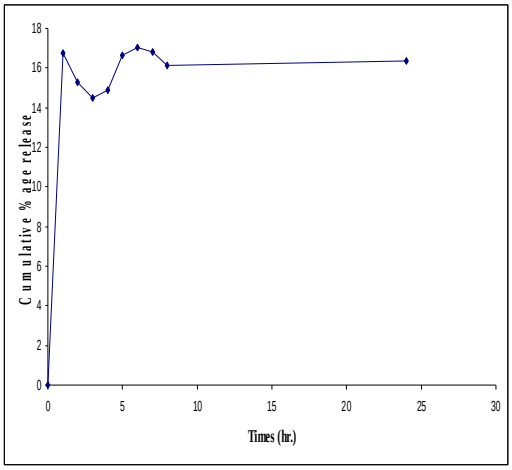


Figure 25: The drug release curve of B10

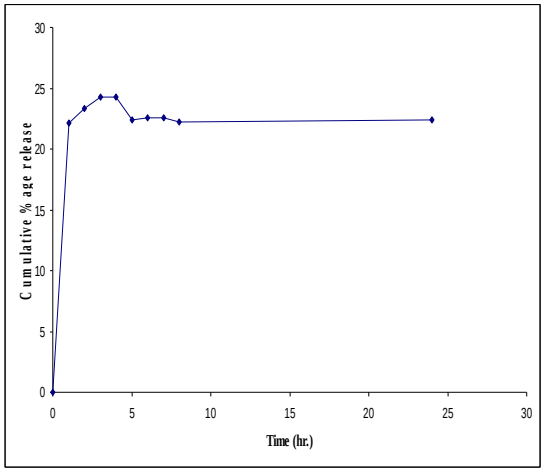


Figure 26: The drug release curve of B11

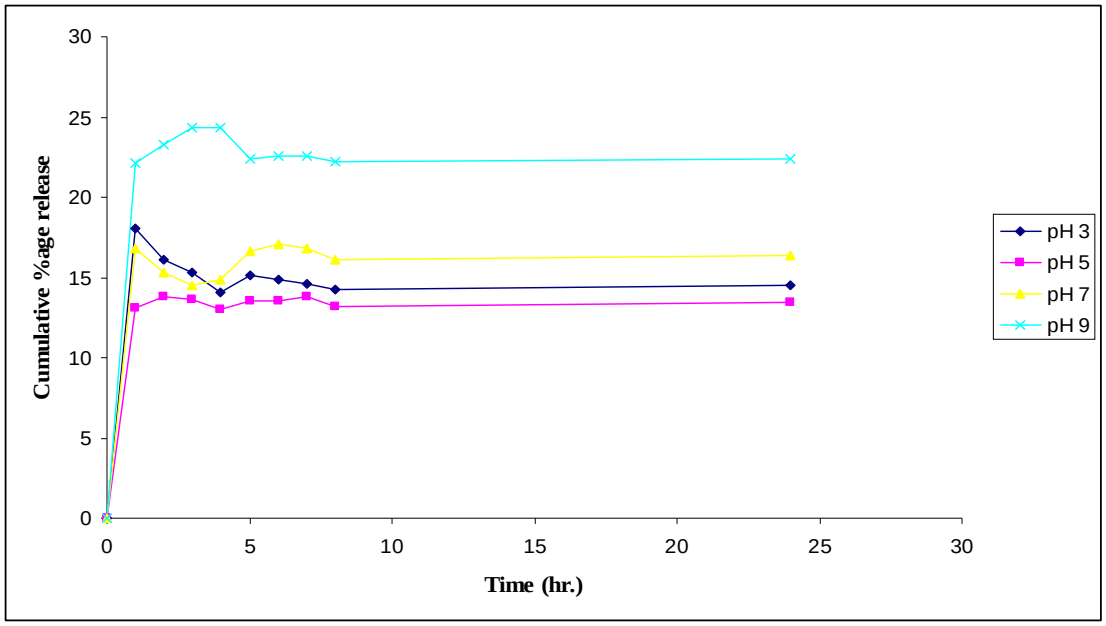


Figure 27: The comparative drug release curves of B7-11

Table 20: CAD data of cross linked chitosan-TPP microspheres prepared by varying pH at various chitosan: drug ratio.

S.No.	Time (hr.)	CAD12	CAD13	CAD14(3)
1.	1	7.78	5.56	8.94
2.	2	12.22	10	11.5
3.	3	13.89	13.33	11.61
4.	4	15.56	14.28	14.94
5.	5	17.08	15.67	17.30
6.	6	17.75	16.13	17.12
7.	7	18.16	16.72	17.65
8.	8	18.18	16.84	17.60
9.	24	17.88	16.33	17.21

Table 21: Cumulative percentage drug release data of cross linked chitosan-TPP microspheres prepared by varying pH at various chitosan: drug ratio.

S.No.	Time (hr.)	B12	B13	B14(3)
1.	1	30.24	24.3	44.7
2.	2	47.19	43.69	57.5
3.	3	53.98	58.24	64.05
4.	4	60.47	62.4	74.7
5.	5	66.38	68.5	85.6
6.	6	70	70.5	86.5
7.	7	70.58	73.04	87.85
8.	8	70.61	73.6	88
9.	24	69.50	71.34	86.05

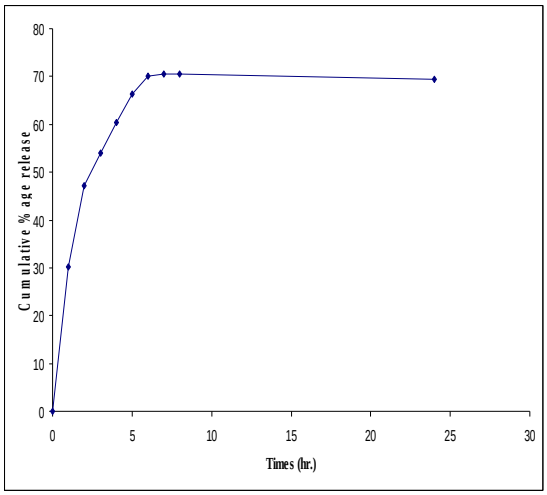


Figure 28: The drug release curve of B12

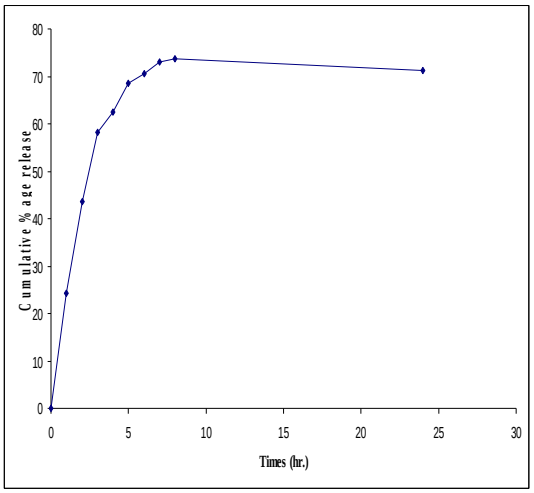


Figure 29: The drug release curve of B13

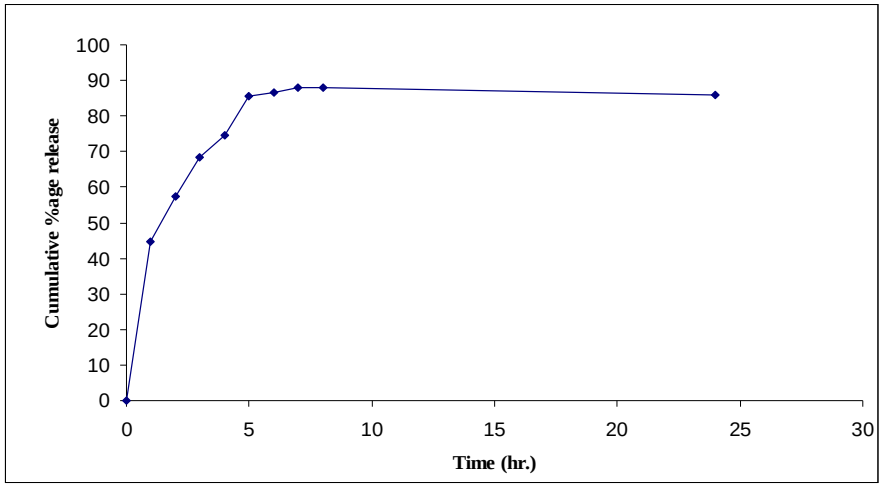


Figure 30: The drug release curve of B14 (3)

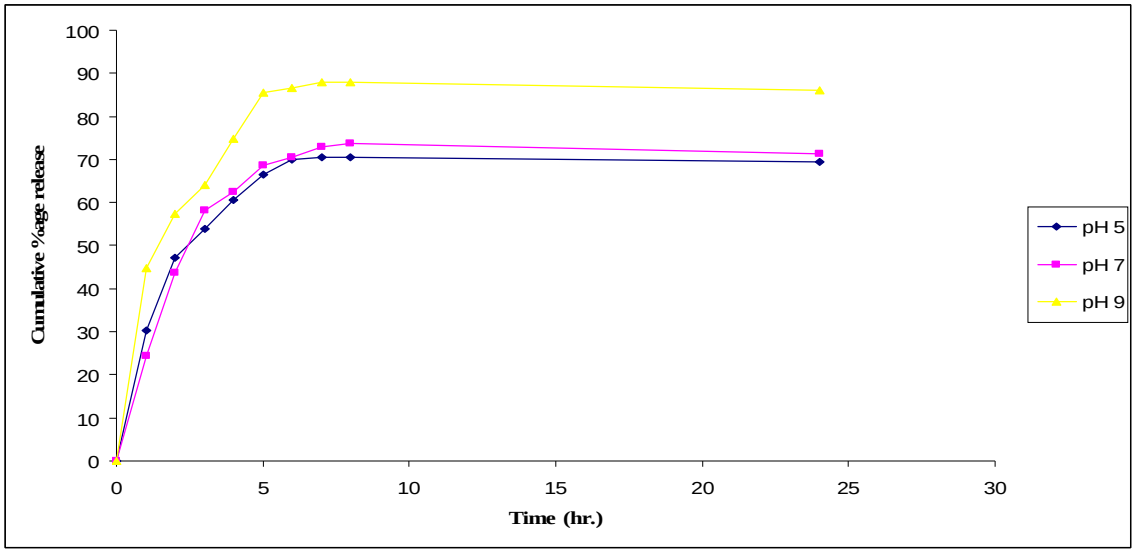


Figure 31: The comparative drug release curves of B12-14(3)

Table 22: CAD data of cross linked chitosan-TPP microspheres prepared by varying pH at various chitosan: drug ratio.

S.No.	Time (hr.)	CAD15	CAD16	CAD17 (2)
1.	1	6.39	6.28	3.94
2.	2	7.06	6.56	4.72
3.	3	7.83	7.22	6.33
4.	4	8.39	7.94	7.22
5.	5	8.80	9.03	7.9
6.	6	8.85	9.13	8.16
7.	7	8.92	9.22	8.3
8.	8	8.97	9.28	8.36
9.	24	8.85	9.17	8.46

Table 23: Cumulative percentage drug release data of cross linked chitosan-TPP microspheres prepared by varying pH at various chitosan: drug ratio.

S.No.	Time (hr.)	B15	B16	B17 (2)
1.	1	33.42	33.53	24.05
2.	2	36.92	35.02	28.82
3.	3	40.95	38.55	38.64
4.	4	43.88	42.39	44.08
5.	5	46.03	48.21	48.23
6.	6	46.29	48.75	49.82
7.	7	46.65	49.23	50.67
8.	8	46.91	49.55	51.04
9.	24	46.29	48.96	51.65

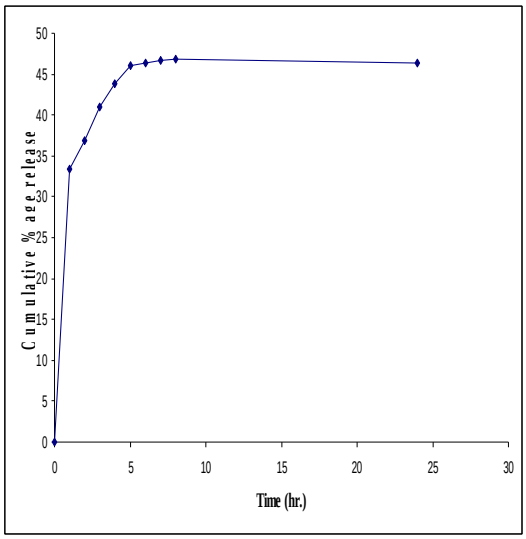


Figure 32: The drug release curve of B15

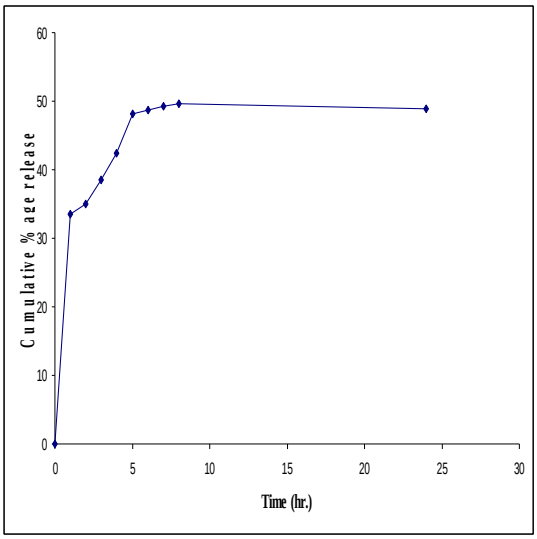


Figure 33: The drug release curve of B16

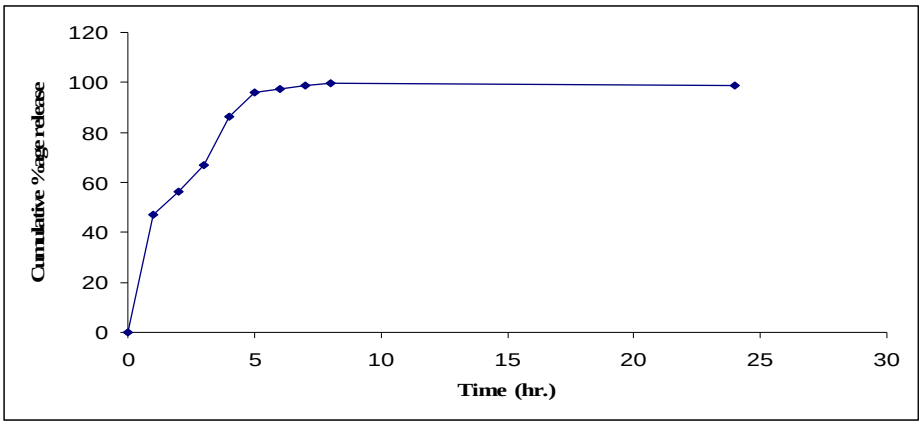


Figure 34: The drug release curve of B17 (2)

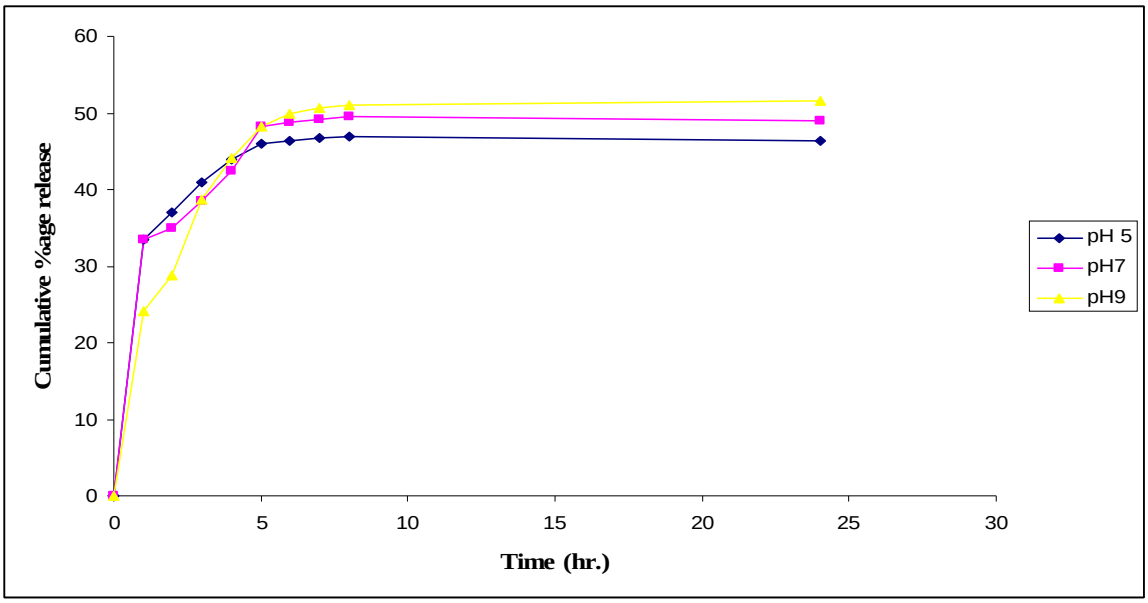


Figure 35: The comparative drug release curve of B15-17(2)

CHAPTER V

RESULT AND DISCUSSION

There are many methods reported in literature for the preparation of microspheres for achieving controlled drug delivery. Some of the important methods are emulsion cross-linking, chemical cross-linking, coacervation, spray drying, precipitation and ionotropic gelation. Some of these methods use organic solvents and chemicals, whose residual amount in the microspheres may cause undesirable effects like irritation to mucosal membranes. Recently cross linked microspheres prepared by ionotropic gelation method (Bodmeier et at., 1989) have gained importance. By this method, the microspheres could be easily prepared using simple instruments and the drug could be entrapped within chitosan microspheres in a completely aqueous environment under mild conditions.

Chitosan, a polycationic polysaccharide is insoluble in alkaline and neutral pH, but soluble in acidic solvents. The amine group undergoes protonation in acidic environment that increases its solubility in acidic solution. The protonated amine group of chitosan interacts with phosphate ions provided by TPP, either by intermolecular or intramolecular linkage and the drug is entrapped within the microspheres.

The objective of this study was to investigate the effect of parameters- drug concentration, drug: polymer concentration and pH of TPP solution at various drug: polymer ratio on the preparation of croos linked chitosan microspheres.The cross linked chitosan microspheres thus prepared by varying the formulation parameters were studied for percent yield, average particle size, encapsulated amount of drug and in vitro drug release rates.

Table 8-10 indicates batches that were prepared by dropping chitosan-drug solution into TPP solution by varying drug concentration, drug: polymer concentration and pH of TPP solution at various drug: polymer ratio.

Percentage yield of different batches of chitosan-TPP microspheres are listed in table 11. It varies from 4.24% to 11.55%. The comparative study of percentage yield was shown by histogram of figure 11. Maximum yield was obtained from batch 7 having 2% TPP, 2% chitosan concentration and 2% drug concentration at pH 9.

The average particle size of the various batches of crosslinked microspheres are listed in table 12. It varies from 586μm-972μm. The comparative study of the average particle size

was shown by histogram of figure 13. Minimum size was obtained from batch 3 having 2% TPP, 1.5% drug conc., and 1% chitosan conc. at pH 9.

The encapsulated amount of drug (EAD) is expressed as the amount of total available drug that is actually entrapped in the microspheres. The EAD varied from 13 µg/ml to 26.84 µg/ml reported in table 13. Maximum EAD was seen in batch 10 prepared at pH 7. It increases with increase in drug concentration from 0.5%-2% (w/v) and decreases with increases in pH for batches 12-17.

The in vitro release studies were performed in buffer (pH 1.2) and subsequently in buffer (pH 6.8) close to the physiological gastrointestinal conditions. The dissolution behaviour of the chitosan-TPP microspheres was dependent on pH. The microspheres swelled in (pH 1.2) while they did not show much change in buffer (pH 6.8). An initial burst release of drug was observed from all batches that can be attributed to two reasons: the leaching of drug on the bead outer surfaces and faster ingress of dissolution medium and subsequent diffusion of drug. However on changing pH from lower to higher level, drug released slowed. The pH responsive release can be explained based on the charge density of beads, which is an important factor in electrostatic interaction and depends on solution pH. In buffer (pH1.2), protonation of phosphate ions causes hydrogen bonds to break, leading to weaker electrostatic interaction. This causes swelling and higher release in buffer (pH1.2), while in buffer (pH6.8) stronger attractive force between phosphate ions and chitosan caused slower release of drug. The change in pH of medium causes swelling (pH1.2) and later deswelling (pH6.8) leading to bimodal drug release.

Cumulative percentage release increased with the increase in drug concentration up to 1.5% (w/v) and then decreases at 2% (w/v) as listed in table 15, maximum at drug conc. 1.5% (w/v), 86.05%. Also, cumulative percentage release increased with the increase in pH from 3-9 at different drug: polymer ratio reported in table 19, 21 and 23 and was maximum at pH 9 in all the batches.

At pH 3 microspheres did not form probably due to their less electrostatic interaction. When drug-polymer ratio was 0.5:0.5 microspheres did not form they clumped together and formed aggregates at this concentration and it was not possible to separate these microspheres. At 2% CTS (w/v) preparation of microspheres became difficult as the

viscosity of 2% CTS solution was high enough for the smooth release of CTS solution from syringe into TPP solution.

CHAPTER VI

CONCLUSION

Chitosan is a natural polymer that possesses biocompatible and biodegradable properties. It is also a water-soluble polymer which has an ideal property as a drug delivery carrier. Employing simple preparation methods, chitosan can be used as a promising drug delivery carrier for a broad category of drugs.

After studying various parameters it was observed that highest encapsulated amount of drug and highest percentage drug release was achieved at drug concentration of 1.5% (w/v), chitosan concentration of 1% (w/v) and at pH 9 of TPP solution having concentration 2% (w/v),. Morphology and stability of these microspheres were also good as compared to others at these optimized values. Thus, ionotropic gelation method, a milder and effective method of preparation of microspheres may be used by optimizing various parameters like drug concentration, drug: polymer concentration and pH of TPP solution. Further, other parameters may also be optimized and studies are to be done extensively so as to achieve an ideal controlled drug delivery system.

CHAPTER VII

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