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RESEARCH ARTICLE

Fabrication and Release Kinetics Studies for Interpenetrating Polymeric Network (IPN) of Chitosan-Amino Acid Beads Loaded with BPM

Bhardwaj H*[1], Jaiswal J[1], Sharma N[2]

[1]*Rakshpal Bahadur College of Pharmacy, Bareilly, India.*

[2]*Research Scholar, CCS University, Meerut, India.*

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ABSTRACT

The present study was designed to synthesize the pH sensitive interpenetrating polymeric network (IPN) beads composed of chitosan-glycine-glutamic acid, cross linked with glutaraldehyde and their use for controlled drug release. The drug was loaded into beads by varying their composition. The beads were characterized by FTIR for cross linking reaction and drug interaction with cross linked polymer in beads and SEM to understand the surface morphology and internal structure and DSC to find out the thermal stability of beads. XRD investigation was carried out to determine the crystalline nature of drug after loading into chitosan-glycine-glutamic acid IPN beads. Results indicated amorphous dispersion of BPM (brompheniramine maleate) in the polymeric matrix. The results indicate that the cross linked IPN beads of chitosan-glycine-glutamic acid might be useful as a vehicle for controlled release of drug. The kinetics of drug release from beads was best fitted by Higuchi's model in which release rate is largely governed by rate of diffusion through the matrix.

KEYWORDS

IPN, FTIR, SEM, DSC, XRD, BPM, Higuchi's model

INTRODUCTION

Recently efforts have been made to design novel drug dosage formulations so that more and more effectiveness could be integrated to the conventional dosage forms. To achieve this goal a methodology was developed by which pre decided and reproducible release of drug up to therapeutic level into a specific environment over a prolonged time period could be maintained and to minimize any possible side effect. Nano and micro beads of polymers have been formulated using polymeric material either synthetic or natural origin. Hence, drug delivery system require polymeric matrix which would be non-toxic, biocompatible, biodegradable.[1,2]

***Address for Correspondence:**
Hemant Bhardwaj
Rakshpal Bahadur College of Pharmacy,
Bareilly, India.
E-Mail Id: hemant1580@gmail.com

Chitosan is such a valuable natural biocompatible polymer, nontoxic, biodegradable mucoadhesive, easily bio absorbable and also possess gel forming ability at low pH.[3,4] Moreover, it has antacid and anti-ulcer activities which prevent or weaken drug irritation in the stomach. All these interesting properties of chitosan make this natural polymer an ideal element for formulating drug delivery devices and this material has been used to form drug carrying systems for several biomedical purposes and also for gene therapy to suture and wound healing materials, vascular grafts and cartilage regeneration among many other applications.[5,6]

Chitosan is obtained by N-deacetylation of chitin which is naturally abundant muco polysaccharide and forms the exoskeleton of

crustaceans, insects etc. It is well known to consist of 2-acetamido 2-deoxy β-D-glucose through a β (1→4) linkage. Thus, chitosan is a hetero polymer having (1→4) 2-amino 2-deoxy β-D-glucose unit with (1→4) 2-acetamido-2 deoxy β-D-glucose units of original chitin in polymeric chain. The ratio of 2-amino-2-deoxy β-D-glucose unit to 2-acetamido-2-deoxy β-D-glucopyranose is an important parameter called as degree of deacetylation which determines its solubility and solution or gel forming properties. Chitosan is highly basic polysaccharide. It is soluble in dilute acids. It possess property of forming hydrogels which are highly swollen hydrophilic polymer network, capable of absorbing large amounts of water and widely used in controlled release system. Recently, pH sensitive hydrogels have potential use in site specific delivery of drug. Some of the most appealing characteristics of chitosan are its bio adhesive properties and its ability to promote cell proliferation and consequently, tissue regeneration. These properties of chitosan are enhanced upon decreasing the polymer's degree of acetylation and are of outmost importance for biomedical engineering.[6]

Beads are solid, spherical, micron or nano sized drug carrier particles constituting a matrix type of structure. Drug may be either absorbed at the spherical beads or entrapped within it. In other words, these are just like vesicular system surrounding a cavity having drug in polymeric solid. These polymeric beads are advantageous over pellets including relatively higher intercellular uptake.

Their charge properties influence the uptake by intestinal epithelia. So nano / micro beads surface charges and increased hydrophobicity of polymeric matrix have been found to be effective for the gastrointestinal uptake in a positive sense.[7-9]

Our study is an attempt to develop cross linked beads composed of chitosan and two amino acids as spacer groups cross linked with glutaraldehyde for sustained release of brompheniramine maleate as a model drug to investigate the swelling behavior and modeling drug release properties.

MATERIALS AND METHOD

Materials

Chitosan was purchased by India Sea Food, Kerala, and was used as received. Its percentage of deacetylation after drying was 89 %. Brompheniramine maleate (BPM), $C_{20}H_{23}BrN_2O_4$ was obtained as a gift sample from Sigma Aldrich Pvt. Ltd.

Glutaraldehyde, glycine and monosodium glutamate were procured from SD Fine Chemicals Ltd., Mumbai, India, Sisco Research Laboratories Pvt. Ltd., India and Reidal Chemicals, India respectively.

All other chemicals used were of analytical grade. Double distilled water was used in throughout the studies.

Methods

Preparation of Semi-Interpenetrating Polymer Network (IPN) Beads

Different IPN beads (G1-G8) varying in composition were prepared separately. Their composition is described in table 1.

Weighed quantity of chitosan and amino acid were dissolved in 40 ml of 2% acetic acid by weight and stirred for three hours using magnetic stirrer at room temperature. The homogeneous mixture was extruded in the form of droplets using a syringe into NaOH-methanol solution (1:20 w/w) under stirring condition at 400 rpm. The beads were washed with hot and cold water respectively.

The resultant beads were allowed to react with glutaraldehyde solution as given in table (1) at 50^{o}C for about 10 minutes. Finally, the cross linked IPN beads were successively washed with hot and cold water followed by air drying.

Drug loaded beads of same composition were also prepared separately by adding a known amount of BPM (150 mg, 200 mg) respectively to the chitosan, amino acid mixture before extruding into the NaOH- methanol solution.

Table 1: Composition of IPN beads

Bead type	Chitosan (g)	Glycine (g)	Glutamic acid (g)	2% Acetic acid (ml)	Glutaraldehyde (%)
G1	1.0	0.5	0.5	40	3.13
G2	1.0	0.5	0.5	40	6.25
G3	1.0	0.5	0.5	40	12.5
G4	1.0	0.5	0.5	40	25.0
G5	0.8	0.5	0.5	40	12.5
G6	1.2	0.5	0.5	40	12.5
G7	1.0	0.4	0.6	40	12.5
G8	1.0	0.6	0.4	40	12.5

Swelling Studies

Swelling behavior of chitosan beads (G1-G8) were studied in different pH (2.0 and 7.4) solutions. The percentage of swelling for each sample at time t was calculated using the following formula-

Percentage of swelling = $\{(W_t - W_o)/W_o\} \times 100$

Where, Wt = weight of the beads at time t after emersion in the solution.

Wo = weight of the dried beads

Drug Loading Assay

Accurately weighed (0.1g) drug loaded sample was kept in 100 ml of 2% acetic acid for 48 hour. After centrifugation the BPM in the supernatant was assayed by spectrophotometer at 193.5 nm.

Drug Release Studies

The drug release experiments were performed at 37^{o}C under unstirred condition in acidic (pH 2.0) and basic (pH 7.4) solution. Beads (0.1 g) containing known amount of the drug were added to the release medium (30 ml). At pre decided intervals, samples of 2 ml aliquots were withdrawn, filtered and assessed by recording the absorbance at 193.5 nm. The cumulative BPM release was measured as a function of time.

Kinetic Analysis of Drug Release

A fair amount of work has been included in literature on kinetics of drug release. A large number of modified release dosage forms contain some sort of matrix system and the drug dissolves from this matrix. The diffusion pattern of the drug is dictated by water penetration rate (diffusion controlled) and thus the Higuchi's equation[10] relationship applies-

$$M_t/M_\infty = k\, t^{1/2}$$

Where, M_t/M_∞ is the fractional drug release at time t and k is a constant related to the structural and geometric properties of the drug release system. According to Higuchi's model, an inert matrix should provide a sustained drug release over a reasonable period of time and yield a reproducible straight line when the percentage of drug released is plotted versus the square root of time.

Characterization of IPN Beads

FTIR Spectra of IPN beads

FTIR spectra of IPN beads were recorded using a thermo Nicolet Avatar 370 FT-IR spectrometer system using KBr pellets.

Scanning Electron Microscopy (SEM)

The shape and surface morphology of the beads were examined using FESEM QUANTA 200

FEG model "(FEI, The Netherlands make)" with operating voltage ranging from 200 V to 30 kV. FESEM micrographs were taken after coating the surfaces of bead samples with a thin layer of gold by using BAL-TEC-SCD-005 Sputter Coater (BAL-TEC AG, Balzers, Liechtenstein Company, Germany) under argon atmosphere. SEM was used to perform textural characterization of full and cross sectioned IPN beads, magnification were applied to each sample in order to estimate the morphology and interior of the bead.

X-ray Diffraction (XRD)

X-ray diffraction studies were performed by using Bruker AXS D8 Advance using CuKα Nickel filter and Copper as target at wavelength of 1.54 Å with goniometer speed 2°/min.

Thermal Analysis

Thermal gravimetric analysis (TGA), Differential thermal gravimetric (DTG) and Derivative thermal analysis (DTA) were carried out simultaneously by using a (PYRIS Diamond). TG/DTA thermal analyzer model DSC-7, supplied by Perkin Elmer and the data was processed and analyzed by PYRIS muse measure and standard analysis software (V. 3.3U; #. 2002 Seiko instruments inc.). The sample was kept in alumina pan, the reference material was alumina powder and study was carried out at heating rate 10°C/min under 200 ml/min flow rate of air or nitrogen atmosphere. Indium and gallium were used as standards for temperature calibration.

RESULTS AND DISCUSSION

Swelling Studies

The effect of pH, concentration of glutaraldehyde crosslinker, chitosan and amino acid on swelling behavior of chitosan-glutamic-glycine beads has been evaluated.

Effect of pH

Swelling studies to evaluate the effect of pH were carried out in solution of pH 2.0 and pH 7.4. It was observed that percentage of swelling is higher in acidic solution (pH 2.0) than in alkaline solution (pH 7.4). We have also performed a comparative study for pure chitosan, chitosan-glycine, chitosan-glutamic acid and chitosan-glycine-glutamic acid systems and observed that percentage of swelling for chitosan-glycine beads in acidic solution was found to be higher than in basic solution which is due to inherent hydrophobicity of the chitosan beads dominating at high pH value, thus preventing faster swelling in neutral and alkaline media but in case of chitosan-glutamic acid beads, percentage of swelling in basic solutions was found to be higher than in acidic solution which may be due to the presence of free carboxylic ends of the chitosan-glutamic acid IPN, more likely to be attacked by basic solution. In case of chitosan-glycine-glutamic acid beads, their rate of swelling was also found to be higher at pH 2.0 than chitosan-glutamic acid and chitosan-glycine beads but at pH 7.4, their rate of swelling were intermediate between chitosan-glutamic acid and chitosan-glycine beads. Thus it was concluded that overall rate of swelling was affected by glycine when chitosan-glycine-glutamic acid beads were subjected to swelling studies.

Effect of Glutaraldehyde

Swelling behavior of crosslinked beads as a function of time in pH 2.0 and pH 7.4 solution having different concentrations of glutaraldehyde have shown in figure 1(a). It was observed that the swelling rate of the crosslinked beads containing varying concentration of glutaraldehyde follows the order G1>G2>G3>G4 i.e. swelling rates increased with the decreased concentration of glutaraldehyde.

When the cross linked beads are placed in the solution, the solution penetrates into the beads and the beads subsequently try to swell. Generally, the swelling process of the beads in pH<6 involves the protonation of amino/imine groups in the beads and mechanically relaxation of the coiled polymeric chains. Initially during the process of protonation, amino/imine groups of the bead surface were protonized which led to dissociation of the hydrogen bonding between amino/imine group and other groups. Afterward,

protons and counter ions diffused into the bead to protonate the amino/imine groups inside the beads and dissociating the hydrogen bonds. It has been observed that the swelling rates are directly proportional to the degree of cross linking. As the higher crosslink density results in higher strength of the beads and lower degree of swelling. Thus, the lowest swelling rate is observed in case of G4 beads.[11,12]

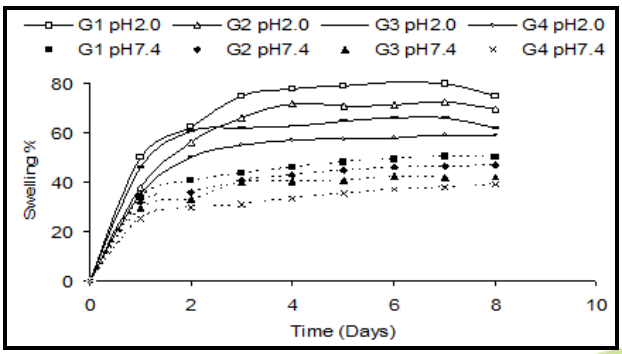

Figure 1(a): Swelling behavior of cross linked beads as a function of time in solution pH 2.0 and pH 7.4 at 37^{0}C having different percentage of glutaraldehyde

Effect of Chitosan

Effect of varying weight ratio of chitosan on swelling behavior of crosslinked beads containing same quantity of glutaraldehyde have been studied in acidic (pH 2.0) and basic (pH 7.4) solutions and results are presented in fig 1(b). The percentage of swelling of the cross linked beads having the same concentration of cross linker decreases with increasing concentration of chitosan i.e. G5>G3>G6. It can be explained as the percentage of chitosan increased from G5(44.4%) to G6 (55.5%) through G3 (50%), the percentage of amino acids which act as a spacer decreased from G6(55.5%) to G5(45.4%) through G3(50%), the pore size of the beads decreases and the penetration of pH solution into the beads became difficult, which resulted in lesser degree of swelling further, swelling percentage was higher in acidic medium than in alkaline medium.

Effect of Amino Acids

The results obtained by changing in amino acids composition i.e. decrease in glutamic acid and increase in glycine concentration of cross linked beads having same concentration of glutaraldehyde are given in figure 1(c) have observed that the increase in concentration of glycine decreased the swelling percentage of cross linked beads in basic solution i.e. G8<G3<G7 while increased in acidic solution (i.e. G7<G3<G8). It may be due to the different behavior of chitosan-glycine and chitosan-glutamic acid beads towards different pH as described earlier.[13]

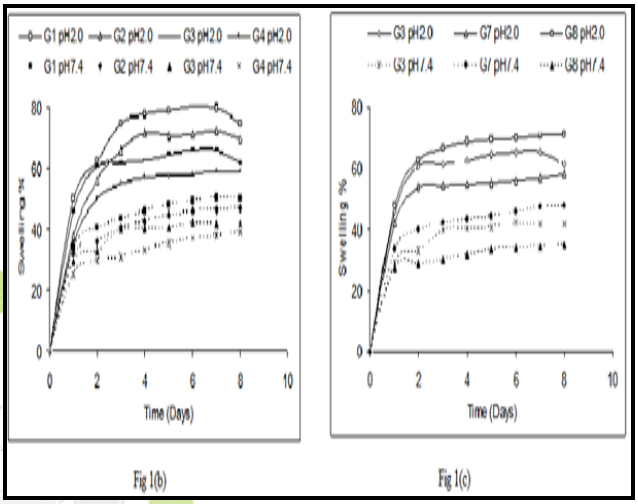

Figure 1(b): Swelling behavior of cross linked beads as a function of time in solution pH 2.0 and pH 7.4 at 37^{0}C having different weight ratio of chitosan

Figure 1(c): Swelling behavior of cross linked beads as a function of time in solution pH 2.0 and pH 7.4 at 37^{0}C having different weight ratio of amino acid

SEM Studies

SEM micrographs of dried beads (G1-G8) and their surface morphology are shown in fig 2. The beads were nearly spherical or somewhat oval in shape this may be due to different composition of cross linked beads due to which solution viscosity varied and beads varied in shape from spherical to oval or elongated as we know that solution with decreased viscosity can be extruded easily as spherical bead through a syringe. The approximate size of beads varied from 0.8 to 1.5 mm.

Cross linked chitosan amino acid beads (G1-G8) had rough, rubbery, fibrous and folded surfaces.

With the highest concentration of cross linker, in case of G4 the chains come closer to each other and exhibit a regular, fibrous structure but with decreasing concentration of glutaraldehyde as in case of G3, G2 and G1 beads the structural morphology changes to layered and big fibrous bunches. Rubbery morphology is observed in case of lowest percentage of glutaraldehyde i.e. in G1 beads. Although having same degree of cross linker (i.e. 12.5 % glutaraldehyde) G5 and G6 beads constituting varied concentration of chitosan, while G7 and G8 beads constituting varied concentration of glycine and glutamic acid.

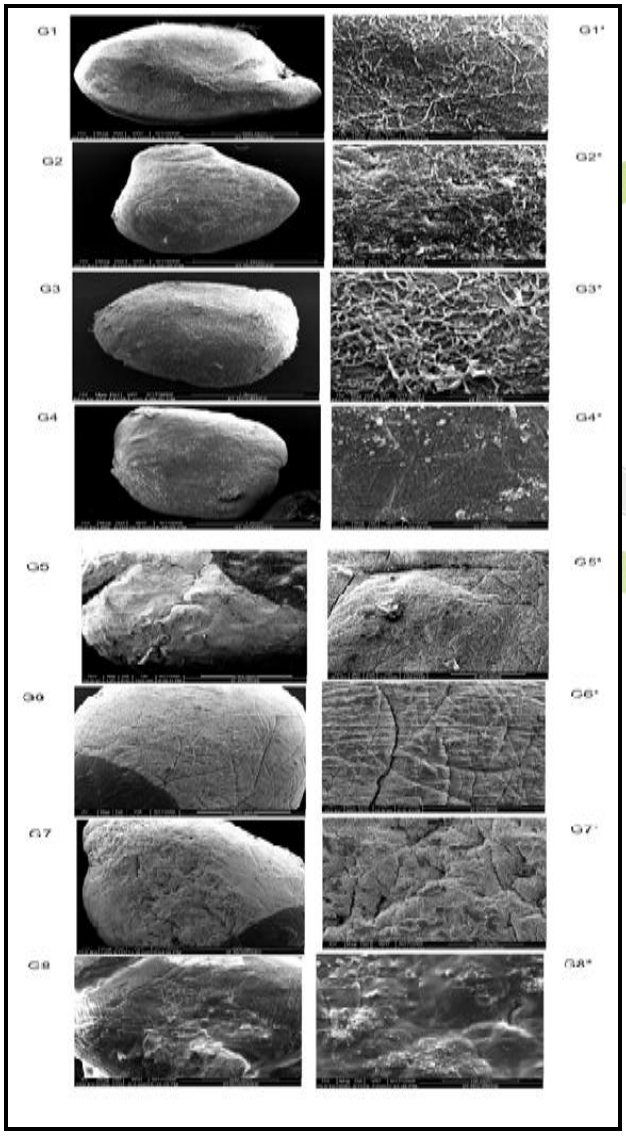

Figure 2: SEM photographs of cross linked beads (G1-G8) and their morphology (G1*-G8*)

FTIR Studies

Fig 3(a) shows the FTIR spectra of chitosan powder, glutamic acid, glycine and G1-G8 drug unloaded beads. FTIR spectra of chitosan powder curve showed two peaks around 894 cm^{-1} and 1171 cm^{-1} corresponding to saccharide structure. The observed peak at 1613cm^{-1} can be assigned as amino absorption peak.

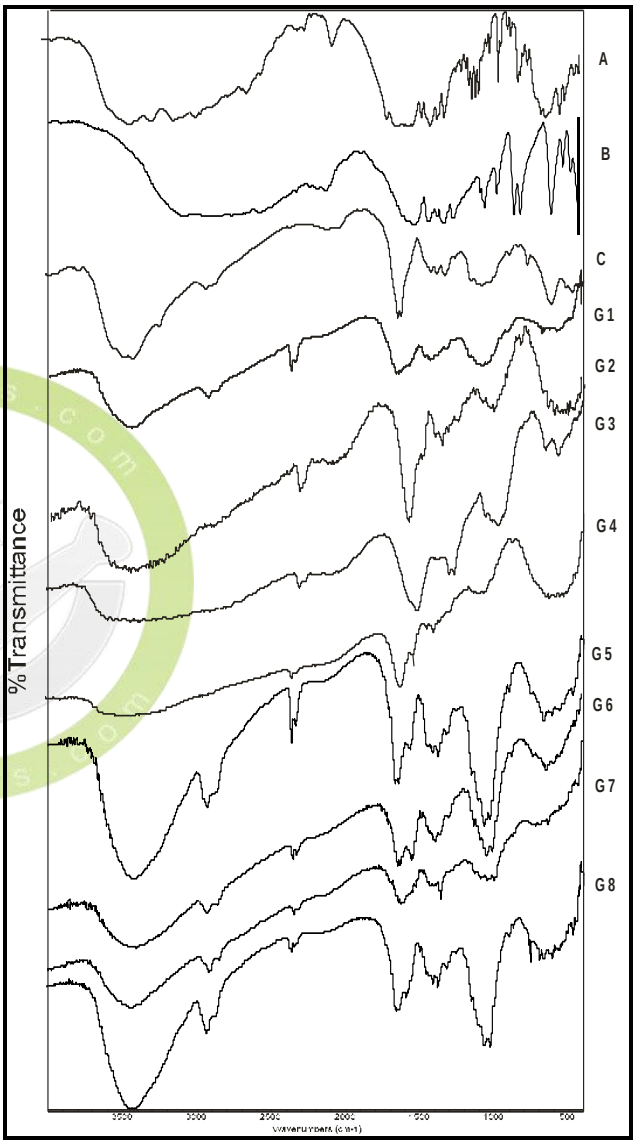

Figure 3(a): FTIR spectra of glutamic acid (A), glycine (B), chitosan powder (C) and drug unloaded cross linked beads (G1-G8)

The absorption peak for amide were observed at 1639 cm^{-1} and 1319 cm^{-1} and observed peak at 1384 cm^{-1} was assigned to CH_3 symmetrical deformation mode.[14] A broad band appearing around 1083 cm^{-1} indicated the $>CO-CH_3$ stretching vibration of chitosan. Another broad

band at 3450 cm^{-1} was due to the amine N-H symmetric stretching vibration which might be due to deacetylation of chitosan. Peak observed at 2924 cm^{-1} is typical of C-H stretching vibration. simultaneously the peak assigned for amino absorption at 1613 cm^{-1} in original chitosan broadened or disappered in cross linked beads and a new peak appearing at about 1567 cm^{-1} due to imine bond (-C=N-) which was formed as a result of cross linking reaction between amino group in chitosan and aldehydic group in glutaraldehyde in curve G1-G8.[15] However, this was due to the overlapping of peaks corresponding to –NH- stretching vibrations in -NH-$COCH_3$ at 1639 cm^{-1} of the original chitosan with that of imino (-C=N-) stretching at 1567cm^{-1} of the newly formed structure between amino group of chitosan and aldehyde group of glutaraldehyde in G1-G8. A reaction taking place in the formation of crosslink is as follows-

-----NH_2 + O=HC------ → ----N=CH-----

Amino aldehyde → imino →

(chitosan) (glutaraldehyde) (cross link) →

On increasing the glutaraldehyde concentration, the peak corresponding to 1567 cm^{-1} was sharpened and distinct in G4. All the curves G1 to G8 showed additional peaks of amino acid.

FTIR spectral data of drug loaded beads in figure 3(b) were used to confirm the chemical stability of BPM in chitosan amino acid beads. FTIR spectra of pure BPM drug (curve D) and BPM loaded cross linked beads (G1-G8) in fig 3(b) were compared with drug unloaded cross linked beads (G1-G8) in figure 3(a). BPM has shown characteristic band at 2966 and 2917 cm^{-1} due to aliphatic C-H stretching. The band at 1619 and 1588 cm^{-1} due to C=N stretching vibration. While those of 1476 and 1432 cm^{-1} are due to aromatic C=C stretching vibration. BPM has also shown characteristic band at around 864 cm^{-1} due to aromatic C-Cl stretching. When drug was incorporated into the cross linked chitosan–amino acid beads, along with all the characteristic band of the crosslinked chitosan and amino acids, additional band have appeared due to the presence of BPM in the matrix. It indicates that BPM has not undergone any chemical change within the beads.

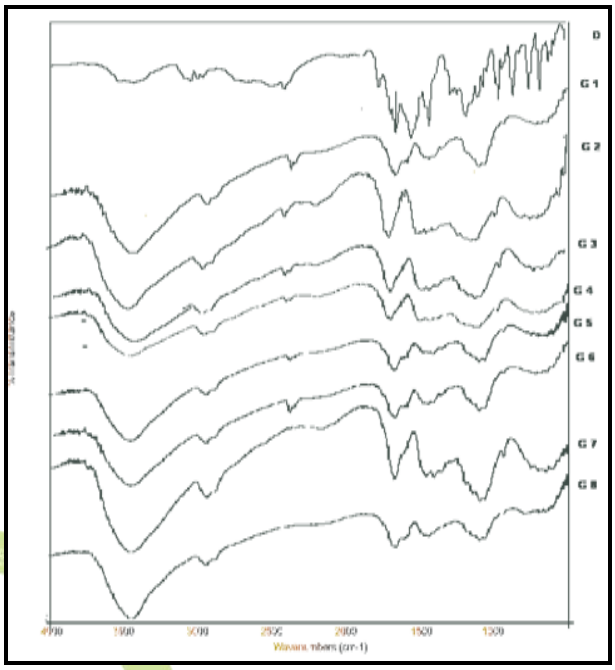

Figure 3(b): FTIR spectra of pure BPM drug (D) and drug loaded cross linked beads (G1-G8)

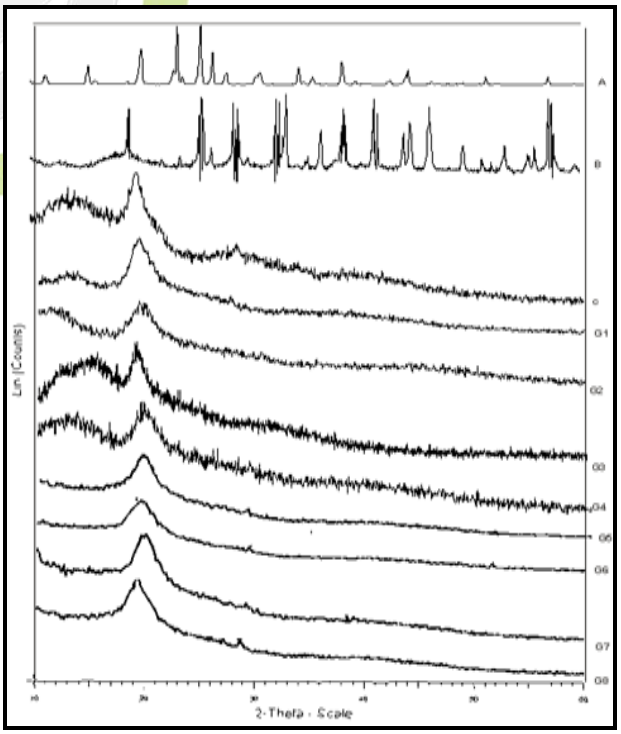

Figure 4(a): XRD graphs of glutamic acid (A), glycine (B), chitosan powder (C) and drug unloaded Cross linked beads (G1-G8)

X-ray Diffraction (XRD)

X ray diffractograms of chitosan, glycine, glutamic acid and drug unloaded beads (G1-G8) are presented in fig 4(a) and also of pure BPM drug and drug loaded beads (G1-G8) are shown in fig 4(b). XRD peaks depend on the crystal size.

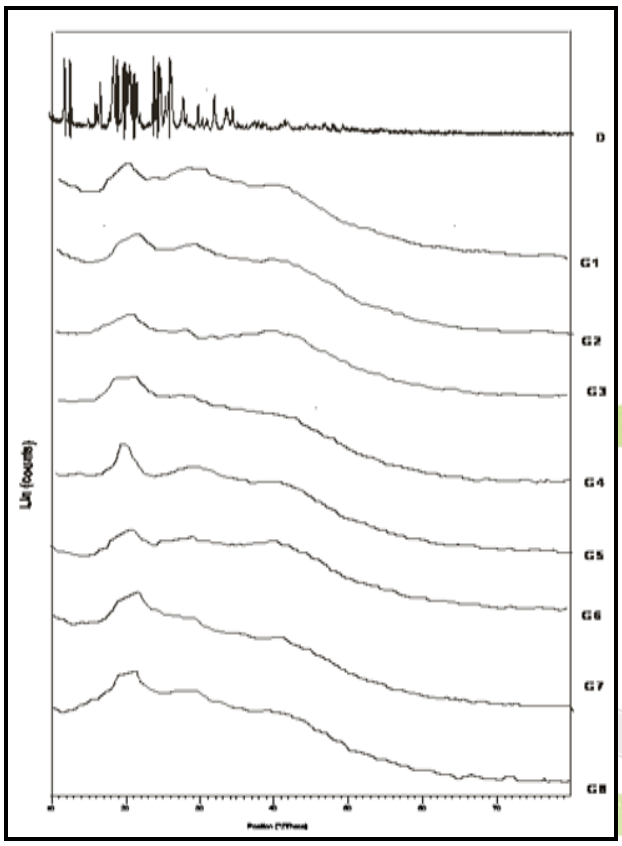

Figure 4(b): XRD graphs of pure BPM drug (D) and drug loaded cross linked beads (G1-G8)

The diffraction pattern of pure chitosan has the characteristic peaks at 2θ of 12 to 16, 20 and 29. All the drug unloaded beads (G1 to G8) in fig 4(a) show the similar peaks as that of chitosan. BPM drug has shown characteristic intense peaks at 2θ of 12 to 35, however, these peaks are not observed in BPM loaded beads (G1-G8) in figure 4(b) but instead, the diffractograms of both the drug loaded and drug unloaded beads are almost identical, indicating the amorphous dispersion of drug after entrapment into polymeric chitosan-amino acid beads. No crystals of drug were found in the drug loaded beads upto the detection limit.[16,17]

Thermal Analysis

TGA experiment were carried out on chitosan, glutamic acid, glycine and cross linked drug unloaded beads G1-G8 and the curve obtained are presented in fig 5(a) which clearly shows that approx 10% weight loss by chitosan powder (curve A) below 100°C due to loss of free water. After this, weight loss remains constant up to 249°C.

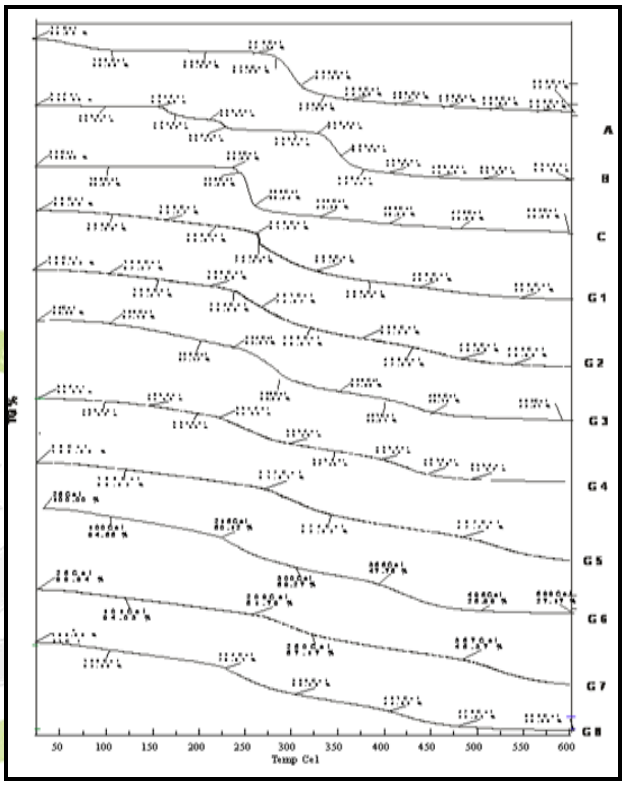

Figure 5(a): TG curves for chitosan powder (A), glutamic acid (B), glycine (C) and drug unloaded cross linked beads (G1-G8)

A sudden weight loss is observed after 249°C and the total weight loss at 400°C is about 60%, while as the cross linking density increased from G1 to G4 the weight loss percent decreased continuously upto 47% at 400°C. This shows that cross linking of chitosan with glutaraldehyde increases its thermal stability. TG curves for BPM model drug (curve D) and drug loaded cross linked beads (G1-G8) are shown in fig 5(b). BPM drug lost about 67% weight between 208°Cand 274°C (curve D) which was due to the decomposition of drug above its melting point. Melting point of BPM is 134°C and such a huge loss in weight was not

shown by drug loaded beads G1-G8 containing drug. This concluded that the drug is quite stable within the beads.

DTG thermograms of pure chitosan, glutamic acid, glycine and cross linked beads G1-G8 are presented in figure 6(a). These indicated the rate of weight loss for chitosan powder was highest at 290^{o}C and cross linked chitosan beads showed lesser rate of weight loss between 222 to 271^{o}C.

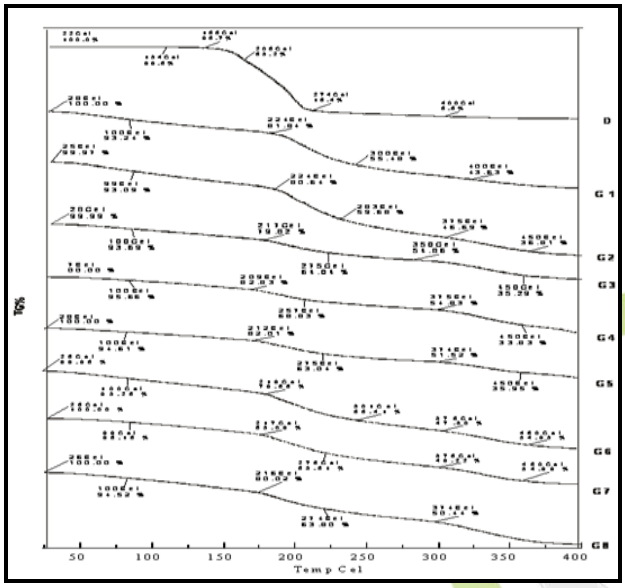

Figure 5(b): TG curves for pure BPM drug (D) and drug loaded crosslinked beads (G1-G8)

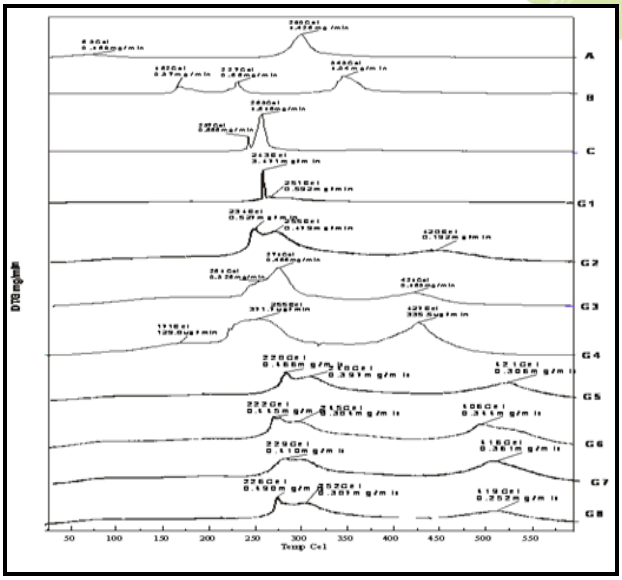

Figure 6(a): DTG curves for chitosan powder (A), glutamic acid (B), glycine (C) and drug unloaded cross linked beads (G1-G8)

On comparing G1-G4 beads it can be seen that G4 beads were found to be most stable as they lose weight at minimum rate approximately 371µg/min at 255^{o}C as compared to G1 beads (3.47 mg/min) at 245^{o}C, this concluded that cross linking made the beads more stable. Variation of chitosan concentration (G5 to G6 beads) and amino acid composition (G7 and G8 beads) give almost equally stable as that of G3 beads. The comparison of drug unloaded beads G1-G8 in figure 6(a) and drug loaded beads G1-G8 in fig 6(b) showed almost similar peaks with approximate same rate of weight lost also proved equally drug stability in the polymeric matrix containing drug.

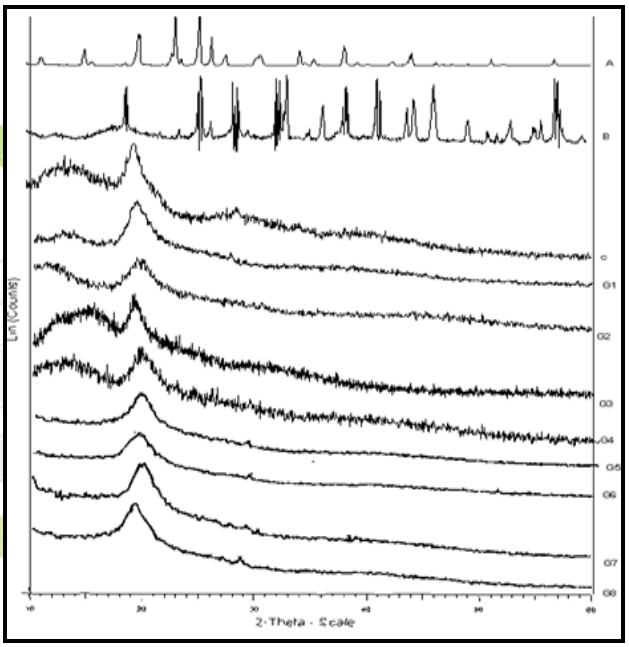

Figure 6(b): DTG curves for BPM drug (D) and drug loaded cross linked beads (G1-G8)

DTA thermograms for pure chitosan, glutamic acid, glycine and cross linked drug unloaded beads (G1-G8) are presented in fig 7(a). Thermograms for chitosan powder showed one endothermic peak at 65^{o}C due to loss of free water and one exothermic peak at 296^{o}C due to chemical transformation. Glutamic acid gives two and glycine gives one endothermic peak in their thermo grams. While in case of (G1-G8) beads only one exothermic peak is observed. It was concluded that cross linked chitosan-glycine-glutamic acid G1-G8 beads are the equally stable.

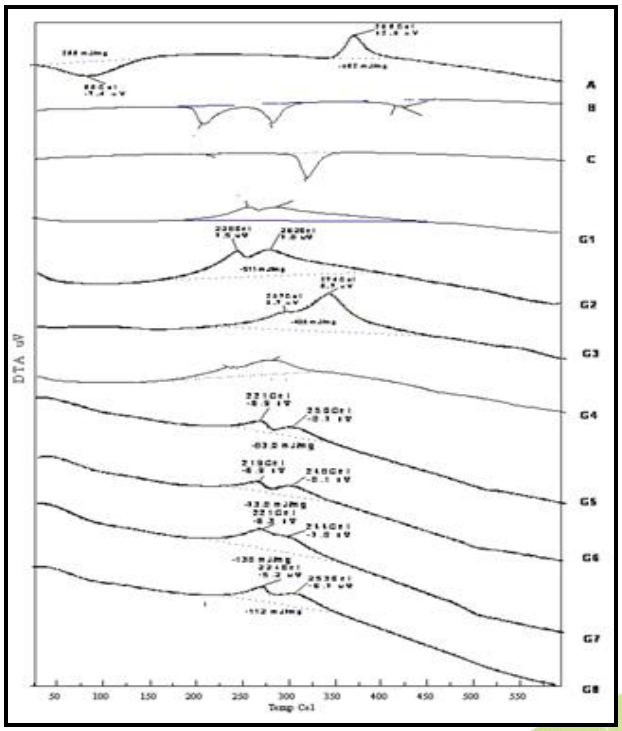

Figure 7(a): DTA curves for chitosan powder (A), glutamic acid (B), glycine (C) and drug unloaded cross linked beads (G1-G8)

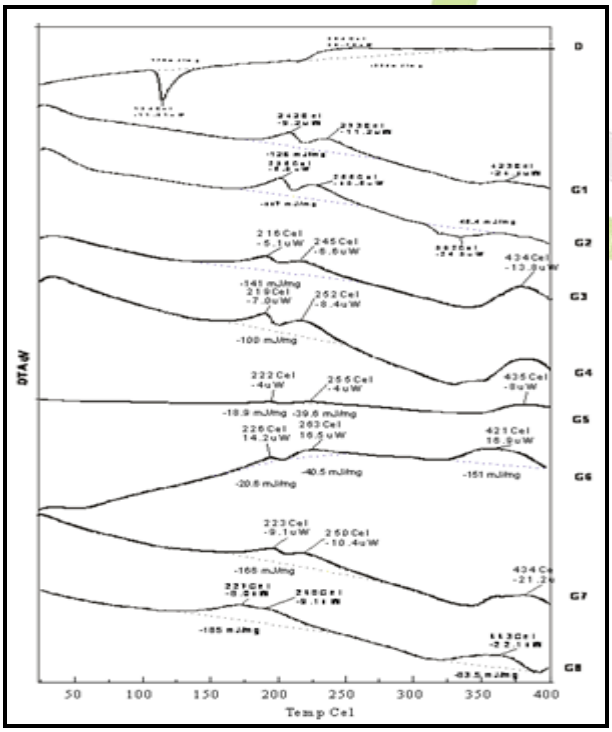

Figure 7(b): DTA curves for BPM drug (D) and drug loaded crosslinked beads (G1-G8)

DTA thermo grams for pure BPM drug and drug loaded beads G1-G8 are represented in fig 7(b) containing drug. In case of BPM drug (curve D) one endothermic peak at 134^{o}C which corresponds to melting process and one exothermic peak at 294^{o}C to chemical transformation were observed. Drug loaded beads (G1-G8) showed almost similar thermo grams in which no peaks were observed at 134^{o}C and 294^{o}C indicating the amorphous dispersion of drug into the beads.[18-20]

Drug Release Studies

The release profile of BPM from chitosan beads loaded (78 µg of drug) at various time intervals in acidic (pH 2.0) and basic (pH 7.4) solutions at 37^{o}C is shown in fig 8(a), 8(b) and 8(c). There was a burst release initially for the first hour in both acidic and basic media followed by a moderate release for next four hours and finally an almost constant release of BPM from the matrix for the studied period of 48 hour. The amount and percentage of drug released followed the order of swelling of beads. It is because the release rate depends on swelling of the beads.

It was noticed that drug release was pH dependent as the amount and percentage of drug released were much higher in acidic medium than in alkaline medium in case of G1 to G8 beads. This can be explained by the fact that the release of drug due to diffusion through the swollen beads depends mainly on the percentage of swelling of beads. Initially the burst release of drug was observed due to the fast penetration of the solvent into the cross linked beads. After few hours, a steady state was reached, due to the equilibrium concentration gradient and then a constant drug release was observed. At pH 7.4 there is less swelling thus drug entrapped in the beads could not be released easily, however, at pH 2.0 the beads were swollen to a higher percentage, leading to faster release of drug. It was observed in fig 8(a) that the drug release rate increases with the decrease in crosslink density. This may be due to the fact that the diffusion of drug from IPN depends on the pore size of the polymer network which will decrease with increase in degree of cross linking. This

may be due to the different chemical structures of glycine and glutamic acid.

The reason may be due to the presence of two carboxylic ends of the cross linked chitosan-glutamic beads. Since carboxylic group is more susceptible to be attacked by the basic solution, the drug release in the acidic medium was less due to the interaction of acidic solution with the polar group of beads.

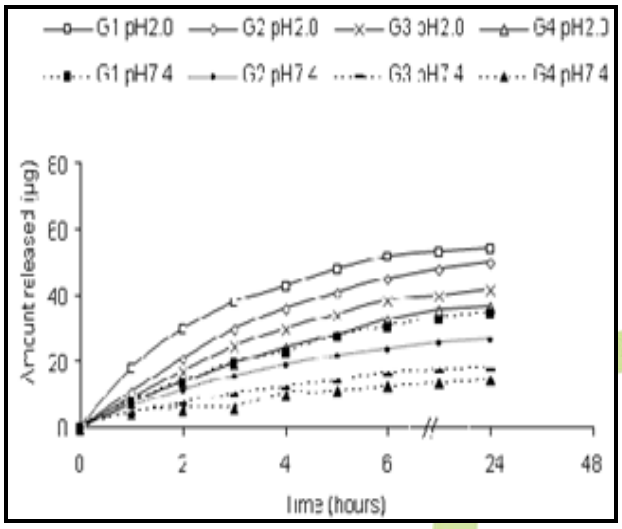

Figure 8(a): Release of BPM from 78 µg BPM loaded beads vs time in solution pH 2.0 and pH 7.4 at 37^{0}C having different percentage of glutaraldehyde

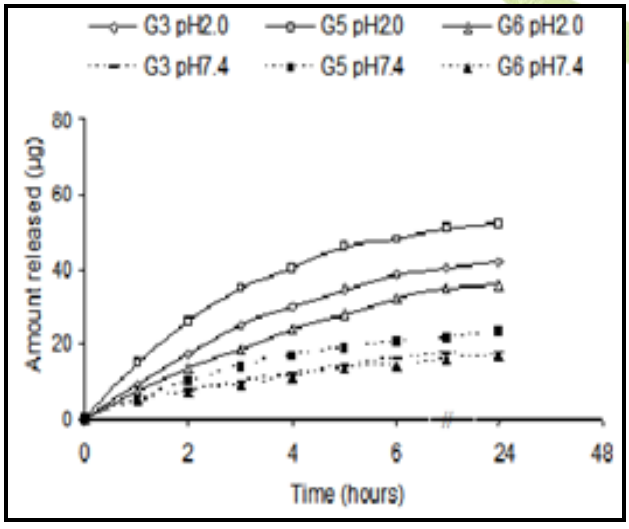

Figure 8(b): Release of BPM from 78 µg BPM loaded beads vs time in solution pH 2.0 and pH 7.4 at 37^{0}C having different weight ratio of chitosan

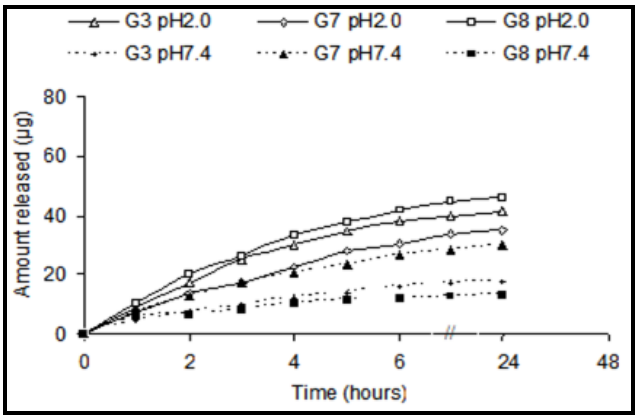

Figure 8(c): Release of BPM from 78 µg BPM loaded beads vs time in solution pH 2.0 and pH 7.4 at 37^{0}C having different weight ratio of amino acid

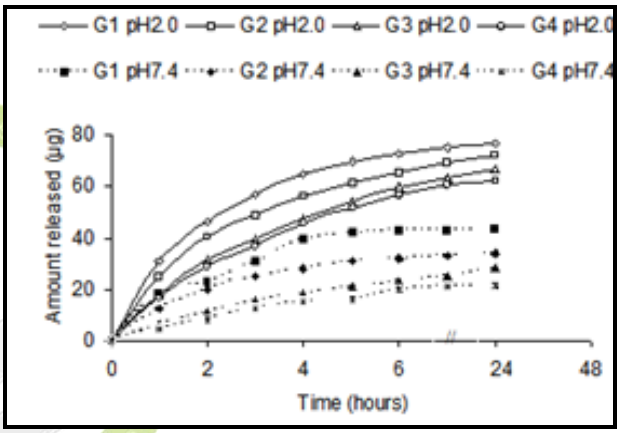

Figure 9(a): Release of BPM from 142 µg BPM loaded bead vs time in solution pH 2.0 and pH 7.4 at 370C having different percentage of glutaraldehyde

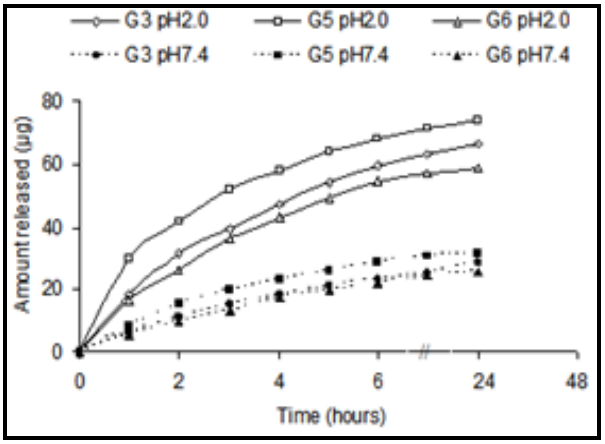

Figure 9(b): Release of BPM from 142 µg BPM loaded bead vs time in solution pH 2.0 and pH 7.4 at 370C having different weight ratio of chitosan

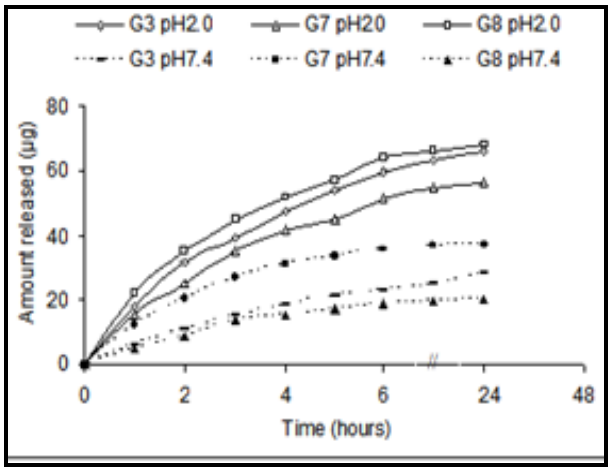

Figure 9(c): Release of BPM from 142 µg BPM loaded bead vs time in solution pH 2.0 and pH 7.4 at 370C having different weight ratio of amino acid

In case of chitosan-glycine-glutamic acid beads, the amount and percentage of drug release were higher in acidic medium than in basic medium. This concluded that glycine showed dominant effect over glutamic acid and overall effect was governed by glycine. The release profile of BPM drug has been checked for the beads having varying amount of chitosan for the same amount of amino acids and glutaraldehyde (12.5%). It was observed from fig 8(b) that the G5 beads having smaller weight of chitosan gave higher release rates and slowest release rate was observed in case of G6 beads containing higher concentration of chitosan. This may be due to containing more chitosan, the amount of available amino acids acting as spacer group became low and there was possibility of formation smaller mesh size volume, which in turn might decrease the rate of swelling as well as drug release. The drug release rates of BPM drug have also been studies for the beads having different composition of amino acids (i.e. glycine and glutamic acid) and results shown in fig 8(c). It was observed that on increasing the amount of glycine or decreasing the amount of glutamic acid in G8 bead increased the rate of drug release in acidic solution while decreased in basic solution. These results are quite similar to the results observed for swelling rate. To check the reproducibility of the result, the release profile of BPM from the chitosan beads loaded with higher amounts of drug (142 µg of drug loaded beads) have also been studied in acidic pH 2.0 and basic pH 7.4 media as shown in fig 9(a), 9(b) and 9(c). The release pattern of the drug loaded beads has been found to be similar irrespective of the amount of the drug loaded. These observations have suggested that the total amount of drug release from the chitosan beads has increased with the increase in concentration of BPM. However, the percentage of BPM released from the beads loaded with a higher amount of drug was found to be lower in comparison to the beads loaded with a lower amount. This concluded that the mechanism of the drug release due to the diffusion through swollen beads depends on the percentage of swelling of beads.

Kinetic Analysis of Drug Release

In order to have an insight into the mechanism of drug release behavior Higuchi's model were best fitted into the kinetic data of drug release. Linear plots of percent cumulative amount release versus square root of time is shown in fig 10 demonstrating that the release from the cross linked polymeric microsphere matrix is diffusion controlled and obeys the Higuchi's model.

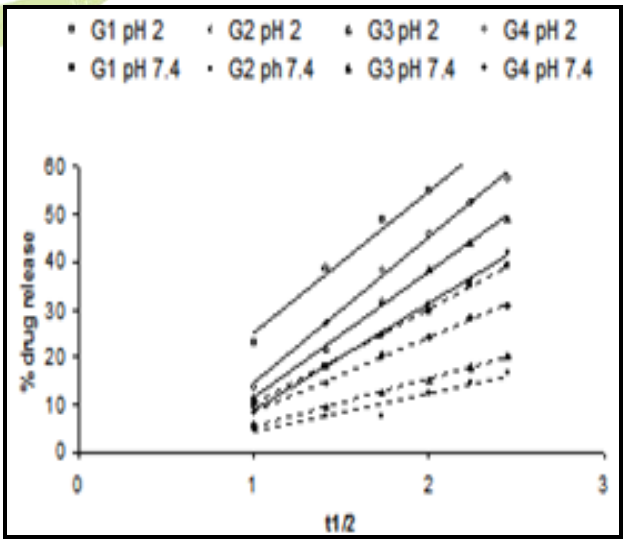

Figure 10 (a): Plots showing drug release profile from 78 µg BPM loaded beads in solution pH 2.0 and pH 7.4 by fitting the Higuchi's equation having different percentage of glutaraldehyde

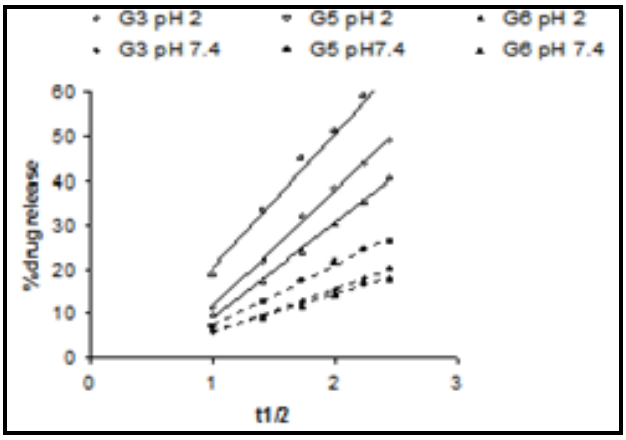

Figure 10 (b): Plots showing drug release profile from 78 µg BPM loaded beads in solution pH 2.0 and pH 7.4 by fitting the Higuchi's equation having different weight ratio of chitosan

The constant k, presented in table-2 was calculated from the slope of the linear portion of plot of percentage of cumulative drug released versus the square root of time. The value of 'k' for the release process has been found to be lower in solution of pH 7.4 than in solution of pH 2.0. However, the values were smaller which indicate mild interaction between the drug and polymeric matrices.[19-21]

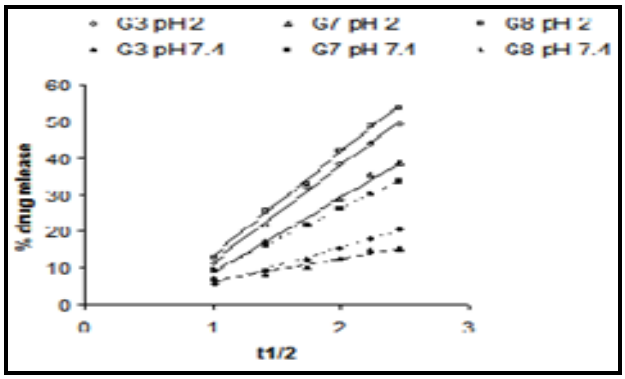

Figure 10 (c): Plots showing drug release profile from 78 µg BPM loaded beads in solution pH 2.0 and pH 7.4 by fitting the Higuchi's equation having different weight ratio of amino acid

CONCLUSION

The observations of the present study have shown that chitosan-glycine-glutamic acid beads possess a pH dependent swelling behavior. It can be used successfully for the formulation of controlled drug delivery devices. They have optimum entrapping capacity for the studied drugs and provide a sustained release of drugs for extended periods which make them appropriate for delivery of drug at a controlled rate.

Table 2: Results of drug release mechanism by fitting data in Higuchi's model for BPM loaded beads

Beads type	pH 2.0						pH 7.4					
	BPM loaded beads with											
	78 µg			142 µg			78 µg			142 µg		
	k	S.D.	R	k	S.D.	R	k	S.D.	R	k	S.D.	R
G1	31	±.035	.99	.205	±.008	.99	.20	±.019	.99	.13	±.008	.99
G2	.26	±.030	.99	.19	±.009	.99	.16	±.015	.99	.095	±.005	.99
G3	.22	±.025	.99	.20	±.015	.99	.10	±.009	.99	.083	±.008	.99
G4	.30	±.026	.99	.195	± .015	.99	.08	±.008	.99	.073	±.009	.99
G5	.22	±.024	.99	.19	± .005	.99	.14	±.014	.99	.097	±.009	.99
G6	.20	±.022	.99	.19	± .015	.99	.085	±.005	.99	.081	±.009	.99
G7	.28	±.031	.99	.175	± .014	.99	.17	±.016	.99	.12	±.009	.99
G8	.31	±.035	.99	.20	+.011	.99	.06	±.005	.99	.069	±.008	.99

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