

## PREPARATION AND CHARACTERIZATION OF FLOATING DRUG DELIVERY SYSTEM OF AZITHROMYCIN

SHAILENDRA WASNIK\*, POONAM PARMAR, DEEPIKA SINGH and ALPANA RAM

SLT Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur – 495 009 (C.G.), India

**Abstract:** The objective of present study was to develop a stomach drug delivery system of azithromycin (AZH) as a model drug for eradication of *Helicobacter pylori* (*H. pylori*). Floating microspheres of AZH were prepared by the solvent evaporation method. The prepared microspheres were subjected to evaluation for particle size, incorporation efficiency, *in vitro* buoyancy and *in vitro* drug release characteristics. The formulations were prepared at a variable stirring rate (300 to 500 rpm) and temperature (30–50°C). Surface morphology characteristics were studied using scanning electron microscopy (SEM). The mean particle size of the microspheres significantly increased with increasing polymer concentration and was in the range 252.26 ± 6.50 to 380.91 ± 4.59 µm. Angle of repose was between 26.42 to 35.83°. Tapped density ranged between 0.493 to 0.612 g/cm<sup>3</sup>. The compressibility index of all formulations was found to be in the range of 12.41 to 17.16%, which was < 20 indicating good flow characteristics. The encapsulation efficiency of the prepared microspheres was in the range of 27.8 ± 4.30 to 66.23 ± 2.08%. The physical state of the drug, before and after formulation was determined by differential scanning calorimetry (DSC). Percentage buoyancy of the microspheres was in the range 45.52 ± 0.69 to 68.71 ± 0.61% for 8 h. *In vitro* drug release studies were performed in simulated gastrointestinal fluid (SGF), pH 2.0 as dissolution medium (900 mL) for 8 h. Effects of stirring rate during preparation, polymer concentration and temperature on the size of microspheres and drug release were also observed. The results of the present studies indicated that the floating microspheres of AZH were formulated to provide site specific delivery of drug with a view to provide an effective and safe therapy for eradication of *H. pylori* with a reduced dose and reduced duration of therapy.

**Keywords:** azithromycin, *H. pylori*, floating microspheres, ethylcellulose, HPMC

In the development of oral controlled drug delivery system, one of the main challenges is to modify the GI transit time. Gastric emptying of pharmaceuticals is highly variable and is dependent on the dosage form and the fed/fasted state of the stomach. This has led to the development of oral gastroretentive dosage form. Floating drug delivery system (FDDS) is of particular interest for drugs, which (a) act locally in the stomach; (b) are primarily absorbed in the stomach; (c) are poorly soluble at an alkaline pH; (d) have a narrow window of absorption; and (e) are unstable in the intestinal or colonic environment (1). FDDSs remain buoyant due to their lower density than the gastric and intestinal fluids. Both single and multiple unit systems have been developed. Multiple unit FDDSs such as microspheres have advantage that they are not subjected to “all or nothing” gastric emptying nature of single unit systems (2, 3). Microspheres are useful as a drug carrier to improve the performance of ther-

apeutic system. Floating drug delivery is able to prolong the gastric retention of microspheres, thereby improving oral bioavailability of drugs.

For eradication of *Helicobacter pylori* (*H. pylori*), therapeutic agents have to penetrate the gastric mucus layer to disrupt and resist the mechanism of colonization. The gastro retentive drug delivery system will release the drug over an extended period in stomach and upper GIT, which will increase the opportunity of drug absorption, bioavailability and be useful in eradication of *H. pylori* by local action.

The objective of present study was to prepare floating microspheres of azithromycin to improve its bioavailability by increasing residence time in the stomach (4). It is macrolide antibiotic that is useful in eradicating *H. pylori* by local action. The prepared microspheres were evaluated for size, *in vitro* CM release, buoyancy and incorporation efficiency. The effect of various formulation variables on the size and drug release was investigated.

\* Corresponding author: Shailendra Wasnik, e-mail: shailendra.wasnik@gmail.com, phone: +91-8878073178, fax: +91-7752260148

Table 1. Batch specification of the floating microspheres.

Batch code	Polymer ratio (HPMC : EC)	Temperature (°C)	Solvent ratio
GC1 <sup>a</sup>	1:1	37	1:1
GC2 <sup>a</sup>	1:2	37	1:1
GC3 <sup>a</sup>	1:3	37	1:1
GC4 <sup>a</sup>	1:4	37	1:1
GC1 <sup>b</sup>	1:1	37	1:1
GC2 <sup>b</sup>	1:2	37	1:1
GC3 <sup>b</sup>	1:3	50	1:1
GC4 <sup>b</sup>	1:4	50	1:1

Stirring rate: <sup>a</sup> = 300 rpm, <sup>b</sup> = 500 rpm.

Table 2. Micromeritics studies of the developed formulations.

Formulation code	Bulk density (g/cm <sup>3</sup> )	Tapped density (g/cm <sup>3</sup> )	Carr's index (%)	Angle of repose (°)
GC1 <sup>a</sup>	0.422	0.493	14.40	27.42
GC2 <sup>a</sup>	0.503	0.596	14.16	31.21
GC3 <sup>a</sup>	0.473	0.571	17.16	35.83
GC4 <sup>a</sup>	0.482	0.589	15.28	32.25
GC1 <sup>b</sup>	0.456	0.527	13.47	30.50
GC2 <sup>b</sup>	0.536	0.612	12.41	26.42
GC3 <sup>b</sup>	0.409	0.472	13.34	28.61
GC4 <sup>b</sup>	0.516	0.605	14.71	30.42

## MATERIALS AND METHODS

Azithromycin was procured from Schon Pharm. Pvt. Ltd., Indore, Madhya Pradesh (India) as a gift sample. Ethyl cellulose (EC) and hydroxypropylmethyl cellulose (HPMC) were obtained from S. D. Fine Chemicals Ltd. (Delhi, India) Ethanol was obtained from Welcome Distillery, Cheraka Bandha, Kota (India) and dichloromethane and Tween 80 from Central Drug House (P) Ltd. (Delhi, India). All other chemicals used were of analytical grade.

### Preparation of microspheres

Drug, HPMC and EC were dissolved in a mixture of ethanol and dichloromethane (1:1, v/v) at room temperature. This was poured slowly as a thin stream into 150 mL of 0.01% Tween 80 maintained at 30–50°C. The emulsion was continuously stirred at a variable rotation speed for 1 h to allow the volatile solvents to evaporate. The floating microspheres

were collected by decantation while the non-floating microspheres were discarded along with polymer residues. The collected microspheres were dried overnight in an oven at 40°C (5). The composition of the floating microspheres is shown in Table 1.

### Characterization of floating microspheres Shape and surface morphology

The external and internal morphology of the floating microspheres were studied by scanning electron microscopy (FEI Quanta – 200 MK2, Netherland) at SAIF, Bose Institute, Kolkata (W.B). The sample for SEM was prepared by sticking the beads on a double adhesive tape, which stuck to an aluminum stub. The samples were then kept inside the vacuum chamber, scanned and photomicrographs were taken. Here it may be noted that the gold coating was not necessary as the samples were operated under low vacuum conditions.

### Differential scanning calorimetry

DSC studies of drug, polymers and optimized microspheres were obtained on Jade differential scanning calorimeter. Samples of 5–10 mg were placed in an aluminum pan and sealed. The probe was heated from 30 to 200°C at 10°C/min. Endotherms of the pure drug and polymers gave sharp peak at particular region corresponding to its melting point.

### Particle size analysis

The particle size of microspheres was determined using an optical microscope with calibrated ocular micrometer. The mean particle size was calculated by measuring 100 particles of each formulation (6).

### Micromeritics studies

#### Angle of repose

The angle of repose of the microspheres was determined by funnel method and was calculated by using the equation:

$$\tan \theta = S/D \quad \theta = \tan^{-1} S/D$$

where S = surface area of the free standing height of the microspheres heap and D = diameter of the heap.

### Bulk density

A weighed amount of microspheres were introduced in a 10 mL measuring cylinder. Bulk density was determined by a ratio of mass of microspheres to bulk volume (6):

$$D_b = M/V_b$$

where  $D_b$  = bulk density, M is the mass of microspheres and  $V_b$  is the bulk volume.

### Tapped density

Weighed amount of microspheres was introduced in a 10 mL measuring cylinder and cylinder was then tapped from height of 2 cm until the time when there was no more decrease in the density and the volume of the microspheres was calculated (6) by the following equation:

$$D_T = M/V_T$$

where  $D_T$  = bulk density, M is the mass of microspheres and  $V_T$  is the bulk volume.

### Carr's (compressibility) index

This parameter was calculated as follows:

$$\text{Compressibility index} = (D_T - D_p)/D_T \times 100$$

where  $D_T$  = bulk density,  $D_p$  is the tapped density of microspheres.

Table 3. Various formulation parameters of floating microspheres.

Formulation code	Particle size ( $\mu\text{m}$ )	Percentage yield	Incorporation efficiency (%)	Buoyancy (%)
GC1 <sup>a</sup>	258.07 $\pm$ 9.86	79.14 $\pm$ 0.06	34.26 $\pm$ 2.80	53.69 $\pm$ 0.99
GC2 <sup>a</sup>	292.16 $\pm$ 9.39	76.34 $\pm$ 0.09	39.86 $\pm$ 1.45	55.38 $\pm$ 1.02
GC3 <sup>a</sup>	320.78 $\pm$ 8.85	86.32 $\pm$ 0.10	53.06 $\pm$ 6.04	62.55 $\pm$ 1.07
GC4 <sup>a</sup>	380.91 $\pm$ 4.59	84.35 $\pm$ 0.12	66.23 $\pm$ 2.08	68.71 $\pm$ 0.61
GC1 <sup>b</sup>	252.26 $\pm$ 6.50	80.46 $\pm$ 0.25	46.23 $\pm$ 1.95	48.56 $\pm$ 1.40
GC2 <sup>b</sup>	279.78 $\pm$ 2.55	74.62 $\pm$ 0.30	27.80 $\pm$ 4.30	54.56 $\pm$ 0.54
GC3 <sup>b</sup>	266.78 $\pm$ 5.86	73.63 $\pm$ 0.20	36.36 $\pm$ 2.48	53.18 $\pm$ 0.85
GC4 <sup>b</sup>	317.03 $\pm$ 8.58	75.69 $\pm$ 0.21	38.36 $\pm$ 4.72	45.52 $\pm$ 0.69

Results are expressed as the mean  $\pm$  SD (n = 3).

Table 4. Effect of storage on various parameters of floating microspheres after 30 days at different temperature.

Parameter	Formulation code	(2–8°C)	Observation at 25 $\pm$ 2°C	45 $\pm$ 1°C
Particle size	GC3 <sup>a</sup>	348.23 $\pm$ 6.87	317.52 $\pm$ 5.95	272.20 $\pm$ 8.54
	GC4 <sup>a</sup>	409.45 $\pm$ 9.34	373.11 $\pm$ 7.06	313.50 $\pm$ 4.92
Buoyancy studies	GC3 <sup>a</sup>	62.34 $\pm$ 1.67	59.86 $\pm$ 0.85	–
	GC4 <sup>a</sup>	71.45 $\pm$ 1.12	66.32 $\pm$ 0.73	–

### Percentage yield

The prepared microspheres were collected and weighed. The measured weight was divided by the total amount of all non-volatile components, which were used for the preparation of the microspheres (7).

$$\% \text{ Yield} = \left( \frac{\text{Actual weight of products}}{\text{total weights of excipients and drug}} \right) \times 100$$

### Drug entrapment efficiency (DEE)

A weighed amount of AZH loaded microspheres was dissolved in small amount of ethanol. The drug was extracted into SGF, pH 2.0. Ethanol was evaporated and the absorbance of the resulting solution was measured at 482 nm using UV-spectrophotometer (Shimadzu UV 1800, Japan) (7). All experiments were performed in triplicate. The percentage drug entrapment was calculated as follows:

$$\% \text{ Drug entrapment} = \left( \frac{\text{Calculated drug conc.}}{\text{Theoretical drug content}} \right) \times 100$$

### In vitro buoyancy studies

The microspheres (about 0.3 g) were spread over the surface of USP basket dissolution test appa-

ratus (Dissolution rate test apparatus USP/IP/ BP STD, Jyoti Scientific Laboratories, Gwalior) which was filled with 900 mL of SGF, pH 2.0 containing 0.02% of Tween 80, in which paddle was rotating at 100 rpm for 8 h. Tween 80 served to mimic the effect of natural surfactants in the stomach. The floating and the settled portions of the floating microspheres were recovered separately, dried and weighed. All experiments were performed in triplicate. Buoyancy was calculated as the ratio of the mass of the microspheres that remained floating and the total mass of the microspheres (7).

$$\text{Buoyancy (\%)} = \frac{Q_f}{(Q_f + Q_s)} \times 100$$

where  $Q_f$  and  $Q_s$  are the weights of the floating and the settled microspheres, respectively.

### In vitro drug release studies

The drug release studies equivalent to 100 mg of AZH were performed using USP basket apparatus (Jyoti Scientific Laboratories, Gwalior) rotating at 100 rpm in SGF, pH 2.0 as dissolution medium (900 mL) maintained at  $37 \pm 0.5^\circ\text{C}$ , microspheres were filled in hard gelatin capsules. Perfect sink conditions prevailed during the drug release study

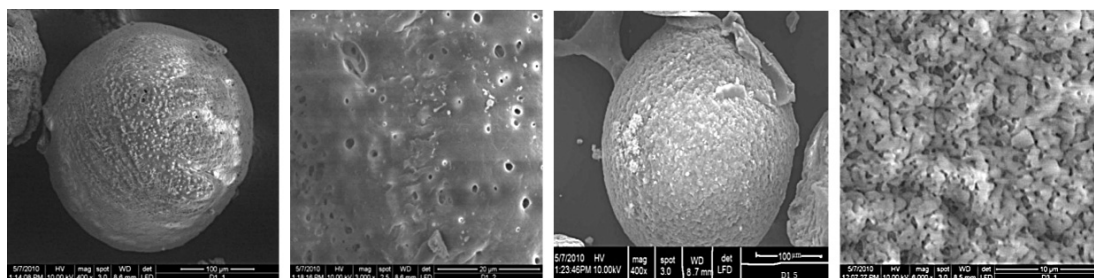


Figure 1. SEM of floating microspheres of different formulations. SEM 1: SEM image of formulation GC3<sup>a</sup>; SEM 2: SEM of surface morphology of formulation GC3<sup>a</sup>; SEM 3: SEM image of formulation GC4<sup>a</sup>; SEM 4: SEM image of surface morphology of formulation GC4<sup>a</sup>

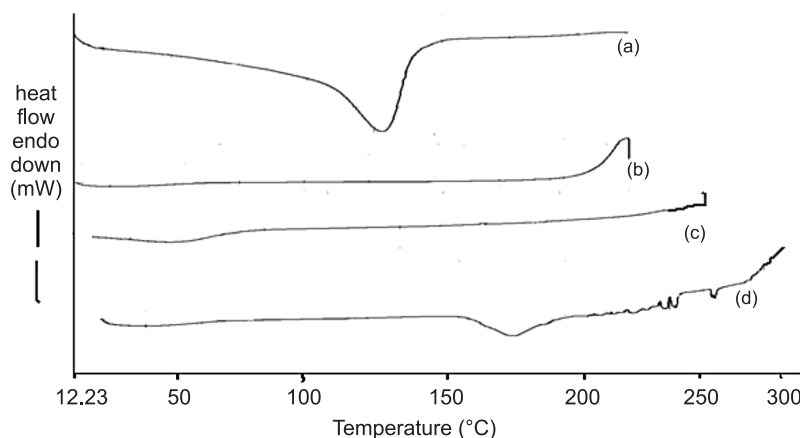


Figure 2. Comparison among DSC thermograms of pure (a) AZH, (b) EC, (c) HPMC and (d) optimized formulation (GC4<sup>a</sup>).

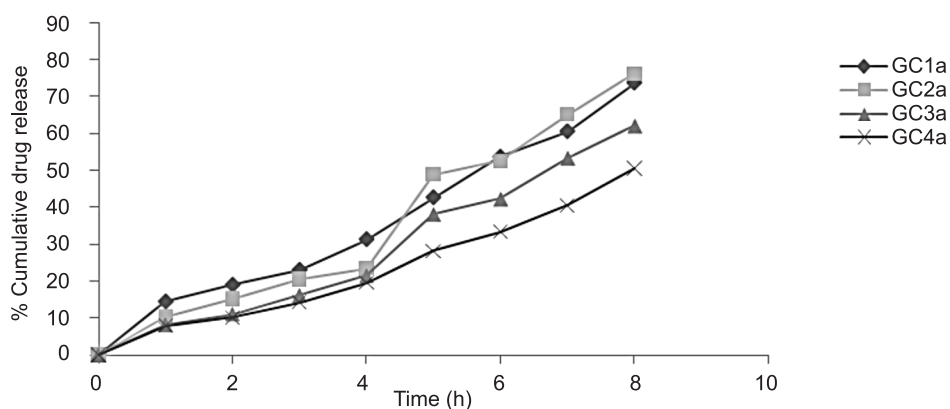


Figure 3. Effect of polymer concentration on *in vitro* release of AZH from floating microspheres

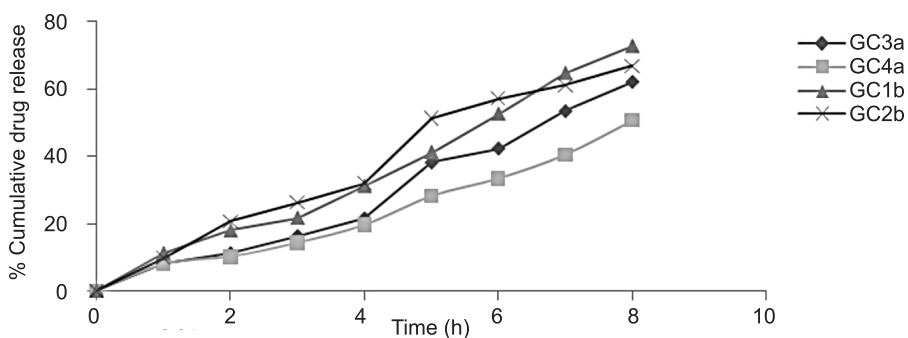


Figure 4. Effect of stirring rate on *in vitro* release of AZH from floating microspheres

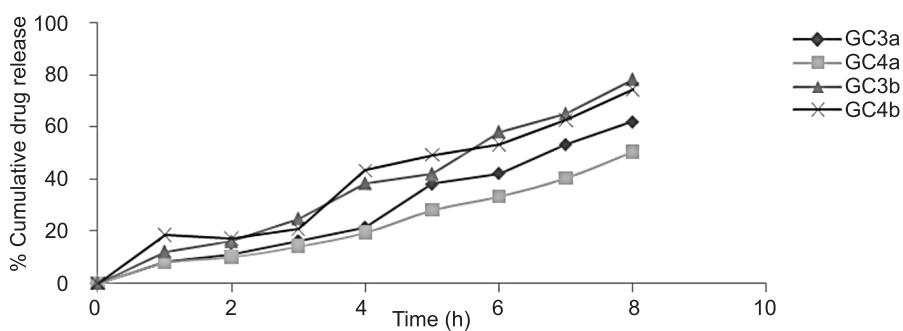


Figure 5. Effect of temperature on *in vitro* release of AZH from floating microspheres

(5). Five mL samples were withdrawn at each 30 min interval, passed through a 0.25  $\mu\text{m}$  membrane filter (Millipore), and analyzed at 482 nm spectrophotometrically after suitable dilution (8).

#### Stability studies

Stability studies were carried out according to ICH guidelines. The stability studies were carried out on two selected formulations i.e., GC3<sup>a</sup> and GC4<sup>a</sup>. Formulations were stored in amber colored

glass bottles for period of 30 days at room temperature ( $25 \pm 2^\circ\text{C}$ ), at refrigeration temperature (2 to  $8^\circ\text{C}$ ) and at  $45^\circ\text{C}$  for one month. The samples were assayed for particle size, *in vitro* floating ability and *in vitro* drug release.

#### RESULTS AND DISCUSSION

Floating microspheres were prepared by the emulsification solvent-evaporation technique using

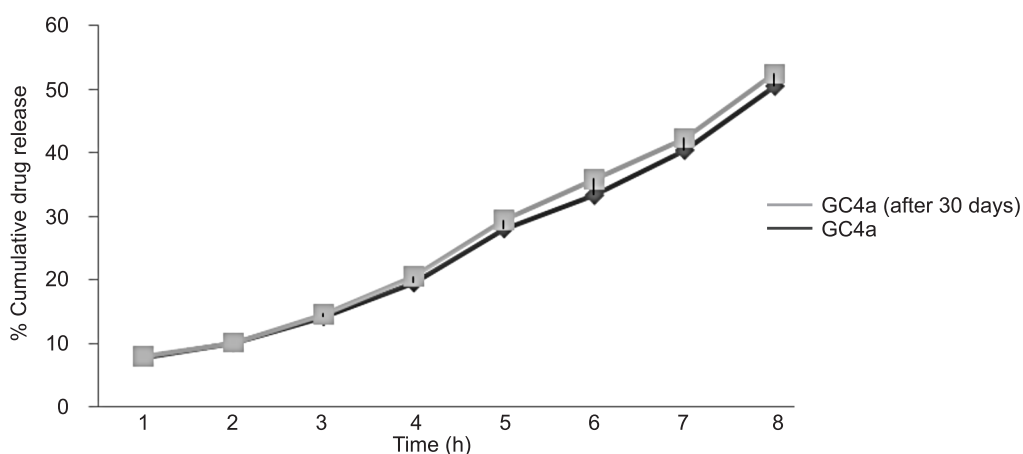


Figure 6. Effect of stability on percent drug release of microspheres after 30 days at room temperature on formulation GC4<sup>a</sup>

EC and HPMC as polymers, their concentration was increased, to assess the effect on the size of microspheres. The batch specifications are shown in Table 1.

The floating microspheres were predominantly spherical in shape from their SEM photographs having rough surface along with pores (Fig. 1). Their sphericity contributed considerably to their very good flow properties. Distinct pores are evident on the surface of microspheres, which will be responsible for the release of drugs or an increase in number and size after dissolution; it shows that the drugs leach out through these channels (9). The photomicrographs also showed the presence of loose crystals of drug on the surface of few microspheres (10).

Thermograms of AZH, HPMC, EC and, optimized floating microsphere formulations were recorded in a differential scanning calorimeter to characterize the solid state of the drug in the microspheres. DSC study was carried out to detect possible interactions between the drug and polymer. The thermograms of AZH showed a sharp melting point at 123.06°C. The endotherms of EC was found to be 43.056°C. Thermograms of HPMC showed peak at 63.068°C. Polymers EC & HPMC used in the preparation did not show any interference with azithromycin in the formulations.

The mean particle size of the microspheres significantly increased with increasing polymer concentration and was found in the range of 252.26 ± 6.50 to 380.91 ± 4.59 μm as shown in Table 3. The variation in mean particle size could be due to variation in drug-polymer ratio. The viscosity of the medium increases at a higher polymer concentration resulting in enhanced interfacial tension. Shearing

efficiency is also diminished at higher viscosities. This results in the formation of larger particles (5). The formulation GC4<sup>a</sup> had the highest particle size. As the stirring rate was increased, the particle size decreased.

Angle of repose was between 26.42 to 35.83°. Tapped density ranged between 0.493 to 0.612 g/cm<sup>3</sup>. High Carr's index is indicative of the tendency to form bridges. The compressibility index of all formulations was found to be in the range of 12.41 to 17.16%, which was < 20 indicating good flow characteristics (Table 2). The good flow property of microspheres indicates that the floating microspheres produced are nonaggregated. Flotation might have been influenced by the low bulk and tapped densities (11).

The production yield of floating microspheres was greater than 73% for all the formulations and was in the range of 73.63 ± 0.20 to 86.32 ± 0.10% as shown in Table 3. Stirring rate and temperature also affected the percentage yield, which may be observed by the values in the table. At a high temperature and stirring speed, the shell was destructed by insufficient diffusion of ethanol into aqueous solution and simultaneous evaporation of dichloromethane.

The drug entrapment of AZH in all formulations was satisfactory at the levels of loading (Table 3). The encapsulation efficiency of the prepared microspheres was in the range of 27.8 ± 4.30 to 66.23 ± 2.08%. The high entrapment efficiency of drug is believed to be due to its poor aqueous solubility which facilitates the diffusion of a part of entrapped drug to surrounding medium during preparation of floating microspheres (12).

Encapsulation efficiency of GC4<sup>a</sup> was found to be the highest ( $66.23 \pm 2.08\%$ ). Encapsulation efficiency rose with an increase in EC concentration, the viscosity of polymer solution also increased and was found responsible for formation of larger microspheres (13). The extent of loading was also influenced by the particle size of microspheres.

The microspheres floated for prolonged time over the surface of the dissolution medium without any apparent gelation. Buoyancy (%) of the microspheres was in the range  $45.52 \pm 0.69$  to  $68.71 \pm 0.61\%$  at the end of 8 h (Table 3). It was observed that larger size microspheres GC4<sup>a</sup> showed the longer floating time. Flotation might have been influenced by the low bulk and tapped densities of the microspheres.

*In-vitro* drug release studies were performed in SGF pH 2.0 for 8 h. The cumulative release of AZH significantly decreased with increasing polymer concentration (batches GC1<sup>a</sup> – GC4<sup>a</sup>) (Fig. 3). The increased density of the polymer matrix at higher concentrations results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres are formed at a lower polymer concentration and have a larger surface area for faster drug release (5).

To observe the effect of agitation speed on the size of the resulting microspheres, formulations (batches GC3<sup>a</sup> – GC2<sup>b</sup>) were prepared at different stirring rate (a = 300 rpm, b = 500 rpm). The size of the resulting microspheres decreased with increasing agitation but the decrease was not statistically significant. It may be inferred that the agitation speed in the studied range was not able to break up the bulk of the polymer into finer droplets and the release rate also was not affected significantly (14). Azithromycin release was higher in the case of microspheres prepared at a higher agitation speed but the difference in drug release was not statistically significant (Fig. 4).

Significant effect of temperature was observed on the *in vitro* release of AZH. Release rate increased with temperature (Fig. 5). This may be due to faster diffusion of solvent in the droplet into aqueous phase and evaporation of ethanol immediately after introducing it into the medium. Size of microspheres decreased with temperature.

Stability studies were carried out with selected formulations GC3<sup>a</sup> and GC4<sup>a</sup> which were stored at different temperature i.e., at room temperature ( $25 \pm 2^\circ\text{C}$ ), at refrigeration temperature ( $2$  to  $8^\circ\text{C}$ ) and at

$45 \pm 2^\circ\text{C}$  for a period of 30 days. The particle size of formulations was determined by optical microscope. Particle size of microspheres varied from its original value at different temperatures, at  $2$  to  $8^\circ\text{C}$ , the particle size of formulation GC3<sup>a</sup> and GC4<sup>a</sup> were in the range of  $348.23 \pm 6.87$  and  $409.45 \pm 9.34 \mu\text{m}$ , which may be attributed to the absorption of the moisture at lower temperature (Table 4). The particle size of fresh formulations GC3<sup>a</sup> and GC4<sup>a</sup> were in the range  $320.78 \pm 8.85$  and  $380.91 \pm 4.59 \mu\text{m}$  (Table 3). After 30 days, the effects of different temperatures were also seen on buoyancy study, the flotation increased at refrigeration temperature, not much difference was observed at room temperature. There was no more change in release rate of formulation stored at room temperature as shown in Figure 6. The rate of drug release for formulations stored at  $25 \pm 2^\circ\text{C}$  was increased as compared to fresh formulations. These storage conditions do not employ more drastic change on the microsphere integrity. After observing all the parameters, the most suitable condition of storage for selected formulations were found to be  $25 \pm 2^\circ\text{C}$  (room temperature) which was used in the study.

## CONCLUSION

In the present work, floating microspheres of AZH were formulated to provide site specific delivery of the drug and reduced duration of therapy. The developed floating microspheres of AZH have excellent buoyant ability, high encapsulation and good release pattern. The slow but complete drug release in the stomach is expected to increase bioavailability of the drug as well as its complete utilization, which may result in lowering the dose and GI side effects.

It can be concluded that optimized multi-unit AZH microspheres (GC4<sup>a</sup>) floated for more than 8 h in stomach. The floating microsphere is a potential system for delivery of AZH in the stomach. So it is expected to provide clinicians with a new choice of an economical, safe and low dose formulation in the management of eradication of *H. pylori*.

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