Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/ajps/default.asp

Original Research Paper

Development and characterization of amoxicillin loaded floating microballoons for the treatment of *Helicobacter pylori* induced gastric ulcer

Rajendra Awasthi [a,b,*], *Giriraj T. Kulkarni* [b]

[a] *Department of Pharmaceutical Science, Jawaharlal Nehru Technological University, Kukatpally 500085, India*
[b] *Laureate Institute of Pharmacy, Tehsil – Dehra, Distt. Kangra, Kathog 177101, Himachal Pradesh, India*

ARTICLE INFO

Article history:
Received 10 April 2013
Received in revised form
22 May 2013
Accepted 10 June 2013

Keywords:
Floating
Gastric ulcer
Gastroretentive
H. pylori
Micromeritic
Microballoons

ABSTRACT

The current communication reports *in vitro* characterization of the optimized hollow floating microballoons of amoxicillin on the basis of micromeritic properties and *in vitro* minimum inhibitory concentration (MIC). Amoxicillin loaded hollow microballoons were prepared by emulsion solvent diffusion method. The morphological characterization was done on the basis of scanning electron microscopy (SEM). Fourier Transformed Infrared Spectroscopy (FTIR) was used to investigate drug–polymer interactions. The correlation between the *in vitro* buoyancy of microballoons and their physical properties, e.g. density and porosity were elucidated. The results of FTIR spectroscopy revealed the absence of any drug–polymer interactions. The porosity values of more than 69% and diameter to thickness ratio greater than 2.90, proved a high cavity volume within the microballoons in all size ranges. The spherical shape of microballoons with hollow internal cavity was confirmed from SEM photomicrographs. The *in vitro* MIC results showed a sustained drug effect from the microballoons. In conclusion, it can be said that the developed microballoons can be used for the effective treatment of *Helicobacter pylori* induced gastric ulcer.

1. Introduction

Marshall and Warren first isolated *Helicobacter pylori* (*H. pylori*), a gram-negative bacterium, in 1982 from the gastric mucus of patients with chronic gastritis and duodenal ulcer [1]. As per the World Health Organization and International Agency for Cancer Research, *H. pylori* is the main etiologic factor in the development of gastric ulcer and gastric carcinoma [2,3]. The complete eradication of *H. pylori* from the deep gastric mucosa has remained a challenge due to the drawbacks of currently available conventional dosage forms as these conventional systems have a short residence time in the stomach. *H. pylori*

* *Corresponding author*. Tel.: +91 9459234530 (mobile).
E-mail address: awasthi02@gmail.com (R. Awasthi).
Peer review under responsibility of Shenyang Pharmaceutical University

ELSEVIER Production and hosting by Elsevier

1818-0876/$ – see front matter © 2013 Shenyang Pharmaceutical University. Production and hosting by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ajps.2013.07.023

infections can be successfully treated if antibiotics are retained in the stomach for 1 h. The absorption of an antibiotic into the mucus through the mucus layer is believed to be more effective for *H. pylori* eradication than absorption through the blood [4]. Eradication of the *H. pylori* resulted in ulcer healing and prevention of peptic ulcer recurrence [5].

Several approaches such as mucoadhesion, swelling, high density, raft forming, expandable, magnetic, gas generating, and superporous hydrogels have been investigated to improve the bioavailability of drugs by increasing the residence time of dosage form in the gastric region [6]. It has been reported that the released drug will have the whole surface area of the stomach and small intestine for absorption and thus the absorption of the drug can be enhanced [7]. Multiparticulate drug delivery systems usually show numerous advantages over monolithic dosage forms such as a higher degree of dispersion in the gastrointestinal tract leads to the reduced risk of systemic toxicity due to dose dumping and avoid the "all or none" emptying pattern of single unit systems [8,9]. A major drawback of these systems is the requirement of sufficient volume of gastric fluid and acid within the stomach to enable the device to float [10–12]. Since, an optimum gastroretentive floating system is expected to retain in the stomach for sufficient time against all the physiological barriers and finally metabolized and eliminated from the body. To fulfill all these requirements, the system should possess physical properties like being smaller in size, high buoyancy, and drug encapsulation along with the controlled release of drug in the gastric environment [6]. Amoxicillin, is rapidly absorbed after oral administration, has a very short half life (~1 h). Thus it possesses all the characteristic drug properties required to develop a gastroretentive controlled release system.

We have previously reported optimization studies on gastroretentive floating system of amoxicillin using central composite design and response surface methodology. A nonlinear quadratic polynomial model was generated for precise evaluation of the effects of independent variables on dependent variables using Design Expert v7.1.5 software (Stat-Ease, Inc., Minneapolis, Minnesota) [13]. The current communication deals with the further *in vitro* characterization of the optimized formulation based on its micromeritic properties and *in vitro* anti *H. pylori* activity.

2. Materials and methods

2.1. Materials

Amoxicillin was a gift from Win-Medicare (Meerut, India). CAP and polyvinyl alcohol were purchased from Central Drug House (New Delhi, India). Eudragit S100 was purchased from Evonik Degussa (Mumbai, India). All the other reagents and solvents used were of analytical grade.

2.2. Optimized method for the preparation of microballoons

Microballoons were prepared by the emulsion solvent diffusion method reported by Awasthi et al. [13]. Briefly, amoxicillin and Eudragit S100 in a ratio of 1:2.5 (200 mg: 500 mg) and CAP (700 mg) were dissolved in a mixture of dichloromethane, ethanol and isopropyl alcohol (7:6:2) at room temperature (15 ml). This solution was introduced to an aqueous solution of polyvinyl alcohol (0.5% w/v, 200 ml) at 40 °C, forming oil in water type emulsion. The resultant solution was stirred, employing a mechanical stirrer (LT400A, Yamato, Japan) at 300 rpm. Finally dispersed droplets were solidified in the aqueous phase via diffusion of the solvent. Dichloromethane, evaporated from the solidified droplets was removed by drying in hot air oven at 40 °C overnight, leaving hollow structure inside the microballoons. After agitating the system for 1 h at 300 rpm, the resulting polymeric particulate systems were filtered and dried overnight at 40 °C in hot air oven to produce hollow microballoons.

2.3. Characterization of the optimized microballoons

Separation of the optimized microballoons into three different fractions was carried out using a mechanical sieve shaker. The British Standard Sieves (BSS) were arranged in ascending order i.e. from sieve no # 85–170 (180–90 μm), # 170–300 (90–53 μm) and the third fraction was collected from the bottom of the sieve no 300 and this fraction consist of particles those were passed through the sieve no 300. The nest of sieves was shaken on sieve shaker for 15 min. All three different fractions were collected, weighed and evaluated for their micromeritic characterization such as bulk density, tapped density, particle density, Housner's ratio, percent porosity, *D/T* ratio, Carr's index and angle of repose. The microballoons were characterized for morphological examination by scanning electron microscopy (SEM). Studies for shape, drug content, percent buoyancy and drug release from different fractions were also conducted to ensure reproducibility of results based on particle size.

2.3.1. Determination of bulk density, tapped density and particle density

Different fractions of the optimized formulation (1 g) were taken into a 10 ml graduated measuring cylinder separately and the volume was noted down. The graduated measuring cylinder was tapped 50 times using USP bulk density apparatus (ETD 1020, Electrolab, Mumbai, India). The bulk density and tapped density were determined using the following formula [14]:

$$\text{Bulk density} = \frac{\text{Weight of the floating balloons}}{\text{Initial volume}}$$

$$\text{Tapped density} = \frac{\text{Weight of the floating microballoons}}{\text{Final volume after tapping}}$$

Particle density of different fractions was determined by the liquid displacement method [15] by suspending the microballoons in a solvent in which the microballoons were insoluble like distilled water.

2.3.2. Determination of Hausner's ratio

The density determinations were used to determine the Hausner's ratio by following formula:

$$\text{Hausners ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

2.3.3. Characterization of hollow structure of microballoons

The porosity (€) and diameter to thickness ratio (D/T) of the microballoons were used as the parameters for characterizing the hollow structure of microballoons.

$$\varepsilon = \frac{1-\rho_p}{\rho_t} \times 100$$

$$\frac{D}{T} = \frac{2}{\left(\frac{1-\varepsilon}{100}\right)^{1/3}}$$

where, ρ_p is particle density and ρ_t is true density.

2.3.4. Angle of repose

For the determination of angle of repose, the microballoons were poured through a funnel, which was fixed at a position such that its lower tip was at a height of 2 cm above the surface. The microballoons were poured till the time when the tip of the microballoons pile surface touched the funnel. The $\tan^{-1}$ of ratio the height of the pile and radius of its base gave the angle of repose. The angle of repose was determined by following formula:

$$\tan^{-1} = \frac{h}{r}$$

2.3.5. Carr's index

The density determinations were used to determine the Carr's index by following formula:

$$\text{Carrs index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

2.3.6. Determination of buoyancy

Fifty milligrams of microballoons were placed in 100 ml simulated gastric fluid (SGF, pH 1.2) containing 0.02% Tween 20. The mixture was stirred at 100 rpm on a magnetic stirrer. After 8 h, the floating and settled microballoons were collected separately, dried at 40 °C and weighed. The buoyancy was determined by the following formula:

$$\text{Buoyancy (\%)} = \frac{\text{Weight of floating microballoons}}{\text{Weight of floating microballoons} + \text{Weight of settled microballoons}} \times 100$$

2.3.7. Determination of drug content

Accurately weighed (10 mg) microballoons were crushed and dispersed into 25 ml phosphate buffer (pH 7.4) without any material loss. After 24 h shaking, the solution was filtered, and the filtrate was analyzed for drug content by a UV-spectrophotometer (UV 3000$^+$, LabIndia Instruments, Mumbai, India) at 227 nm after suitable dilution.

2.3.8. Fourier transform infrared (FTIR) spectroscopy

The optimized formulation was subjected to FTIR spectroscopy and spectra taken by an FTIR spectrophotometer (IR affinity-1, Shimadzu, Japan). The formulation was mixed with suitable amount of KBr and converted into pellets by using KBr press at 15 tons hydraulic pressure. The IR scanning of samples was done in between 4000 and 400 cm^{-1} and spectrum observed for any occurrence and disappearance of characteristic drug peak.

2.3.9. Scanning electron microscopy (SEM)

The external and internal morphology of the microballoons was studied by scanning electron microscopy (SEM) using a scanning election microscope (EVO-50, ZEISS, United Kingdom). The microballoons were adhered to an aluminum stub using a double adhesive tape. The stubs were then coated with silver under an argon atmosphere using a high-vacuum evaporator (Polaron SEM coating system). The photomicrographs of coated samples were taken randomly.

2.3.10. In vitro drug release study

The release rate from different fractions of the optimized formulation was determined using USP type II apparatus. Dissolution medium (SGF, pH 1.2, 500 ml) containing 0.02% Tween 20 was filled in the dissolution vessel and stirred at 50 rpm. The temperature was maintained at 37 ± 0.5 °C. A weight of microballoons equivalent to 50 mg of amoxicillin was placed in the dissolution vessel. Aliquots were withdrawn at every 15 min of the first hour and then at every hour till 4 h followed by 6 and 82 h. Samples were then analyzed by UV-spectrophotometer (UV 3000$^+$, LabIndia Instruments, Mumbai, India) at 228 nm. All the dissolution runs were conducted in triplicate. The release behavior of all the three fractions was compared with the optimized formulation (reference formulation) [13]. The mechanism of drug release from the microballoons was confirmed by Peppas' equation. Differences in *in vitro* drug release of amoxicillin from different fractions of the optimized formulation were statistically analyzed by one-way analysis of variance (ANOVA) with posttest (Tukey's multiple comparison test). Statistically significant difference between *in vitro* amoxicillin release from different fractions was defined as $p < 0.05$ level. Calculations were performed using GraphPad prism v5 software (GraphPad prism software Inc, San Diego, CA) [16].

2.3.11. Determination of minimum inhibitory concentration and duration of action

Minimum inhibitory concentration (MIC) of the optimized formulation was determined using ATCC 43504 culture of *H. pylori*. The strain was grown on Mueller-Hinton agar medium supplemented with 5% Fetal Calf Serum. The test sample was suspended in distilled water and added to tubes containing 1 ml of Mueller-Hinton broth at 5, 10, 20, 30, 40 and 50 μg/ml concentrations. Tubes were inoculated with 10 μl *H. pylori* suspension prepared in saline and incubated in the microaerophilic environment (CO_2 10%, O_2 5%, N_2 85%) at 37 °C for 5 days. The inoculum was prepared to contain 10^8 CFU/ml by adjusting the suspension to match the McFarland No 0.5

turbidity standard. The growth was observed as turbidity in the samples (+) and inhibition of growth was confirmed by the transparent appearance of medium (−). The duration of growth inhibition was also determined for a period of 84 h.

3. Results and discussion

Controlled release system of amoxicillin was designed and optimized to increase its residence time in the stomach without contact with gastric mucosa for effective eradication of *H. pylori* infection. Novel hollow microballoons were prepared via emulsion solvent diffusion method. The major advantage of preparation technique includes short processing time, the lack of exposure of drug to high temperature, due to which the drug stability increased during the processing and which ultimately lead to high encapsulation efficiencies of drug in the prepared system. The results show that the particle size of microballoons was closely related to the particle density, porosity and buoyancy as shown in Fig. 1. The satisfactory *in vitro* buoyancy may be attributed to the low bulk density (0.124–0.144) and optimum porosity of the microballoons (67.55–69.31). Fig. 1 shows that the true density and porosity of the microballoons increased with an increase in particle size. On the other hand the buoyancy of the microballoons decreased with an increase in particle size. This might be due to the increased porosity of the microballoons. This causes increased fluid uptake and leads to increased density of microballoons and thus microballoons sunk to the bottom.

3.1. Determination of bulk density, tapped density and particle density

The bulk density and tapped density were determined using USP bulk density apparatus and the results are represented in Table 1. The bulk density and tapped density of fraction F_2 and F_3 were found to be almost similar, indicating both the fractions have similar flow properties. The differences in bulk density and tapped density was only 0.01 and 0.02 in case of fraction F_2 and F_3 indicating that the change volume is very less even after 50 tapping, which confirms the small particle size range and reproducibility in drug content, whereas fraction F_1 has comparatively a large range of particle size. The particle density was measured by liquid displacement method by suspending the microballoons in distilled water. The observed particle density ranged from 0.063 to 0.049 g/cm^3, which is less than the specific gravity of the gastric fluid (1.004 g/cm^3), substantiating the buoyant properties of the microballoons. The effect of true density on the buoyancy of microballoons is represented in Fig. 1. The percent buoyancy of microballoons was increased from 88 to 94.72% as the particle size decreased.

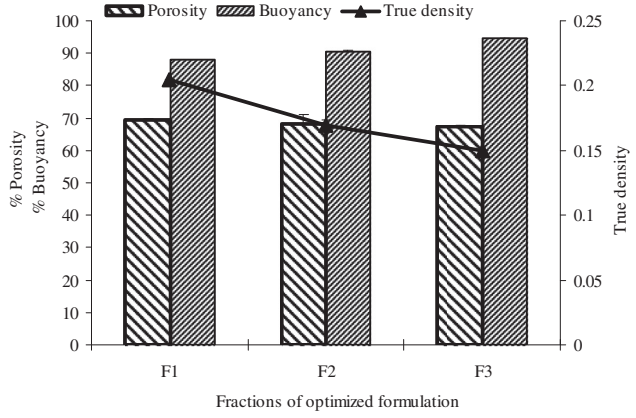

Fig. 1 – Relationship between particle size and micromeritic properties of the microballoons (mean ± SD, $n = 3$).

Table 1 – Evaluation parameter of fractioned formulation.

Parameters	Fractions		
	F_1 (180–90 μm)	F_2 (90–53 μm)	F_3 (Particle passed through sieve no 300)
Bulk density (g/cm^3)	0.124 ± 0.002	0.134 ± 0.001	0.144 ± 0.002
Tapped density (g/cm^3)	0.205 ± 0.002	0.169 ± 0.002	0.150 ± 0.001
Particle density (g/cm^3)	0.063 ± 0.00	0.054 ± 0.005	0.049 ± 0.0002
Hausner's ratio	1.650 ± 0.021	1.270 ± 0.021	1.046 ± 0.009
Percent porosity	69.31 ± 0.346	68.13 ± 2.83	67.55 ± 0.094
D/T ratio	2.95 ± 0.02	2.92 ± 0.12	2.90 ± 0.00
Angle of repose (θ)	33.25° ± 0.42	29.21° ± 0.35	28.21° ± 0.22
Carrs' index	39.51 ± 1.36	20.71 ± 0.56	14.40 ± 0.24
Percent buoyancy	88.00 ± 0.27	90.59 ± 0.42	94.72 ± 0.82
Drug content (mg/10 mg)	0.926 ± 0.05	1.023 ± 0.04	1.054 ± 0.03

3.2. Determination of Hausner's ratio

Hausner's ratio is related to inter-particle friction. Hausner's ratio is indirect measures of bulk density, size and shape, surface area, moisture content and cohesiveness of microballoons. A higher Hausner's ratio and more fine particles indicate greater cohesion between particles while a low range of Hausner's ratio indicates good flowability. The desirable value of Hausner's ratio is <1.25 for good flow of materials. The Hausner's ratio of three different fractions was determined and found to be in the range of 1.05–1.65. The fractions F_2 and F_3 exhibited good flow property. On the other hand fraction F_1 exhibited poor flow property. The flow properties can be arranged as $F_3 > F_2 > F_1$. An increase in Hausner's ratio is due to an increase in particle size, and this might be due to increased void space between the particles.

3.3. Characterization of hollow structure of microballoons

The microballoons were characterized by a cavity enclosed within a hard polymer shell due to the evaporation of dichloromethane. Diameter thickness ratio (D/T) is the significant parameter for defining the thickness of the wall of microballoons. Diameter to thickness ratio and percentage

porosity decreased as the size range decreased and this could be interpreted as a decrease in the size range resulted in an increase in thickness of the wall and hence the rigidity which consequently effected percentage buoyancy at the end of 8 h. This might be the reason for the increase in buoyancy of microballoons of smaller size range. The porosity values of more than 69% and *D/T* ratio greater than 2.90, proves a high cavity volume within the microballoons in all size ranges. Fig. 1 shows the relationship between percent porosity and percent buoyancy with respect to particle size of the microballoons. The percent porosity decreased and percent buoyancy increased as the particle size of the microballoons decreased.

3.4. *Angle of repose*

It is well known that particle size and shape influences flowability. The fine particles (<100 μm) tend to be more cohesive and therefore less free-flowing, whereas larger denser particles tend to be free-flowing. The rougher and more irregular the surface of the particles, the higher will be the angle of repose. In the present study, the angle of repose increased from 28.21° ± 0.22 to 33.25° ± 0.42 as the particle size increased, indicating the decrease in flowability of microballoons. This also supported by the results of Hausner's ratio study. Characterizing the flow property, angle of repose values of all microballoons did not exceed 35° and the microballoons were accepted as free-flowing.

3.5. *Carr's index*

A high Carr's index is indicative of the tendency to form bridges between the particles. Smaller the Carr's index, better will be the flow properties, for example, a value of 5–15 indicates excellent, 12–18 good, 19–21 fair and 22–35 poor flow, 36–40 very poor and >40 extremely poor. The results show that the fraction F_2 and F_3 had good and excellent flow property, respectively, which is also supported by Hausner's ratio. On the other hand fraction F_1 exhibited poor flow property.

3.6. *Determination of buoyancy*

The buoyancy test was carried out to investigate the floatability of the prepared microballoons as it is an important factor responsible for sustained drug delivery for the gastric region. The floating ability varied according to the fraction tested. The buoyancy of microballoons, till the end of 8 h in SGF (pH 1.2) containing 0.02% w/v Tween 20, was found to be 88.00%, 90.00% and 94.72%, respectively, when the average particle size was 1180–425 μm, 425–180 μm and 180–90 μm. In fraction F_3, Eudragit S100 presented in lowest amount as compared to F_2 and F_3, resulting higher value of percentage buoyancy for fraction F_3. The low buoyancy of fraction F_1 might be due to the large particle size, high particle density and more *D/T* ratio which leads to easy penetration of media. The percentage buoyancy is a factor of polymer concentration in the wall of microballoons.

3.7. *Determination of drug content*

The drug content increased as the size range of the microballoons decreased. It was found that fraction F_1 had the lowest amount of drug as compared with fraction F_2 and F_3. This might be due to the diffusion of drug in the aqueous medium, as a result of increased permeability of the wall of microballoons in fraction F_1. These results again confirmed the effect of Eudragit S100 on the drug content.

3.8. *Fourier transform infrared (FTIR) spectroscopy*

FTIR spectroscopy studies were carried out to ascertain that the processing condition has not led to any interaction between the drug and the polymer in the formulation. The formulation was subjected to FTIR spectroscopy to find out any possible interaction due to the process condition. The FTIR spectrum of drug, polymers, physical mixture and formulation are shown in Fig. 2. No sign of any loss or generation of any characteristic peak in the FTIR spectrum of physical mixture or formulation was observed; on a comparison with the FTIR spectrum of pure amoxicillin. This

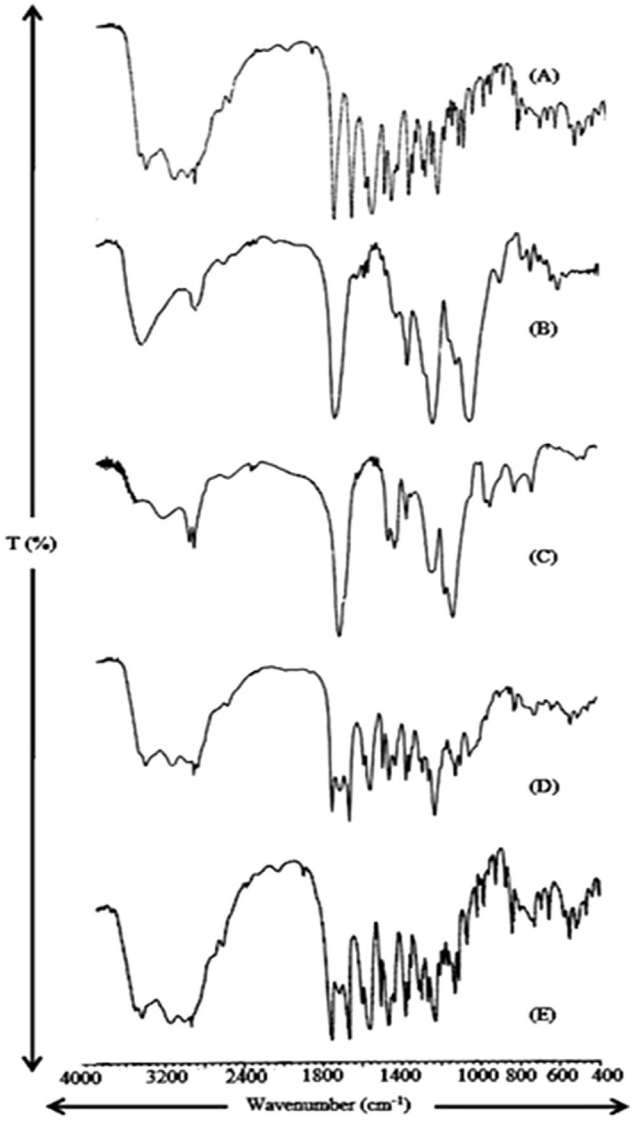

Fig. 2 – FTIR spectra of amoxicillin, Eudragit S100, cellulose acetate phthalate, physical mixture and formulated microballoons.

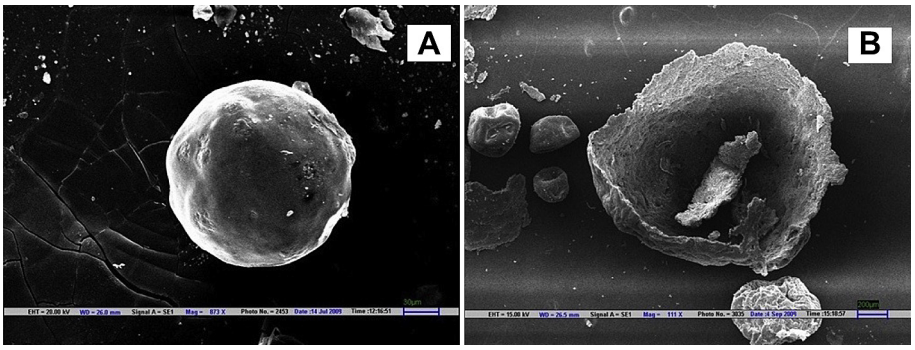

Fig. 3 – Scanning electron micrographs of floating microballoons showing general appearance (A) and internal hollow structure (B). Scales are given on individual micrograph.

suggested that all the process conditions were relevant to the formation of microballoons and which in turns showing the absence of any kind of interaction in between the process condition, drug and excipients.

3.9. *Scanning electron microscopy (SEM)*

From the SEM micrographs it is apparent that the amoxicillin loaded microballoons were predominately spherical in appearance. The surface was observed to be smooth, dense and less porous, whereas the internal core was highly porous and irregular with numerous depressions that are expression of evaporation of water, ethanol and dichloromethane (Fig. 3). The smooth outer surface and highly porous internal core was mainly influenced by the diffusion of ethanol and evaporation of dichloromethane from the polymer matrix. The evaporation of dichloromethane appeared to be related to cavity formation in microballoons. The less porous outer surface and highly porous internal surface supported controlled release of drug from the microballoons and good buoyancy.

3.10. In vitro *drug release*

In vitro drug release studies were carried out for all the three fractions (F_1, F_2 and F_3) using USP type II apparatus. Fig. 4 shows the drug release profiles of fraction F_1, F_2 and F_3. Both the polymers (Eudragit S100 and cellulose acetate phthalate) in combination showed excellent control on the release of drug from the microballoons in simulated gastric fluid (pH 1.2) due to their enteric nature. The mechanism of drug release from the microballoons was confirmed by Peppas' equation. According to the logarithmic form of Peppas' equation, the rate of drug release can be expressed as:

$$\log Q = \log K + n \log t$$

where Q is the amount of drug released, t is the time, K is a kinetic constant that incorporates the structural and geometric characteristics of the release device and n is the slope of the linear plot. If the value of n is less than or equal to 0.5, the mechanism of drug release is diffusion without swelling. If the value is greater than 0.5 and less than 1, the release is through diffusion with swelling and if it is above 1, the release mechanism is anomalous diffusion, not confirming to Fick's laws (non-Fickian) [13,16]. In this study, the Peppas' plots (log percent release against log time) were straight line for all the fractions, with correlation coefficients ranging between 0.987 and 0.992 and slope "n" values less than 0.5 (Table 2). This confirms that the release mechanism of drug from the prepared microballoons was Fickian diffusion without swelling. On application of one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, there was no significant difference observed between the *in vitro* drug release profiles of amoxicillin from the different fractions (F_1, F_2 and F_3) at 95% confidence interval ($p < 0.05$) as the calculated F value (0.006213) was found smaller than the tabulated value (2.21). The results of Tukey's multiple comparison test showed that there was no significant difference between the *in vitro* drug release profile of amoxicillin from the different fractions (F_1, F_2 and F_3) at 95% confidence interval ($p < 0.05$) because the calculated q values are smaller than the tabulated values.

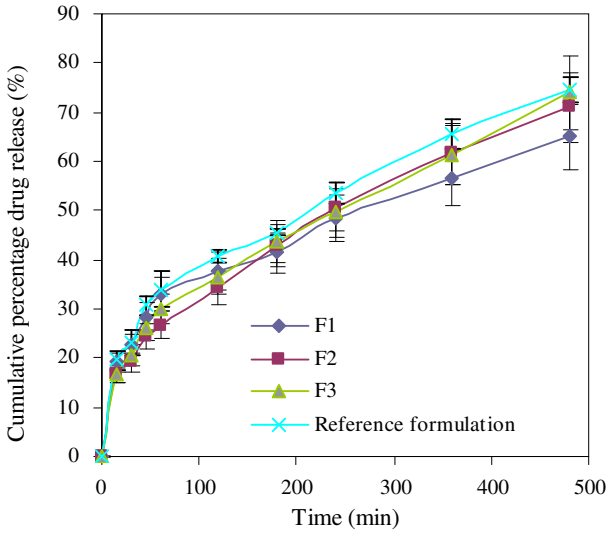

Fig. 4 – Drug release profiles of fractions F_1, F_2, F_3 and reference formulation in simulated gastric fluid (pH 1.2) at 37 °C (mean ± SD, $n = 3$).

Table 2 – Results of Peppas' equation kinetics parameters for drug release from different fractions of the optimized formulation.

Fraction code	Correlation coefficient (*r*)	Slope (*n*)	*K* value
F_1	0.986	0.342	0.875
F_2	0.987	0.429	0.676
F_3	0.992	0.420	0.711

Table 3 – Results of *in vitro* anti *H. pylori* study using ATCC 43504 culture.

Minimum inhibitory concentration (MIC)	Concentrations (μg/ml)	UT	5	10	20	30	40	50
	Test 1: amoxicillin	+	+	–	–	–	–	–
	Test 2: optimized formulation	+	+	+	–	–	–	–
Duration of action (20 μg/ml)	Time (h)	12	24	36	48	60	72	84
	Test 1: amoxicillin	–	–	–	+	+	+	+
	Test 2: optimized formulation	–	–	–	–	–	–	+

UT untreated; (+) Turbidity of the medium, indicating growth of the microorganism; (–) Transparent appearance of the medium, indicating inhibition of growth.

3.11. *Minimum inhibitory concentration (MIC) and duration of action*

The results of *in vitro H. pylori* growth inhibition study indicate that the optimized formulation exhibited lower inhibition (MIC 20 μg/ml) than the free drug (MIC 10 μg/ml) but for a long period of time (72 h). The results of MIC and duration of action of developed optimized formulation are shown in Table 3.

4. Conclusion

In the present work, hollow floating microballoons of amoxicillin were formulated. The optimized formulation was further characterized to ascertain the properties of microballoons to be used as a gastroretentive drug delivery system for local availability of drug. In consideration of Carr's index and Hausner's ratio, microballoons of fraction F_3 were appeared predominant in flowability. The developed microballoons exhibited excellent properties to be used as a gastroretentive dosage form. Also, *in vitro* anti *H. pylori* study confirmed that the developed system had good antimicrobial activity for a prolonged time period. Thus, from the results it can be concluded that the amoxicillin loaded hollow microballoons appears to have promising potential for localize drug release for effective treatment of *H. pylori* induced peptic ulcer.

REFERENCES

[1] Marshall BJ. Unidentified curved bacillus on gastric epithelium in active chronic gastritis. Lancet 1983;321(8336):1273–1275.

[2] Park S, Kim WS, Choi UJ, et al. Amelioration of oxidative stress with ensuing inflammation contributes to chemoprevention of *H. pylori* associated gastric carcinogenesis. Antioxid Redox Signal 2004;6:549–560.

[3] Sunanliganon C, Ngam DT, Tumwasorn S, et al. Lactobacillus plantarum B7 inhibits *Helicobacter pylori* growth and attenuates gastric inflammation. World J Gastroenterol 2012;18(20):2472–2480.

[4] Umamaheshwari RB, Ramteke S, Jain NK. Anti-*Helicobacter pylori* effect of mucoadhesive nanoparticles bearing amoxicillin in experimental gerbils model. AAPS Pharm Sci Tech 2004;5(2):60–68.

[5] Sepulveda AR, Coelho LGV. *Helicobacter pylori* and gastric malignancies. Helicobacter 2002;7:37–42.

[6] Pawar VK, Kansal S, Garg G, et al. Gastroretentive dosage forms: a review with special emphasis on floating drug delivery systems. Drug Deliv 2011;18(2):97–110.

[7] Awasthi R, Kulkarni GT. Development of novel gastroretentive floating particulate drug delivery system of gliclazide. Curr Drug Deliv 2012;9(5):437–451.

[8] Badhan AC, Mashru RC, Shah PP, et al. Development and evaluation of sustained release gastroretentive minimatrices for effective treatment of *H. pylori* infection. AAPS Pharm Sci Tech 2009;10(2):459–467.

[9] Varshosaz J, Tabbakhian M, Zahrooni M. Development and characterization of floating microballoons for oral delivery of cinnarizine by a factorial design. J Microencapsul 2007;24(3):253–262.

[10] Shah SH, Patel JK, Patel NV. Gastroretentive floating drug delivery systems with potential herbal drugs for *Helicobacter pylori* eradication: a review. J Chin Integr Med 2009;7(10):976–982.

[11] Waterman KC. A critical review of gastric retentive controlled drug delivery. Pharm Dev Technol 2007;12:1–10.

[12] Awasthi R, Pawar V, Kulkarni GT. Floating microparticulate systems: an approach to increase gastric retention. Indian J Pharm 2010;1(1):17–26.

[13] Awasthi R, Kulkarni GT, Pawar VK, et al. Optimization studies on gastroretentive floating system using response surface methodology. AAPS Pharm Sci Tech 2012;13(1):85–93.

[14] Wells JI, Aultom ME. Pharmaceutical preformulation. In: Aulton ME, editor. Pharmaceutics: the design and manufacture of medicines. 3rd ed. London: Elsevier, Churchill Livingstone; 2007. p. 336–360.

[15] Martin A. Physical pharmacy: physical chemical principles in pharmaceutical science. 4th ed. New Delhi: B.I. Waverly Pvt. Ltd.; 1998.

[16] Awasthi R, Kulkarni GT. Development of novel gastroretentive drug delivery system of gliclazide: hollow beads. Drug Dev Ind Pharm 2013:1–11. http://dx.doi.org/10.3109/03639045.2013.763817. Early Online.