IJPSR (2019), Volume 10, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 January 2019; received in revised form, 28 July 2019; accepted, 18 August 2019; published 01 October 2019

CHARACTERIZATION AND EVALUATION OF NIZATIDINE FLOATING MICROSPHERES BASED DRUG DELIVERY SYSTEM FOR ANTI-ULCER ACTIVITY

Sanjay Kumar Mishra * and M. K. Gupta

Oriental College of Pharmacy & Research, Oriental University, Indore - 453555, Madhya Pradesh, India.

Keywords:

Floating drug delivery systems, Gastric residence time, *in-vitro* and *in-vivo*

Correspondence to Author:
Sanjay K. Mishra

Research Scholar, Oriental College of Pharmacy & Research, Oriental University, Indore - 453555, Madhya Pradesh, India.

E-mail: mishra_sanjay87@rediffmail.com

ABSTRACT: **Objective:** The purpose of the present study to develop gastro-retentive drug delivery formulation for enhancing GRT, including the physiological and formulation variables affecting gastric retention. It is a widely employed approach to retain the dosage form in the stomach for an extended period and release the drug slowly that can address many challenges like poor bioavailability. **Methods:** Floating microspheres were prepared by solvent evaporation (oil-in-water emulsion) technique. In this 225 mg poly(methyl methacrylate) (PMMA) were dissolved in a mixture of dimethylformamide and dichloromethane (1:1) at room temperature. And 75 mg nizatidine hydrochloride was added in the above mixture. This was poured into 250 ml water containing 0.02% tween 80, maintained at a temperature 30 - 40 ºC and subsequent stirred at ranging agitation speed for 20 min to allow the volatile solvent to evaporate. The microspheres formed were filtered, washed with water and dried in vacuum. **Results:** The prepared floating microspheres were characterized in a different way like size distribution 131.4 ± 1.6 µm and 89.5 ± 1.4% entrapment efficiency was found, an *in-vitro* floating test of optimized floating microspheres formulation was studied in SGF (pH 1.2). The percent cumulative amount of drug release was found 87.2 ± 2.6% in SGF (pH 1.2), 90.2 ± 3.5% in SIF (pH 6.8) and 93.2 ± 3.5% in PBS (pH 7.4) up to 24 h. The ulcer protection of the microspheres formulation was 79.84% as compared to the nizatidine pure drug (66.05%) in ulcer induced rats. The C-max value of nizatidine as obtained from the graph was 575.14 ± 55.43 mg/ml with T-max value 2 h and for the formulation was 206.58 ± 7.71 mg/ml. **Conclusion:** Floating microspheres drug delivery system provides the possibility of enhancing the bioavailability and control the release of formulation exhibiting absorption window by prolonging the gastric emptying time of the dosage form ensuring availability of the drug at the absorption site for the desired period.

INTRODUCTION: Floating systems, first described by Davis in 1968, are low-density systems that have sufficient buoyancy to float over the gastric contents and remain in the stomach for a prolonged period.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(10).4557-67

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(10).4557-67

While the system floats over the gastric contents, the drug is released slowly at the desired rate, which results in increased GRT and reduces fluctuation in plasma drug concentration.

An ulcer is a round or oval-shaped hole (also called parietal defect), 2 to 4 cm in diameter with perpendicular borders and a smooth base. A peptic ulcer is an ulcer in the gastrointestinal tract that is characteristically acidic and thus extremely painful [3, 17]. The oral route is the predominant and most preferred route for drug delivery, but drug absorption is unsatisfactory and highly variable in

the individuals despite excellent *in-vitro* release pattern. These aspects lead to developing a drug delivery system which will remain in the stomach for prolonged and predictable time [18].

The genuinely effective ulcer healing agents histamine H_2 receptor antagonists nizatidine hydrochloride requires frequent dosing due to short biological half-life (1.9 ± 0.1 h) and absorbed only in the stomach and in the initial part of the small intestine, with 35% absolute bioavailability. The traditional oral sustained release formulation releases most of the drug in intestine and colon thus the drug has a narrow absorption window and the colonic metabolism of is partly responsible for poor bioavailability from the colon, therefore, sustained release dosage form of nizatidine hydrochloride prepared by the conventional technology may not be very successful and clinically acceptable. So that controlled release intragastric floating microspheres of nizatidine hydrochloride eliminate the problems associated with conventional dosage forms. The major objectives of the present study are: -

- To develop an intragastric floating and sustained release floating microspheres for gastric retention using polymethyl methacrylate (PMMA) as a floating carrier.
- To study the effect of important formulation and processing variables on the floating and drug release behavior of these systems.

Gastro retentive drug delivery systems have made it possible to deliver the drugs in GIT for a prolonged period in a controlled manner. Thus it is envisaged to develop a floating drug delivery system, which can be retained in the stomach for a prolonged period of time by virtue of their floating properties. Hence, it is advantageous to prepare small-sized floating microspheres which could float and simultaneously adhere to directly to the mucous network where the absorption window of H_2 receptor antagonist can exist. Floating microspheres of nizatidine could localize the drug within the peptic region to enhance the drug absorption process in a site-specific manner. Developed floating system of nizatidine increases the local drug concentration by prolonging the residence time of the formulation in the stomach [19].

To develop an oral drug delivery system, it is necessary to optimize both the residence time of the system within the gastrointestinal tract and the release rate of the drug from the system. Various attempts have been made to prolong the residence time of dosage forms within the stomach. The prolongation of gastric residence time (GRT) of delivery devices could be achieved by adhesion to the mucous membranes by preventing their passage through the pylorus, using high-density systems, delayed gastric emptying devices or by maintaining them in buoyant fashion in gastric juice [10].

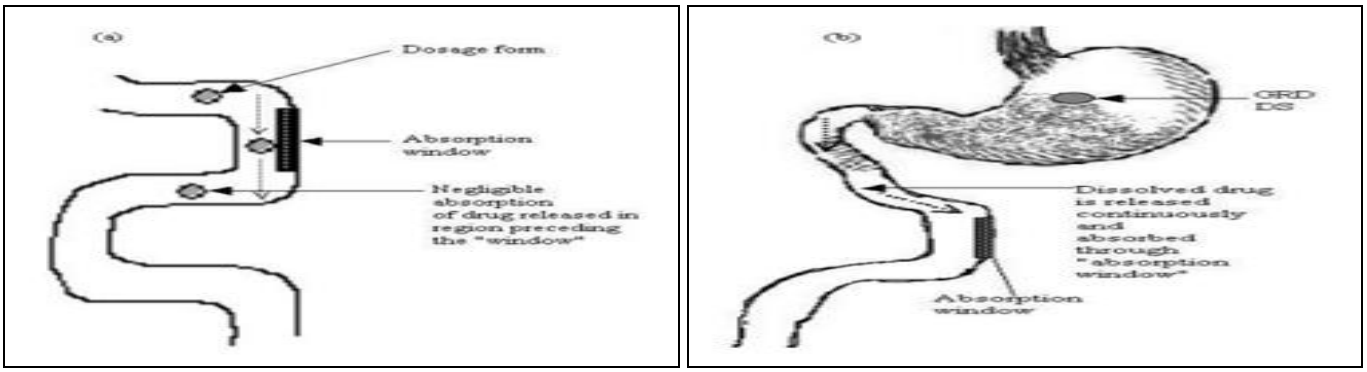


FIG. 1: DRUG ABSORPTION FROM (A) CONVENTIONAL DOSAGE FORMS AND (B) GASTRO RETENTIVE DRUG DELIVERY SYSTEM

MATERIALS AND METHODS:

Materials: Nizatidine hydrochloride was generously supplied as a gift sample by Dr. Reddy Laboratories Hyderabad. Polymethyl methacrylate, dichloromethane, and dimethylformamide were purchased from CDH India. All other chemicals and reagents were used for an analytical grade.

Methods:

Preparation of Floating Microspheres by (Solvent Evaporation Method): Floating microspheres were prepared by solvent evaporation (oil-in-water emulsion) technique. In this 225 mg polymethyl methacrylate (PMMA) were dissolved in a mixture of dimethylformamide and dichloro-

methane (1:1) at room temperature. And 75 mg nizatidine hydrochloride was added in the above mixture. This was poured into 250 ml water containing 0.02% tween 80, maintained at a temperature 30 - 40 °C and subsequently stirred at ranging agitation speed for 20 min to allow the volatile solvent to evaporate [16]. The microspheres formed were filtered, washed with water and dried in vacuum.

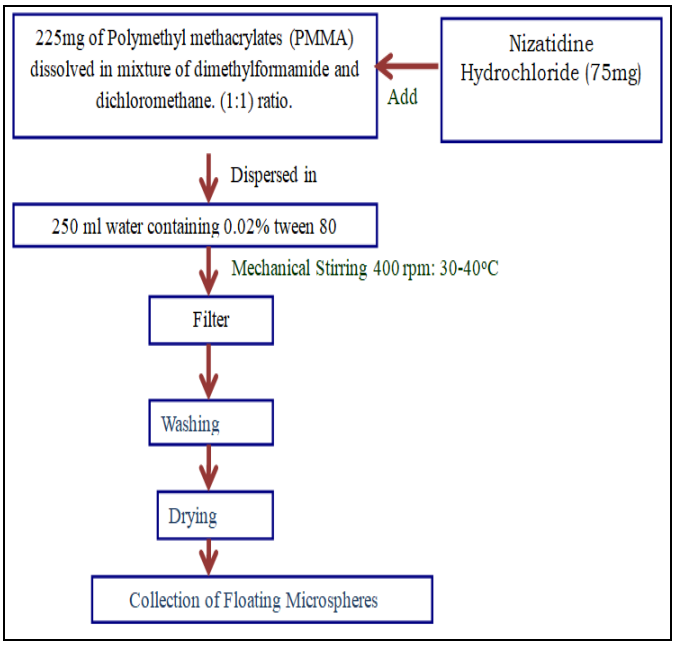


FIG. 2: SCHEMATIC REPRESENTATION OF METHOD OF PREPARATION OF FLOATING MICROSPHERES

Characterization of Prepared Floating Microspheres: The prepared floating microspheres were characterized for shape and surface morphology, size, percent drug loading and *in-vitro* drug release in different GIT PH.

Shape and Surface Morphology: In order to examine the surface morphology, the formulations were viewed under scanning electron microscopy. The samples for SEM were prepared by lightly sprinkling the floating microspheres powder on a double adhesive tape, which stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 300 Å using sputter water. The samples were then randomly scanned for studying surface morphology but show the images of coating to prove internal surface [4, 6].

Particle Size Determination: The particle size of the formulation was determined by optical microscopy using a calibrated ocular micrometer [9].

% Drug Entrapment: 100 mg of floating microspheres were dissolved in 3 ml of dichloromethane and shaken vigorously for 2 min. The solution was then filtered through a 0.45 µm syringe filter (Millipore Millex HN, USA). After suitable dilution with PBS (pH 7.4) solution was assayed for combined drug spectrophotometrically [9]. The percent of drug entrapped was calculated.

% DE = Amount of drug actually present / Theoretical drug load expected × 100

***In-vitro* Buoyancy of Floating Microspheres in SGF (pH 1.2):**

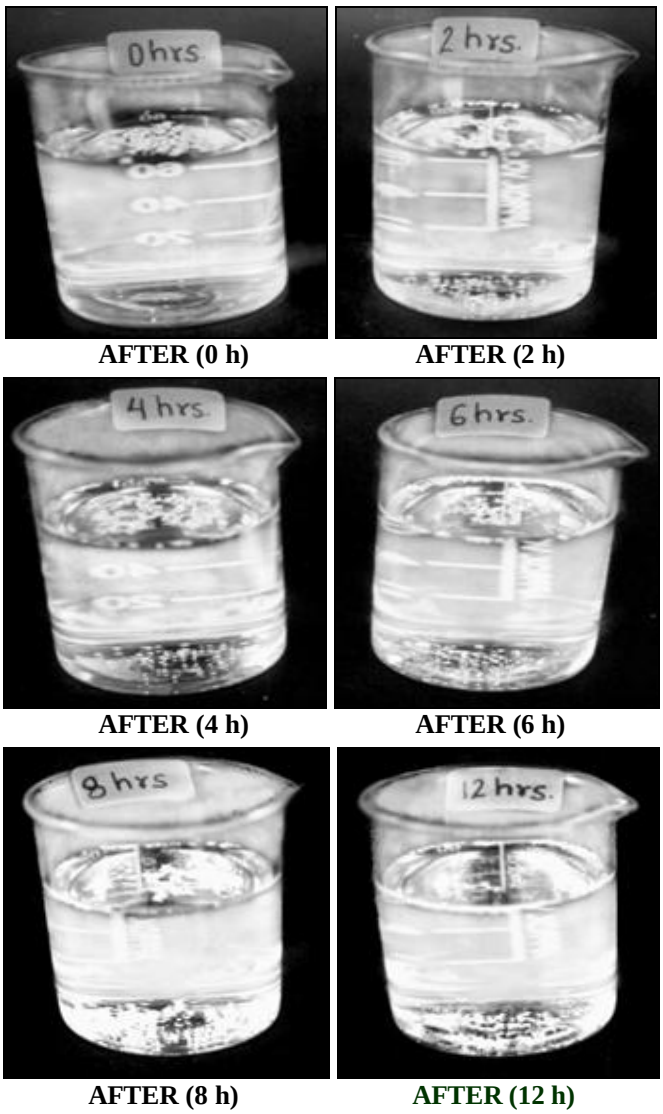


FIG. 3: PHOTOGRAPHS OF *IN-VITRO* BUOYANCY OF FLOATING MICROSPHERES IN SGF (pH 1.2)

***In-vitro* Buoyancy Test of Optimized Floating Microspheres Formulation:** The floating test of the prepared optimized floating microspheres formulation was carried out using dissolution test

apparatus USP XXII method II. 500 mg of floating microspheres were immersed in 900 ml simulated gastric fluid SGF (pH 1.2) maintained at 37 ± 2 ℃, which was agitated by a paddle rotated at 100 rpm. The paddle blades were positioned at the surface of dissolution medium.

The floating microspheres floating on the surface of SGF (pH 1.2) were recovered with a sieve no. 120 (34 µm) at every 1 h time interval for 8 h. The floating microspheres so collected were dried and weighed. The floating percentage of the floating microspheres was defined as the weight ratio of the floating microspheres against the total weight of floating microspheres in the floating test [7, 4]. The buoyancy of the floating microspheres was calculated by the following equation:

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

Where, Qf and Qs are the weights of the floating and settled floating microspheres respectively.

In-vitro **Drug Release in Different Gastrointestinal Fluids:** Optimized formulation was evaluated for the *in-vitro* drug release study in different GIT fluids. The dissolution test of floating microspheres was carried out by the paddle type dissolution apparatus specified in USP XXIII under perfect sink condition [9]. 500 mg of floating microspheres was weighed accurately and gently spread over the surface of 500 ml of dissolution medium. The media was rotated at 100 rpm and thermostatically controlled at 37 ± 2 ℃. Perfect sink condition was prevailed during the drug dissolution. The release was tested in dissolution medium of pH 1.2, pH 6.8 and pH 7.4 solutions [10]. An aliquot of the release medium was withdrawn at every 1 h time interval and an equivalent amount of fresh medium was added to the release medium. The collected samples were filtered through 0.45 µm-syringe filter (Millipore millex HN) and after suitable dilution sample were analyzed spectrophotometrically. % cumulative drug release are calculated.

Stability Studies: The stability of a preparation is usually defined as the capacity of the formulation to remain within defined limits over a predetermined period of time and is known as shelf life of the product. Stability of a formulation may also be defined as the capability of a particular formulation packaged in a specific container to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications. A stable drug delivery system should maintain its integrity and morphology, and at the same time should preserve various characteristics such as nature of the entrapped drug, drug content and release rate *etc*. In most of the stability studies, the major emphasis has been directed towards the accelerated stability studies but the stability studies of aged products have been of greater pharmaceutical significance [8].

The stability of the drug-loaded floating microspheres during storage is undoubtedly another important prerequisite for its successful clinical application. Degradation is likely to occur under tropical conditions of higher ambient temperature and humidity. Hence, the prepared floating microspheres were subjected to accelerated stability testing.

Effect of Storage on Structural Integrity of Optimized Floating Microspheres Formulation: The optimized formulation was stored in amber colored glass bottles at 4 ± 1 ℃, 25 ± 1 ℃ and 40 ± 1 ℃ for a period of 45 days and observed for any change in particle size (optical microscopy) and surface morphology by phase contrast microscope (Leica MPS, Germany) [7, 9].

Effect of Storage on Residual Drug Content: Stability of floating microspheres formulations on storage is of great concern as it is the major factor in their development as marketed preparation. The prepared formulation was tested for stability at 4 ± 1 ℃, 25 ± 1 ℃ and 40 ± 1 ℃ temperatures. Formulation was stored in amber colored glass vials, and then it was evaluated after 15, 30 and 45 days for change in residual drug content [7].

For the determination of residual drug content floating microspheres formulation were dissolved in 3 ml dichloromethane filter through polycarbonate membrane (Millipore, USA) of 200 nm pore size than after suitable dilution with PBS (pH 7.4) the drug content estimated spectro-photometrically using UV-visible spectro-photometer (Shimdazu 1800, Japan) [21].

In-vivo **Radiographical Study:** In order to assess the gastro retentive efficacy of floating

formulations, the percent buoyancy in a biological system was determined by using barium sulfate X-ray contrast medium containing 15% barium sulfate as a contrast agent was prepared for radiographical study. The study was carried out with one healthy male rabbits free of detectable gastrointestinal diseases or disorders. The study was carried out under the guidelines compiled by CPCSEA (Committee for the purpose of control) supervision of experiments on the animal, ministry of culture, the government of India and the local institutional animal ethics committee approved all the study protocols. The rabbits have fasted overnight. The rabbits were administered optimized floating microspheres formulation with 25 ml of water and X-ray photograph was taken after every one hour of administration and intragastric behavior of the floating microspheres was observed by taking a series of X-ray photographs at different time intervals [5].

***In-vivo* Studies:** Albino rat of either sex weighing 400 – 450 gm were chosen for the present studies. All *in-vivo* studies on animals were approved by Animal Ethical Committee of the Adina Institute Of Pharmaceutical Sciences, Sagar, (MP), India constituted under the guidelines of CPCSEA, New Delhi, India through their vide letter no. animal eths. Comm. 1546/PO/E/S/11/CPCSEA dated 21/05/2016.

Induction of Gastric Ulcer: The experiment was conducted on Albino rat, whose average body weight of 400 - 450 gm and age nearly 03 months. Animals were kept in standard cages for constant room temperature at 25 ± 1 ℃. Rats were kept in Fasted condition for 18 h where no food but the water was provided *ad-libitum*. Gastric Ulcers were induced by administered ethanol in the range of (95%, 01ml/200 gm body weight) orally through a feeding tube [20, 22].

Experimental Design: The anti-ulcer activity of the formulation was carried out in Albino rat. The oral dose of 20 mg/kg was chosen for this purpose. The healthy rats were divided into four groups with five animals each. The animals in the test groups were administered 1 ml / 100 gm of the rat with the necrotizing agent (80% ethanol) orally which is known to produce gastric lesions. The dosage schedule for the study is as follows:

Group 1: Animals were given the normal saline with a dose of 10 ml/kg and served as negative control.

Group 2: Animals were administered with ethanol (80%) orally and served as positive control.

Group 3: Animals were administered ethanol 1 ml / 100 gm and treated with pure nizatidine 20 mg/kg.

Group 4: Animals were administered with ethanol and treated with formulation (equivalent 20 mg nizatidine) and ulcer index (UI) was estimated [20].

$$UI = \text{Ulcerated area } (mm^2) / \text{Total stomach area } (mm^2)$$

***In-vivo* Bioavailability Study:** The bioavailability study was carried out in albino rats of either sex weighing 200 - 250 gm. The animals were divided into three groups of five animals each and were fasted overnight before starting the experiment with free access to water. The pure nizatidine and prepared microspheres formulation was administered orally with dose 20 mg/kg body weight with the help of cannula after anesthetizing for a very short period of time with diethyl ether, after administration 0.3 ml blood samples were collected from retro-orbital plexus into the heparinized tubes at preset period of 0.5, 1, 2, 4, 8, 12, 24 h. The blood samples were centrifuged at 4000 rpm for 10 min and the separated samples were stored at -20 °C until analysis had completed.

Estimation of Nizatidine in Plasma Sample by RP-HPLC Analysis: The amount of nizatidine in blood samples was measured by RP-HPLC method Haque *et al.*, 2011. The method was validated before estimation. The measurement was carried out at 280 nm. The mobile phase used consist of a mixture of 0.1(M) orthophosphoric acid (pH 3.0) and methanol in the ratio of 30:70 and the pump flow rate was 1 ml/min, and C18 (250 mm × 4.6 mm) column was used. The mobile phase was filtered with a nylon membrane filter and degassed before use. To 0.1 ml plasma 50 µl of standard nizatidine (50 ng/ml) was added in a microcentrifuge tube, and volume was made up to 2 ml with acetonitrile to precipitate the protein.

Then the sample was centrifuged at 4000 rpm for 25 min. The supernatant was collected and transferred into an Eppendorf tube and was dried.

The residue was dissolved in 200 µl of mobile phase and 10 µl was injected to the HPLC system. The analysis was carried out by RP-HPLC method using a flow rate of 1.0 ml/min and measurement was made at 280 nm. The amount of the nizatidine in the sample was determined from the peak area ratio correlated with a standard curve prepared under the same condition.

Pharmacokinetic Analysis:

Determination of C_{max} and T_{max}: The peak plasma concentration (C_{max}) and the time of peak plasma concentration (T_{max}) were determined from the plasma drug concentration *vs.* time plot for the pure drug and prepared microspheres.

Determination of Area under Curve (AUC): The area under the time versus plasma concentration curve (AUC) was measured by applying the trapezoidal rule. (AUC) 0-α was calculated as given below

$$(AUC)_{0-t} = \int_0^t C(t)\,dt$$

$$(AUC)_{0-\alpha} = (AUC)_{0-t} + C_t / K_{el}$$

Determination of Relative Bioavailability: The relative bioavailability (Fr) of nizatidine was calculated using the following equation:

Fr (%) = (AUC (Nizatidine microspheres)) / (Pure nizatidine suspension)

RESULTS AND DISCUSSION: Floating microspheres were prepared by the solvent evaporation method. Poly(methyl methacrylate) (225 mg) was dissolved in a mixture of dimethylformamide and dichloromethane (1:1) at room temperature and drug (Nizatidine Hydrochloride – 75 mg) was dispersed in the above mixture. This drug-polymer mixture was poured into 250 ml water containing 0.02% tween 80, maintained at a temperature 30 – 40 °C, and subsequently stirred at ranging agitation speed 300 – 400 rpm to allow the volatile solvent to evaporate.

The microsphere formed were filtered, washed, and dried in vacuum. For this floating microspheres formulation were prepared with varying drug concentration *viz.* 25, 50, 75 mg. It was observed that on increasing the concentration of the drug, the entrapment efficiency increased. While on further increasing drug concentration, the entrapment efficiency gradually decreased. The average particle size of floating microspheres reduces with increasing temperature. Narrow size distribution 131.4 ± 1.6 µm and 89.5 ± 1.4% entrapment efficiency was found to the formulation at 37 °C temperature. *In-vitro* floating test of optimized floating microspheres formulation was studied in SGF (pH 1.2).

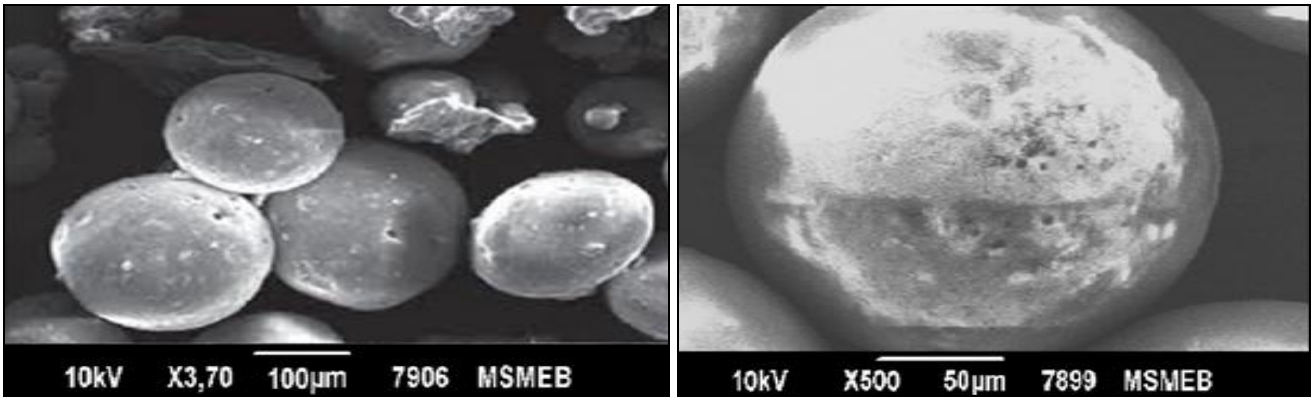


FIG. 4: SEM PHOTOGRAPH OF NIZATIDINE HYDROCHLORIDE FLOATING MICROSPHERE

The results showed that the percentage buoyancy of floating microspheres formulation was significantly decreased after 5 h. The buoyancy (%) of optimized nizatidine hydrochloride floating microspheres formulation in SGF (pH 1.2) are reported. *In-vitro* drug release from optimized floating microspheres was carried out in SGF (pH 1.2), SIF (pH 6.8) and PBS (pH 7.4) by dissolution test of floating microspheres were carried out by the paddle method specified in the U.S.P. XXI. No initial burst release was observed in any medium suggested that the nizatidine hydrochloride molecules are entrapped over the floating microspheres. The percent cumulative amount of drug release was found 87.2 ± 2.6% in SGF (pH 1.2), 90.2 ± 3.5% in SIF (pH 6.8) and 93.2 ± 3.5 % in PBS (pH 7.4) up to 24 h. The results clearly suggest that floating microspheres formulation

could also be utilized for sustained and drug delivery purpose.

TABLE 1: PERCENT BUOYANCY OF OPTIMIZED NIZATIDINE HYDROCHLORIDE FLOATING MICROSPHERES FORMULATIONS IN SGF (pH 1.2)

S. no.	Time (h)	% Buoyancy
1	0	100
2	1	95
3	2	89
4	3	81
5	4	71
6	5	68
7	6	44
8	7	31
9	8	26

(n=3) mean ± SD

TABLE 2: PERCENT CUMULATIVE DRUG RELEASE FROM OPTIMIZED FLOATING MICROSPHERES FORMULATION IN SFG (pH 1.2)

S. no.	Time interval (h)	% Cumulative drug released
1	1	13.4 ±1.4
2	2	22.8 ± 1.5
3	3	31.6 ± 1.3
4	4	40.3 ± 1.9
5	5	49.8 ± 3.2
6	6	54.2 ± 2.8
7	7	61.6 ± 3.1
8	8	69.7 ± 2.7
9	24	87.2 ± 2.6

(n=3) mean ± SD

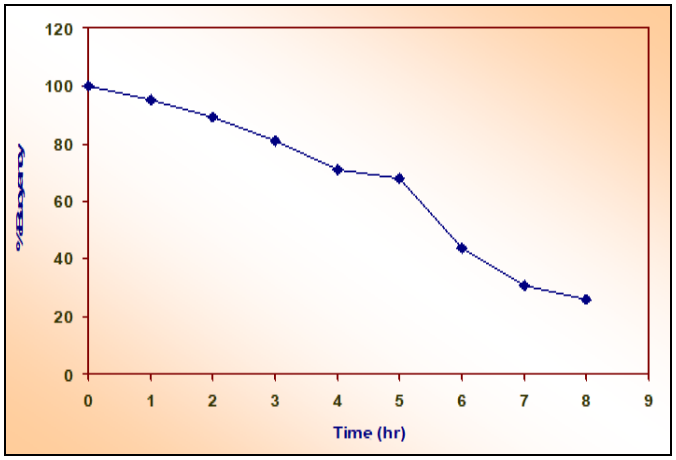


FIG. 5: PERCENT BUOYANCY OF OPTIMIZED NIZATIDINE HYDROCHLOROIDE FLOATING MICROSPHERES FORMULATIONS IN SGF (pH 1.2)

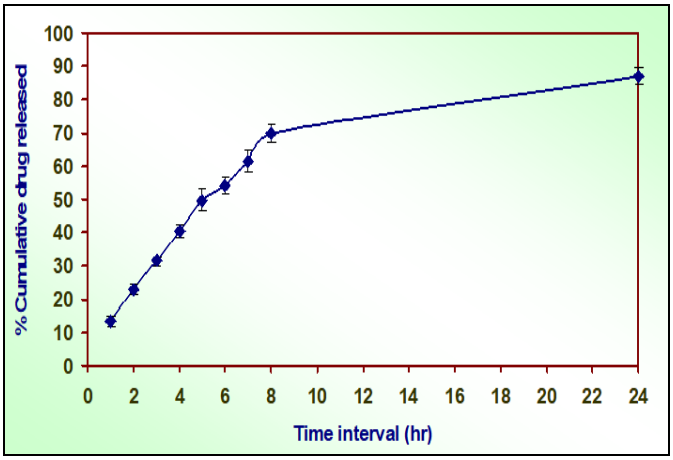


FIG. 6: PERCENT CUMULATIVE DRUG RELEASE FROM OPTIMIZED FLOATING MICROSPHERES FORMULATION IN SFG (pH 1.2) SGF= Simulated gastric fluid

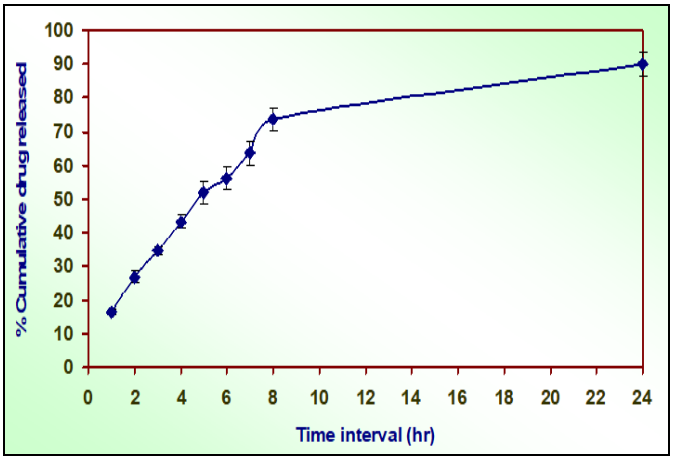


FIG. 7: PERCENT CUMULATIVE DRUG RELEASE FROM OPTIMIZED FLOATING MICROSPHERES FORMULATION IN SFG, SIF (pH 6.8) *SGF - Simulated Gastri Fluid *SIF – Simulated Intestinal Fluid

Stability studies were carried out with optimized floating microspheres formulation which was stored for a period of 45 days at 4 ± 1 °C, 25 ± 1 °C and 40 ± 1 °C. The particle size of formulation was determined by optical microscopy using a calibrated ocular micrometer. The particle size of the floating microspheres was found to increase at 25 ± 1 °C, which may be attributed to the aggregation of floating microspheres at a higher temperature.

TABLE 3: PERCENT CUMULATIVE DRUG RELEASE FROM OPTIMIZED FLOATING MICROSPHERES FORMULATION IN SIF (pH 6.8)

S. no.	Time interval (h)	% Cumulative drug released
1	1	16.4 ± 0.8
2	2	26.8 ± 1.7
3	3	34.6 ± 1.2
4	4	43.3 ± 2.1
5	5	51.8 ± 3.4
6	6	56.2 ± 3.3
7	7	63.6 ± 3.7
8	8	73.7 ± 3.4
9	24	90.2 ± 3.5

(n=3) mean ± SD, *SIF – Simulated Intestinal Fluid

At 40 ± 1 °C, the floating microspheres aggregated and a no change in a spherical shape. To ellipsoidal shape with irregular observed, *i.e.* these floating microspheres were unstable at a higher temperature like 40 ± 1°C. The selected optimized floating microspheres formulation was stored at 4 ± 1 °C, 25 ± 1 °C and at 40 ± 1 °C and the residual drug content of the formulation was determined after 15, 30 and 45 days. It was observed that the formulation stored at 4 ± 1 °C and 25 ± 1 °C was quite stable as fewer drugs were degraded on storage for 45 days while it was quite unstable at 40 ± 1 °C for 45 days.

TABLE 4: PERCENT CUMULATIVE DRUG RELEASE FROM OPTIMIZED FLOATING MICROSPHERES FORMULATION IN PBS (pH 7.4)

S. no.	Time interval (h)	% Cumulative drug released
1	1	13.5 ± 0.7
2	2	19.6 ± 0.9
3	3	30.3 ±1.2
4	4	38.6 ± 1.4
5	5	49.3 ± 2.3
6	6	61.6 ± 3.1
7	7	72.2 ± 3.4
8	8	77.6 ± 3.7
9	24	93.2 ± 3.5

(n=3) mean ± SD *PBS – Phosphate Buffer Solution

TABLE 5: EFFECT OF STORAGE TEMPERATURE ON PARTICLE SIZE AND SURFACE MORPHOLOGY OF OPTIMIZED FLOATING MICROSPHERES FORMULATION

S. no.	Formulations	Storage temperature	Particle size (µm)				Vesicles shape after 45 days
			Initial	15 days	30 days	45 days	
1	MFD	4± 1°C	131.4 ± 3.4	134.7 ± 2.9	138.8 ± 3.1	141.4 ± 3.3	Spherical
		28 ± 1°C	131.4 ± 3.4	139.6 ± 3.4	146. ± 3.7	154.5 ± 2.9	Spherical
		40 ± 1°C	131.4 ± 3.4	146.4 ± 2.7	153. ± 2.1	162.2 ± 2.6	No change in shape

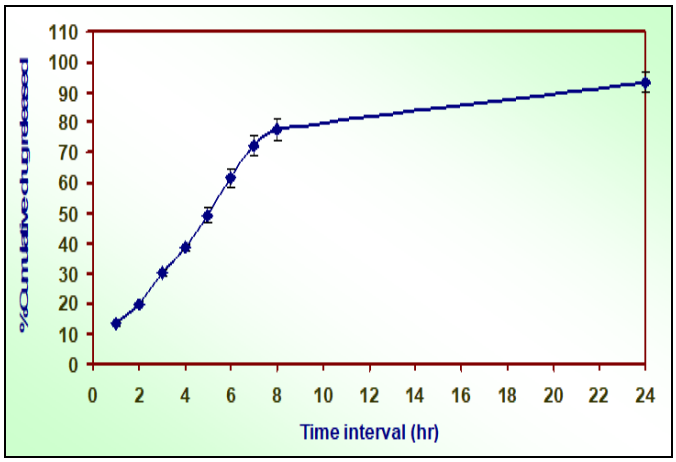


FIG. 8: PERCENT CUMULATIVE DRUG RELEASE FROMOPTIMIZED FLOATING MICROSPHERES FORMULATION IN PBS (pH 7.4) * PBS - Phosphate Buffer

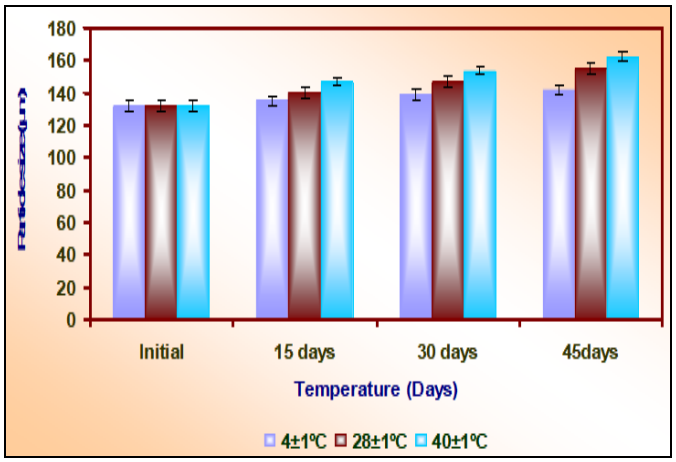


FIG. 9: EFFECT OF STORAGE TEMPERATURE ON PARTICLE SIZE OF OPTIMIZED FLOATING MICROSPHERES FORMULATION Mean SD ± (n=3**)**

TABLE 6: PERCENT RESIDUAL DRUG CONTENT IN OPTIMIZED FLOATING MICROSPHERES FORMULATION STORED AT DIFFERENT TEMPERATURES

S. no.	Time in days	% Residual drug content		
		4 ± 1 °C	4 ± 1 °C	4 ± 1 °C
1	Initial	100	100	100
2	15	98.3 ± 1.2	96.8 ± 2.7	92.2 ± 2.2
3	30	96.2 ± 2.5	93.2 ± 2.3	86.4 ± 2.6
4	45	92.2 ± 2.3	84.7± 2.1	78.4 ± 2.9

(n=3) mean ± SD

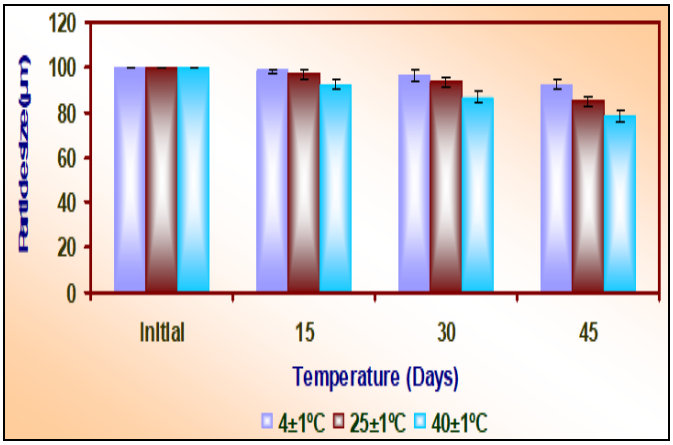


FIG. 10: PERCENT RESIDUAL DRUG CONTENT IN OPTIMIZED FLOATING MICROSPHERES FORMULATION STORED AT DIFFERENT TEMPERATURES

The *in-vivo* study with X-ray contrast medium containing floating microspheres was conducted to determine the *in-vivo* floating performance of optimized floating microspheres formulation. X-ray photograph taken after each 1 h interval shows intragastric behavior of the floating microspheres. It is clear from the X-ray photographs that floating microspheres remained buoyant even after 4 h which is a satisfactory time for a gastro retentive property obtained by floating microspheres formulation. In the case of *in-vivo* study the ethanol-induced ulcer model, the oral administration of 95% ethanol in the control group, produce characteristic lesions in the stomach which shows as the bands of broad red lesions. The *in-vivo* evaluation showed the Ulcer Index (UI) were, 1.090 ± 0.04 for Group 1 (Normal saline-treated group), 23.92 ± 0.58 for Group 2 Ethanol Induction, 8.12 ± 0.28** for Group 3 (Nizatidine solution) and 4.83 ± 0.86* for Group 4 Nizatidine loaded microspheres. Microspheres treated group showed significant ($p<0.01$) ulcer protection index as compared to the free drug-treated group.

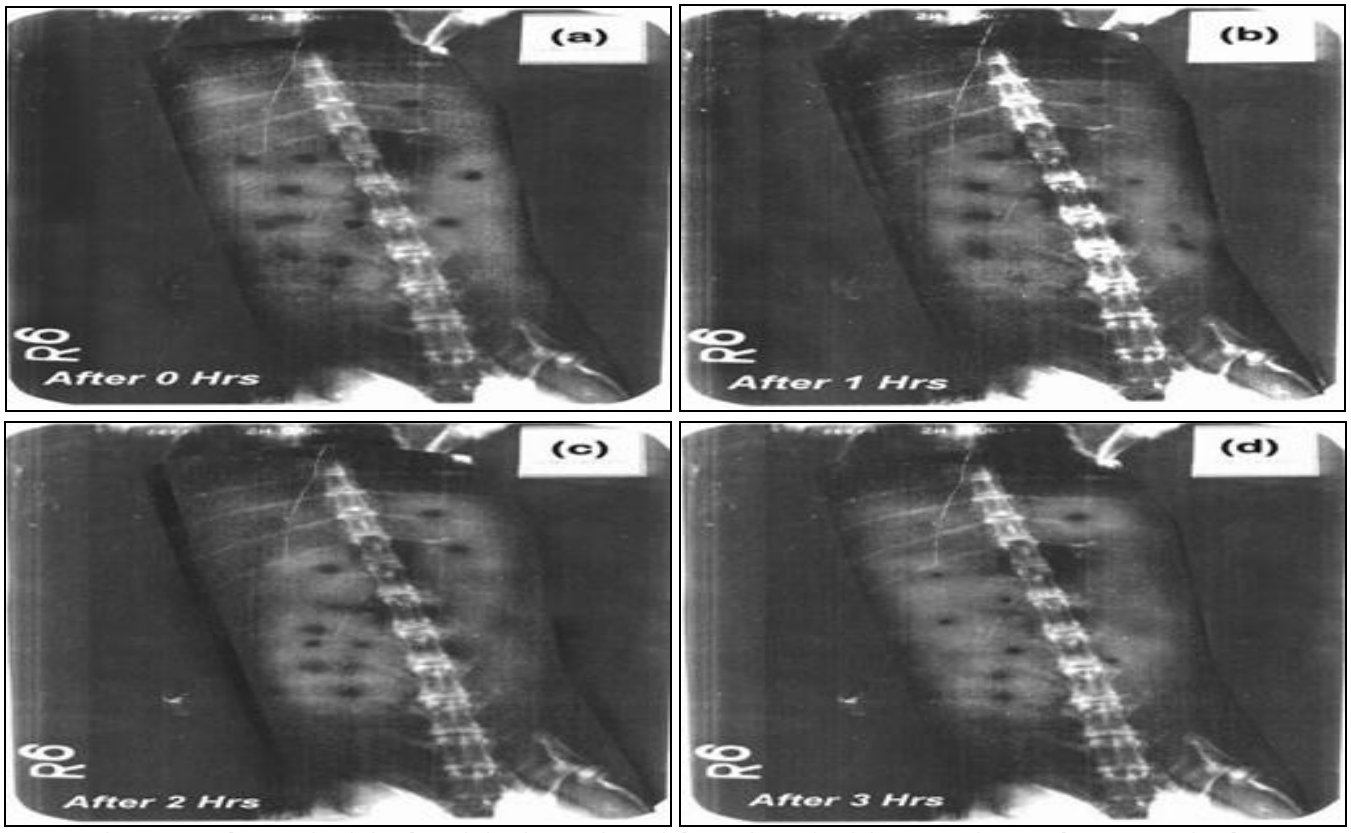
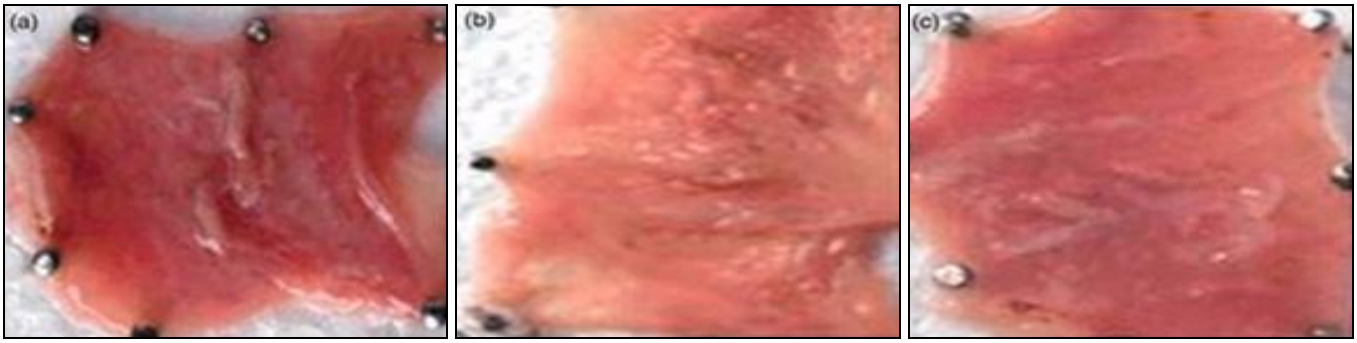


FIG. 11: X-RAY PHOTOGRAPHS SHOWING FLOATING MICROSPHERES REMAINED BUOYANT

TABLE 7: ANTI-ULCER ACTIVITY OF THE NIZATIDINE FORMULATION IN ETHANOL INDUCED ULCER IN RAT

Groups	Induction	Dose	Ulcer index
Group – I	Normal Saline	10ml/kg	1.090 ± 0.04
Group –II	Ethanol	1 ml/gm	23.92 ± 0.58
Group –III	Nizatidine	20 mg/kg	8.12 ± 0.28**
Group – IV	Nizatidine Formulation	20 mg/kg	4.83± 0.86*

Note: Values express mean ± SEM; n=4; *p < .0.05 versus control, **p < 0.01 *vs.* control



CONTROL GROUP SHOWING NORMAL GASTRIC INTEGRITY | **NIZATIDINE SOLUTION – TREATED GROUP (20 mg/kg)** | **NIZATIDINE LOADED MICRO-SPHERES TREATED GROUP**

FIG. 12: EVIDENCE FOR THE PROTECTIVE EFFECT OF NIZATIDINE LOADED MICROSPHERES IN RATS TREATED WITH ETHANOL

TABLE 8: DRUG PLASMA CONCENTRATION *vs.* TIME DATA FOLLOWING ORAL ADMINISTRATION OF PURE NIZATIDINE (STANDARD) TO RAT

Plasma concentration in mg/ml *vs.* Time		
Time (h)	**Pure nizatidine**	**Nizatidine formulation**
0	0	0
1.5	269.65 ± 42.31	45.250 ± 8.42
1	391.72 ± 56.25	69.250 ± 9.81
2	575.14 ± 55.43	138.26 ± 13.94
4	283.5 ± 33.960	189.250 ± 7.03
8	61.790 ± 7.720	206.58 ± 7.71*
12	16.430 ± 4.570	97.750 ± 10.38

TABLE 9: PHARMACOKINETIC PROFILE OF PURE NIZATIDINE AND NIZATIDINE LOADED MICRO-SPHERES AFTER ORAL ADMINISTRATION IN RATS

Pharmacokinetics parameters	Units	Nizatidine standard	Nizatidine formulation
C_{max}	ng / ml	575.14 ± 55.43	206.58 ± 7.71
T_{max}	H	2 ± 0	8 ± 0
AUC (0-24)	h x (ng / ml)	2064.07	2272.22
Fr	(%)	-	110.07

CONCLUSION: The result obtained from all the experiments performed as a part of project work suggested that it is possible to prepare an intragastric floating and sustained release floating microspheres preparation using poly(methyl methacrylate), solvent evaporation method. Floating microspheres drug delivery system provides the possibility of enhancing the bioavailability and control the release of formulation exhibiting absorption window by prolonging the gastric emptying time of the dosage form ensuring availability of the drug at the absorption site for the desired period of time. As the floating microspheres showed a good buoyancy and drug release properties so that it has a great potential for its use both in powder form for dry suspension and granular form for tableting.

ACKNOWLEDGEMENT: I would like to thank the department of pharmaceutics, ADINA Institute of Pharmaceutical Sciences, Sagar Madhya Pradesh, India for providing all the facilities and special thanks to Chaitanya hospital and medical research for providing the *in-vivo* examination of animal, apart from these like to thanks digital journals, library and support during this research paper writing.

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Siddique O, Ovalle A, Siddique AS and Moss SF: *Helicobacter pylori* infection: an update for the internist in the age of increasing global antibiotic resistance. Am J Med 2018; 131: 473-79.
2. Yamasaki A, Yoshio T, Muramatsu Y, Horiuchi Y, Ishiyama A, Hirasawa T and Suchid TT: Vonoprazan is superior to rabeprazole for healing endoscopic submucosal dissection. Induced Ulcers Digestion 2018; 97: 170-76.
3. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D and Malfertheiner P: Global prevalence of *helicobacter pylori* infection: systematic review and meta-analysis. Gastroenterology 2017; 153: 420-29.
4. Maes ML, Fixen DR and Linnebur SA: Adverse effects of proton-pump inhibitor use in older adults: a review of the evidence. Ther Adv Drug Saf 2017; 8: 273-97.

5. Mössner J: The indications applications and risks of proton pump inhibitors. Dtsch Arztebl Int 2016; 113: 477-83.
6. Kagawa T, Iwamuro M, Ishikawa S, Ishida M, Kuraoka S, Sasaki K, Sakakihara I, Izumikawa K, Yamamoto K and Takahashi S: Vonoprazan prevents bleeding from endoscopic submucosal dissection induced gastric ulcers. Aliment Pharmacol Ther 2016; 44: 583-91.
7. Chen PY, Wu MS, Chen CY, Bair MJ, Chou CK, Lin JT and Liou JM: Gastrointestinal disease and *Helicobacter* consortium systematic review with meta-analysis: the efficacy of levofloxacin triple therapy as the first or second-line treatments of *Helicobacter pylori* infection. Aliment Pharmacol Ther 2016; 44: 427-37.
8. Mariadi, Bangun H and Karsono: Formulation and *in-vitro* evaluation of gastroretentive drug delivery system of antacids using alginate-chitosan films. Int J Pharmtech Research 2015; 8: 1-12.
9. Søreide K, Thorsen K, Harrison EM, Bingener J, Møller MH, Ohene-Yeboah M and Søreide JA: Perforated peptic ulcer. Lancet 2015; 386: 1288-98.
10. Vinod M, Jitendra N, Priyenka S and Navin S: Formulation and *in-vitro* evaluation of cimetidine microsphere as gastroretentive floating drug delivery system. Int J Chem Environ Bio Science 2013; 1: 701-06.
11. Patel R, Patel N, Patel NM and Patel M: A novel approach for dissolution enhancement of ibuprofen by preparing floating granules. Int Res Pharm Science 2010; 1: 57-64.
12. Ramji A, Kumar A, Rao GCS and Reddy VP: Formulation and evaluation of swellable and floating gastroretentive ciprofloxacin hydrochloride tablets. AAPS Pharm Science Technology 2009; 10: 124-28.
13. Jain A and Jain C: Buoyant microspheres of famotidine an approachable dosage form for gastric treatment. Asian Journal of Pharmaceutics 2009; 10(4): 20-23.
14. Jagdale S, Agavekar A, Pandya S, Kuchekar B and Chabukswar A: Formulation and evaluation of gastroretentive drug delivery system of propranolol hydrochloride. AAPS Pharm Sci Tech 2009; 10: 3-5.
15. Tuorkey M and Karolin K: Anti-ulcer activity of curcumin one experimental gastric ulcer in rats and its effect on oxidative stress/antioxidant, IL-6 and enzyme activities. Biomed Environ Sci 2009; 22: 488-95.
16. Basavaraj B, Deveswaran R, Bharath S, Abraham S, Furtado S and Madhavan V: Hollow microspheres of diclofenac sodium a gastroretentive controlled delivery system. Pak J Pharm Science 2008; 21(4): 451-54.
17. Gattani YS, Bhagwat DA and Maske AP: Formulation and evaluation of intragastric floating drug system of diltiazem hydrochloride. Asian Journal of Pharmaceutics 2009; 228-31.
18. Jang S, Lee J and Park S: Gastroretentive drug delivery system of DA-6034, a new flavonoid derivative for the treatment of gastritis. Int J Pharm 2008; 2: 88-94.
19. Sahoo SK, Mallick AA, Barik BB and Senapati PC: Formulation and *in-vitro* evaluation of eudragit® microspheres of stavudine. Tropical J Pharm Science 2005; 4: 369-75.
20. Jain SK, Awasthi AM, Jain NK and Agrawal GP: Calcium silicate-based microspheres of repaglinide for gastroretentive floating drug delivery: preparation and *in-vitro* characterization. J Control Release 2005; 3: 8-10.
21. Uma Maheshwari RB, Jain S and Jain NK: Investigation of a new approach in gastroretentive drug delivery system in h. pylori treatment. Int J Pharma 2003; 5: 136-43.
22. Kim BK, Hwang SJ and Park JB: Preparation and characterization of drug-loaded polymethacrylate microspheres by an emulsion solvent evaporation method. J Microencapsul 2002; 19: 811-22.

How to cite this article:
Mishra SK and Gupta MK: Characterization and evaluation of nizatidine floating microspheres based drug delivery system for anti-ulcer activity. Int J Pharm Sci & Res 2019; 10(10): 4557-67. doi: 10.13040/IJPSR.0975-8232.10(10).4557-67.