Design, Development and Characterization of Hydrogel Beads for Colon-Specific Delivery of an Anti-Inflammatory Agent

Mithlesh Kumar[1], D.C. Bhatt[2], Bhawna Sharma[3]

1. *Department of Pharmaceutical Sciences, Guru Jambheshwar University, India, Hisar-125001*
mkgaur143@gmail.com
2. *Department of Pharmaceutical Sciences, Guru Jambheshwar University, India, Hisar-125001*
bhatt_2000@yahoo.com
3. *P.D.M. College of Pharmacy, India, Bahadurgarh- 124507*
bhawnasharma033@gmail.com

Abstract: **Site specific delivery systems offer several advantages over traditional therapyincluding enhanced pharmacological response, omission of first pass metabolism and lowered side effects etc. Colon-specific drug-delivery systems also offer several potential therapeutic advantages not only in a number of colonic diseases such as Inflammatory Bowel Disease, Colorectal Cancer and Spastic colon, but also in the systemic delivery of protein and peptide drugs. Mesalamine (5-ASA) has well established anti-inflammatory activity and it is the first line drug for the management of ulcerative colitis. The drug is to be delivered to the colon for its local action against inflammation. But, 5-ASA is rapidly absorbed from the small intestine and there is little localization of Mesalamine in the colon relative to the small intestine. The main objective of the present study was to design and evaluate oral colon targeted delivery system based on utilizing specific biodegradability of natural polymers like sodium alginate. Sodium alginate when used alone, results in drug leaching during hydrogel preparation and rapid dissolution at higher pH, resulting in very low entrapment efficiency and burst release of entrapped drug, once it enters the intestine. To overcome these limitations, another natural polysaccharide, guargum was included in the alginate matrix along with a cross linking agent, gluteraldehyde to ensure maximum encapsulation efficiency and controlled drug release. The beads having an alginate to guar gum (7:1) showed desirable characters like better encapsulation efficiency and bead forming properties in the preliminary studies. The glutaraldehyde concentration giving maximum (83.6±2.04%) encapsulation efficiency and the most appropriate swelling characteristics was found to be 0.5% (w/v). Presence of guar gum and glutaraldehyde crosslinking increases entrapment efficiency and prevents the rapid dissolution of alginate in higher pH of the intestine, ensuring a controlled release of the entrapped drug.**

Keywords: **Polysaccharide, Alginate; Guargum; Hydrogel; Mesalamine; Colon-specific drug delivery.**

I. INTRODUCTION

Drug delivery by oral route is preferable due to various reasons like ease of administration, patient compliance and flexibility in dosage form design etc. However, the limitations associated with oral delivery of drug molecules e.g. first pass metabolism, gastric irritation and fluctuations in drug concentration can be overcome bydeveloping site-specific drug delivery. Colon-specific drug delivery systems offer several potential therapeutic advantages not only in the treatment of a number of colonic diseases such as colorectal cancer, Crohn's disease, ulcerative colitis, spastic colon, but also in the delivery of therapeutic proteins and peptides. Colon-specific drug delivery approaches include the use of prodrugs, pH-sensitive polymers, time-release dosage forms, bacterial degradable polymers, multi-particulate systems (including pellets, microspheres, hydrogel beads, nanoparticles etc.) and multi-coatingtime-dependent delivery systems.

Many natural polysaccharides such as chondroitin sulphate, pectin, dextran, guar gum etc. have been investigated for their potential in designing colon specific drug delivery. These are broken down by the colonic microflora to simple saccharides. Most of the polysaccharide based delivery systems protect the bioactive from the hostile conditions of the upper GIT. Hydrolysis of the glycosidic linkages on arrival in the colon triggers the release of the entrapped bioactive. The main saccharolytic species responsible for this biodegradation are Bacteroides, Bifidobacterium, Eubacterium, Peptococcus, Peptostreptococcus, Ruminococcus, Propionibacterium, and Clostridium[1].

Hydrogels are polymeric networks with three-dimensional configuration capable of imbibing high amounts of water or biological fluids[2]. Their affinity to absorb water is attributed to the presence of hydrophilic groups such as –OH, –CONH–, –CONH2–, and –SO3H in polymers forming hydrogel structures. Due to the contribution of these groups and domains in the network, the polymer is thus hydrated to different degrees (sometimes, more than 90%wt.), depending on the nature of the aqueous environment and polymer composition[3,4]. The preparation of a hydrogel based drug formulation system is done either by its crosslinking of polymers or polymerization of monomers and cross linking with poly functional monomers[5]. Synthetic, semi synthetic or natural source based polymers can be used to make hydrogels. Normally polymers having hydroxyl, amine,

amide, ether, carboxylate and sulfonate as their functional groups in its side chain are employed in this method[6]. Alginic acid is an anionic biopolymer consisting of linear chains of α-L-glucuronic acid and β-D-mannuronic acid with properties such as a high degree of aqueous solubility, a tendency for gelation in proper condition with high porosity of the resulting gels, biocompatibility, and non-toxicity[7]. Generally speaking, Sequential cross-linking and formation of polymeric networks, results in hydrogel structured drug delivery carriers such as micro- and nanoparticles upon the addition of counter-ions to alginate. Any possible cationic species can initiate the reaction sequence, but calcium chloride is favorably utilized by most researchers. The methods of preparation are usually determined with the aim to control the gelification phenomenon, which leads to desired size ranges depending on various factors including alginate concentration/ viscosity, counter-ion concentration, the speed of adding counter-ion solution onto the alginate solution, etc.

Mesalamine (5-ASA) has well established anti-inflammatory activity and it is the first line drug for the management of ulcerative colitis. The drug is to be delivered to the colon for its local action against inflammation. But, 5-ASA is rapidly absorbed from the small intestine and there is little localization of Mesalamine in the colon relative to the small intestine[8]. Thus, it is necessary to develop a colon-specific delivery system for Mesalamine in the treatment of ulcerative colitis.

In present study a colon specific delivery system was developed using combination of natural polysaccharides, sodium alginate and guar gum. The system consists of cross-linked hydrogel beads of sodium alginate and guar gum which releases Mesalamine locally in the physiological environment of the colon.

II. MATERIALS AND METHODS

Mesalamine USP was obtained as a gift sample from CosmePharma Pvt. Ltd., Goa, manufactured by Symed Laboratories Ltd. All other chemicals, reagents and solvents used were of analytical grade and used directly as supplied by the manufacturer.

A. Preparation of Meselamine Hydrogel Beads

Meselamine loaded hydrogel beads were prepared using Cross linkingmethod using hydrogel forming polysaccharides as shown in Table 1. Cross linking method involves extrusion of mixture through a syringe having a diameter of 0.1 nm. In the first scheme of formulation (F1), meselamine gel beads were prepared by using sodium alginate only followed by addition of drug meselamine. F2 was prepared by addition of both sodium alginate and guar gum with addition of drug meselamine. But F3 to F10 formulations were prepared by addition of sodium alginate and guar gumfollowed by addition of glutaraldehyde[0.25, 0.3, 0.4 and 0.5%(w/v)]to this mixture. Meselamine was then added to a final conc. of 0.2% and blended well. Beads were made in 5% $CaCl_2$ solution by extrusion through a needle having a diameter of 0.1nm, cured for 1 h in the same solution. Beads were removed by filtration and washed with distilled water which were then air dried and stored in the air tight container[9].

TABLE I
FORMULATION OF DIFFERENT BATCHES OF HYDROGEL BEADS

Formulation	Drug (g)	Sodium Alginate (g)	Guar Gum(g)	Gluteraldehyde Sol (%)	Calcium Chloride Sol (%)
F1	0.5	2	0	0	5
F2	0.5	1.75	0.25	0	5
F3	0.5	1.75	0.25	0.2	5
F4	0.5	1.75	0.25	0.3	5
F5	0.5	1.75	0.25	0.4	5
F6	0.5	1.75	0.25	0.5	5
F7	0.5	1.25	0.25	0.25	5
F8	0.5	1.25	0.25	0.3	5
F9	0.5	1.25	0.25	0.4	5
F10	0.5	1.25	0.25	0.5	5

B. Characterization of Meselamine Hydrogel Beads

1) *% Yield:* The percentage yield of the hydrogel beads was determined from total weight of beads obtained and total weight of drug and polymers used.
2) *% Moisture content:* Moisture content was calculated from the difference in weight of freshly prepared beads and beads dried over-night of a fixed number (20) of beads.
3) *Particle size:* Size of the hydrogel beads was determined by optical microscope fitted with stage micrometer and ocular micrometer. A minimum of 50 dried beads per batch were counted for the determination of particle size and mean diameter is calculated.
4) *Optical Microscopy:* Hydrogel beads were placed on a slide using forceps and visualized under optical microscope at different magnification to check the shape and surface smoothness of the beads. Photographs of the selected batches were also recorded using camera fitted on the microscope.
5) *Differential Scanning Calorimetory(DSC):* The physical state of drug inside the hydrogel beads was investigated by DSC. The thermogram of the drug loaded beads were obtained using DSC (TA instruments, Model no. Q10). For this, the small amount (2-5 mg) of sample was sealed in the aluminium pan and the temperature was raised at 20^0C/min from 40 to 300^0 C.
6) *FT-IR (Fourier Transform Infrared Spectroscopy) Spectral Analysis:* Infrared spectroscopy of the different formulations was studied to confirm the drug loading and drug-excipient interaction using KBr pellet method.
7) *X-Ray Diffraction Studies (XRD):* In order to determine change in physical state of the drug in formulation, whether it is in crystalline or amorphous form the XRD studies were carried out.

C. Evaluation of Meselamine Hydrogel beads

1) *Drug Entrapment Efficiency:* 100 mg of the dried beads of each formulation containing drug weighed and dispersed in 100 ml Phosphate Buffer (pH 7.4) and allowed to stand for 24 hours with intermittent shaking to ensure complete extraction of drug. The solution was diluted suitably, filtered and absorbance was measured using at 330 nm UV-Visible Spectrophotometer. The drug content was estimated using calibration curve.

$$\textbf{Entrapment Efficienciency} = \frac{\textbf{Total amont of drug added} - \textbf{Amount of free drug}}{\textbf{Total amount ofdrug added}} \textbf{X100}$$

2) *In-Vitro Swelling Study:* The swelling characteristics of the test alginate-guar gum beads were determined by a method described previously (Chen *et al.*, 2004). The dried test samples were immersed in 5mL of a solution of pH 1.2 (HCl–KCl buffer) or 7.4 (phosphate buffer) for 3 h. At specific time intervals, the samples were taken out and were blotted with a paper towel to absorb excess water on the surface. The swelling ratios (Q_s) of test samples were calculated from the equation: $Q_s = W_s - W_d/W_d$, Where, W_s = weight of the swollen sample and W_d = weight of the dried test sample.

III. RESULT AND DISCUSSION

In the present study the mesalamine loaded hydrogel beads were prepared as enteric coated system to achieve the specific drug delivery. The hydrogel beads were prepared by cross-linking method which involved extrusion of mixture through a syringe having a diameter of 0.1 mm.

A. FTIR-Spectra

The FT-IR spectra of the drug give rise to broad peak at 2983.8 due to Aryl C-H streching, at 1481.3 due to skeletal vibration of benzene ring, at 690.5 due to C-C out of plane bending in benzene ring and at 1085.9 due to C-O streching of carboxylic acid. The combination of drug with sodium alginate and guar gum shows peak at 2982.8 due to Aryl C-H streching, at 1483.2 due to skeletal vibration of benzene ring, at 681.7 due to C-C out of plane bending in benzene ring and at 1085.4 due to C-O streching of carboxylic acid. These data explained confirms that during the formulation no chemical reaction has taken place between drug and the polymer.

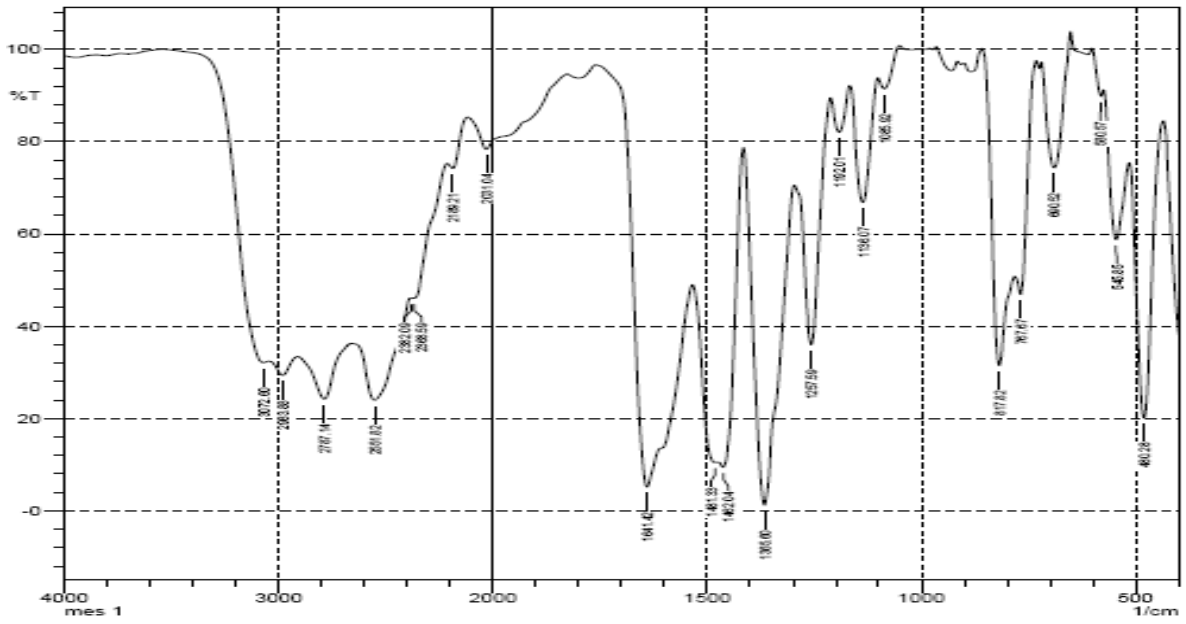


Fig. 1 FT-IR (Fourier Transform Infrared) of Mesalamine

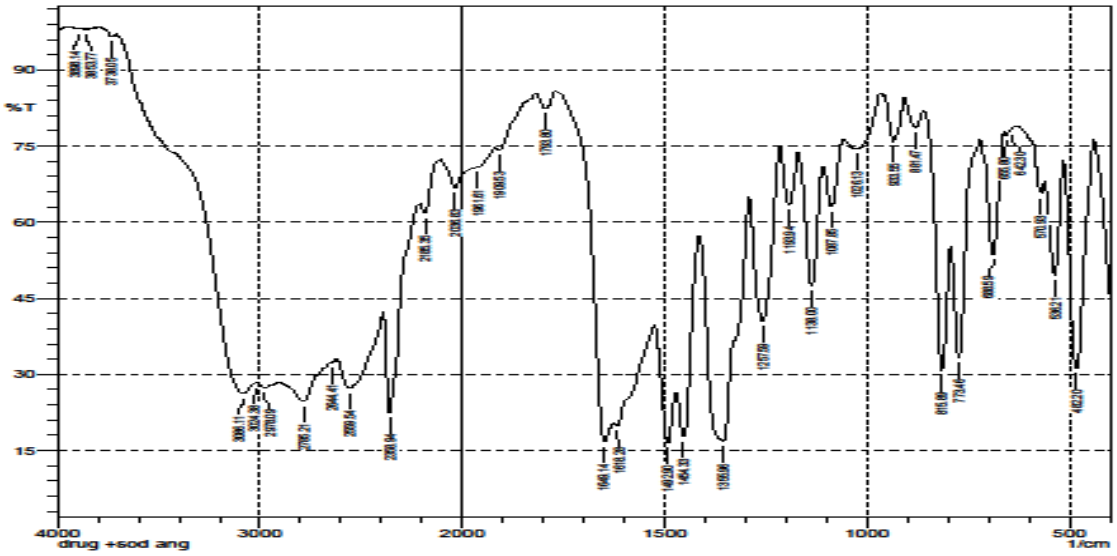
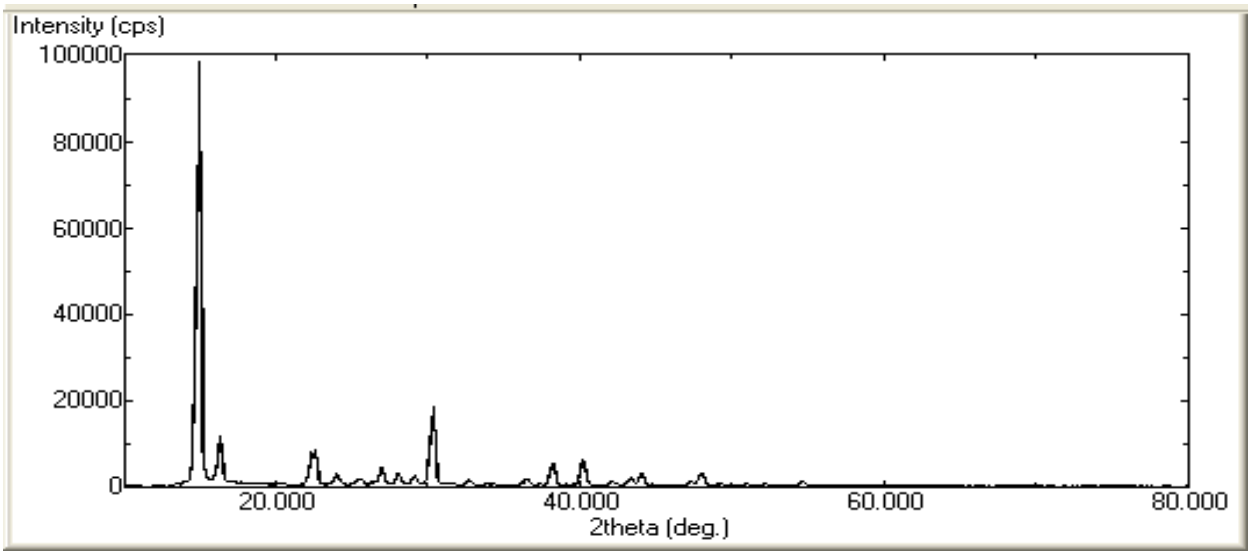


Fig. 2 FT-IR Spectra of Drug and Excipient

B. X-Ray- Diffraction Studies

X-Ray diffraction (XRD) studies were carried out to determine the physical state of the drug, whether amorphous or crystalline

Fig. 3 XRD of Mesalamine USP



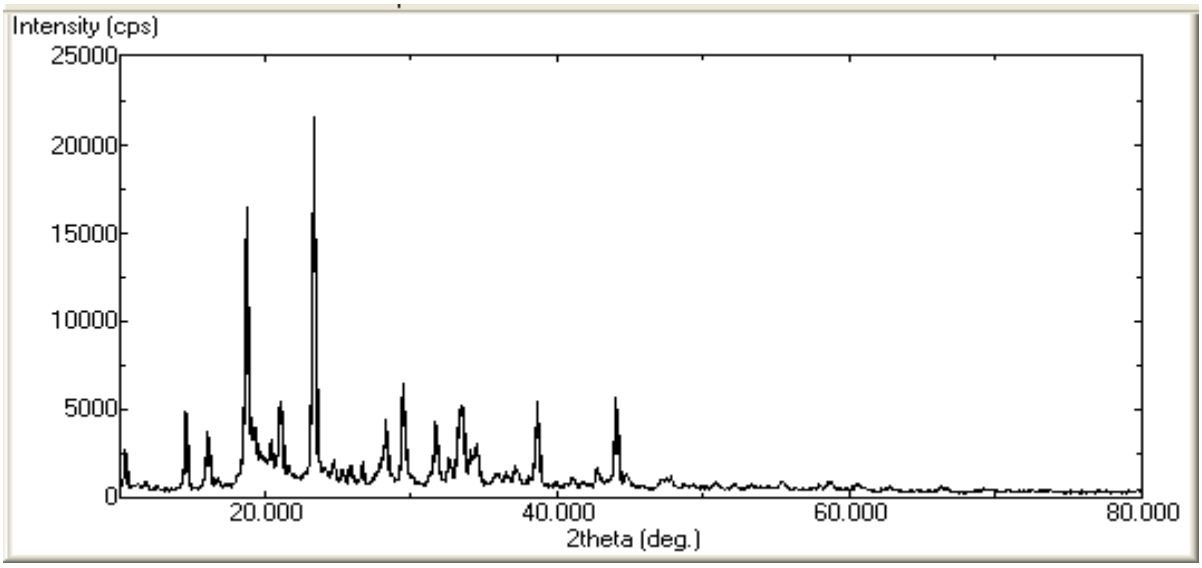


Fig. 4 XRD Spectra of Sodium Alginate and Guar Gum Hydrogel Beads (F6)

C. Differential Scanning Calorimetory (DSC)

DSC thermogram of the drug loaded beads were obtained using DSC (TA instruments, Model no. Q10). For this, the small amount (2-5 mg) of sample was sealed in the aluminium pan and the temperature was raised at 20^0C/min from 40 to 300^0 C.

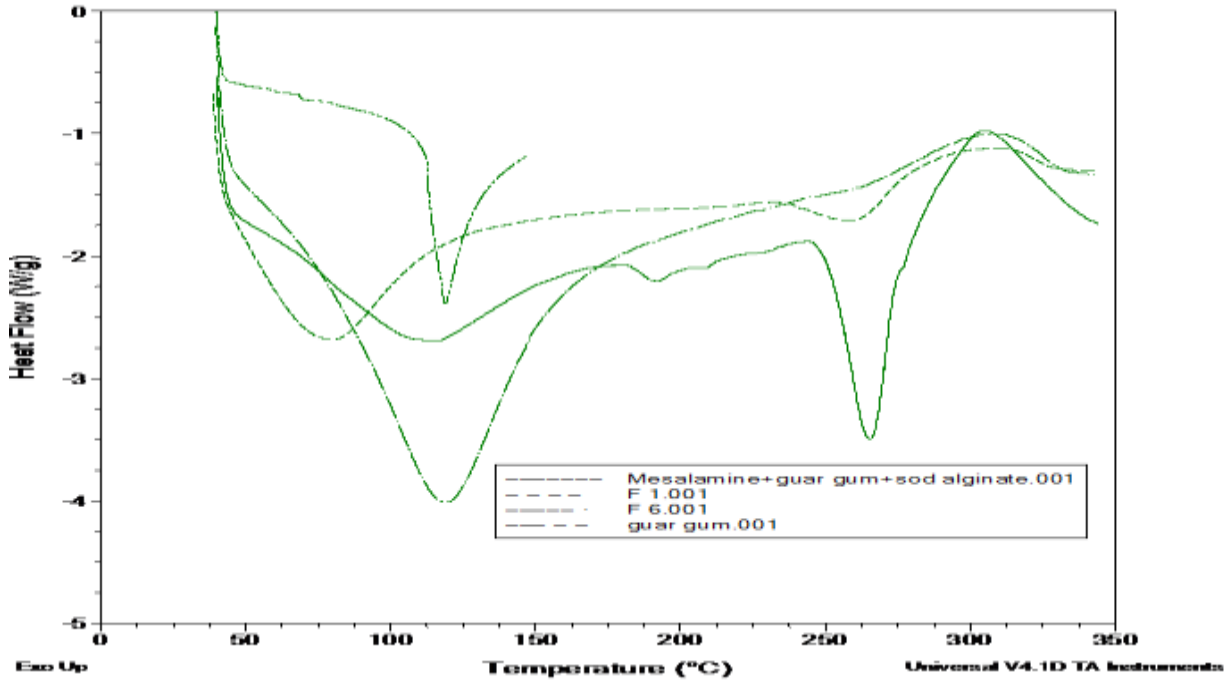


Fig. 5 DSC Spectra Mesalamine, Excipients and Formulation F1 and F6

From the DSC spectra of drug, excipients and formulation, it was observed that there is no interaction between drug and excipients. It was also observed that there is no sharp peak near 283 ^{0}C (melting point of Mesalamine) which indicate

that means the most of the drug has entrapped in the matrix of sodium alginate and guar gum.The suppression of endothermic peak of Mesalamine indicated that drug is in amorphous form rather than crystalline form in the formulation.

D. % Yield, % Moisture Content and Average Size

From the observations it was concluded that bead forming property was enhanced with increased use of guar gum and gluteraldehyde (up to 0.5%). Particle size was observed more in hydrogel beads with high polymer content i.e., particle size of formulation with 4% w/v polymer was found greater than that containing 3%. It was also found that average size also decreases when concentration of gluteraldehyde was increased from 0.2- 0.5%.

TABLE II

% YIELD, % MOISTURE CONTENT AND AVERAGE YIELD OF DIFFERENT FORMULATIONS

Formulation	Yield (%)	Moisture Content (%)	Average size (µm)
F1	56.3 ±0.144	10.75±0.042	1152±2.43
F2	62.4 ±0.299	10.05±0.037	1134±1.74
F3	63.9 ±0.305	8.86±0.035	1070±1.65
F4	65.4 ±0.281	9.95±0.03	1020±2.38
F5	69.3 ±0.281	11.48±0.018	1002±1.25
F6	73.6 ±0.472	12.42±0.116	956±1.95
F7	54.4 ±0.307	9.25±0.025	1044±3.75
F8	59.7 ±0.338	10.25±0.041	1000±1.85
F9	56.5 ±0.404	11.36±0.007	963±1.63
F10	61.5±0.167	12.13±0.008	934±1.72

E. Shape and Surface Smoothness of Hydrogel Beads

Shape and surface smoothness of beads was determined using optical microscope. It was found to be nearly spherical and surface was found rough and dense along with surface folds.

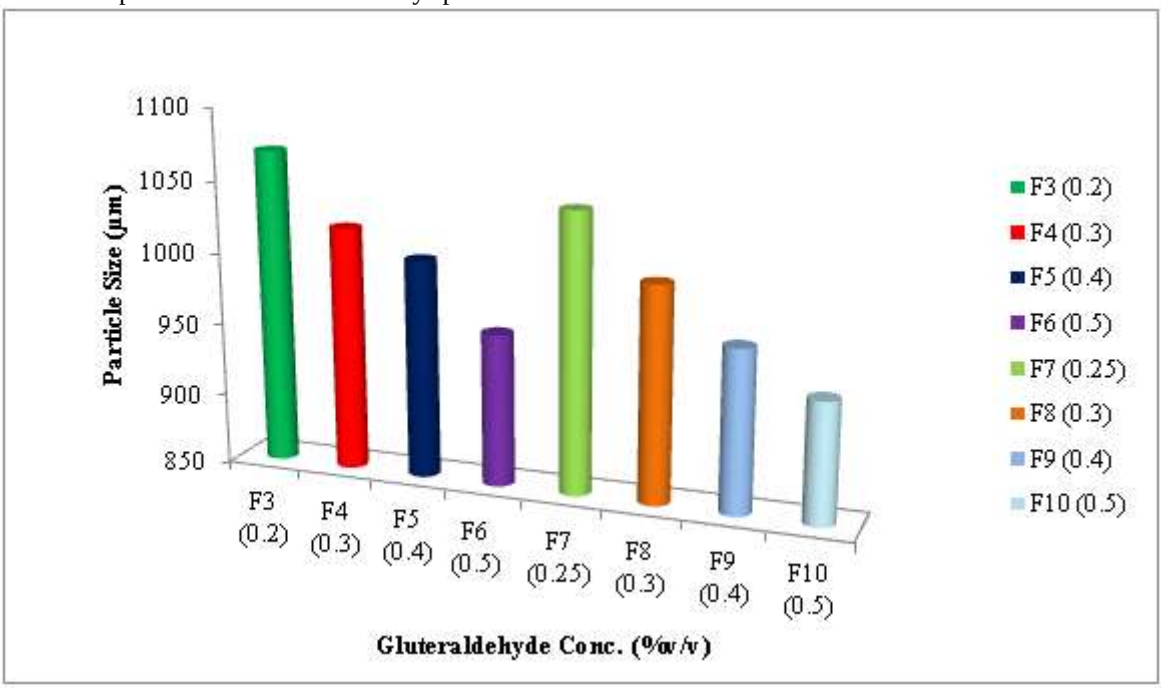


Fig. 6 Effect of Gluteraldehyde and Polymer Concentration on Bead Size

Fig. 7 Photograph of Dried Hydrogel Bead (Formulation F6)

F. Encapsulation Efficiency: Encapsulation Efficiency of the dried beads of each formulation was determined using UV-Visible Spectrophotometer at 330 nm.

From the observations (Table III) it was concluded that the encapsulation efficiency increases with the addition of guar gum and gluteraldehyde as cross-linking agent upto 0.5% w/v. It was also concluded that encapsulation efficiency is higher in case of polymer conc. of 4% w/v as compared to that of 3% w/v. The formulation F6 with 4% w/v polymer and 0.5% w/v of gluteraldehyde showed best encapsulation efficiency.

TABLE III
ENCAPSULATION EFFICIENCY OF DIFFERENT FORMULATIONS

Formulation	Encapsulation Efficiency (%)
F1	54.84±0.219
F2	63.44±0.69
F3	67.90±0.28
F4	73.15±0.11
F5	82.98±0.40
F6	88.00±0.32
F7	66.12±0.16
F8	72.48±0.26
F9	78.53±0.21
F10	85.46±0.21

G. Swelling Study: The swelling characteristics of the test alginate-guar gum beads were determined in different media mimicking simulated gastric and intestinal media of pH 1.2 and 7.4 respectively. The swelling characteristics of all batches of bead was determined at pH 1.2 (Fig. 2&3) and at pH 7.4 (Fig. 4&5).
From the swelling ratio studies of different alginate-guar gum combinations and different gluteraldehyde concentrations studied (0.2, 03, 0.4 and 0.5%), the one giving the most suitable swelling characteristics in gastric and intestinal media was found to be 7:1 and 0.5% respectively. It showed very low swelling at pH 1.2 and 7.4; while others showed high swelling at both pH 1.2 and 7.4. The rapid swelling exhibited by other groups of beads will tend to limit their efficiency for controlled release at intestinal pH. This is because of the increased chance of entrapped drug for a rapid release in the intestinal pH. Thus the beads with alginate to guar gum ratio of 7:1 and crosslinked with 0.5% gluteraldehyde(i.e. F6) was selected for further studies.

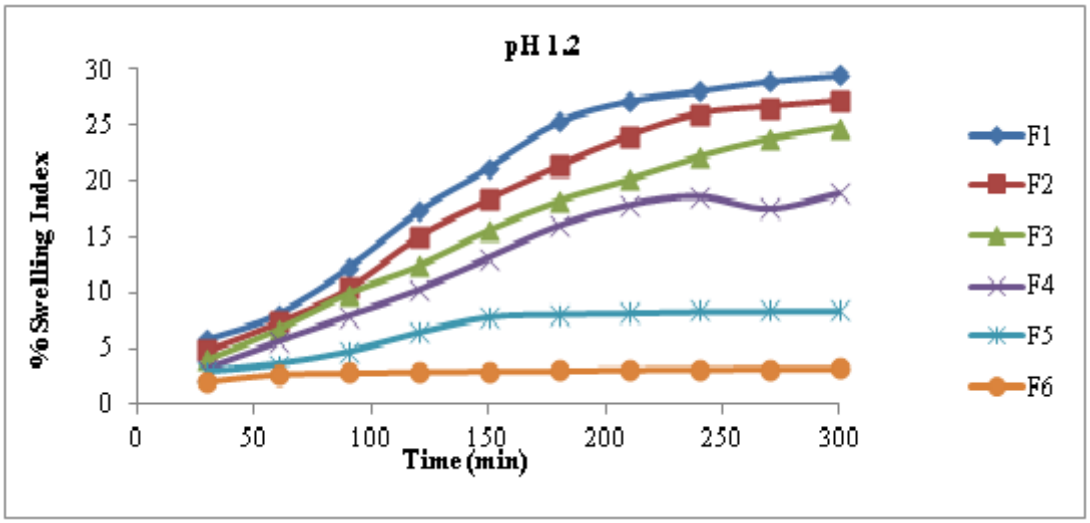


Fig. 8 Swelling Index of Formulations (F1-F6) at pH 1.2

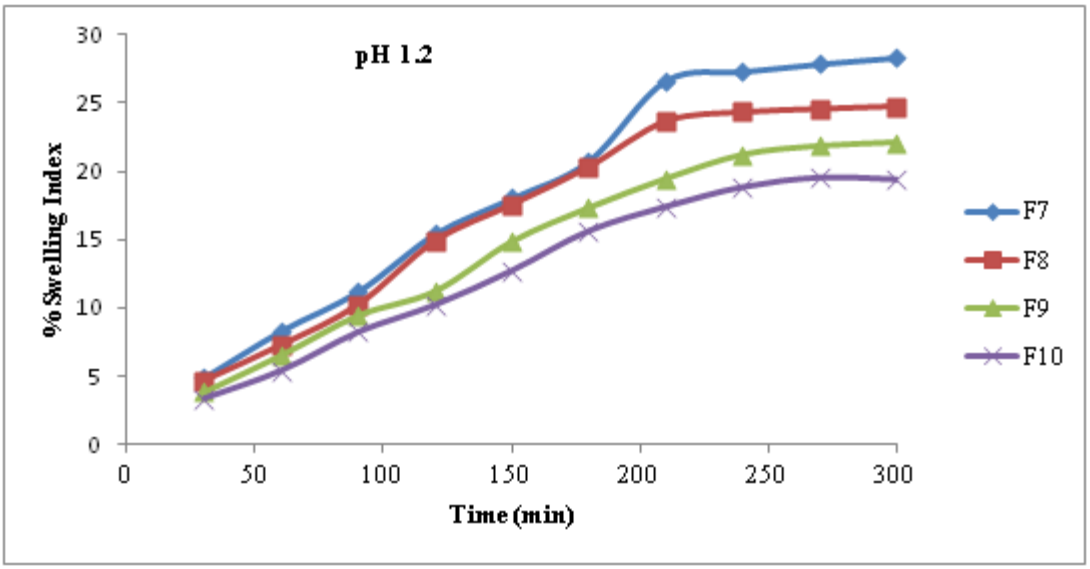


Fig. 9 Swelling Index of Formulations (F7-F10) at pH 1.2

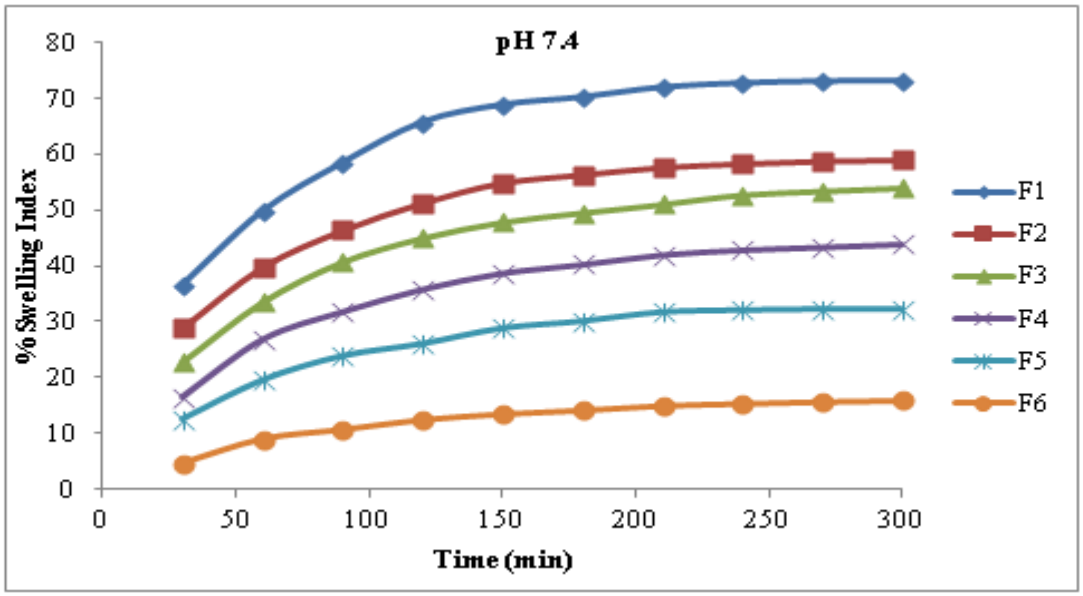


Fig. 10 Swelling Index of Formulations (F1-F6) at pH 7.4

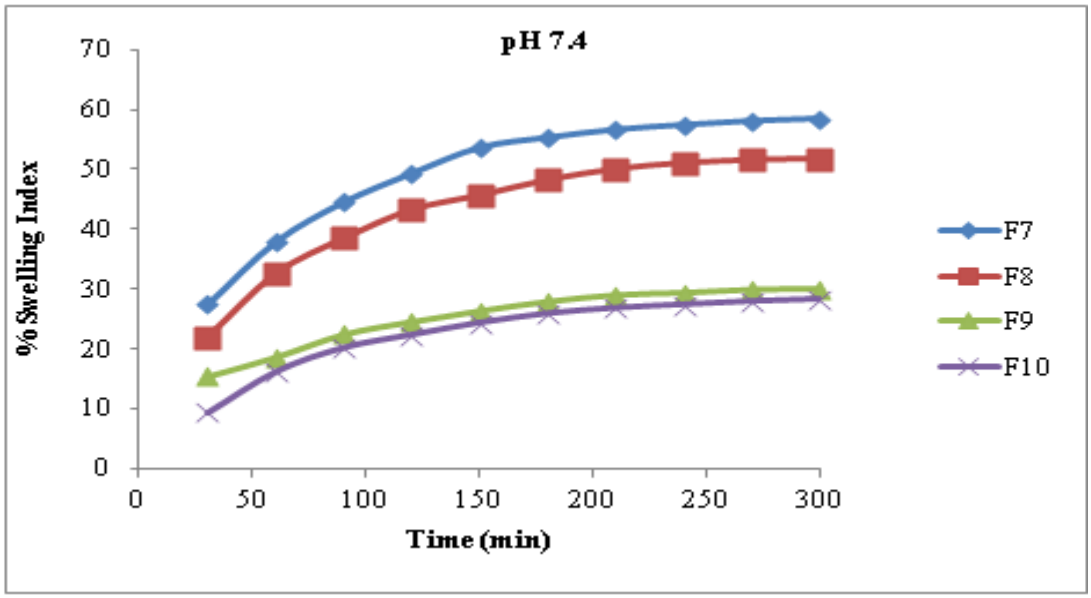


Fig. 11 Swelling Index of Formulations (F7-F10) at pH 7.4

IV. CONCLUSION

Hydrogel beads were prepared by varying different parameters using cross-linking method and evaluated for Yield, Moisture Content, Particle Size, shape and surface morphology, Encapsulation Efficiency, Swelling index. DSC, FT-IR, X-RD analysis of the selected formulations was also carried out.

The bead forming property was found optimum with 4 % w/v polymer solution having sodium alginate to guar gum (7:1) and gluteraldehyde can be used as cross-linking agent upto 0.5% w/v of final solution. Beads can also be prepared with 3% polymer solution having sodium alginate to guar gum (5:1) with gluteraldehyde. Particle size of hydrogel beads was found in the range of 960-1160 µm. The particle

size of beads decreases with increase in cross-linker and average size increases with increase in polymer concentration. The obtained beads were spherical in shape having rough and dense surface. The encapsulation efficiency of beads vary from 54.8-88.0 % .encapsulation efficiency increases with addition of guar gum and gluteraldehyde as cross-linker. Increase in polymer conc. also shows an increase in encapsulation efficiency. Swelling studies carried out at pH 1.2 and 7.4 revealed that swelling of formulation F6 with sodium alginate to guar gum 7:1 and gluteraldehyde concentration of 0.5% was very low at pH1.2 and 7.4 (3.16 & 15.89 respectively) while all other formulation showed higher swelling index at both pHs.

V. ACKNOWLEDGEMENT

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