

## Preparation, Evaluation and Optimization of Famotidine-Alginate Microspheres Using (3)<sup>2</sup> Full Factorial Design

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**Abstract:** The present study aims to formulate and optimize alginate microspheres by employing 3<sup>2</sup> factorial designs for treatment of chronic ulceritis. In this investigation famotidine was selected as the drug of choice for preparing microspheres by ionic-gelation technique using sodium alginate as polymer and calcium chloride: calcium carbonate as cross linking agent. The prepared microspheres were characterized in terms of drug-excipient compatibility study, % yield, micromeritics study, swelling index, particle size analysis, shape analysis, drug content, drug encapsulation efficiency and *in vitro* drug release study. Results signified the fact that, microspheres formed had sufficient good surface and size to be utilized as a dosage form responsible for slow release of drug from matrix through erosion. Among all the nine batches formed, B8 was selected as the optimized batch in terms of all parameters evaluated.

**Key words:** Ionic-gelation • Sustained • Optimized • Ulceritis • Alginate

### INTRODUCTION

Recently, considerable focus regarding the local as well as systemic administration of poorly absorbed dosage formulations is being conducted on the use of mucoadhesive polymers [1]. These mucoadhesive polymers have the property of mucoadhesion to the gastric mucus layer until removed by several factors from the surface. The present investigation deals with the preparation of sodium alginate microspheres [2, 3]. Alginate, obtained from brown seaweed, is a linear unbranched polysaccharide composed of alternating blocks of 1-4 linked  $\alpha$ -L-guluronic and  $\beta$ -D-mannuronic acid residues. Being naturally originated water soluble, anionic polymer with carboxyl end groups, it is a good biodegradable and biocompatible mucoadhesive agent widely used in drug delivery. This characteristic of alginate is applied in the formulation of microspheres for effective absorption, enhanced bioavailability and site specific targeting of drugs [4-8]. Low pH gastric fluid environment converts the hydrated sodium alginate into porous and insoluble alginic acid skin that shrinks at low pH and the encapsulated drugs are not released. When reaches to the higher pH intestinal fluid environment, the shrunk alginic acid skin gets converted to a soluble

viscous layer. To modify these pH dependent characteristic of natural alginate, several approaches are used to prolong the release pattern of encapsulated drugs. Alginate salts form reticulated structure when comes in contact with various polyvalent ions such as Ca<sup>2+</sup>, Sr<sup>2+</sup>, or Ba<sup>2+</sup>. Most widely used divalent ion is calcium ion which forms matrix particulate system by gel formation with sodium alginate to produce sustained release for a variety of dosage forms [9, 10]. The mechanism of gelation and cross-linking of alginate is concerned with the exchange of sodium ions from the glucuronic acid with divalent calcium ions to form an insoluble salt [11]. These inter-chain associations can be either temporary or permanent depending on the amount of calcium present. Highly viscous, thixotropic temporary associations are formed with low levels of calcium while gelled permanent associations are formed at high calcium levels. This property of alginate is used in present investigation by external/ionic gelation method, involving the drop wise addition of drug-alginate dispersion into a calcium chloride solution [12, 13].

Famotidine, a histamine-2 receptor antagonist is beneficially encapsulated in ionic gelled microspheric beads for the treatment of gastric reflux and hypersecretory states. Oral bioavailability of famotidine

is 40-45% with low biological half-life of 2.0-4.0 hrs. Thus, to reduce the dose frequency and dose size, microspheric formulation was prepared that retards its release profile and thereby aims to achieve prolonged action [14, 15].

## MATERIALS AND METHODS

**Materials:** Drug Famotidine was purchased as a gift sample from Zydus-Cadila, Ahmedabad, India. Sodium alginate was procured from Central Drug House (P) LTD, New Delhi, India. Calcium chloride and calcium carbonate was obtained from M/S Loba Chemie Pvt Ltd., Mumbai, India. All other materials and reagents used were of pharmaceutical grade.

**Experimental Design:** Drug loaded sodium alginate microspheres were prepared by ionic-gelation method. A 2.5% (w/v) sodium alginate solution was prepared in distilled water by properly mixing along with slight heating. This concentration of sodium alginate solution remains to be fixed for all the nine batches prepared. Similarly, 5.0% (w/v) aqueous concentration of calcium chloride and calcium carbonate in 1:1 ratio was prepared which also remains to be constant for all the nine batches during the experiment. Two independent variables that need to be optimized for the preparation of microspheres are the mixing speed and curing time. The adding time of drug-sodium alginate mixture to the calcium chloride solution was fixed at 8 minutes for all the batches. The nine batches are prepared as per the three level- two factor ( $3^2$ ) design shown in table 1.

**Preparation and Solidification of Famotidine-Sodium Alginate Microspheres:** To the 2.5% (w/v) aqueous sodium alginate solution, accurately weighed (200 mg) quantity of famotidine was dispersed and mixed properly.

This drug-sodium alginate solution was then added drop wise to the continuously stirred 5% (w/v) aqueous solution of calcium chloride-calcium carbonate at the speeds mentioned in table 1 in constant time using syringe of #20 gauze needle size. Thereafter, the microspheric beads formed of the added drug-sodium alginate in the calcium salt solution were further cured for specified time mentioned in the table 1. This curing of microspheres ensures their solidification as shown in figure 1. Thus, nine batches were prepared according to the formulation table with varying independent variables.

**Filtration and Washing of Drug Loaded-alginate Microspheres:** The microspheric beads obtained were subjected to filtration through Whatman's filter paper immediately after the completion of curing time. These microspheres were further washed several times (3-4 approx.) with distilled water to completely remove any traces of calcium chloride stuck on the microspheres surface.

**Collection and Drying of Microspheres:** The finally washed microspheres were then collected in petridish and dried fully in air at room temperature as shown in figure 2(a and b). Each batch of dried microspheres were weighed properly and packed in individual containers for further evaluation.

**Evaluation of Dried Famotidine-alginate Microspheres Drug-excipient Compatibility Study:** Polymer-drug interactions were determined by FTIR spectroscopy. The FTIR spectra was obtained for famotidine, sodium alginate and famotidine-loaded sodium alginate microspheres at room temperature in KBr pellets by applying 6000 kg/cm<sup>2</sup> pressure, using a Fourier transform infrared (FTIR) spectrophotometer (FTIR-8400 S, Shimadzu, Japan) between ranges 400 to 4500 cm<sup>-1</sup>. This study uses air as a reference.

Table 1: Preparation of sodium alginate microspheres using ( $3^2$ ) factorial designs

Formulation	Ingredients	
	Mixing speed ( $X_1$ )	Curing time ( $X_2$ )
B1	-1	-1
B2	0	-1
B3	+1	-1
B4	-1	0
B5	0	0
B6	+1	0
B7	-1	+1
B8	0	+1
B9	+1	+1

X1: mixing speed (rpm), Level: -1(150), 0(250), +1(350)

X2: curing time (minutes), Level: -1(15), 0(30), +1(45)



Fig. 1: Curing of microspheric beads in calcium salt (5% w/v) solution



Fig. 2: Filtered microspheric beads (a) before drying and (b) after drying

Table 2: Data showing results of different micromeritic parameters

Formulations	Percent Yield (%)	Micromeritic Properties				
		Bulk Density (g/ml)	Tapped Density (g/ml)	Carr's Index (%)	Hausner's Ratio	Angle of Repose (°)
B1	76.36 ±0.01	0.45 ±0.01	0.53 ±0.01	15.09 ±0.03	1.18±0.04	16.37 ±0.01
B2	84.85 ±0.00	0.54 ±0.02	0.58 ±0.02	6.90 ±0.01	1.07 ±0.04	10.44 ±0.03
B3	98.79 ±0.16	0.74 ±0.01	0.75 ±0.03	1.33 ±0.11	1.01 ±0.08	8.84 ±0.11
B4	75.15 ±0.10	0.62 ±0.03	0.69 ±0.01	10.14 ±0.02	1.11 ±0.01	7.95 ±0.10
B5	84.85 ±0.00	0.64 ±0.01	0.65 ±0.11	1.54 ±0.01	1.02 ±0.10	10.68 ±0.00
B6	88.79 ±0.01	0.64 ±0.13	0.69 ±0.00	7.25 ±0.13	1.08 ±0.03	9.34 ±0.06
B7	88.48 ±0.00	0.56 ±0.01	0.61 ±0.00	8.20 ±0.06	1.09 ±0.01	17.26 ±0.10
B8	98.80 ±0.00	0.62 ±0.02	0.67 ±0.08	7.46 ±0.01	1.08 ±0.03	13.28 ±0.02
B9	89.70 ±0.00	0.49 ±0.01	0.57 ±0.03	14.04 ±0.00	1.16 ±0.00	17.20 ±0.05

± Shows standard deviation of triplicate readings

**Percent Yield:** The percent yield was estimated after weighing thoroughly dried microspheres as shown in table 2.

$$\text{Percent Yield (\%)} = \frac{\text{Practical Yield}}{\text{Theoretical yield}} \times 100$$

**Micromeritic Studies:** The micromeritic evaluation of the microspheres was done to estimate the flow properties, packing properties, porosity, average particle sizes and diameter of the resultant microspheres. Different micromeritic studies performed are determination of bulk density, tapped density, carr's index, hausner's ratio and angle of repose as represented in table 2.

**Bulk Density:** Bulk density was determined by pouring accurately weighed microspheres in measuring cylinder and determined by the below mentioned formula.

$$\text{Bulk density (g/ml)} = \frac{\text{Weight of microspheres}}{\text{Volume}}$$

**Tapped Density:** Tapped density was determined by pouring accurately weighed microspheres in a well dried measuring cylinder and tapping 100 times from a constant height.

$$\text{Trapped Density (g/ml)} = \frac{\text{Weight of microspheres}}{\text{Volume}}$$

**Carr's Index:** Carr's index was determined from the values of bulk and tapped density.

$$\text{Carr's index} = \frac{\text{Trapped density} - \text{Bulk Density}}{\text{Trapped Density}}$$

**Hausner's Ratio:** Hausner's ratio was again determined from the values of bulk and tapped density.

$$\text{Hausner's Ratio} = \frac{\text{Trapped Density}}{\text{Bulk Density}}$$

**Angle of Repose:** It was used to determine the flow properties of famotidine loaded microspheres by fixed funnel flow method.

$$\text{Angle of repose } (^{\circ}) = \tan^{-1} \frac{\text{Height}}{\text{Radius}}$$

**Swelling Index:** Swelling index of microspheres was measured by determining the extent of their swelling in a given buffer through increase in weight. Accurately weighed amount of microspheres (250 mg) were suspended in 5 mL of buffer solution. The study was done for 12 hours with first two hours in 0.1 N HCl and rest in 6.8 pH phosphate buffer. At specific time intervals of 15, 30, 60, 120, 240, 480 and 720 minutes, the swelled microspheres were taken out, patched dry on filter paper to remove excess water and then weighed. Swelling index was calculated according to the formula mentioned below in percent.

$$\text{Swelling Index (\%)} = \frac{W1 - W2}{W1} \times 100$$

where,

W1 = Weight of swelled microspheres (mg)

W2 = Weight of dried microspheres (mg)

**Particle Size Analysis:** Particle size determines the release behavior of microspheres. The particle size of microspheres of each batch was analyzed through optical

microscopy method fitted with an ocular and stage micrometer. At least 50 particles must be examined in all measurements in 5 different fields.

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Practical weight of drug in sample}}{\text{Theoretical weight of drug}} \times 100$$

**Drug Content:** Different batches of microspheres were checked for drug content uniformity. Accurately weighed amount of dried microspheres were taken in a pestle and mortar and powdered. The powdered microspheres were then separately dissolved in adequate quantity of 0.1 N HCl and 6.8 pH phosphate buffer and kept for 24 hrs. The solution was then filtered, scanned for absorbance and then final absorbance was noted down at 266nm using UV spectrophotometer (Shimadzu Model 1601, Japan). Each process was repeated in triplicate and average was calculated.

**Drug Encapsulation Efficiency:** Drug encapsulation efficiency was determined thereafter using the following formula.

**In vitro Drug Release Profile:** *In vitro* drug release studies are determined by filling specified quantity of microspheres in capsule shells and collecting samples (5ml) at specified intervals using release at 0.1 N HCl (pH 1.2) for first 2 hrs and 6.8 pH phosphate buffer for next 24 hrs. The drug content of the withdrawn samples was estimated through UV spectroscopy at 266 nm and subsequently sink condition was maintained by adding a 5 ml of fresh medium maintained at  $37 \pm 0.5^{\circ}\text{C}$  after the removal of each test aliquot. The whole dissolution study was conducted at  $35 \pm 0.5^{\circ}\text{C}$  in USP XII dissolution test apparatus (Electrolab, India) basket type at the speed of 50 revolutions per minute. Each dissolution tests of all nine batches were performed in triplicate and the dissolution profile was used to determine the percentage drug released after 24 hrs.

## RESULTS AND DISCUSSION

All the evaluation parameters are performed in triplicate and data estimated was calibrated in different graphic and tabular representation for better correlation and to select the best optimized batch.

**Drug Excipient Compatibility Study:** The prepared batches of microspheres were analyzed by IR spectroscopy in order to determine the drug-polymer interaction, if any. Figure 3 shows spectra of Famotidine

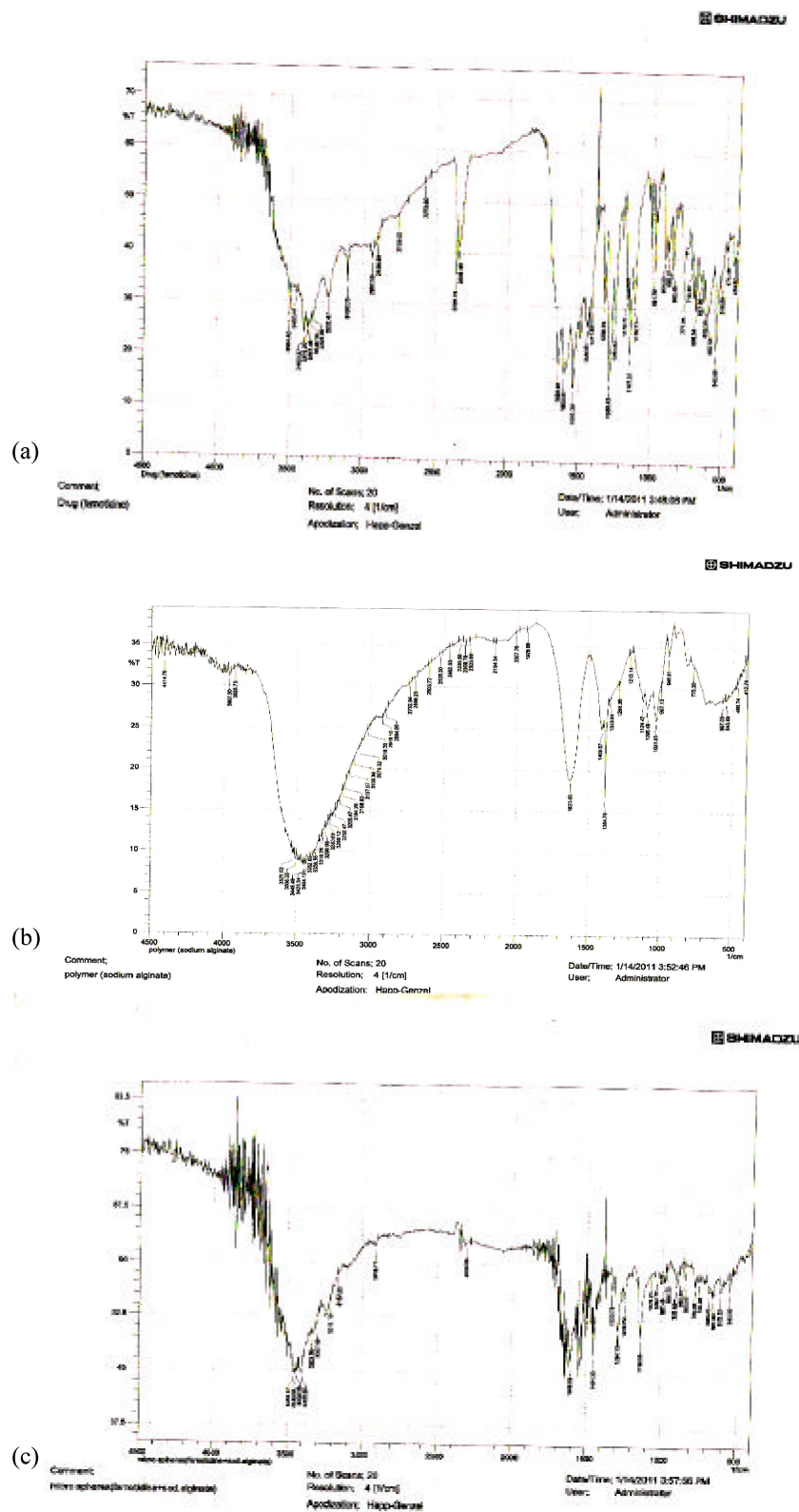


Fig. 3: IR Spectra of (a) Famotidine (drug), (b) Sodium alginate (polymer) and (c) Famotidine-sodium alginate microspheres (drug-polymer)

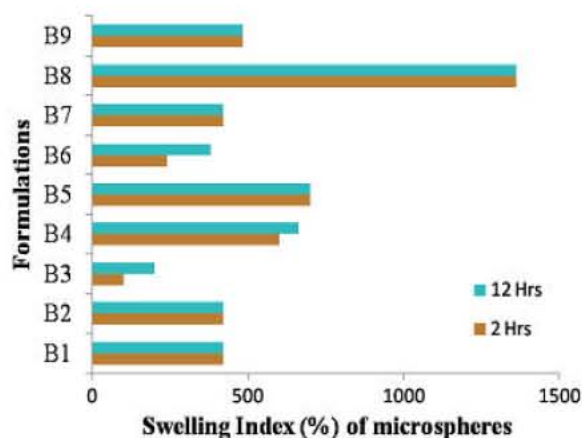


Fig. 4: Swelling index (%) of microspheres of all nine batches

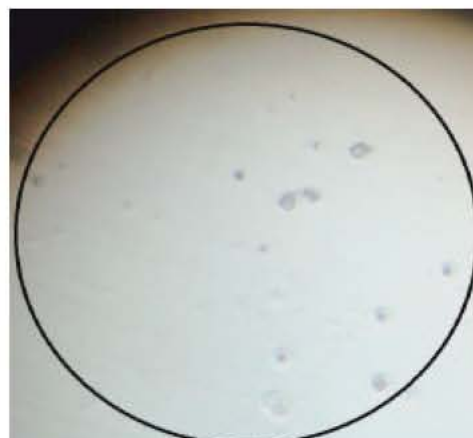


Fig. 6: Photomicrographic picture of microspheres of B8 batch

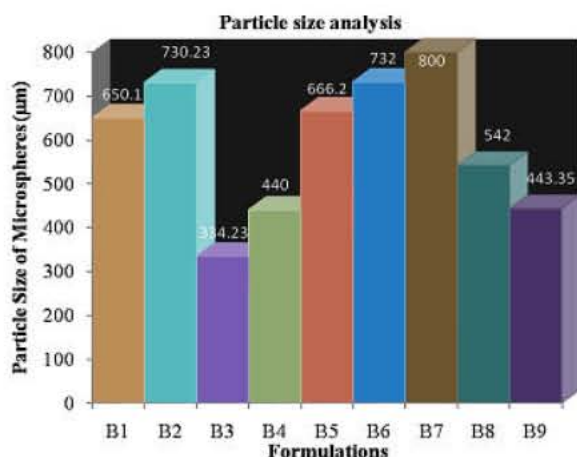


Fig. 5: Determination of particle size (µm) of all nine batches microspheres

(drug) (a), Sodium alginate (polymer) (b) and physical mixture of Famotidine-sodium alginate (drug-polymer) (c). From the IR spectra, it was found that there were no drug-polymer interaction and thus the drug could be stable in the formulation.

**Percent Yield:** All the prepared nine batches were evaluated for percent yield and it was found to range from  $75.15 \pm 0.10$  to  $98.80 \pm 0.00$  as shown in table 2. From the study, B8 showed maximum percent yield whereas B4 showed lowest percent yield indicating that this method could be very useful for adoption in the formulation of famotidine microspheres.

**Micromeritic Properties:** The prepared famotidine-alginate microspheres were then evaluated for micromeritic parameters such as bulk density, tapped

density, carr's index, hausner's ratio and angle of repose as shown in table 2. Carr's index was evaluated to determine compressibility characteristics of the microspheres and found to range between  $1.33 \pm 0.11$  to  $15.09 \pm 0.03$ . This showed that all the nine batches of microspheres were having good compressibility. Accordingly, hausner's ratio was evaluated and found to range between  $1.01 \pm 0.08$  to  $1.18 \pm 0.04$ . Values for angle of repose were found in the range of  $7.95 \pm 0.10$  to  $17.26 \pm 0.10$  showing that the different batches of microsphere were free flowing. As the result showed that the microspheres of all batches have good flow property and compressibility so can be used for large industrial production.

**Swelling Index:** From the swelling index study of microspheres, it was found from the figure 4 shown below that B8 showed highest swelling capabilities as depicted which is best optimized in terms of sustained release characteristics.

**Particle Size Distribution:** From the present investigation it was found that the particle size of all nine batches ranges from  $334.23 \mu\text{m}$  to  $800 \mu\text{m}$ . B3 showed smallest particle size whereas B7 showed maximum particle size. Other batches ranges between them in terms of particle size as shown in figure 5. The smallest particle size of B3 batch might be due to high mixing speed and less curing time [16].

**Shape of Drug Loaded Alginate Microspheres:** Shape of the microspheres was visualized through microscope and

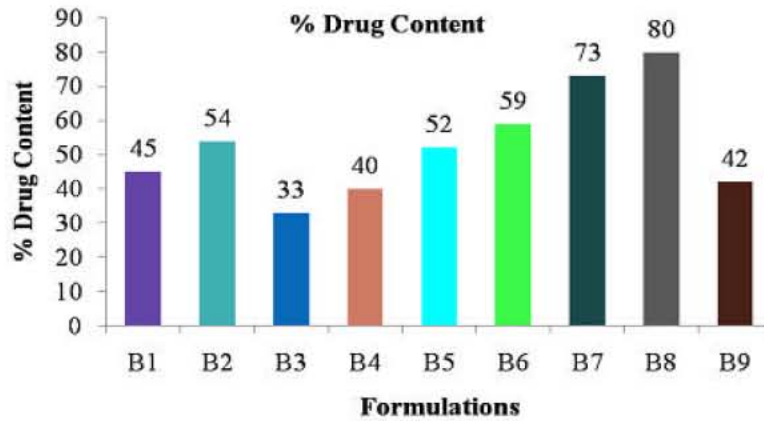


Fig. 7: Diagrammatic representation of drug content (%) of microspheres

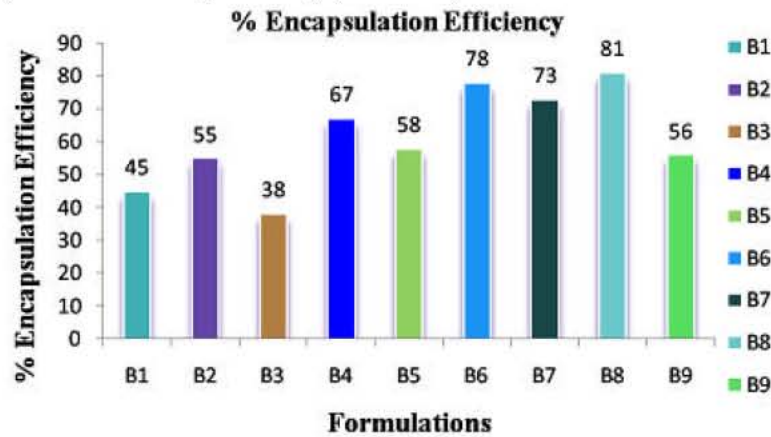


Fig. 8: Encapsulation efficiency (%) of famotidine loaded alginate microspheres

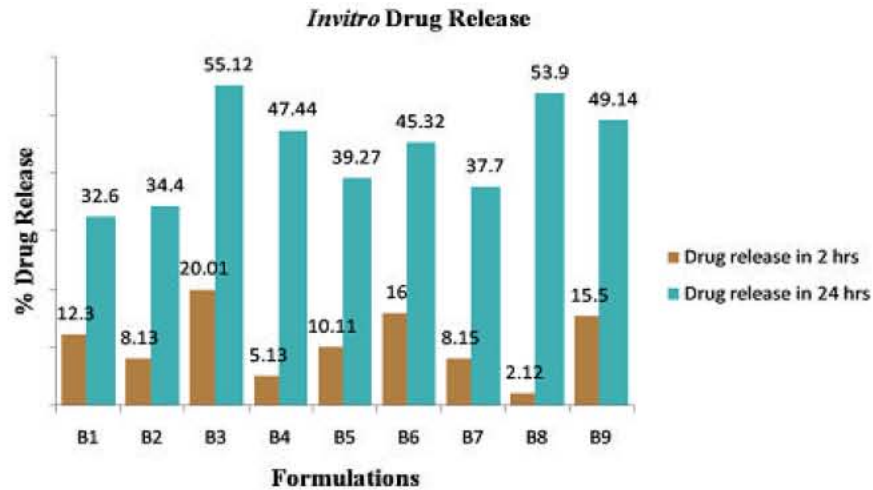


Fig. 9: Determination of % drug release from famotidine–alginate microspheres in 2 hrs and 24 hrs

was found to be spherical under the particle size range mentioned in figure 5. This was further confirmed through photomicrographic picture of the microspheres of one of the batch as shown in figure 6.

**Drug Content:** From the drug content study it was determined that B8 has the highest percentage of drug content as compared to other batches as shown in figure 7. It might be due to highest percentage yield in this ratio [16].

**Encapsulation Efficiency:** It was found from the present study that % drug encapsulation efficiency ranges from 38% to 81% as shown in figure 8. The maximum drug encapsulation was found to be for B8 and minimum for B3 batch. As the mixing time and curing time increases the encapsulation efficiency also increases. Moreover, the higher percentage yield of B8 also increases its encapsulation efficiency [14].

**In vitro Drug Release:** *In vitro* drug release study was carried out for 24 hrs and the comparison was made between the percentage of drug released in 2 and 24 hrs as shown in fig. 9. Famotidine-alginate microspheres of all nine batches showed slow initial drug release in 2 hrs which ranges from 2.12% to 20.01%. The release was found to be slowest for B8 and maximum for B3 within 2 hrs. Further also, the release was slow and found to range from 32.6% to 55.12% in 24 hrs. The release was found to be slowest for B1 and maximum for B3 within 24 hrs. The present investigation represented that B8 was found to be optimum in terms of *in vitro* drug release as maximum drug release was shown in intestinal part and very less in stomach region as hypothesized with residence time in body. This may be due to better encapsulation efficiency and optimum particle size as compared to other batches. Whereas on the other hand though B3 showed maximum (55.12%) drug release in 24 hrs yet it showed somewhat more drug release in stomach part which led to wastage of drug [17].

## CONCLUSION

On the basis of all the studies it can be concluded that the famotidine-alginate microspheres were successfully prepared by ionic gelation technique and confirmed that it is one of the reliable methods for preparing famotidine loaded microspheres. It served to be an efficient means of delivering an antiulcer drug for the course of long time therapy in inhibiting the production of acid. The formulated microspheres showed good flow properties and compressibility as depicted from various micromeritic parameters. In the present investigation the batch B8 has highest percentage of drug content followed by other formulations. The percentage of encapsulation of B8 batch was found to be 81%, also B6 and B7 showed good drug encapsulation efficiency in the range of 73 to 78%. The particle size of microspheres was determined by optical microscopy and all the nine batches of microspheres showed uniformity in size distribution

within their respective batches. The particle size was found to be in the range of 334.23-800nm. The prepared microspheres had good spherical shape as determined by photomicrographs. The *in vitro* dissolution studies showed that famotidine microspheres formulation B8 showed better drug release (53.9%) over a period of 24 hours than other formulations and much more left to be released thus showing sustained effect for even three to four days. Thus, B8 was selected as the optimized batch in terms of all evaluated parameters. To maintain a sustained blood level for a period of over 24 hrs and to avoid repetitive dosing, alginate microspheres would be an efficient and successful means of delivering drugs which can control repetitive production of acid and chronic ulceritis.

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