CROSSLINKED BIODEGRADABLE ALGINATE HYDROGEL FLOATING BEADS FOR STOMACH SITE SPECIFIC CONTROLLED DELIVERY OF METRONIDAZOLE

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Abstract

The retention of drug delivery systems in the stomach for longer time is required to improve the bioavailability and therapeutic efficacy of the drugs used for the diseases associated with the stomach. In the present work, gastro-retentive floating beads of sodium alginate (SA) were prepared through ionotropic gelation with divalent Ca^{++} ions and covalent cross-linking with glutaraldehyde (GA). Metronidazole (MZ) was successfully encapsulated into beads by varying the amount of SA, xanthan gum, magnesium stearate, and GA. Encapsulation of MZ was up to 79.17%. However, with an increasing amount of GA in the matrix, the encapsulation efficiency was found to decrease significantly. Beads prepared without GA released 50% of the drug in 2.76h. GA treatment suppressed the drug release significantly. Compatibility of the drug with the polymers was examined using Fourier transform infrared (FTIR) spectroscopy. Differential scanning colorimetry (DSC) and X–ray diffraction studies (XRD) were carried out to examine the crystalline nature of the encapsulated drug. The drug was relatively stable and amorphous in the beads.

Rezumat

Retenţia sistemelor de transport a medicamentelor la nivelul stomacului este foarte importantă pentru biodisponibilitatea şi eficacitatea terapeutică.

În această lucrare au fost preparate pelete gastrorezistente flotante cu alginat de sodiu, prin metoda solidificării la rece cu ioni de Ca^{2+} si cross-linking covalent cu glutaraldehidă.

Substanta activa încorporată a fost metronidazolul şi s-au variat cantităţile de alginat de sodiu, gumă xantan, stearat de magneziu şi glutaraldehidă. Compatibilitatea substanţei active cu polimerii a fost evaluată prin spectroscopie în infraroşu cu transformata Fourier, iar natura cristalină a substanţei active încorporate a fost evaluată prin colorimetrie diferenţială şi difracţie cu raze X.

Rezultatele au evidenţiat faptul că substanţa activă medicamentoasă este relativ stabilă şi amorfă în formulările realizate.

Keywords: gastroretentive; beads; biodegradation, release kinetics, metronidazole

Introduction

Helicobacter pylori (*H.pylori*) is a gram negative bacterium that colonizes in human stomach. It is one of the most common pathogens, infecting greater than 50% of the population and also recognized as a risk factor in the development of gastritis, gastric ulcer and gastric carcinoma [26]. *H.pylori* resides mainly in the gastric mucosa or at the interface between the mucous layer and the epithelial cells of the antral region of the stomach [25]. The major reasons for the failure of *H. pylori* eradication with conventional dosage forms of antibiotics include: low concentration of the antibiotics reaching the bacteria under the mucosa, instability of the drug in gastric fluid (low pH), and short residence time of the drug in the stomach [27]. One way to improve the efficacy in eradicating the infection is to deliver the antibiotic locally in the stomach for a longer period of time that will allow more of the antibiotic to penetrate through the gastric mucus layer to act on *H. pylori.* Gastroretentive drug delivery system is an approach to prolong gastric residence time, thereby improving the oral bioavailability of the basic drugs. Various approaches used to increase the gastric residence time include bioadhesive systems [20, 22], modified shape systems [2, 3]**,** high-density systems [8], swelling and expanding systems [21, 37], magnetic [14] and floating systems [23]. Floating delivery systems seem to offer a greater safety for clinical uses than other approaches. Various dosage forms like tablets, capsules, beads, microparticles, pellets, granules have been evaluated for floating systems. Single unit system such as tablet or capsule shows a higher inter and intrasubject variability and is generally unreliable and non-reproducible in prolonging the gastric retention time. However, multiparticulate systems like beads and microparticles may be more suitable because they show reduced intersubject variability in absorption and also often a better dispersion in the gastrointestinal fluid with reduced localized mucosal damage [9, 10]. The use of natural polymers is the most common method for controlling the release of drugs from oral formulations. Among the various natural polymers, SA, a hydrophilic biopolymer obtained from marine brown algae, appeared to be highly promising due to its non-toxic, biocompatible, biodegradable characteristics [16]**.** Its unique property of forming water insoluble calcium alginate gel through ionotropic gelation with Ca^{++} ions in a simple, mild and ecofriendly condition has been utilized to encapsulate various therapeutic agents like aceclofenac [12], theophylline [13]**,** meloxicam [33], furosemide [7], losartan [4], clarithromycin [24]**,** rosiglitazone maleate [29] etc.

However, various floating SA beads suffered from rapid drug release [5]. Therefore improved SA-based floating beads system is still desired and no reports are available on the development of glutaraldehyde cross-linked SA floating beads for the stomach specific sustained release of MZ. The objective of the work was to develop novel cross-linked SA hydrogel floating beads using glutaraldehyde as a covalent cross linking agent in order to suppress the erosion of beads and to control the drug release rate from the hydrogel beads. Magnesium stearate (Mg. St.) is commonly used, up to 5%, as tablet and capsule excipient. It is of low bulk density and hydrophobic in nature. Magnesium stearate was used in the study to impart buoyancy of the beads. MZ has been used as a model drug in this study. It has been reported that MZ is an active therapeutic agent in the treatment of *H. pylori* with common side effects including anorexia, nausea, vomiting, and epigastric pain [36].

Materials and methods

Materials

Metronidazole (MZ) was a gift sample from Arti Pharmaceutical Company, Orissa, India. SA, xanthan gums (XG), magnesium stearate (Mg St.), calcium chloride dihydrate ($CaCl_2,2H_2O$) were purchased from Loba Chem., Mumbai, India. GA (25% v/v) were purchased from SD Fine Chem., Mumbai, India. All other chemicals were of pharmaceutical grade.

Methods

Preparation of Metronidazole (MZ) loaded floating beads

MZ floating beads were prepared by ionotropic gelation method. In this method an accurately weighed quantity of MZ was dispersed in an aqueous solution of SA, XG and Mg St. of different concentration and mixed homogeneously, using a magnetic stirrer. The homogeneous mixture was extruded dropwise using a 22G flat tipped needle into slightly agitated 100mL of $CaCl_2$ solution of different concentrations. The $CaCl_2$ solution was presaturated with MZ in order to avoid drug leakage from the beads thereby increasing drug entrapment efficiency [15]. The formed beads were then separated after a gelation period of 15 min and washed with distilled water repeatedly to make them free from unreacted ions and dried at room temperature for 24 h. Then the beads are dried at 40^{o}C till constant weight. Further, the beads incubated in $CaCl_2$ solution for 15 min, were transferred to solution of 3:1 proportion of acetone and water containing different concentrations of GA to introduce the covalent crosslink. The cross linked beads were removed and washed with distilled water repeatedly to remove non reacted GA. The complete removal of the unreacted GA was confirmed

by the negative test of the washings with Brady's qualitative reagent (2, 4-dinitrophenyl hydrazine). The same procedure was adopted for the preparation of blank beads (without drug). Figure1 shows some dry and wet beads.

Figure 1
Photomicrograph of MZ loaded floating beads: (a) before drying (b) after drying

Bead size analysis

Bead size analysis was performed by sieving method. Several British Standard Sieves ranging 10-80 meshes were arranged in a nest with the coarse sieve (sieve No. 10) at the top. A weight amount of samples were placed on the top and the sieve set was shaken with a mechanical sieve shaker (Labtech Instrument, India) for 10 min. The samples retained on each sieve were collected and weighed. The arithmetic mean diameter was calculated.

Drug Entrapment Efficiency (DEE)

Accurately weighed, 20 mg of dried beads were crushed with a mortar-pestle and were transferred into 250 mL of phosphate buffer (pH 7.4) solution. After 2h, the MZ was assayed using a spectrophotometer (UV1800, Shimadzu, Japan) at 319 nm, after filtration. The DEE was calculated according to the following equation:

$$\text{Entrapment efficiency (\%)} = \text{(actual drug content / theoretical drug content)} \times 100$$

Fourier-transform infrared spectroscopy study (FTIR)

FTIR spectra of pure drug, SA, drug free beads and drug loaded beads were recorded in a FTIR spectrophotometer (Varian 640-IR, USA) using KBr pellets. The spectra were recorded within 4000 – 400 cm^{-1} wave numbers.

Differential scanning colorimetry (DSC) study

DSC thermograms of pure drug, drug free and drug loaded beads were obtained in an atmosphere of nitrogen. 2–5mg of samples were kept in hermetically sealed aluminum pans and heated at a scan speed of 20^{o}C/min over a temperature range of 30-300^{o}C in a differential scanning calorimeter (DSC-8500 with Hyper DSC, Perkin Elmer, USA).

X-ray diffraction (XRD) study

XRD analyses were performed using an X-ray diffractometer (Regaku ultima IV, Japan). Pure drug, drug free bead and drug loaded bead were scanned from 0^{o} to 60^{o} diffraction angle (2θ) range under the following measurement conditions: voltage, 40kv; current, 20mA; scan speed 20^{o}C/min.

Scanning electron microscopy (SEM)

The floating beads were mounted onto stubs using double sided adhesive tape and sputter coated with a palladium layer in a JFC-1600 autofine coater, in order to make them conductive. The coated beads were observed under SEM (JSM-6701F, JEOL, Japan) at room temperature. Pictures were taken at an excitation voltage of 20kV and with magnification of 5000x.

Floating properties

Floating properties of the beads were evaluated using the USP II dissolution apparatus (TDT-08L, Electro lab, Mumbai, India) at 50 rpm, in 500 mL of simulated gastric fluid (pH 1.2). Temperature was maintained at 37±0.5°C. The percentage of floating beads was calculated by the following equation.

Buoyancy (%) = weights of floating beads/(weight of floating beads + weight of settled beads) × 100

Swelling study

The swelling of the beads was studied in 20 mL of acidic medium (pH 1.2). The beads were removed at different time intervals by filtration and blotted carefully to remove excess surface water. The swollen beads were weighed. The swelling ratios were calculated as follows:

Swelling ratio= (final weight-initial weight) / initial weight

In vitro biodegradation study

The *in vitro* biodegradation of the beads was carried out in 0.2% lysozyme dissolved in phosphate buffer solution, pH 7.4 [10]. Predetermined weights of beads were transferred to test tubes containing 5mL of lysozyme solution and incubated with shaking (120 rpm) in an incubator (CIS-24BL, Remi, Mumbai, India) at 37^{o}C for 3h until

equilibrium swelling was attained. The weights of the swollen beads were determined after removing the solvent. Then 5 mL fresh lysozyme solution was added to the swollen beads. The percent weight remaining of the samples after enzymatic degradation were calculated as follows:

Percent weight remaining of the beads = 100-[(weight of sample after 3h swelling in lysozyme solution- weight of the sample after incubating with fresh lysozyme for a given time)/ weight of sample after 3h swelling in lysozyme solution]

In vitro drug release study

In vitro drug release study was carried out in acidic solution (KCl/HCl buffer solution, pH1.2) using USP-II dissolution rate test apparatus (TDT-08L, Electro lab, Mumbai, India). Accurately weighed, 20mg of dried beads were placed in 500 mL of dissolution medium and maintained at 37±0.5^{o}C. The paddle was rotated at 50 rpm. Aliquots were withdrawn at different time intervals and replenished the medium immediately with the same volume of fresh solution. Suitably diluted samples were analyzed spectrophotometrically at 277 nm. Each sample was tested and analyzed in triplicate.

Statistical analysis

The statistical analyses were accomplished using Graphed instant package. The one way analysis of variance (ANOVA) was used to determine the statistically significant differences between the results. Differences were considered significant when $p < 0.05$.

Results and Discussion

Development and characterization of beads

Beads composed of sodium alginate (SA) were prepared by ionotropic gelation process using $CaCl_2$ as a cross-linking agent. SA contains anionic groups i.e., carboxyl and hydroxyl groups in its structure, both of them exhibit the property of electrostatic interaction.

When this polymer comes in contact with some divalent metal ions (Ca^{++}), an ionic interaction occurs between the Ca^{++} ions and COO^{-} groups present in SA chain. This leads to the formation of solid gel. Mg St. was incorporated in the preparation of beads to impart buoyancy. The prepared beads were spherical in shape as evidenced by photomicrograph (Figure 1) and in the size range of 346.25±1.34 to 1375.08±2.56µm (Table I).

Table I

Composition, drug entrapment efficiency (DEE) and floating percentage of different formulations

Formulation Codes	SA (%w/v)	XG(%w/v)	Mg St. (%w/v)	$CaCl_2$ (%w/v)	DEE (%) Mean±SD, n=3	Floating (%)	GA (%v/v)	Mean bead size (µm) ± SD
F1	2	-	1	1	60.43±0.31	-	-	439.58±3.08
F2	2	-	1	2	52.56 ±0.14	-	-	399.58±5.09
F3	2	-	1	3	46.85±0.05	-	-	359.58±3.09
F4	2	-	1	4	34.14 ±0.03	-	-	346.25±1.34
F5	2	-	2	1	58.12±0.10	48.03	-	495.61±5.89
F6	2	-	3	1	56.40±0.37	58.91	-	570.41±7.79
F7	2	-	4	1	54.42±0.37	71.93	-	602.50±2.12
F8	2	-	5	1	50.12±0.14	100	-	627.92±4.02
F9	2	0.3	5	1	52.27±0.31	100	-	653.33±4.02
F10	2	0.4	5	1	56.47±0.40	100	-	678.75±2.52
F11	2	0.5	5	1	60.53±0.13	100	-	749.50±2.76
F12	2	0.6	5	1	64.15±0.18	100	-	936.67±7.66
F13	2.5	0.6	5	1	68.23±1.39	100	-	1201.25±3.34
F14	3	0.6	5	1	71.80±1.83	100	-	1259.17±1.31
F15	3.5	0.6	5	1	73.24±3.32	100	-	1317.08±2.35
F16	4	0.6	5	1	79.17±6.40	100	-	1375.08±2.56
F17	4	0.6	5	1	54.98±0.79	100	0.5%	1143.33±1.37
F18	4	0.6	5	1	54.41±0.5	100	1 %	1085.42±1.39
F19	4	0.6	5	1	53.41±0.36	100	1.5 %	1060.52±4.78
F20	4	0.6	5	1	41.31±0.16	100	2 %	1027.50±5.51

Increasing the Ca^{++} concentration, there was a reduction in the mean size of the beads when compared to the beads obtained with low Ca^{++} concentration. This would be due to shrinkage of the gel bead. Similar results were also observed by other authors [35]. Increasing the concentration of Mg St. led to increase in the mean size of the beads (Table I). While other factors were kept constant, the beads size was found to increase with increasing SA and XG concentration. The proportional rise in the mean size of the beads with increasing the amount of SA and XG could be attributed to an increase in the relative viscosity at higher concentration of polymer and due to formation of larger droplets during addition of the polymer solution to the cross-linking agent. Similar observations were made by other authors [19, 30]. Further, with an increase in concentration of GA, the decrease in size of the beads was observed. This may be due to rapid shrinking leading to the formation of smaller and rigid matrix at higher crosslink densities. A similar finding was reported previously [18].

Drug Entrapment Efficiency (DEE)

It was found that increasing $CaCl_2$ concentration (1-4% w/v) decreased the DEE in the beads (Table I). Among four concentrations of $CaCl_2$, 1 % w/v solution produced highest DEE. Thus concentration of $CaCl_2$ was maintained at 1% w/v in next formulations. Low DEE value of the SA beads cross-linked with higher concentrations of $CaCl_2$ resulted the formation of porous beads with

Ca^{2+}, for which the drug diffused out of the beads at the time of preparation**.** Das *et al*. had reported similar observations [6]. Increasing the concentration of Mg St. from 1 to 5% (w/v) decreased the DEE from 60.43±0.31 to 50.12±0.14% respectively due to competition for encapsulation between the amount of drug powder and Mg St. However, 100% floating was not achieved up to 4% (w/v) concentration of Mg St.(Table I). By increasing the concentration of Mg St. to 5% (w/v) complete floating of the beads were found. Thus in the subsequent formulations concentration of Mg St. was maintained at 5% (w/v). To improve the DEE of SA gel beads, an attempt was made to prevent the diffusion of the drug by dissolving XG in the gelation medium. It was observed that the addition of XG (0.3 - 0.6% w/v) to the polymer solution significantly ($p<0.05$) increased the DEE (Table I). When the beads were formed and allowed to cure in an aqueous medium, the coagulation fluid diffused to the external phase containing a part of the dissolved drug resulting drug loss. Such loss of drug was reduced by addition of XG, which increased the viscosity of the polymeric solution and thus, impeded the diffusion of the dissolved drug. Therefore, the highest concentration (0.6% w/v) of XG was chosen in all the subsequent formulations. The DEE of the beads increased from 60.43 ± 0.31 % to 79.17 ± 6.40 % by increasing SA concentration from 2 to 4% w/v at a given gelation time (0.25h) and at a fixed concentration (1% w/v) of $CaCl_2$ (Table I). Such difference in drug entrapment efficiency was statistically significant ($p<0.05$). Concentration of SA beyond 4% w/v increased the viscosity of the solution to an extent that the formation of drops was strongly hindered. Thus the 4% w/v SA was maintained in all subsequent formulations. The effect of GA on the DEE of beads was also studied. It was observed that with an increasing amount of GA in the matrix, DEE decreased significantly ($p<0.05$) due to lesser free volume space available in the matrix. It was found that an increase in GA concentration decreased the DEE in the beads. This could be due to higher cross-link density and increased rigidity of the beads. A similar finding was reported previously [1]**.**

Fourier- transforms infrared spectroscopic studies (FTIR)

Figure 2 depicts the FTIR spectra of (a) SA, (b) drug free bead, (c) MZ, and (d) drug loaded bead. In case of SA, a broad band at 3297.70 cm^{-1} is attributed to O-H stretching vibrations. Band at 2927.43 cm^{-1} represents the C-H stretching of cyclic aldehyde. Two bands at 1596.78 cm^{-1} and 1415.50 cm^{-1} indicate the presence of asymmetric and symmetric carboxylate anions. The formation of beads could have resulted due to ionic linking between Ca^{++} ion and carboxylate groups of SA. In case of drug free bead, two bands due to O-H stretching and C-H stretching of SA were observed. However, asymmetric and symmetric carboxylate peaks, at 1596.78 cm^{-1} and 1415.50 cm^{-1}, of SA shifted

to a higher absorption frequency 1669.78 cm^{-1} and 1457.93 cm^{-1}, indicating ionic interaction between $–COO^–$ and Ca^{++} ions.

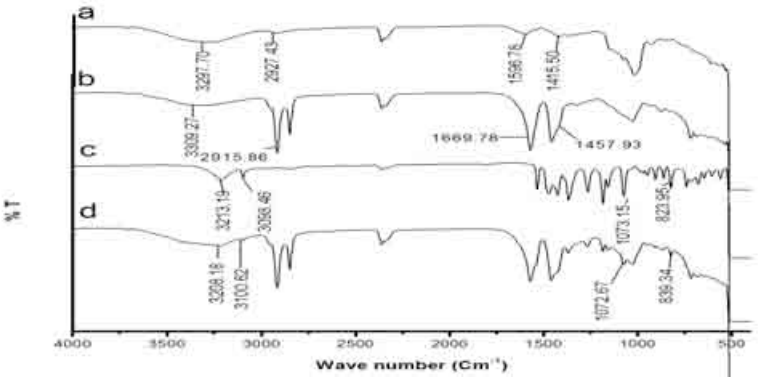

Figure 2

FT–IR spectra of (a) SA, (b) drug free bead, (c) MZ, and (d) drug loaded bead

The frequencies of pure MZ at 3213.19 cm^{-1}, 3098.46 cm^{-1}, 1073.15 cm^{-1} and 823.95 cm^{-1} could be assigned to the O-H stretching, C-H stretching, C-O stretching, C-NO_2 and C-N stretching respectively (Figure 2c). The similar bands were identified at 3208.18 cm^{-1}, 3100.62 cm^{-1}, 1072.67 cm^{-1} and 839.34 cm^{-1} in drug loaded SA beads with minor differences in frequencies (Figure 2d). Hence, it could be suggested that the drug was relatively stable in drug loaded SA beads.

Differential scanning colorimetry study (DSC)

DSC thermograms of MZ, drug free beads, and drug loaded beads are depicted in Figure 3. DSC of pure MZ exhibited a sharp endothermic peak at 162.62°C corresponding to its melting point (Figure 3a). Drug free bead showed peaks at 120.10°C and 160.18°C due to endothermic transition of the polymer matrix. However, there was no peak corresponding to MZ in drug loaded bead, indicating the amorphous dispersion of MZ into polymer matrix.

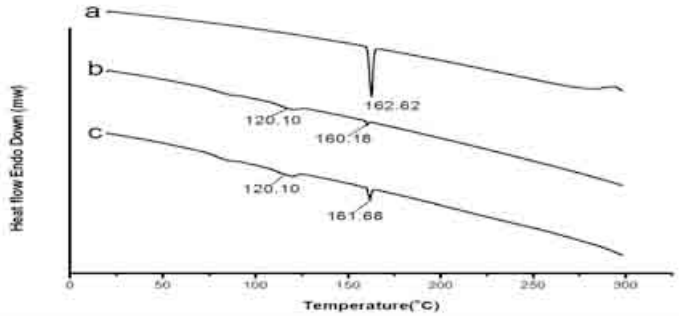

Figure 3

DSC curves of (a) MZ, (b) drug free beads, and (c) drug loaded beads

X-ray diffraction studies (XRD)

The X-ray diffractograms of MZ, drug free beads, and drug loaded beads are presented in Figure 4. MZ has shown characteristic intense peaks between 2θ of 0^o and 60^o due to its crystalline nature. X-ray diffractograms recorded for drug free bead and drug-loaded bead did not show any characteristic peak of the drug, indicating that the encapsulated drug is in the amorphous state.

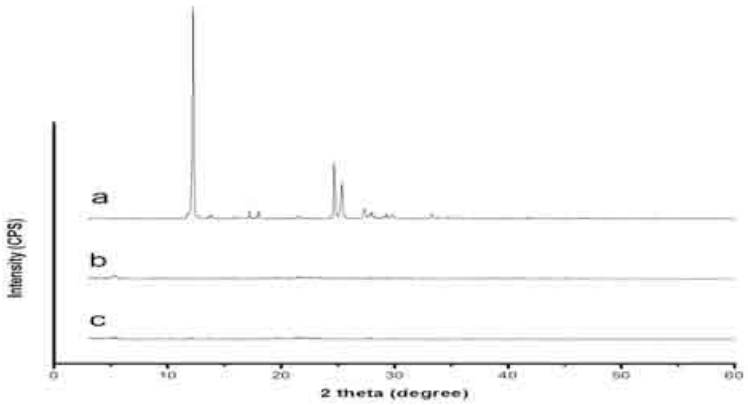

Figure 4

XRD of (a) MZ, (b) drug free beads, and (c) drug loaded beads

Scanning electron microscopic study (SEM)

Scanning electron microscopic study revealed that the beads were more or less spherical having rough surface (Figure 5). GA treated beads appeared to be wrinkled and possessing more rough surface. This might be due to a higher degree of crosslinking of matrix.

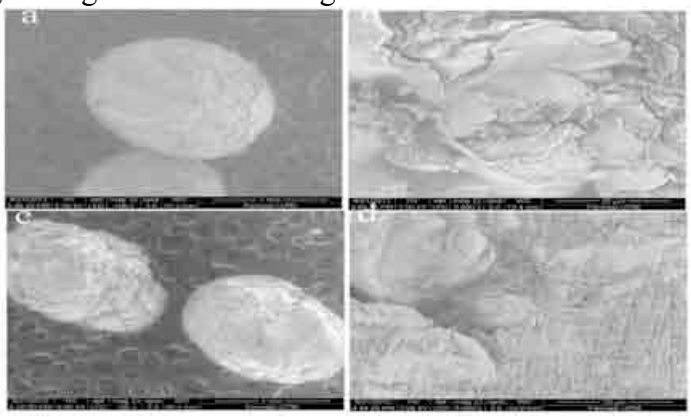

Figure 5

SEM micrograph of (a) without GA treated beads, (b) surface morphology of without GA treated beads, (c) GA treated beads and (d) surface morphology of GA treated beads

Percent of Floating Beads

The floating property of the beads in pH 1.2 buffer was studied by visual observation. Time required for sinking of the beads was observed. All the beads showed complete floating except those of formulations F1to F4 which contained lower concentration of Mg.St. The use of 5% Mg St. had significantly increased the extent (%) of floating of beads (Table II).

Table II

Swelling ratio and drug release behaviors of MT loaded beads in pH 1.2 KCl/HCl buffer solutions

Formulation Codes	Swelling ratio in 1.5 h Mean ± SD	Drug release in acidic medium Mean± SD		Korsemeyer-Peppas model	
	Acidic medium	% drug release in 2h	$t_{50\%}$ (h)	Acidic solution	
				n	r^2
F1	0.92 ± 0.22	78.71 ± 0.12	0.86±0.41	1.07	0.93
F2	0.84 ± 0.43	80.19 ± 0.20	0.80±0.11	0.85	0.96
F3	0.78 ± 0.21	82.44 ± 0.17	0.54±0.19	0.76	0.92
F4	0.65 ± 0.12	86.99 ± 2.25	0.44±0.21	0.75	0.78
F5	0.87 ± 0.23	77.41 ± 7.12	0.89±0.13	0.97	0.94
F6	0.85 ± 0.08	73.80 ± 2.82	0.95±0.15	1.02	0.95
F7	0.80 ± 0.13	68.20 ± 0.20	1.08±0.23	1.12	0.95
F8	0.77 ± 0.27	60.88 ± 0.46	1.27±0.13	1.21	0.95
F9	0.71 ± 0.35	58.29 ± 0.97	1.58±0.21	0.98	0.97
F10	0.67 ± 0.41	52.85 ± 3.44	1.81±0.19	1.26	0.97
F11	0.61 ± 0.19	49.10 ± 0.04	2.05±0.09	1.31	0.98
F12	0.57 ± 0.26	47.55 ± 1.61	2.15±0.15	1.64	0.96
F13	0.56 ± 0.17	46.54 ± 2.08	2.17±0.08	1.47	0.98
F14	0.55 ± 0.29	45.66 ± 0.13	2.26±0.25	1.70	0.98
F15	0.54± 0.37	42.25 ± 0.12	2.51±0.06	1.84	0.98
F16	0.52 ± 0.33	38.41 ± 0.07	2.76±0.08	2.04	0.98
F17	0.51 ± 0.36	35.47 ± 0.11	2.89±0.14	1.93	0.98
F18	0.46 ± 0.07	33.24 ± 7.35	3.31±0.35	2.02	0.98
F19	0.38 ± 0.28	30.14 ± 5.28	4.06±0.18	2.02	0.98
F20	0.30 ± 0.23	26.51 ± 6.45	5.02±0.11	2.02	0.99

Swelling Behaviors

The release of a drug from a polymeric matrix is controlled by the swelling behavior of the polymer. To explain the drug release behavior of the beads, the swelling study was conducted in buffer solution (pH 1.2 KCl/HCl) for 1.5h (Table II).

It was estimated that increase in $CaCl_2$ concentration from 1 to 4%, reduced the swelling ratio by 29.35% (Table II). Higher concentration of Ca^{++} ions in the gelation medium increased the availability of Ca^{++} ions which increased the number of interactions with COO^- groups present in SA. This enhanced crosslinking density appeared as a barrier for inward diffusion of swelling medium. Further, an increase in Mg St. concentration

from 1 to 5% reduced the swelling ratio by 16.30 % (Table II). This is due to the hydrophobicity of Mg St. which hindered the penetration in swelling medium. It was further observed that increase in XG concentration from 0.3 to 0.6% w/v decreased the swelling of beads (Table II). At low XG concentration, the polymeric network is loose with a greater hydrodynamic free volume which allows more of the liquid to be absorbed leading to greater swelling.

It was observed that decrease in swelling occurred with increase of SA concentration from 2 to 4 % w/v in the beads. This may be due to increased number of interactions of COO^- groups present in the SA chains with the calcium ions during the synthesis of beads. This increases the number of cross-linking sites within the beads which form a more rigid gel with higher crosslinking density and, thus the swelling decreases. A similar finding was reported earlier [34].

It was further observed that swelling of the beads decreased with increasing amount of GA. Increase in GA concentration from 0.5 to 2% v/v reduced the swelling ratio by 41.18% in acidic medium. At low cross-link density, the hydrogel network is loose with a greater hydrodynamic free volume and can absorb more of the solvent resulting in higher swelling. Ranjan *et al*, had similar observations [28].

In Vitro Biodegradation Study

An *in vitro* biodegradation study of the prepared SA beads was carried out in presence of lysozyme. The percent of remaining weight of bead was determined as a function of time and considered as a measure of degradation. Figure 6 shows the degradation patterns of the beads prepared using GA as a crosslinking agent in addition to the control formulation prepared without using GA. The beads prepared without GA showed higher degradation extent (lower % remaining weights) than the beads prepared with GA (Figure 6).

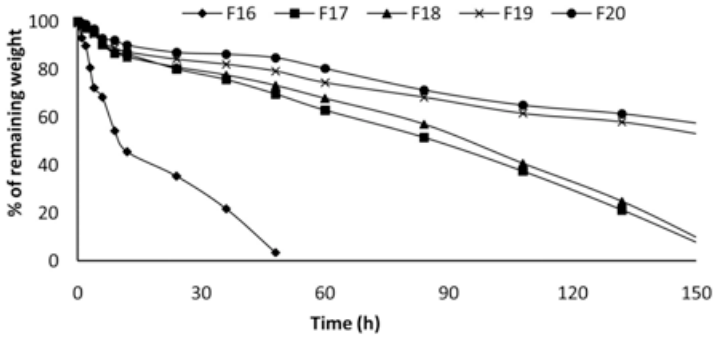

Figure 6
Biodegradation study of without GA treated beads and with GA treated beads

The beads prepared using GA showed less swelling than the beads prepared without using GA (Table II). This decrease in swelling hinders the diffusion of enzyme through the hydrogel matrix and consequently decreases the rate of degradation. Further, it was observed that increasing the GA content in the beads decreased their degradation rate. This is due to the increase of crosslinking density upon increasing the GA content which tends to impede the diffusion of enzyme through the beads and consequently retards their degradation.

Release of Drug

The drug release was studied in pH 1.2 KCl/HCl buffer solutions. Release of MZ from beads, prepared using increased $CaCl_2$ concentration have been presented in figure 7.

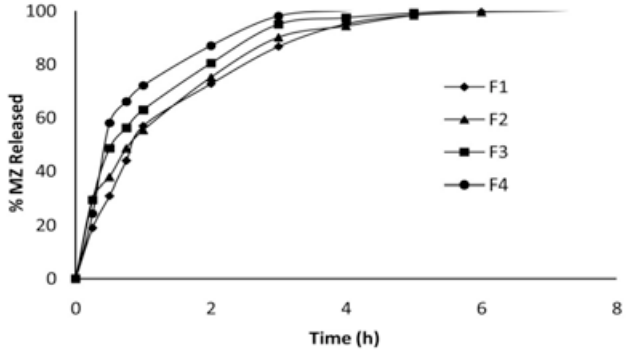

Figure 7
Release profiles of MZ Beads in acidic solution prepared by different concentration of $CaCl_2$

In acidic medium, the drug release was 78.71±0.12%, 80.19±0.20%, 82.44±0.17% and 86.99±2.25% in 2h respectively with increasing $CaCl_2$ concentration (Table II) and resulted in a statistically significant difference in their drug release rate ($p<0.05$). In order to compare the drug release rate, a time point approach was adopted. The values of $t_{50\%}$ (i.e. time required for the release of 50% drug) were calculated, and were 0.86±0.41, 0.80±0.11, 0.54±0.19 and 0.44±0.21 h, respectively in the order of the increasing $CaCl_2$ concentration (Table II). Since an increase in the concentration of $CaCl_2$ increases the thickness of the gelled membrane, the imbibitions as well as comparative swelling of the beads decrease and consequently the release has been found to decrease. Mg St. produced the beads with higher $t_{50\%}$ with the higher concentration (5% w/v) (Table II). The significant ($p<0.05$) retarding effect in drug release was observed when 5% Mg St. was used (Figure 8). Due to its hydrophobic nature hindered the penetration of dissolution medium and consequently decreased the release.

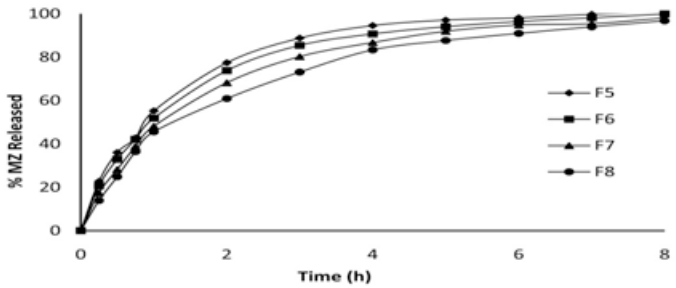

Figure 8
Release profiles of MZ beads in acidic solution prepared by different concentration of Mg St.

Release of MZ from beads, prepared using increased XG concentrations have been shown in Figure 9. The drug release was 58.29±0.97%, 52.85±3.44%, 49.10±0.04 and 47.55±1.61 in 2h, respectively with increasing XG concentration (Table II) and resulted in a statistically significant difference in their drug release rate ($p<0.05$). Values of $t_{50\%}$ were 1.58±0.21, 1.81±0.19, 2.05±0.09, 2.15±0.15 h, respectively in the order of the increasing XG concentration (Table II). This retardation of drug release could be attributed to the formation of a thick gel structure that delayed drug release from the matrix. Release of MZ from beads, prepared using increased SA concentrations (2 to 4% w/v) have been presented in Figure 10. Increase of SA concentration from 2 to 4% (w/v) decreased the drug release in the dissolution medium. Similarly, the time required for 50% drug release increased with increase of SA concentration in the beads (Table II). Increase of SA concentration resulted in a more entangled or more compact gel system with a greater cross linking density in the matrix. As a result the rigidity of gel matrix increased and free volume of the matrix decreased. This hinders easy transport of drug molecules through the matrix and reduces drug release from the matrix. The results are in agreement with the reports of the other workers [31].

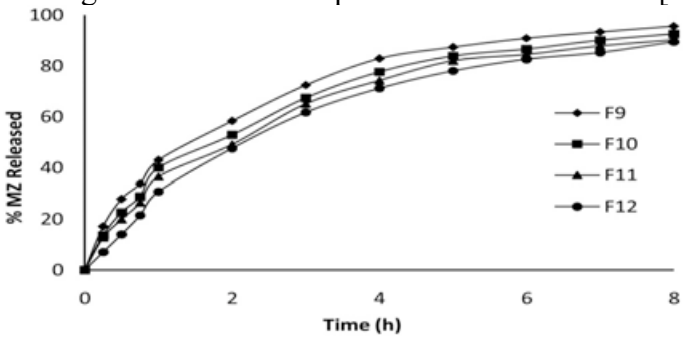

Figure 9
Release profiles of MZ beads in acidic solution prepared by different concentration of XG

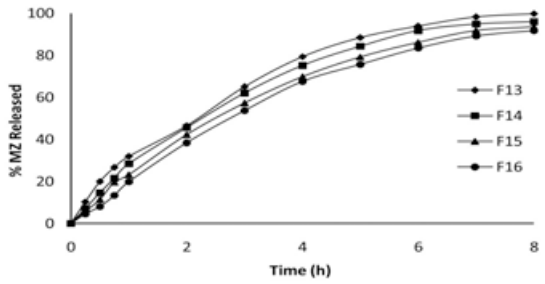

Figure 10
Release profiles of MZ beads in acidic solution prepared by different concentration of SA

With a same time frame of 2h, the GA treatment of the beads suppressed the drug release in dissolution medium (Figure 11).
In acidic medium, the drug release were 35.47±0.11, 33.24±7.35, 30.14±5.28 and 26.51±6.45 in 2h, respectively with increasing GA concentration (Table II) and resulted in a statistically significant difference in their drug release rate ($p<0.05$). In addition to ionic cross-links, GA brought about covalent acetal linkages and showed the retarded drug release in dissolution media than those having ionic linkages only. This could be due to the fact that at higher cross-linking, free volume of the matrix will decrease, thereby hinders the transport of drug molecules through the matrix. This could also reduce the swelling as well as release rate from the matrix. A similar result has been explained by Ranjan *et al*., 2011 [28].

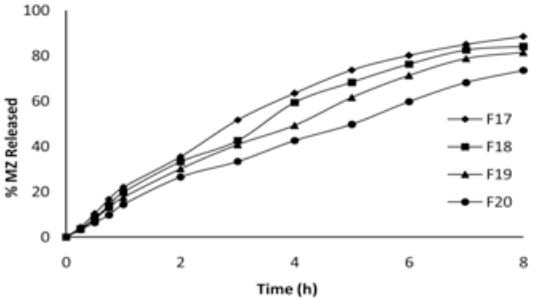

Figure 11
Release profiles of MZ beads in acidic solution prepared by different concentration of GA

To understand the mode of drug transport through the beads, the release data was fitted to the following empirical equation [17].

$$\mathbf{Mt/M\alpha = kt^n,}$$

Where, Mt/Mα is the functional drug release at time t, k is a constant which incorporates the structural and geometric characteristics of the device and n

is diffusion exponent. The mechanism of drug release from spherical polymeric devices shall be Fickian diffusion when the value of n= 0.43 or less, anomalous (non-Fickian) transport when the value of n lies between 0.43 and 0.85, and case II transport when n= 0.85. The values above 0.85 indicate case II transport that relates to polymer relaxation during swelling [32]. The n values have been calculated and given in table II along with correlation coefficients. The n values of beads confined within the range from 0.75 to 2.04, indicating that the drug release in the beads follows anomalous and super case II transport.

Conclusions

Metronidazole was successfully encapsulated into the beads and percentual encapsulation efficiency was up to 79.17%. The *in vitro* biodegradation study of the beads was carried out in pH 7.4 phosphate buffer solution at 37^{o}C in presence of lysozyme and showed promising rate of degradation. The preliminary investigation of the beads prepared in this study showed a consistent swelling pattern, high entrapment efficiency and promising controlled release profiles of the drug. At the same time floating nature of the beads was found to extend the retention of beads in the stomach for longer time. This may improve the bioavailability and therapeutic efficacy of the drugs when used for the diseases associated with the stomach.

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