BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902022e191009

Article

Formulation of stomach-specific floating microparticles of nizatidine and their radiographic evaluation

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Nizatidine is an anti-secretogogue and a gastroprotective drug with a half-life of 1-2 h and is well absorbed in the stomach. This study aimed to optimize the process and develop floating microparticles of nizatidine that are based on low methoxyl pectin. Oil-in-oil dispersion method and Taguchi orthogonal array design were employed, and the prolonged residence time of the microparticles in the stomach was demonstrated. The constraints for independent variables, viz. A-polymer, B-internal solvent volume, C-surfactant, D-stirring rate and E-stirring time were set to generate the experimental runs. Particle size, percentage yield, micromeritic properties, entrapment efficiency, *in vitro* buoyancy and *in vitro* release were characterized. Surface morphology, zeta potential, *in vitro* release kinetics and *in vivo* floating performance of the optimized formulation was examined. The microparticles were free-flowing, irregular in shape and had a mean particle size distribution of 73–187 µ. Low methoxyl pectin played a predominant role in achieving buoyancy and optimum gastric retention for the modified release of the drug, suggesting Korsmeyer–Peppas model as the possible release mechanism. *In vivo* radiographic study in rabbits revealed that the drug was retained in the stomach for a period of 6 h. These results indicate that nizatidine floating microparticulate system provides modified drug release for the effective treatment of gastric ulcer.

Keywords: Floating microparticles. Nizatidine. Low methoxyl pectin. Oil-in-oil dispersion. Solvent evaporation. Modified release.

INTRODUCTION

In recent times, research endeavours have given impetus to controlled release, site-specific drug delivery systems (Thakur *et al.*, 2010). Drugs with a short half-life are rapidly eliminated from systemic circulation and hence require frequent dosing. Oral sustained/controlled release formulations capable of maintaining steady-state plasma concentration for a long period have been developed to circumvent this problem and improve patient compliance with the therapeutic regimen (Gholap *et al.*, 2010). However, oral controlled drug delivery systems face several challenges, viz. physiological limitations such as low gastric retention time, variable pH in different regions of the gastrointestinal tract as well as inter- and intra-subject variations in gastric emptying time, which may affect the performance of the product and lead to reduced efficacy of the administered dose (Vyas, Khar, 2002). Recent advances in drug delivery systems recommend the use of high-density systems (Redniek, Hill, Tucker, 1970), superporous hydrogel systems (Chen *et al.*, 2000), magnetic systems (Fujimori *et al.*, 1995), floating drug delivery systems (FDDS) (Choi *et al.*, 2002), expandable, unfoldable and swellable systems (Kalusner *et al.*, 2003), bioadhesive or mucoadhesive drug delivery systems (Liu

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et al., 2011) and modified shape systems (Kedzierewicz *et al*., 1999) to overcome the aforementioned limitations. Among these systems, FDDS is gaining popularity as it involves a simple manufacturing process suitable for commercial production (Stops *et al*., 2008). Nevertheless, single-unit floating delivery systems may exhibit high variations in bioavailability owing to the all-or-none gastric emptying effect (Arora *et al*., 2005). On the contrary, multi-unit particulate dosage forms, viz. microparticles, microspheres, and microbeads are preferred since they offer several benefits such as better dispersibility in gastrointestinal fluids, predictable gastric emptying time, reproducible pharmacokinetics and reduced inter-subject variability in absorption as a result of sustained release of the drug in the stomach (Singh *et al*., 2015). FDDS is most suitable for administering anti-secretagogues such as antacids as they are required to act in a site-specific manner in the stomach.

Nizatidine, a competitive H2 receptor antagonist, acts specifically on the gastric parietal cells and is widely used in the treatment of peptic ulcer, reflux esophagitis and other conditions where inhibition of gastric acid secretion is beneficial. At present, nizatidine is the drug of choice for the long-term treatment of hyperacidity and for the management of recently healed active duodenal ulcers.

Nizatidine is well absorbed (>70%) from the upper gastrointestinal tract following oral administration, and peak plasma concentration is reached within 0.5-3 h. A short half-life of 1-2 h, rapid clearance, susceptibility to metabolism by colonic bacteria, chemical and enzymatic stability, sparingly soluble nature and better absorption profile in the stomach make nizatidine an ideal candidate for the formulation of modified release gastroretentive dosage forms. Overall reduction in dosage and frequency of administration can be achieved, which enhance patient compliance and treatment efficacy (Wozniak, 1984).

In this study, we selected nizatidine since extensive research has not been carried out in the field of floating drug delivery. This work intended to design, develop and optimize floating microparticles of nizatidine (FMN) with respect to particle size, floating ability, drug entrapment efficiency and *in vitro* drug release. Furthermore, we aimed to perform radiographic studies for establishing the prolonged residence time of the drug in the stomach.

MATERIAL AND METHODS

Material

Nizatidine samples were gifted by Strides Shasun Ltd., Bangalore. Low methoxyl pectin (LMP) was purchased from SD Fine-Chem Ltd., Boisar. Acetone and petroleum ether were procured from SD Fine-Chem Ltd., Mumbai. Liquid paraffin (light) was obtained from Hi Media Laboratories, Mumbai. All other chemicals and solvents used were of analytical grade.

Experimental design

Taguchi orthogonal array design with five factors and four levels was used to prepare various batches of floating microparticles (Table I). The independent variables selected were polymer concentration (A), internal phase volume (B), Span 80 (C), stirring speed (D) and stirring time (E). The response variables chosen were particle size, floating ability, drug entrapment efficiency and *in vitro* drug release.

TABLE I - Taguchi orthogonal array design in different formulation

Formulation	Polymer (LMP) (%)	(Internal phase volume) Acetone (ml)	Surfactant (%)	Stirring rate (rpm)	Stirring time (min)
FMN1	5.5	20	1.5	1200	60
FMN2	0.5	30	1	600	60
FMN3	3	20	1	900	180
FMN4	5.5	50	1	300	120
FMN5	0.5	40	1.5	900	120
FMN6	3	30	0.5	1200	120
FMN7	8	20	2	600	120
FMN8	3	50	1.5	600	30
FMN9	5.5	40	0.5	600	180
FMN10	3	40	2	300	60

TABLE I - Taguchi orthogonal array design in different formulation

Formulation	Polymer (LMP) (%)	(Internal phase volume) Acetone (ml)	Surfactant (%)	Stirring rate (rpm)	Stirring time (min)
FMN11	5.5	30	2	900	30
FMN12	0.5	20	0.5	300	30
FMN13	8	50	0.5	900	60
FMN14	0.5	50	2	1200	180
FMN15	8	40	1	1200	30
FMN16	8	30	1.5	300	180
FMN1 (Optimized)	5.5	20	1.5	1200	60

FMN- Floating microparticles of Nizatidine, LMP- Low methoxyl pectin

Preparation of floating microparticles

Microparticles containing nizatidine were prepared using oil-in-oil dispersion followed by solvent evaporation. Nizatidine and varying proportions of LMP were dissolved in acetone at room temperature with agitation. The mixture was then emulsified at ambient temperature by transferring it into light liquid paraffin containing Span 80 at the rate of 2.5 mL/min using a syringe. Continuous stirring was done at a specified speed and time (Table I) to allow the evaporation of acetone and the formation of microparticles. The formed microparticles were filtered and washed with petroleum ether to remove excess paraffin and finally dried at 40°C using a vacuum dryer. The dried microparticles were stored in a desiccator until further evaluation.

Characterization of the microparticles

Percentage yield

The percentage yields of FMNs were calculated for all the batches by considering the final weight of the product after vacuum drying in comparison with the total weight of the drug and the polymer used in preparing the respective formulations. The percentage yield of the product was calculated using the following formula (equation 1).

$$PY=\frac{Wo}{W_T}\times 100 \quad (1)$$

Where, Wo is the practical mass of the dried floating microparticles, WT is the theoretical mass of the drug and polymer and PY is the percentage yield.

Micromeritic properties

The micromeritic properties such as bulk density, tapped density, true density and Carr's compressibility index of the microparticles were determined.

The bulk density was estimated by adding a weighed amount of microparticle sample into a 100-mL graduated cylinder. The cylinder was dropped at 2-second intervals onto a hardwood surface three times from a height of 1 inch. Subsequently, the bulk volume of the sample was gauged, and the bulk density was calculated using the formula given below (equation 2):

$$\text{Bulk density}=\frac{\text{Mass of microparticles}}{\text{Volume of microparticles}} \quad (2)$$

The tapped density was computed by tapping a 100-mL graduated cylinder containing a known mass of microparticles from a height of 1 inch at 2-second intervals with 1000 tappings. The tapped density, Hausner's ratio and Carr's compressibility index were derived using the following formulae (equations 3, 4 and 5):

$$\text{Tapped density}=\frac{\text{Mass of microparticles}}{\text{Volume of microparticles after tapping}} \quad (3)$$

$$\text{Hausner's ratio}=\frac{\text{Tapped density}}{\text{Bulk density}} \quad (4)$$

$$\text{Carr's compressibility index (\%)}=\frac{\text{Tapped density}}{\text{Bulk density}}\times 100 \quad (5)$$

True density

The true density of the floating microparticles was determined by the liquid displacement method using n-hexane as the solvent. A specific gravity bottle was used

for this purpose in the following manner: The weight of the bottle was noted (a); 25 mL of n-hexane was added to it, and the weight was recorded (b); the bottle was emptied, a weighed amount of floating microparticles was added, and the net weight was noted (c); n-hexane was added to occupy the void spaces within the floating microparticles, and the weight was noted (d). True density was calculated according to the following formulae (equations 6 and 7):

$$\text{Density of liquid } (\rho) = \frac{b-a}{25} \cdot \quad (6)$$

$$\text{True density} = \frac{c-a}{25-[d-c/\rho]} \quad (7)$$

Angle of repose

The angle of repose (θ) is an important flow characteristic of the microparticles, and was determined by the fixed funnel method. An accurately weighed amount of the microparticles was added into a funnel. The height of the funnel was adjusted in such a way that its tip just touched the peak of the heaped blend of microparticles. The blend was allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured, and the angle of repose was calculated in triplicate using the following formula (equation 8):

$$\text{Tan } \theta = \frac{h}{r} \quad (8)$$

Where, h is the height of the pile and r is the radius of the base of the pile on the graph.

Particle size analysis

The particle size of the microparticles in each formulation was determined by optical microscopy. The optical microscope (Labomed Vision 2000 Lab Microscope)was fitted with an ocular and a stage micrometer. The ocular micrometer was calibrated using the stage micrometer. The average particle size was calculated after measuring the diameter of 500 microparticles.

Entrapment efficiency

A high performance liquid chromatography method was developed and validated in-house as per ICH (International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use) guidelines to estimate the amount of nizatidine in the bulk drug and microparticles (Geetha *et al.*, 2018). The optimized conditions of the chromatographic system included RP-C18 Phenomenex column, isocratic pump mode with a flow rate of 1 mL/min, UV detection at 314 nm and injection volume of 5 µL. The mobile phase consisted of water and methanol in the ratio of 70:30 (v/v). The column oven temperature was maintained at 35°C. All the solutions were degassed by ultrasonication. The mobile phase was filtered through a 0.45-µm membrane filter.

Microparticles equivalent to 100 mg of nizatidine were extracted into 25 mL of methanol by sonication for 30 min and then filter-transferred into a 50-mL volumetric flask. The residue was washed with two 10-mL portions of methanol, and the washings were added to the filtrate. The filtrate was then diluted to obtain a final concentration of 1 mg/mL. Further dilutions were made to get a concentration of 75 µg/mL, and the sample was injected into the column to record the chromatogram. The entrapment efficiency (%) was calculated by using the following formula (equation 9):

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100 \quad (9)$$

In vitro floating ability

Microparticles amounting to 50 mg were transferred to a beaker containing 10 mL of HCl (0.1 mol/L) and 0.02% Tween 20 and were shaken for 20 h in a water bath maintained at 37.0 ± 0.1ºC. The floating particles were collected after 20 h and then dried in a desiccator to a constant weight. The percentage buoyancy was calculated using the following formula (equation 10):

$$\% \text{ Buoyancy} = \frac{\text{Weight of floating microparticles}}{\text{Initial weight of microparticles}} \times 100 \quad (10)$$

In vitro drug release studies

The rate of drug release from the floating microparticles was determined using the USP XXIII basket-type dissolution apparatus. A weighed amount of floating microparticles equivalent to 100 mg of the drug was filled in a capsule and placed in the basket. Simulated gastric fluid (SGF, pH 1.2) (900 mL) containing Tween 20 (0.02% w/v) was used as the dissolution medium and maintained at a temperature of 37 ± 0.5ºC and a rotation speed of 50 rpm. Sink condition was maintained during the drug release study. Five millilitres of the sample were withdrawn at specified time intervals, passed through a 5-µm membrane filter (Millipore), diluted to 25 mL with SGF and analysed spectrophotometrically at 314 nm to estimate the concentration of the drug in the dissolution medium to further calculate the percentage cumulative drug release (%CDR). The initial volume of the dissolution fluid was kept constant by replenishing with fresh medium as required.

Optimization

Analysis of variance was performed for the response variables by using commercially available Design Expert software, version 9. Each parameter was assessed using an F-test ($p = 0.05$), and the responses were subjected to multiple regression analysis to generate polynomial equations which explain the relationship between the factors and the responses. The constraints for all the independent and dependent variables were set and subjected to multiple response optimization. The numerical optimization technique was applied to identify the optimum combination of factors to simultaneously optimize multiple responses.

Evaluation of the optimized formulation

Infrared spectroscopy

Fourier transform infrared (FTIR) spectra of the optimized FMN and the drug were obtained using the Shimadzu FTIR spectrophotometer (FTIR-8400S). The potassium bromide (KBr) pellet method was employed for the study. The samples were thoroughly blended with dry KBr crystals, and the mixture was compressed to form a disc. The disc was placed in the sample cell, and the spectra were recorded between 400 cm^{-1} and 4000 cm^{-1}.

Differential scanning calorimetry

The drug and optimized FMN were subjected to differential scanning calorimetry (DSC, Mettler 7, Germany). In this analysis, 1-3 mg of the sample was placed on an aluminium pan and the lid was crimped using Shimadzu crimper. An empty pan sealed in the same way was used as the reference. The thermal behaviour of the sample was investigated by heating it in a nitrogen atmosphere at the rate of 20°C/min over a temperature range of 25°C–200°C.

Scanning electron microscopy

The shape and surface characteristics of the prepared microparticles were evaluated using scanning electron microscopy (SEM, JSM 840 A). The samples were prepared by sprinkling the microparticles on a double-sided adhesive tape stuck to a stub. Gold–palladium coating was done on the prepared stub by using a sputter coater. The coated substances were then scanned, and photomicrographs were captured using a SEM (JSM 840 A).

Zeta potential

The zeta potential of the optimized formulation was measured using Zetasizer Nano ZSP, Malvern Instrument Ltd., USA. The microparticles were dispersed in 10-mL Millipore water, mixed for a minute and then measured.

In vitro drug release kinetics

The *in vitro* release data were fitted into various kinetic models such as zero order, first order, Higuchi and Korsmeyer–Peppas. The slope value and regression co-efficient (r^2) were calculated to determine the release mechanism.

In vivo radiographic study

The experimental procedure for *in vivo* radiographic study was approved by the Institutional Animal Ethics Committee (Ref No. DCD/GCP/20/E.C/ADM/2015-2016 dated 05-03-2016), Government College of Pharmacy. Four healthy albino rabbits weighing 2.0-2.5 kg were selected for the study. The animals were maintained under standard husbandry conditions; they were fasted for 24 h before the start of the study, with water provided *ad libitum*. Radiopaque microparticles were prepared by incorporating 2.5 g of barium sulphate in the polymeric solution, and the method was the same as that adopted for the optimized formulation. X-ray photographs of the untreated animals were taken to ensure the absence of radiopaque materials in the stomach. The animals were treated with the optimized formulation; they were fasted during the study period, but water was provided *ad libitum*. At varying time intervals, X-ray photographs of the gastric region were acquired for monitoring the location of the microparticles in the stomach.

RESULTS AND DISCUSSION

Experimental design

Taguchi orthogonal array design, a regular factorial design, was used to optimize the process of microparticulate formulation. This design aids in the development of a cost-effective and optimized formulation since it uses minimum number of formulations to estimate the important effects of key variables as well as their interaction effects within a short span of time (Singh, Kumar, Ahuja, 2004).

Preparation of microparticles

Floating microparticles were prepared using the oil-in-oil dispersion and solvent evaporation method as it is more advantageous in producing porous microparticles with improved entrapment efficiency than the oil-in-water emulsification technique which has low encapsulation efficiency for moderately water-soluble drugs. LMP is a gel-forming hydrocolloid polymer, which swells when it comes in contact with the gastrointestinal fluid but retains its initial shape and buoyancy. As reported in an earlier study (Bogataj *et al*., 1991), while preparing microparticles using liquid paraffin, the solvent used must be partially miscible and have a dielectric constant in the range of 20–30. For the present study, acetone was chosen since it is partially miscible with liquid paraffin and has a favourable dielectric constant of 20.7 in preparing oil-in-oil emulsion. Moreover, acetone is a volatile solvent and can be easily evaporated during the process of microparticle recovery. Span 80 acts as a stabilizer and prevents coalescence by forming a thin film around the particles (Huang *et al*., 2016).

Percentage yield

The percentage yields of all the formulations were found to be in the range of 75.46%–86.77%, with no substantial difference among them. Nevertheless, formulations having high LMP levels of 3%, 5.5% and 8% and surfactant concentrations of 1%, 1.5% and 2% provided enhanced yields (Table II). Specifically, 5.5% LMP gave a satisfactory yield, which could be due to effective dispersion of the polymer solution at the said concentration in liquid paraffin in the presence of 1.5% surfactant. On the other hand, very low and very high polymer concentrations may not allow effective dispersion in liquid paraffin because of increased viscosity of the polymer solution at high concentrations and inadequate dispensability at low concentrations. This hypothesis is in agreement with the results reported (Zhao *et al*., 2010) for the preparation of hollow microspheres based on polyvinyl pyrrolidone and ethyl cellulose.

TABLE II - Results of % yield, particle size, bulk density and tapped density

FORMU LATION	% YIELD	PARTICLE SIZE ± SD (µm)	BULK DENSITY±SD (g/cm^3)	TAPPEDDENSITY±SD (g/ cm^3)
FMN1	84.61	86.67 ±0.03	0.312 ±0.01	0.361 ±0.02
FMN2	75.46	90.51 ±0.08	0.348 ±0.02	0.439 ± 0.03
FMN3	84.25	128.89 ±0.07	0.407 ±0.06	0.493 ± 0.09
FMN4	82.3	97.88 ±0.05	0.332 ±0.03	0.393 ± 0.03
FMN5	79.03	105.64 ±0.19	0.344 ±0.02	0.401 ± 0.004
FMN6	85.25	115.96 ±0.08	0.21±0.01	0.231 ± 0.05
FMN7	78.11	114.2 ±0.06	0.262 ±0.01	0.305 ± 0.05
FMN8	76	106.66 ±0.18	0.357 ±0.03	0.435 ± 0.05
FMN9	77.98	119.3 ±0.09	0.435 ±0.03	0.532 ± 0.16
FMN10	80.75	74.57 ±0.06	0.32 ±0.04	0.372 ± 0.01
FMN11	85.38	108.61 ±0.05	0.53 ±0.11	0.636 ± 0.09
FMN12	80.05	97.2 ±0.06	0.354 ±0.01	0.426 ± 0.04
FMN13	83	101.79 ±0.08	0.453 ±0.06	0.53 ± 0.06
FMN14	79.98	187.05 ±0.09	0.346 ±0.01	0.385 ± 0.03
FMN15	83.22	79.53 ±0.07	0.407 ±0.05	0.457 ±0.05
FMN16	86.77	130.14 ±0.05	0.374 ±0.04	0.41 ±0.08

SD- Standard deviation

Micromeritic properties

The bulk density values of the formulations ranged between 0.21 g/mL and 0.53 g/mL (Table II), while their tapped density values were between 0.23 g/mL and 0.64 g/mL (Table II). The true density of the microparticles were in the range of 0.18–0.45 g/mL (Table III), which indicates their ability to float in the gastric fluid. The flow property of all formulations was determined by measuring the angle of repose, Carr's index value and Hausner's ratio, which were in the range of 20.91°–36.31°, 8.33%–28.57% and 1.09–1.23, respectively, thus suggesting the free-flowing nature of the microparticles.

TABLE III - Results of true density, Hausner's ratio, Carr's index and angle of repose

FORMU LATION	TRUE DENSITY±SD(g/ cm^3)	HAUSNER'S RATIO±SD	CARR'S INDEX±SD (%)	ANGLE OF REPOSE±SD(θ)
FMN1	0.22 ±0.04	1.157 ±0.009	12.5 ±0.0	24.13 ±1.09
FMN2	0.24 ±0.07	1.238 ±0.124	25 ±1.08	22.86 ±0.95

TABLE III - Results of true density, Hausner's ratio, Carr's index and angle of repose

FORMULATION	TRUE DENSITY±SD(g/cm^3)	HAUSNER'S RATIO±SD	CARR'S INDEX±SD (%)	ANGLE OF REPOSE±SD(θ)
FMN3	0.3 ±0.05	1.205 ±0.04	14.28 ±4.98	21.46 ±1.74
FMN4	0.26 ±0.07	1.182 ±0.01	16.66 ±1.76	20.91 ±3.78
FMN5	0.29 ±0.04	1.168 ±0.01	28.57 ±1.67	24.12 ±0.16
FMN6	0.35 ±0.17	1.099 ±0.18	8.33 ±1.45	20.41 ±1.90
FMN7	0.181 ±0.07	1.122 ±0.05	15 ±5.89	23.7 ±1.67
FMN8	0.34 ±0.05	1.217 ±0.02	16.66 ±2.56	24.26 ±2.87
FMN9	0.138 ±0.05	1.22 ±0.02	16.66 ±2.76	32.08 ±1.67
FMN10	0.19 ±0.03	1.169 ±0.01	12.5 ±2.34	30.97 ±1.59
FMN11	0.35 ±0.07	1.202 ±0.04	16.66 ±4.87	36.31 ±2.06
FMN12	0.27 ±0.18	1.12 ±0.06	28.57 ±1.09	25.58 ±0.78
FMN13	0.45 ±0.05	1.165 ±0.17	12.5 ±2.89	21.29 ±1.65
FMN14	0.3 ±0.08	1.14 ±0.05	22.22 ±1.98	22.02 ±1.89
FMN15	0.3 ±0.04	1.12 ±0.03	11.11 ±4.67	30.61 ±2.94
FMN16	0.43 ±0.06	1.096 ±0.05	10 ±2.56	29.46 ±3.45

SD- Standard deviation

Statistical optimization

Particle size

The particle size of all the 16 batches of microspheres prepared in the study was in the range of 71.83–187.05 µm (Table II). The polynomial equation alluded that the particle size decreased with an increase in LMP concentration.

Particle size = 106.66-10.25*A[1]+24.50*A[2]+5.28*A[3]-0.62*C[1]+25.59 *C[2]-11.80 *C [3] ---- (11)

Increase in LMP (A) (equation 11) caused a slight increase in the particle size, which was indicated by the positive coefficient of A[2]; however, at higher concentrations, the particle size decreased considerably, as inferred from the low positive coefficient of A[3]. This effect could be attributed to the prevention of agglomeration by the repulsive negative charges on the surface of the particles, which was confirmed by a zeta potential of -35.7 mV. These findings agree with the existing reports (Wijaya *et al*., 2019). The particle size of the microparticles depends upon the size of the emulsion droplets, which in turn depends upon the dispersive and surface tension forces. The former tends to disperse the emulsion, while the latter causes it to coalescence. To maintain the particles in a well-dispersed state, a factor C, i.e. surfactant was used, which had a negative effect on particle size; the decrease in particle size could be due to stabilizing effect of Span 80 at a high concentration by the prevention of coalescence (Jelvehgari *et al*., 2010). The average particle size was found to be 86.67 µm. The factors of internal phase volume (B), stirring speed (D) and stirring time (E) had negligible influence on particle size; hence, they were excluded from the model.

Entrapment efficiency

Entrapment efficiency can be explained by the following polynomial equation:

% Entrapment efficiency = +91.77–6.22*A[1]–1.21*A[2]+1.71*A[3]+0.74*C[1]–0.87*C[2]+1.83*C[3] (12)

Entrapment efficiency was found to increase with an increase in polymer concentration (equation 12) (Table IV). Increased entrapment efficiency, denoted by a positive coefficient at higher levels of LMP (A) and constant drug amount, may be due to the availability of excess polymer, which forms a dense matrix to encapsulate the drug. The entrapment efficiency of the drug depends on drug solubility, the stability of the dispersion, the method of preparation and the affinity between the drug and the polymer (Yang *et al.*, 2001). The high incorporation efficiency may be attributed to the use of liquid paraffin as a continuous phase (Ramesh, 2009). Increase in surfactant levels enhanced the entrapment efficiency by improving the stability of the emulsion. Similar conclusions have been drawn in another study (Dinarvand *et al.*, 2005), but the internal phase volume did not exert any influence on the entrapment efficiency; hence, it was excluded from the model.

TABLE IV - Results of drug entrapment efficiency and floating ability

FORMULATION	ENTRAPPMENT EFFICIENCY (%)	FLOATING ABILITY (%)
FMN1	95±0.00	96±2.00
FMN2	83.33±0.57	80.67±1.52
FMN3	89.67±1.15	84±1.00
FMN4	91±1.00	94±1.00
FMN5	87.67±1.52	82±1.00
FMN6	91.67±2.08	92.33±1.54
FMN7	95.33±0.57	90±2.00
FMN8	94.67±1.52	88±1.00
FMN9	93.33±0.57	94.33±0.57
FMN10	86.33±1.15	90±1.00
FMN11	94.67±0.57	90±1.52
FMN12	87.33±1.52	80.67±1.54
FMN13	97.67±1.52	82±1.00
FMN14	84.33±1.52	78.67±1.54
FMN15	99±0.00	90±1.00
FMN16	97.33±1.52	84±1.15

Floating ability

In vitro floating ability can be described by the following polynomial equation:

Floating ability = +87.13–7.12*A[1]+1.37*A[2]+ 6.37*A[3]–0.12*D[1]+0.87*D[2]–2.63*D[3] (13)

In the above polynomial equation, coefficients with positive and negative sign signify positive and negative influence on the response, respectively. The formation of a porous, low-density gel matrix of microparticles is favoured in an acidic environment. LMP exhibited improved floating ability in a concentration-dependent manner (equation 13) (Table IV). These observations on non-effervescent systems containing only LMP polymer are comparable to the floating behaviour described using effervescent *in situ* gel systems containing LMP in the presence of calcium carbonate and sodium citrate (Ghare, Mundada, 2017). However, the floating ability was not affected by variations in internal phase volume (B). Furthermore, the moderate stirring rate in combination with a high concentration of LMP had a positive influence on floating ability. This observation could be due to a reduction in particle size at moderate stirring speeds, with the simultaneous generation of charged particles and the production of repulsive forces that contribute to better floatability (Junyaprasert, Pornsuwannapha, 2008). Succinctly, the use of liquid paraffin-based oil-in-oil emulsification method and LMP with moderate shearing rate played a pivotal role in producing low-density microparticles.

In vitro drug release study

LMP concentration in the formulation was found to influence the release pattern. Fast release was seen at low concentrations, whereas sustained or slow release was noted at high concentrations.

The polynomial equations for 15 min, 2 h, 7 h and 24 h are given below:

% CDR 15 min = +0.57+0.22*A[1]+0.18* A[2]+ 0.047*A[3]–0.31*C[1]+0.45*C[2]+0.047*C[3] (14)

% CDR 2 h = +17.29+9.78*A[1]+0.11*A[2]– 3.77*A[3]–0.50*C[1]+8.66*C[2]–2.62*C[3 (15)

% CDR 7 h = +86.44+3.36*A[1]+0.45*A[2]– 5.41*A[3]–0.48*E[1]-3.61*E[2]+3.62*E[3] (16)

% CDR 24 h = +96.26–3.76*A[1]+1.83*A[2]+0.81 *A[3]– 1.19*C[1]–0.60*C[2]-0.11*C[3] (17)

It is evident from equation 14 that % CDR at a time interval of 15 min was high at lower levels of LMP, as indicated by a high positive coefficient value, and low at higher levels of LMP, as denoted by a low positive coefficient value. This result could be attributed to the formation of a polymer matrix of microparticles. At subsequent timepoints, such as 2 h (equation 15) and 7 h (equation 16), the decrease in drug release was signified by low positive coefficient values with increase in LMP concentration. A further decline in drug release was shown by very high negative coefficient values at higher concentrations of LMP, which may be due to the release-retardant characteristics of LMP. As reported in an earlier study (Patel, Srinatha, Sridhar, 2014), these release-retardant features are due to the formation of hydrogen bonds between the partially ionized carboxyl groups of LMP and the water molecules of simulated gastric fluid, which transforms the microparticles into swellable network matrices for sustained release of the drug. However, at the end of 24 h (equation 17), % CDR remained almost constant (Figure 1A, 1B, 1C, 1D and 2) and a drop in drug release was noted at augmented levels of LMP. Furthermore, it was observed that prolonged stirring (equation 16) resulted in enhanced drug release when compared with a short stirring time. This result may be ascribed to the existence of the particles as individual entities without agglomeration upon shearing for long hours. The internal phase volume (B) and stirring rate (D) played a negligible role on the *in vitro* release profile; hence, these factors were omitted from the model.

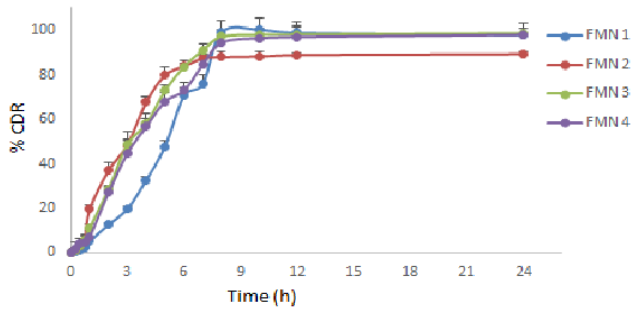

Figure 1(A) - In vitro release profile of formulation F1- F4

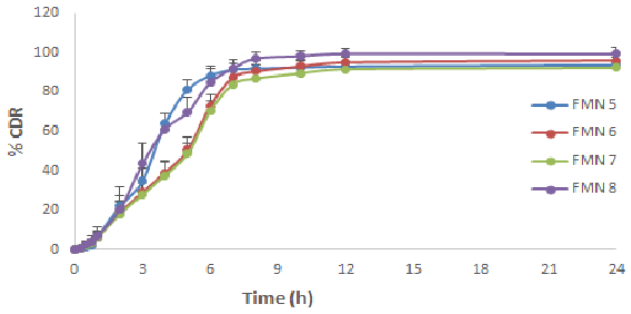

Figure 1(B) - In vitro release profile of formulation F5-F8.

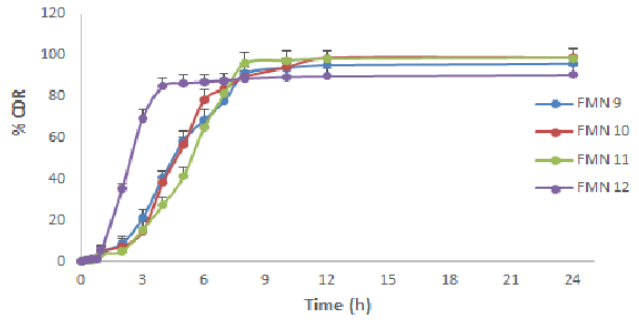

Figure 1(C) - IIn vitro release profile of formulation F9-F12.

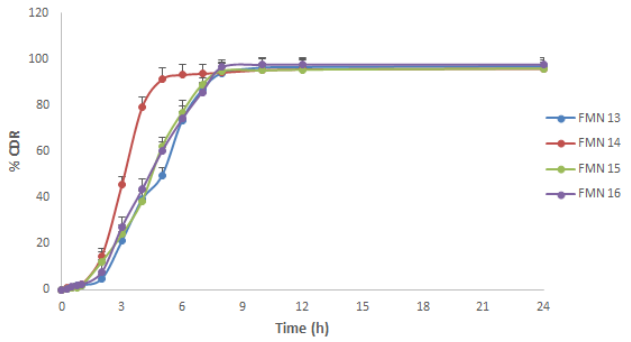

Figure 1(D) - In vitro release profile of formulation F13-F16.

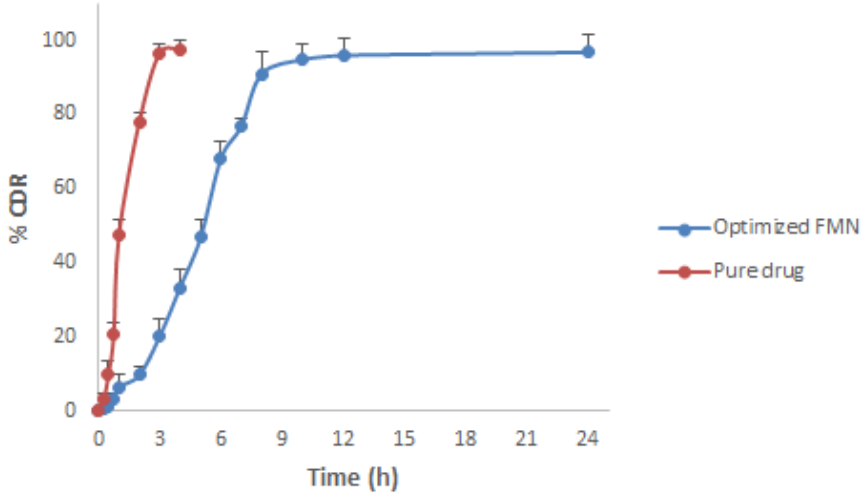

FIGURE 2 -In vitro release profile of pure drug and optimized FMN.

Regression analysis

The formulations were optimized using a numerical optimization technique. The optimum settings for dependent and independent variables were defined. All the response variables were analysed using a factorial model and were subjected to regression analysis to determine the regression coefficients. Model F values

(p values) (Table V) of 5.15 (0.0146) for particle size, 12.14 (0.0007) for entrapment efficiency, 9.22 (0.0020) for floating ability, 1.90 (0.1852) for % CDR 15 min, 2.94 (0.0710) for % CDR 2 h, 3.24 (0.055) for % CDR 7 h, and 5.44 (0.0123) for % CDR 24 h were obtained. These findings imply that the model was significant, with p values < 0.0500, except for % CDR 15 min, 2 h and 7 h. The R^2 values of the responses for particle size, entrapment efficiency, floating ability and % CDR 15 min, 2 h, 7 h and 24 h were determined to be 0.775, 0.890, 0.860, 0.559, 0.662, 0.683 and 0.784, respectively. High R^2 values denote significant influence of the factors on the responses. The optimized formulation was prepared as per the predicted model and evaluated for the said responses. The results suggested that all the independent factors played an important role in the preparation of nizatidine microparticles. The actual and predicted values of the optimized formulation were compared and found to be in close agreement with each other (Table VI).

TABLE V - Results of ANOVA

Response	Source	Sum of square	df	Mean square	F-value	p- value
Particle size	Factorial model	8328.01	6	1388.00	5.15	0.0146*
	A-Polymer	4457.10	3	1485.70	5.52	0.0199*
	C-Surfactant	3870.91	3	1290.30	4.79	0.0292*
	Residual	2424.43	9	269.38	-	-
	Cor Total	10752.44	15	-	-	-
Drug entrapment efficiency	Factorial model	333.68	6	55.61	12.14	0.0007*
	A-Polymer	303.71	3	101.24	22.10	0.0002*
	C-Surfactant	29.97	3	9.99	2.18	0.1601NS
	Residual	41.23	9	4.58	-	-
	Cor Total	374.91	15	-	-	-
Floating ability	Factorial model	419.50	6	69.92	9.22	0.0020*
	A-Polymer	374.75	3	124.92	16.47	0.0005*
	D-Stirring rate	44.75	3	14.92	1.97	0.1896NS
	Residual	68.25	9	7.58	-	-
	Cor Total	487.75	15	-	-	-
%CDR 15min	Factorial model	2.48	6	0.41	1.90	0.1852NS
	A-Polymer	1.13	3	0.38	1.74	0.2286NS
	C-Surfactant	1.35	3	0.45	2.07	0.1750NS
	Residual	1.96	9	0.22	-	-
	Cor Total	4.44	15	-	-	-

%CDR 2h	Factorial model	1040.35	6	173.39	2.94	0.0710*
	A-Polymer	589.29	3	196.43	3.33	0.0700*
	C-Surfactant	451.06	3	150.35	2.55	0.1208NS
	Residual	530.29	9	58.92	-	-
	Cor Total	1570.64	15	-	-	-
%CDR 7h	Factorial model	279.46	6	46.58	3.24	0.0555*
	A-Polymer	173.21	3	57.74	4.02	0.0454*
	E-Stirring time	106.25	3	35.42	2.47	0.1287NS
	Residual	129.25	9	14.36	-	-
	Cor Total	408.72	15	-	-	-
%CDR 24h	Factorial model	99.09	6	16.51	5.44	0.0123*
	A-Polymer	77.43	3	25.81	8.51	0.0054*
	C-Surfactant	21.66	3	7.22	2.38	0.1374NS
	Residual	27.30	9	3.03	-	-
	Cor Total	126.39	15	-	-	-

*Significant, NS Not Significant, CDR- Cumulative drug release

TABLE VI - Actual and predicted values of optimized formulation

Variables	Predicted	Actual
Particle size (μm)	100	86.67±0.03
% Drug entrapment efficiency	95.31	95±0.00
% Floating ability	95.37	96±2.00
% CDR 15 min	0.66	0.473±0.02
% CDR 2 h	10.98	9.531±0.64
% CDR 7 h	77.43	76.821±3.89
% CDR 24 h	96.95	97.294±5.02

CDR- Cumulative drug release

Evaluation of the optimized formulation

Infrared spectroscopy

The FTIR spectra of nizatidine and optimized FMN were recorded and compared. The spectrum of FMN (Figure 3) exhibited principal peaks of pure nizatidine (Figure 3) along with some other peaks, which are the characteristics of LMP, thereby indicating the compatibility of the polymer with the drug.

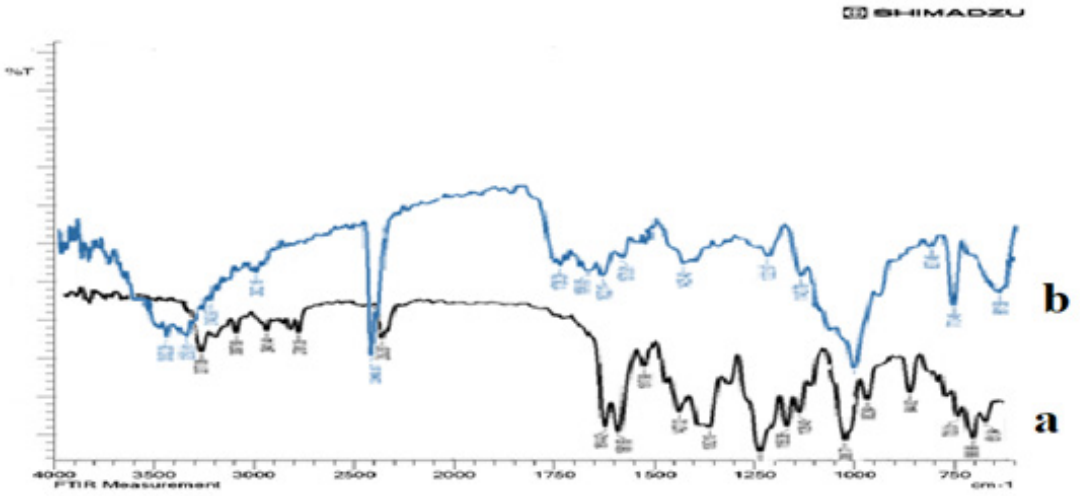

Figure 3 - FTIR spectra of pure drug (a) and optimized formulation (b). 3 -

Differential scanning calorimetry

The DSC thermograms of pure nizatidine and optimized FMN were evaluated, and nizatidine was found to have an endothermic melting peak at 136.5°C (Figure 4). The melting peak of the optimized FMN formulation was retained at the same temperature as that of the drug. It was evident that the drug had not undergone any chemical change during the formulation process; however, the decrease in the intensity of peak suggested the entrapment of the drug in the polymeric matrix. Hence, the excipients used in the formulation were inferred to be compatible with pure nizatidine.

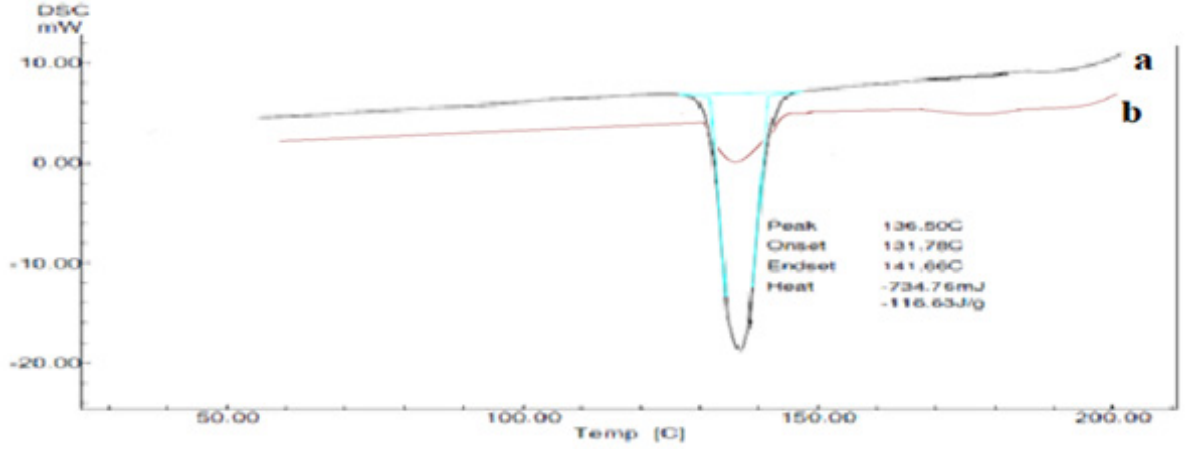

FIGURE 4 - DSC thermograms of pure drug (a) and optimized formulation (b).

Scanning electron microscopy

The morphology of the microparticles was investigated by SEM. The photomicrographs of the optimized formulations, which revealed the presence of irregular microparticles with a highly undulated surface, are presented in Figure 5 (A and B).

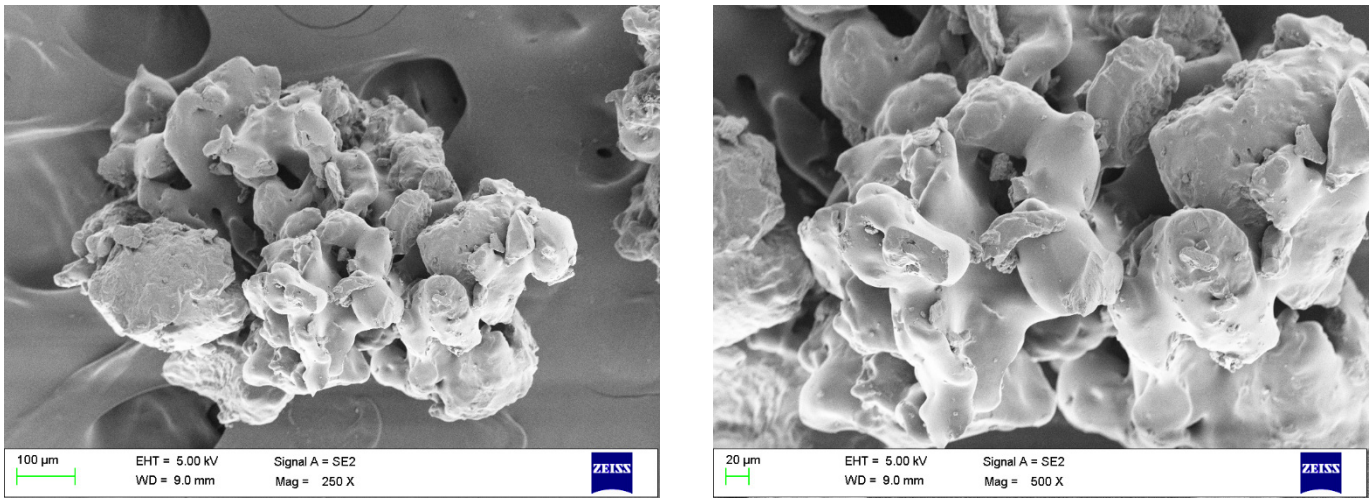

Figure. 5 - SEM photographs of optimized FMN,(A) Magnification 250 X and (B) Magnification 500 X.

Zeta potential

The magnitude of zeta potential, which ranged from ±40 to ±60 mV, indicated the potential stability of the colloidal dispersion owing to the presence of repulsive forces between the particles. The zeta potential of the microparticles was found to be -35.7 mV (Figure 6), which implied that the produced microparticles were moderately stable with no agglomeration.

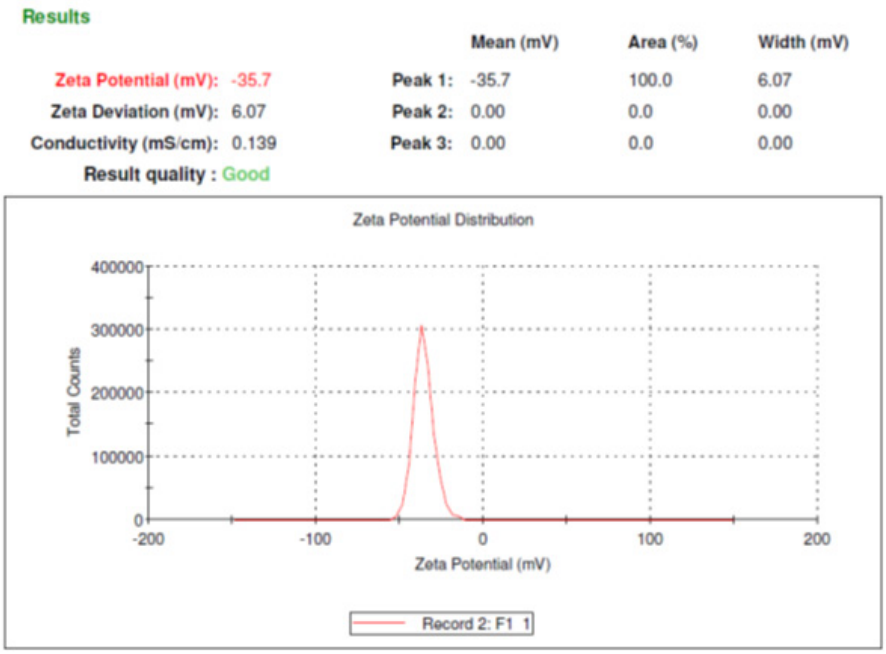

FIGURE 6 - Zeta potential report of optimized formulation.

In vitro drug release kinetics

The *in vitro* release data of the optimized formulation was fitted into various kinetic models such as zero-order (Figure 7A), first-order (Figure 7B), Higuchi (Figure 7C) and Peppas (Figure 7D). The highest regression coefficient was obtained for Peppas, followed by Higuchi, first-order and zero-order. The release exponent value (n) of 1.34 signified that non-Fickian diffusion (super case-II transport) was the release mechanism. During the sorption process, the polymer gets stretched and the outer gel layer acts as a permeation barrier to the vitreous core matrix (Bruschi, 2015). Over a period of time, the gel vitreous interface moves from the periphery towards the core, causing an increase in the stress–strain forces. This increase triggers the collapse of the core matrix, slows down the diffusion and dissolution process, and ultimately results in a modified drug release pattern.

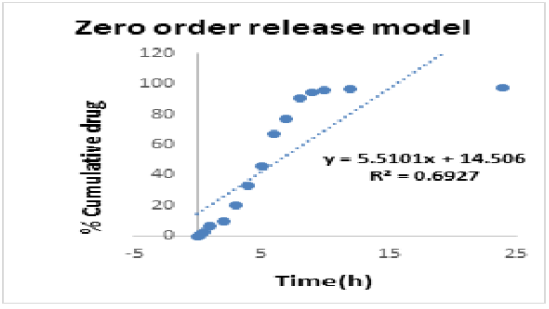

Figure 7(A) - *In vitro* release kinetics for Zero order

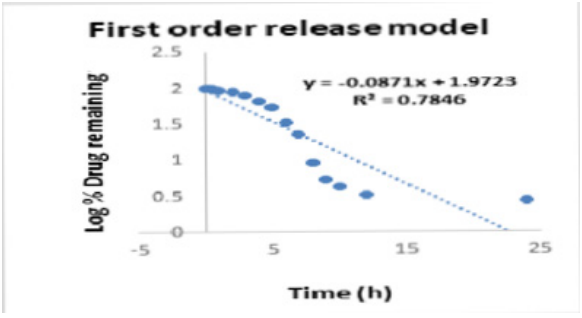

Figure 7(B) - *In vitro* release kinetics for First order

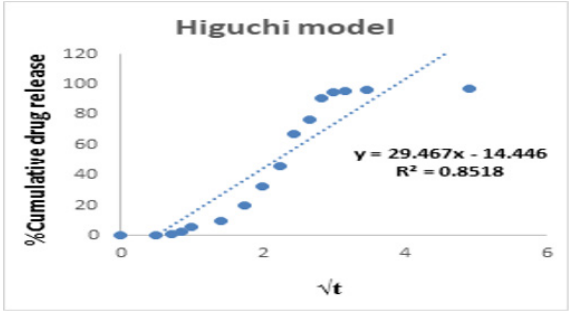

Figure 7(C) - *In vitro* release kinetics for Higuchi model.

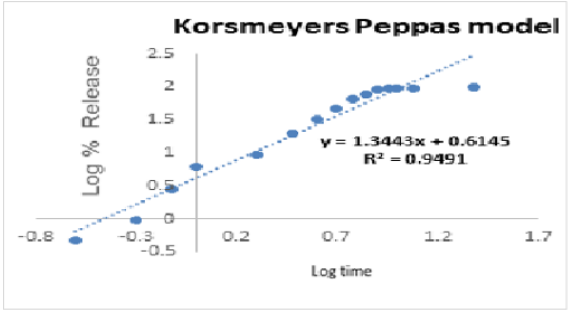

Figure 7(D) - *In vitro* release kinetics for Higuchi model.

In vivo radiographic studies

The optimized FMN was selected for examining the *in vivo* floating efficiency by the radiological method. A series of X-ray photographs of the stomach were taken at different intervals (Figure 8A–8G). Following oral administration of the suspension, the microparticles were seen floating in the upper part of the stomach after 1 h (Figure 8B). Most of the microparticles were found to be retained in the stomach for as long as 6 h (Figure 8G). Hence, the X-ray images of the rabbit stomach supported the prolongation of the gastric residence time in the fasted state. Improved floatability and prolonged retention of the microparticles in the stomach may be due to the formation of small particles in the acidic environment because of the interaction of LMP with various ions such as sodium, potassium, calcium, magnesium, copper and zinc by a cross-linking reaction to form a porous *in situ* gel matrix based on the egg–box model (Sundar *et al.*, 2012; Lofgren, Guillotin, Hermansson, 2006).

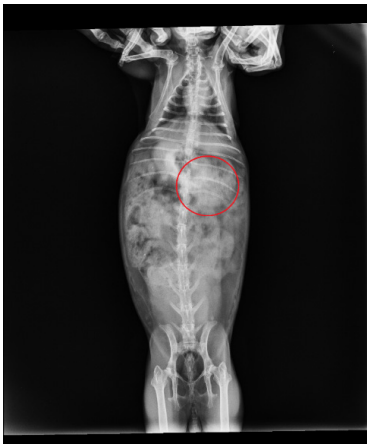

Figure 8(A) - X- ray image of Rabbit at 0h.

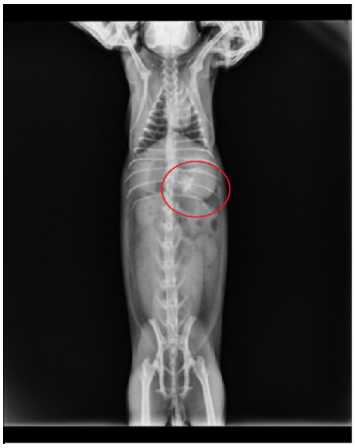

Figure 8(B) - X-ray image of Rabbit at 1h.

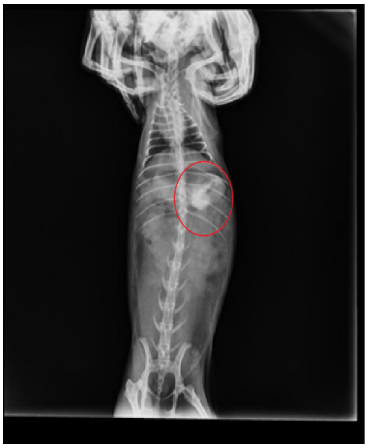

Figure 8(C) - X-ray image Rabbit at 2h of.

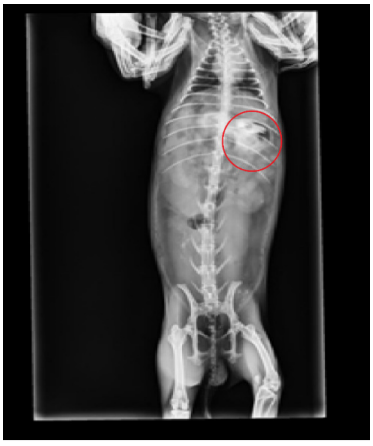

Figure 8(D) - X-ray image of Rabbit at 3h.

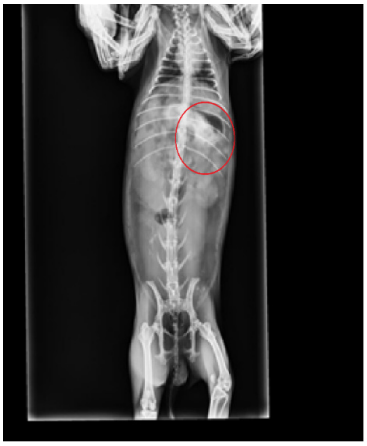

Figure 8(E) - X-ray image of Rabbit at 4h.

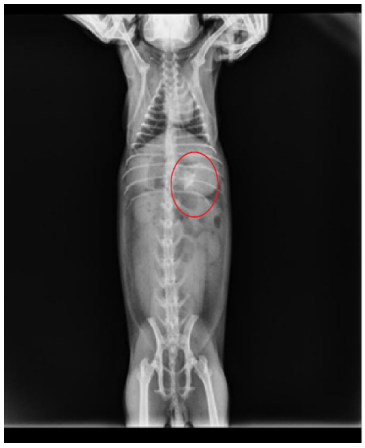

Figure 8(F) - X-ray image of Rabbit at 5h.

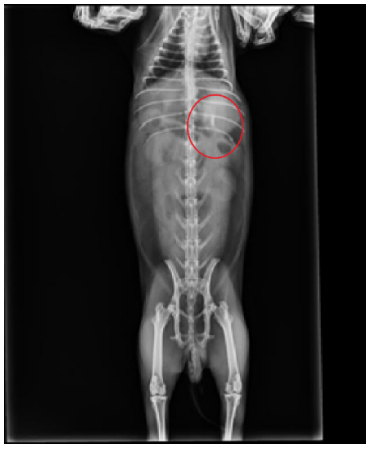

Fig. 8(G) - X-ray image of Rabbit at 6h.

CONCLUSION

Literature on the use of LMP in the absence of divalent or monovalent ion salts for the preparation of floating microparticles is scant. In this scenario, we have developed a novel, simple and economical oil-in-oil dispersion method for preparing floating microparticles using a single polymer in the presence of a surfactant and have optimized it for stomach-specific drug delivery. FMN1 was observed to be the optimized formulation, with an average particle size of 86.6 µm, floating ability of 96% and drug entrapment efficiency of 95%. % CDR at 15 min, 2 h, 7 h and 24 h were found be 0.473%, 9.53%, 76.82% and 97.29%, respectively. It was concluded that the developed drug delivery system had an extended gastro-retention time of 6 h and excellent floating ability, characteristics which are most amenable for stomach-specific drug delivery. The modified drug release pattern exhibited by LMP microparticles may be exploited to develop novel formulations for effective treatment of gastric ulcer.

ACKNOWLEDGMENT

The authors wish to thank the Government College of Pharmacy and the Drugs Control Department, Government of Karnataka, for providing the necessary facilities to carry out this research. The authors are also thankful to Strides Shasun, Bangalore and Chennai, for providing nizatidine samples as a gift. The authors are further indebted to CV Raman Institute, Indian Institute of Science, and Cessna Life Care, Bangalore, for providing the required facilities to perform the work. The authors express their heartfelt gratitude to the American Association of Government College of Pharmacy Alumni (AAGCPA) for sponsoring this project.

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Received for publication on 03rd January 2019
Accepted for publication on 11th April 2021