RESEARCH ARTICLE

Interpenetrating polymeric network hydrogel for stomach-specific drug delivery of clarithromycin: Preparation and evaluation

Gupta Anish Kumar, Siddiqui Abdul Wadood, Maurya Sheo Datta[1], Dhakar Ramchand[1]

Department of Pharmacy, Lloyd Institute of Management and Technology, [1]IEC College of Engineering and Technology, Greater Noida, India

The aim of this study was to develop a controlled release system targeting antibiotic delivery to the stomach. The hydrogels were synthesized by using chitosan, poly (acrylic acid) and poly (vinyl pyrrolidone) polymers crosslinked with glutaraldehyde and *N*,*N*'-methylenebisacrylamide. Interpenetrating polymeric network (IPN) hydrogels were prepared by varying the concentration of crosslinking agent (glutaraldehyde). The amount of chitosan, poly (acrylic acid), poly (vinyl pyrrolidone) and *N*,*N*'-methylenebisacrylamide were kept constant in all formulations. The effect of glutaraldehyde concentration on the swelling and release characteristics were evaluated. Modalities used to assess the most optimal hydrogel formulation included high liquid chromatography, FTIR analysis, differential scanning calorimetry, swelling studies, *in vitro* drug release study, mucoadhesive study and scanning electron microscopy. The result showed that IPN hydrogels were greater in swelling, more mucoadhesive and released more drug at lower pH values. Thus, it is believed that the antibiotic concentration in the stomach might be sustained through this formulation.

Key words: *Controlled release, glutaraldehyde, IPN, stomach targeted*

INTRODUCTION

Site-specific controlled release systems offer many distinctive advantages over classical method of drug delivery. These include localized delivery of the drug to a particular part of the body. Controlled release systems that have been developed so exhibit pH-dependent drug release.[1] Hydrogels are three dimensional, hydrophilic, polymer networks capable of imbibing large amounts of water or biological fluids. Interpenetrating polymeric networks (IPN) comprise two or more independent polymer networks that are formed in presence of one another. IPN properties such as porosity, elasticity, degree of swelling and responsive behavior to a stimulus can be tuned by the appropriate choice of network forming polymers and suitable crosslinking agent and its proportion. IPN hydrogels have emerged as useful materials for certain applications such as localized antibiotic delivery in the acidic environment of gastric fluid.[2]

Address for correspondence:
Mr. Anish Kumar Gupta,
Department of Pharmacy, Lloyd Institute of Management and Technology, Greater Noida–201 306, U.P., India.
E-mail: anishpharma02@yahoo.co.in

Chitosan, a natural polysaccharide, exhibits favorable biological properties such as biocompatibility, biodegradability and nontoxicity. For several years chitosan has been largely evaluated as a potential vehicle for oral dosage forms. Chitosan, poly (acrylic acid) and poly (vinyl pyrrolidone) have been much investigated as a stimulus sensitive drug release system and glutaraldehyde is the most common crosslinking agent chosen for chitosan-based hydrogels.[3]

Helicobacter pylori lives deep within gastric mucus layer and prolonged local application of drug is needed sufficiently to diffuse bacteria. It has been demonstrated that *H. pylori* is one of the major causative micro-organisms for peptic ulcer disease. This bacterium release enzyme urease, which convert urea into ammonia and bicarbonate, which aids in neutralizing acidic medium and allow the bacteria to colonize in gastric mucosa.[4]

Access this article online

Quick Response Code:

Website: www.asiapharmaceutics.info

DOI: 10.4103/0973-8398.76738

In the present study, an attempt has been made to formulate IPN hydrogels of clarithromycin using chitosan, poly (acrylic acid) and poly (vinyl pyrrolidone) polymers. Chitosan hydrogels provide pH-responsive release profile by swelling in gastric fluid (low in pH value) to release drug over a prolonged period of time.

MATERIALS AND METHODS

Clarithromycin was gift sample from Ranbaxy Laboratories, Gurgaon, India. Chitosan (MW=3.5×10^5, >80% deacetylated) was gift sample from Central Institute of Fisheries Technology, Cochin. Poly (vinyl pyrrolidone) and glutaraldehyde were purchased from Central Drug House, New Delhi. Poly (acrylic acid) and *N*,*N*'-methylenebisacrylamide were purchased from Loba Chemie, Mumbai. All other chemicals were of analytical grade and double distilled water was used throughout the experiment.

Preparation of interpenetrating hydrogels

IPN hydrogel containing clarithromycin was prepared by chemical crosslinking process.[2] Chitosan gel was prepared in 1% acetic acid solution. A known amount of drug was added to chitosan solution and stirred for 15 min using mechanical stirrer. Poly (vinyl pyrrolidone), acrylic acid and crosslinkers were added to the solution of chitosan under continuous mixing. Chitosan, poly (vinyl pyrrolidone) and acrylic acid was used in 1:1:1 (w/w) ratio. The glutaraldehyde added in the IPNs was 0.25, 0.5, 1.0 and 2.0% w/v. Amount of *N*,*N*'-methylene-bis-acrylamide added was 1% to the weight of acrylic acid. Ammonium persulfate solution (25 ml) was lastly added. The mixture was placed in petridish and placed in room temperature to form gel. Gel was cut into equal dimensions (2×2mm) and washed with distilled water to remove any unreacted monomer or crosslinking agent. The washings were collected and preserved for analysis. They were then dried in air and vacuum, and stored for further use. Figure 1 showed schematic preparation of hydrogel.

Determination of amount of drug entrapped

The amount of drug entrapped: in the IPN hydrogels was determined by an indirect method. After the gel preparation washings are collected, filtered with a 0.45 μm milipore filter and analysed by reversed-phase high performance liquid chromatography (RP-HPLC). The detection was done on RP-

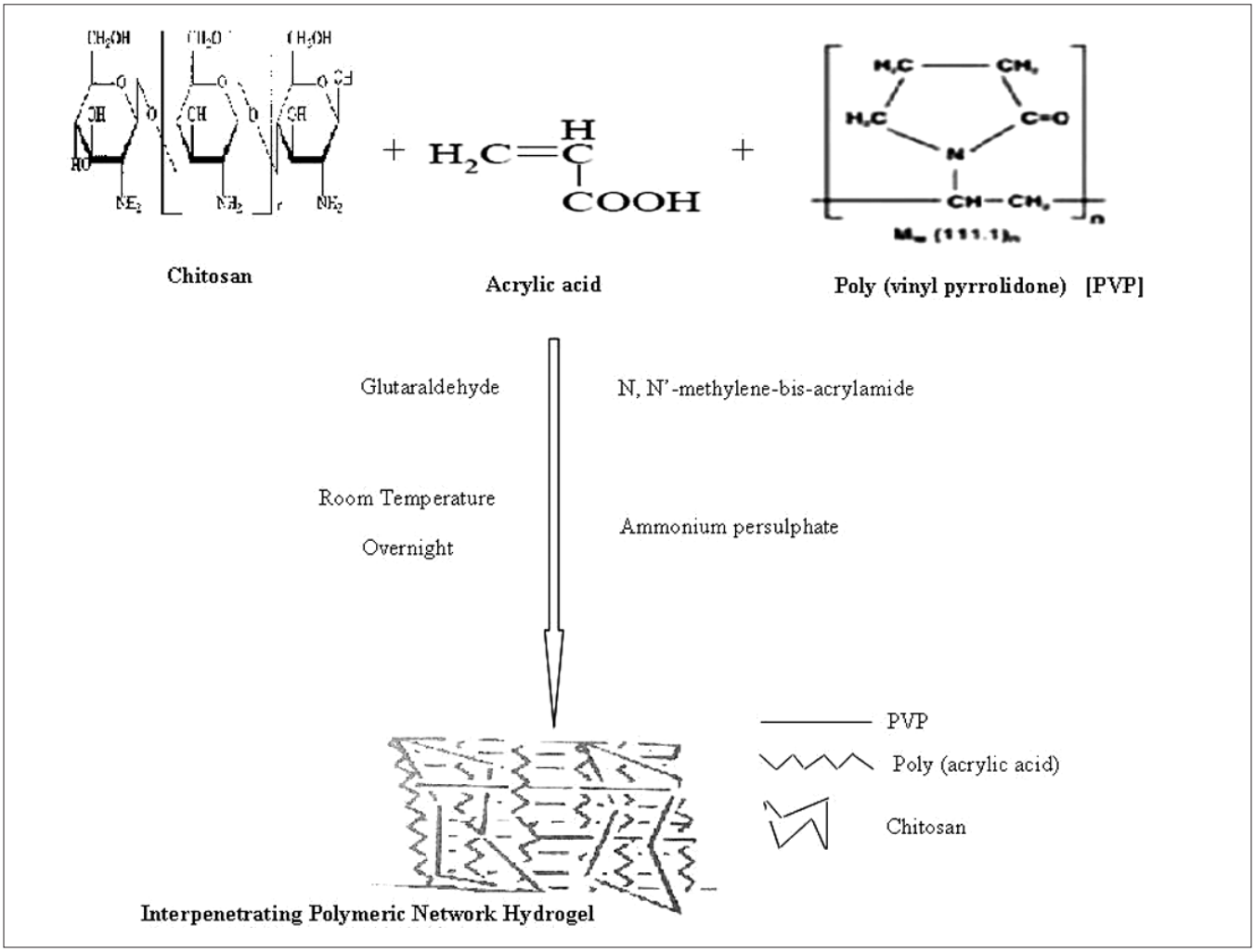

Figure 1: Schematic preparation of IPN hydrogel

HPLC with the detection chosen at 210 nm. The difference between the amount of drug initially employed and the drug content in the washing is taken as an indication of the amount of drug entrapped.

% Drug entrapment = A2 / A1 × 100
where,
A1 – Amount of drug initially loaded.
A2 – Amount of drug in washings.

Fourier transform infrared spectral analysis
The prepared IPN hydrogel pieces were subjected to fourier transform infrared (FTIR) analysis by KBr pellet method using FTIR spectrophotometer, [8201 PC (4000-400/cm), Shimadzu, Japan]. This was employed to ascertain the compatibility of drug with excipients.

Differential scanning calorimetry
Differential scanning calorimetry (DSC) was performed on pure sample of carithromycin and its formulation using STARe SW 9.00 apparatus. DSC thermogram of 4-5 mg sample was recorded at a heating rate of 5°C/min. over a temperature range of 50-500°C.

Surface morphology/scanning electron microscopy
The surface morphology of IPN hydrogel in unswollen state and kept in acidic pH (pH 2.0) was determined separately by using PHILIP 505 scanning electron microscope. Swollen hydrogel sample was dried in vaccum. Hydrogel samples were mounted on a scanning electron microscope holder (aluminium sample mount) with a double-sided adhesive tape and coated with gold palladium. The hydrogel samples were analyzed with PHILIP 505 scanning electron microscope at different magnifications and an acceleration voltage of 14.9 KV.

Swelling studies
Swelling studies of IPNs were done as dynamic equilibrium swelling. In dynamic swelling experiment IPNs were placed in 0.01 N HCl (pH 2.0) at 37±1°C. During swelling, gels were removed from the swelling bath at regular time intervals and their surface dried with filter paper and weighed; thereafter they were returned in to the same swelling bath.

The swelling ratio can be calculated as function of time and is calculated from the following relationship:

$$\text{Swelling ratio (\%)} = \left(\frac{Ws - Wd}{Wd}\right) \times 100$$

$$\text{Equilibrium water content (EWC\%)} = \left(We\,\frac{Wd}{Wd}\right) \times 100$$

Where Ws represents the weight of the swollen state at a given time, Wd is the weight of sample at dry state, and We is the weight of the swollen state of a sample at equilibrium.

Mucoadhesion study
The mucoadhesive property of prepared IPN hydrogel was evaluated by *in vitro* mucoadhesive testing method known as washoff method as reported previously by Shantha and Harding.[5,6] A rat stomach mucosa was tied on the glass slide using a thread. About 50 hydrogel pieces were spread on to wet rinsed tissue specimen and prepared slide was hung on to one of the grooves of a USP tablet disintegration apparatus. By operating the disintegrating test apparatus the tissue specimen was given a slow regular up and down movement in the test fluid at 37±1°C. At every 1 hr-interval the equipment was stopped and the number of pieces still adhering to tissue was counted. Percent mucoadhesion was given by the following formula:[7]
% Mucoadhesion = P1/P2 × 100
where,
P1- no. of adhered hydrogel pieces
P2- no. of applied hydrogel pieces

***In vitro* drug release study**
The release of clarithromycin from hydrogel was determined as described by Miyazaki *et al*.[8] using USP dissolution test apparatus with a paddle stirrer at 50 r/min. The dissolution medium used was 500 ml of 0.01 N HCl (pH 2.0), and temperature was maintained at 37±1°C. Hydrogel were transferred to the dissolution media. At specific time interval, a precisely measured sample of the dissolution medium was removed and replenished with prewarmed (37°C) fresh medium to maintain sink condition. Samples were withdrawn at predetermined time intervals and neutralized with sodium hydroxide (0.05 M) solution prior to analysis by RP-HPLC using a mobile phase consisting of acetonitrile-aqueous 0.05 M phosphate buffer solution of pH 4.0 (40/60 v/v). The apparatus used for HPLC analysis was an Agilent 1100 quarternary pump, with a variable wavelength detector, thermostatted autosampler and column. A Hypersil ODS C_{18} column (250×4.5 mm ID, 5 μm Thermo, UK) was fitted with a Phenomenex guard column packed with octadecyl C_{18}. The column temperature was maintained at 40°C and flow rate at 1 ml/min. The detector was set at 210 nm.

RESULT AND DISCUSSION

Chitosan, a natural polymer which exhibits pH sensitivity, was chosen to develop IPN hydrogels for oral-controlled drug delivery to the stomach region in combination with poly (vinyl pyrrolidone) and poly (acrylic acid). Acrylic acid is converted to poly (acrylic acid) by free radical polymerization using ammonium persulfate as a free radical polymerization initiator. Free radical polymerization is a process in which there is generation of free radical acts as a catalyst for the polymerization of monomers.[9,10]

Drug entrapment efficiency
The entrapment efficiency of different IPN hydrogel formulation was calculated as percent total drug entrapped.

The entrapment efficiency of clarithromycin in different formulation of IPN hydrogel was found to be 94.2±2.4%, 95±2.1%, 95.4±2.7% and 96.8±1.9% for FC1, FC2, FC3 and FC4 respectively. According to the method of preparation of IPN hydrogel the entrapment efficiency should be 100%, but the observations shows that entrapment efficiency is <100% in all formulations. This may be due to loss of drug during washing of hydrogel. [Table 1] The data indicate that entrapment efficiency depends on crosslinker ratio, at lower crosslinker ratio entrapment efficiency decreases because lower crosslinker ratio results into higher swelling during washing of hydrogel that will cause greater amount of drug loss during washing, and higher crosslinker ratio results in greater entrapment efficiency because of lesser swelling during washing that will results in lesser amount of drug loss during washing.

FTIR spectral analysis

The chitosan spectrum exhibits band at 3379.05 (OH stretching) and 3436.91 (-NH_2 stretching). The absorption band at 1118.64 (antisymmetric stretching of C-O-C bridge) and 2923.88 (-CH2 stretching). The band at 1652.88 due the chitosan spectrum was attributed to the formation of C=N, due to imine reaction between amino group of chitosan and aldehyde group in aldehyde [Figure 2].

DSC

From the DSC thermogram, it was observed that a sharp endothermic peak at 226°C was seen for clarithromycin (corresponds to its melting point) as well as for its formulation. Since the peaks are identical, it proves that there is no chemical interaction between the drug and the polymer employed for the preparation of IPN hydrogels [Figure 3].

Swelling studies

Swelling curves of IPN hydrogel with different amount of crosslinking agent shown that swelling increased with time and it will also show that swelling decreased with increasing crosslinking agent in IPN hydrogel. The degree of swelling of IPN hydrogels depend on its network structure, which is controlled by the concentration of crosslinking agent. Results indicate that IPN hydrogels exhibited greater swelling at lower crosslinker ratio [Figure 4]. This can be explained in terms of ionic interaction of amino group of chitosan in stomach pH condition. The protonation of amino group in chitosan ensures chain relaxation, thus leads to faster hydrogen bond dissociation and efficient solvent diffusion.[1,11]

Table 1: Batch specifications of prepared IPN hydrogel

Batch specifications of prepared IPN hydrogel				
Composition	**FC1**	**FC2**	**FC3**	**FC4**
Chitosan	1	1	1	1
PVP	1	1	1	1
AAC	1	1	1	1
GLD	0.25%	0.50%	1%	2%
MBA	1%	1%	1%	1%
APS	25µl	25µl	25µl	25µl
Drug	0.5	0.5	0.5	0.5

PVP, Poly vinyl pyrrolidone; AAC, acrylic acid; GLD,glutaraldehyde; MBA, *N,N*-methylene-bis-acrylamide; APS, ammonium persulfate

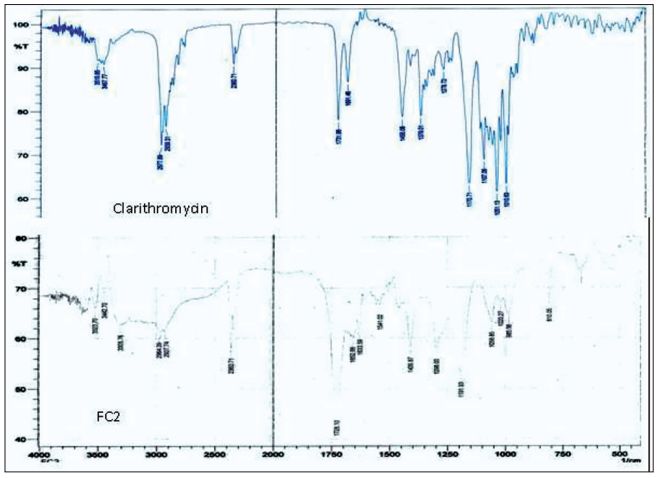

Figure 2: Fourier transform infrared spectra showing compatibility of drug with polymers in formulation FC2

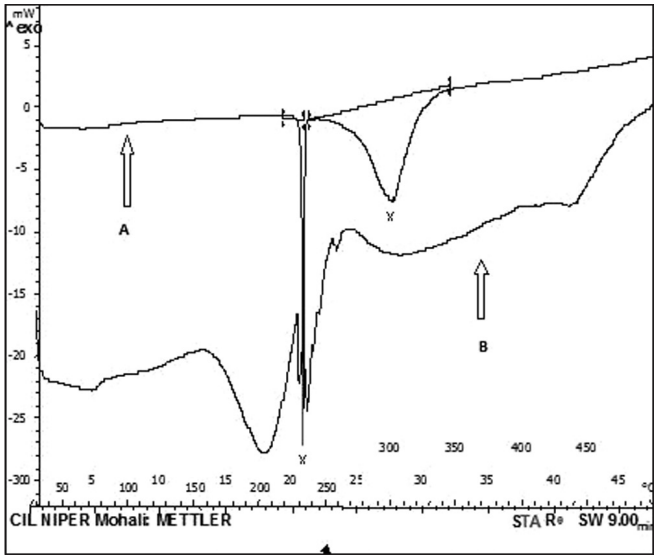

Figure 3: Comparative differential scanning calorimetric thermogram: DSC thermogram of clarithromycin (A) and IPN hydrogel formulation FC2 (B)

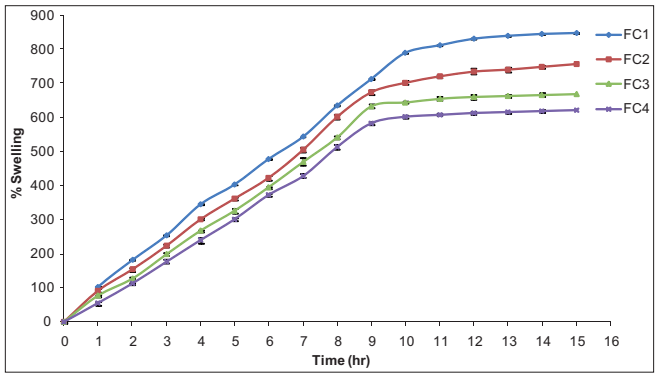

Figure 4: Percentage swelling of interpenetrating polymeric hydrogels prepared

An increase in amount of crosslinking agent leads to denser crosslinking bridges. Such structures can be characterized by lower and slower penetration of solvent through the chain structure of polymer, suggesting that the swelling ratio and release characteristics of the IPN hydrogel can be controlled by varying crosslinking agent concentration used during manufacturing process. Swelling of hydrogels may result in to mobility of crosslinking chains, facilitating solvent diffusion and rapid release of drug through polymer. Since glutaraldehyde is responsible for the formation of crosslinks, increasing the concentration of glutaraldehyde will increase the network density, resulting in reduction of chain mobility and formation of more stable and rigid hydrogel that show a slower tendency to swell. Sahin *et al*, reported that a decrease in the swelling ratio in terbutaline sulfate loaded albumin microsphere was observed with an increase in the glutaraldehyde concentration.[12] Raymond *et al* found out that the release of phenytoin sodium from gelatine microsphere can be delayed by the addition of glutaraldehyde to the microsphere formulation.[13]

In vitro drug release study

The release of clarithromycin from IPN hydrogel was studied in 0.01 N HCl (pH 2.0) at 37±1°C. The release of drug significantly increased with decreasing crosslinking agent concentration. The diffusion of drug molecule out of IPN hydrogels containing chitosan was enhanced because of swelling at lower pH. The extent of release increase as the hydrogel swelling increase at lower pH, which leads to ionization of amino groups. This selective release will ensure maximum availability of the drug in the stomach thereby maintaining bactericidal concentration of the antibiotic in the stomach. The formulation FC1 shows best releases profile and it will release about 88.54±4.1% drug respectively in 12 hr, so justifying itself as an optimized formulation in terms of drug release profile [Figure 5].

Mucoadhesion study

The mucoadhesive property of the hydrogels was evaluated by wash-off method. At the end of 5 hr, % mucoadhesion was found to 80±2.15%, 60±3.40%, 62±3.67%, 62±2.68% for FC1, FC2, FC3, FC4 formulations [Figure 6]. Formulation FC1 shows highest % mucoadhesion. The basis of mucoadhesion that a dosage form can stick to the mucosal surface. A salt bridge effect has been proposed for the interaction of the positively charged mucoadhesive hydrogel particles with the negatively charged mucous glycoprotein.[14] Chitosan possesses OH and NH_2 groups that can give rise to hydrogen bonding. These properties are considered essential for mucoadhesion. Further cationic polyelectrolyte nature of chitosan could provide a strong electrostatic interaction with mucosal surface.[5] The rank order of mucoadhesion for formulations was to be FC1 > FC2 > FC3 > FC4.

Surface morphology/scanning electron microscopy

Surface morphology of the IPN hydrogels was examined by scanning electron microscopy (SEM) by using PHILIP 505 scanning electron microscope [Figure 7]. It was observed that surface of the unswollen hydrogels (FC1) showed no surface pores [Figure 8a]. The IPN hydrogel pieces kept in 0.01N HCl (pH 2.0) showed sponge like porous surface [Figure 8b].

Thus, IPN hydrogels of clarithromycin could be prepared using chitosan, poly (vinyl pyrrolidone) and acrylic acid monomer, and glutaraldehyde and N, N'-methylene bis acrylamide as crosslinking agents. The prepared IPN hydrogel particles showed swelling in acidic pH. Formulation FC1 with low crosslinking agent showed better swelling and release of drug.[15,16]

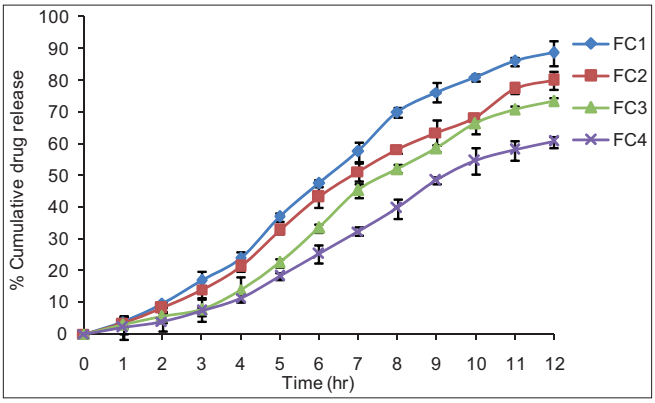

Figure 5: *In vitro* drug release profile of IPN hydrogels

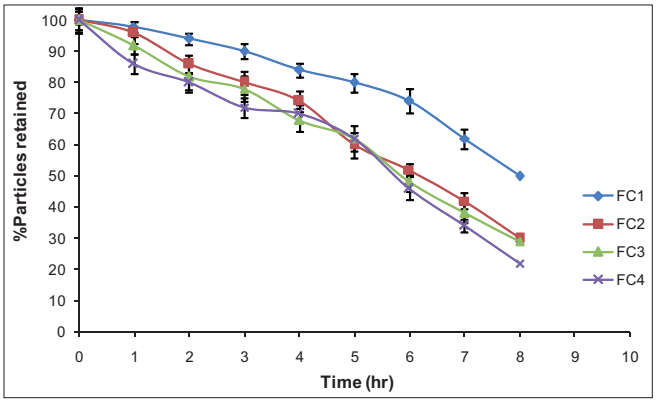

Figure 6: Percentage mucoadhesion of IPN hydrogels

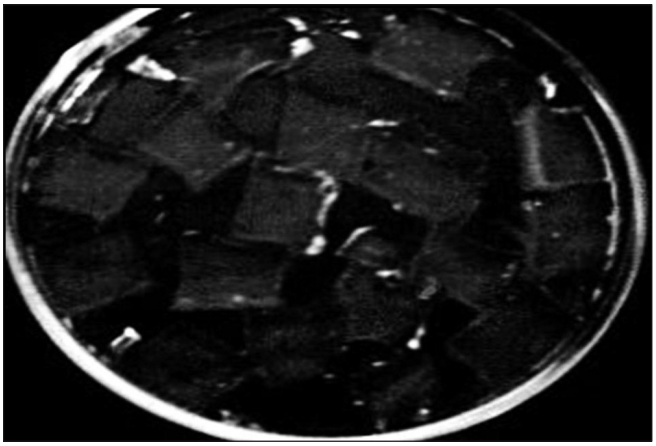

Figure 7: Swollen IPN hydrogel pieces

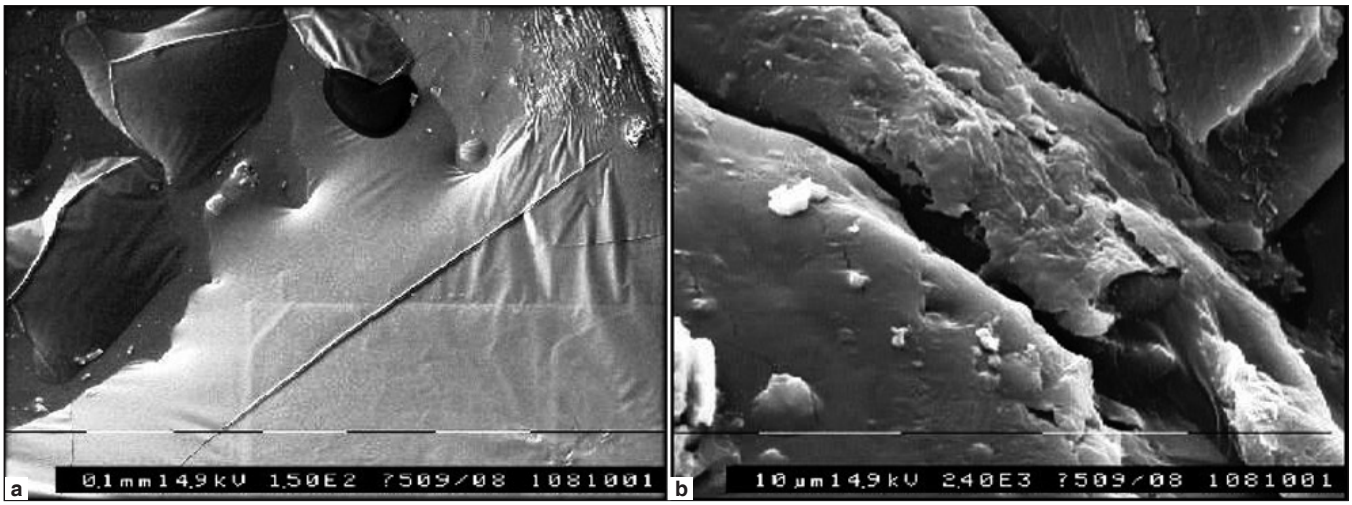

Figure 8: Scanning electron micrograph of unswollen (a) and swollen (b) IPN hydrogel

CONCLUSIONS

A Stomach retentive IPN hydrogel was prepared successfully by chemical crosslinking process.[17] The results of *in vitro* studies showed that by appropriate modification of crosslinking agent concentration the extent of swelling and rate of drug release can be modulated. Mucoadhesive study showed that, "IPN hydrogel prepared" have good mucoadhesion property and retained in gastric environment of stomach for prolonged period of time. Thus IPN hydrogel prepared maintain antibiotic concentration in stomach for prolonged period of time, can be used as a drug delivery system for treatment of *H. pylori* infection and in management of peptic ulcer.[18] Further modification of these interpenetrating hydrogels can lead to the successful application for the targeting a drug delivery system (or device) to the stomach with extended release.

REFERENCES

1. Risbud MV, Hardikar AA, Bhat SV, Bhonde RR. pH-sensitive freeze-dried chitosan-polyvinyl pyrrolidone hydrogels as controlled release system for antibiotic delivery. J Control Release 2000;68:23-30.
2. Sema E, Dursun S. Synthesis, characterization and evaluation of IPN hydrogels for antibiotic release. Drug Deliv 2004;11:381-8.
3. Khurma JR, Nand AV. Temperature and pH sensitive hydrogels composed of chitosan and poly (ethylene glycol). Polymer Bull 2008;59:805-12.
4. Lee YM, Kim SH, Cho CS. Synthesis and swelling characteristics of pH and thermo-responsive interpenetrating polymer network hydrogel composed of poly (vinyl alcohol) and poly (acrylic acid). J App Poly Sci 1996;62:301-11.
5. Shantha KL, Harding DR. Preparation and *in-vitro* evaluation of poly [N-vinyl-2-pyrrolidone-polyethylene glycol diacrylate]-chitosan interpolymeric pH-responsive hydrogels for oral drug delivery. Int J Pharm 2000;207:65-70.
6. Chowdary KP, Rao YS. Mucoadhesive microcapsules of glipizide: Characterization, in-vitro and in-vivo evaluation. Indian J Pharm Sci 2003;65:279-84.
7. Chun MK, Sah H, Choi HK. Preparation of mucoadhesive microspheres containing antimicrobial agents for eradication of *H.pylori*. Int J Pharm 2005;297:172-9.
8. Yong Q, Park K. Environmental-sensitive hydrogels for drug delivery. Ad Drug Del Rev 2001;53:321-39.
9. Kim SJ, Park SJ, Kim SI. Synthesis and characterization of interpenetrating polymer network hydrogels composed of poly(vinyl alcohol) and poly(N-isopropylacrylamide). React Funct Polym 2003;55:61-7.
10. Miyazaki S, Kubo W, Attwood D. Oral sustained delivery of theophylline using in-situ gelation of sodium alginate. J Control Release 2000;67:275-80.
11. Torre PM, Enobakhare Y, Torrado G, Torrado S. Release of amoxicillin from polyionic complexes of chitosan and poly (acrylic acid), study of polymer/polymer and polymer/drug interaction within the network structure. Biomaterials 2003;24:1499-506.
12. Sahin S, Selek H, Ponchel G, Ercan MT. Preparation, characterization and *in vivo* distribution of terbutaline sulfate loaded albumin microspheres. J Control Rel 2002;82:345-58.
13. Raymond G, Degennaro M, Mikeal R. Preparation of gelatin phenytoin sodium microspheres: An *in-vitro* and *in-vivo* evaluation. Drug Dev Ind Pharm 1990;16:1025-51
14. Gupta N, Srivastava AK. Interpenetrating polymer networks: A review in synthesis and properties. Poly Int 2003;35:109.
15. Hoffman AS. Hydrogels for biomedical applications. Adv Drug Del Rev 2002;54:3-12.
16. Lin CC, Metters AT. Hydrogels in controlled release formulations: Network design and mathematical modeling. Ad Drug Del Rev 2006;58:1379-408.
17. Yadav KS, Satish CS, Shivakumar HG. Preparation and evaluation of chitosan-poly(acrylic acid) hydrogels for stomach specific delivery for amoxicillin and metronidazole. Indian J Pharm Sci 2007;69:91-5.
18. Bumsang K, Youngchan S. pH-sensitive swelling and release behaviours of anionic hydrogels for intelligent drug delivery system. J App poly Sci 2007;105:3656-61.

Source of Support: Nil, **Conflict of Interest:** None declared.