Formulation and Evaluation of Gastroretentive Floating Microspheres of Lafutidine

Anand Panchakshari Gadad*, Sneha Shripad Naik, Panchaxari Mallappa Dandagi and Uday Baburao Bolmal

Department of Pharmaceutics, KLE University's College of Pharmacy, JNMC Campus, Belagavi-590010, Karnataka, INDIA.

ABSTRACT

Background: Gastroretentive floating microsphere containing Lafutidine, a second generation histamine H_2–receptor antagonist were prepared by ionotropic gelation technique by using sodium alginate, HPMC K4M, ethyl cellulose as polymers, sodium bicarbonate as gas generating agent and calcium chloride as cross linking agent. **Objective:** To formulate a system to remain in the stomach for prolonged and predictable period in order to enhance the drug bioavailability. **Method:** They were evaluated for micromeritic study, percentage yield, drug entrapment efficiency, *in-vitro* buoyancy, surface morphology, *in-vitro* drug release, *in-vivo* floating study and stability studies. **Results:** The micromeritic parameters of floating microspheres were found to be within the acceptable limits. The particle size of microspheres containing HPMC K4M was found to be in the range 85-312 μm and that of ethyl cellulose containing microspheres was in the range of 167-329 μm. The entrapment efficiency was found to be in the range of 61.5%-79.0%. The floating microspheres were spherical in shape with distinct pores, slightly rough surface when observed under scanning electron microscopy. The percentage yield was found to be in the range of 75%-83.72%. The *in vitro* buoyancy was found to be in the range of 67.3%-87% and a total buoyancy time of more than 10 h. The *in vitro* dissolution studies showed a cumulative % release in the range of 57.15%-87.43%. The optimized formulation F4 was floating in rabbit stomach for almost 8 h. All the formulations followed Korsemeyer-Peppas kinetics indicating drug release by non-fickian release mechanism. The stability studies showed that floating microspheres were stable at 40 ± 2°C. **Conclusion:** The optimized formulation showed good floating for 8 h in stomach of rabbit. The formulation was stable at the end of 60 days with stability study.

Key words: Lafutidine, HPMC K4M, Sodium alginate, Ethylcellulose, Floating, Microsphere.

INTRODUCTION

Oral route of drug administration is the most convenient and commonly used method of drug delivery but this route usually produces gastric emptying rate that varies from person to person with a short stomach transit time and the existence of large absorption window in the upper small intestine for several drugs.[1]

Floating systems are low-density systems that have sufficiently buoyancy to float over the gastric content and remain buoyant in the stomach without affecting gastric emptying rate for a prolonged period of time, which results in a increased gastric retention time and a better control of fluctuation in plasma drug concentration. After release of drug, the residual system is emptied from the stomach.[2,3]

These difficulties have prompted researchers to design a drug delivery system which can stay in the stomach for prolonged and predictable period. Attempts are being made to develop a drug delivery system which can provide therapeutically effective plasma drug concentration for a longer period, thereby reducing the dosing frequency and minimizing fluctuation in plasma drug concentration at steady state by delivering the drug in a controlled and reproducible manner.[4]

Lafutidine is newly developed second generation histamine H_2–receptor antagonist, having poor water solubility and short elimination half-life up to 3.0 hours, belonging to BCS class-II drugs. It is absorbed more in the small intestine than in stomach.

Submission Date : 12-05-15
Revised Date : 26-06-15
Accepted Date : 13-02-16

DOI: 10.5530/ijper.50.2.21

Correspondence Address
Anand Panchakshari Gadad,
KLE University's College of Pharmacy, Department of Pharmaceutics,
JNMC Campus,
Belagavi-590010,
Karnataka, INDIA
Mobile: +91-9448309472
Fax: 08312472387
Email: gadadap@rediffmail.com

www.ijper.org

So our intention is to make drug remain in the stomach for prolonged period of time and thereby increase its residence time. Drug rapidly binds to gastric cell histamine H_2 receptors, thereby inhibiting the stimulation of cAMP and a resultant decrease in acid production (anti secretory action).[5]

As per the literature study, it shows Ionotropic gelation technique is best adopted method for development of microspheres and it is one of the approaches to formulate as GRDDS. Most of the literature study revealed a promising study of sodium alginate with use of effervescent gas generating agents to design the formulation to float in the stomach media.[6,7]

MATERIALS AND METHOD

Materials

Lafutidine was procured as a gift sample from Enaltec labs Pvt. Ltd., Nashik, Maharashtra. HPMC K4M was procured as a gift sample from Sanofi India Ltd, Goa. Ethyl cellulose was procured from West coast lab, USA. Sodium alginate was procured from SD Fine chem. Ltd., Mumbai. Calcium chloride was procured from Loba Chemie Pvt. Ltd, Mumbai. Sodium bicarbonate was procured from RFCL limited, New Delhi. All other chemicals used were of analytical grade.

Preparation of floating microspheres

Floating microspheres were prepared by Ionotropic gelation method. Accurately weighed amount of drug was dispersed uniformly in aqueous mucilage of sodium alginate with stirring. To this dispersion, desired polymer in different concentrations was mixed in suitable proportion and stirring is continued. Required amount of sodium bicarbonate was added to above solution. The resulting solution was then added drop wise through 26 gauge needle into calcium chloride solution. The formed microspheres were kept suspended in the solution for 1 h to improve their mechanical strength and then collected, washed with distilled water and air dried. The composition of floating microspheres is given in (Table 1).

CHARACTERIZATION OF FLOATING MICROSPHERE

Micromeritic properties

The microspheres were characterized by their bulk density, tapped density, compressibility index, Hausner's ratio and angle of repose.[8]

Determination of Percentage yield

The percentage yields of microspheres were calculated as the weight of the final product after drying to the initial weight of the drug and polymer used for the preparation of microspheres.[9]

The percentage yield was calculated by using the following formula:

$$\% \text{ yield} = \frac{\text{Practical mass(microspheres)}}{\text{Theoretical mass(drug+polymer)}} \times 100$$

Determination of drug content and entrapment efficiency

Accurately weighed 100 mg of microspheres and crushed in a mortar, 100 ml of simulated gastric fluid (pH 1.2) was added, the aqueous suspension was then sonicated for complete dissolution. From the above suspension, aliquot of 1 ml was taken and diluted to 10 ml. The mixture was then filteredand assayed spectrophotometrically using Shimadzu UV spectrophotometer (Japan), at 286 nm for the estimation of free drug.[10,11] The entrapment efficiency was calculated by using the following formula:

$$\text{DEE}\% = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Determination of In-vitro Buoyancy

Accurately weighed 300 mg of microspheres were taken and spread over the surface of USP type II dissolution apparatus filled with 900 ml 0.1N HCl containing Tween 80 (0.02%). The medium was agitated with a paddle at 100 rpm for 12 h. The floating and settled portions of microspheres were recovered separately. The microspheres were then dried and weighed.[12] The buoyancy percentage was calculated by using the following formula:

$$\text{Buoyancy}(\%) = \frac{Q_F \times 100}{Q_F + Q_S}$$

Where,

Q_F= Weight of the floating microspheres

Qs = Weight of the settled microspheres.

Surface morphology

Shape and surface morphology of floating microspheres were studied using scanning electron microscopy. The microsphere formulations were scanned randomly, and photographs were taken at suitable magnification.[13]

In-vitro drug release study

In-vitro drug release studies of floating microspheres were performed using USP type I dissolution apparatus in 900 ml of 0.1N HCl (pH 1.2) dissolution media at 100 rpm and 37°C. At each specified interval 5 ml of the sample was withdrawn and was replaced by equal volumes of fresh dissolution medium on each occasion.

The sample was analyzed by UV spectrophotometer at 286 nm.[12]

Release kinetics

The mechanism of drug release and release rate kinetics from the floating microspheres were studied by subjecting *in vitro* drug release studies into various kinetics models, Zero order, First order, Higuchi matrix and Korsmeyer Peppas model.[14]

In-vivo floating behaviour

Floating study was carried out on a New Zealand rabbit by fasting the animal for 12 h and X-ray photograph was taken to ensure absence of radio opaque material in the stomach. The rabbit was made to swallow barium sulphate loaded floating microspheres with 30 ml water. At predetermined time intervals the radiograph of abdomen was taken using an X-ray machine.[15]

Stability study

The stability studies were conducted according to ICH guidelines. The stability of floating microspheres were determined by keeping the optimized formulation (F4) at 25ºC ± 2ºC and 40ºC ± 2ºC. The samples were tested after 30th and 60th day for percentage buoyancy drug, entrapment efficiency and *in vitro* drug release.

RESULTS AND DISCUSSION

Eight formulations of floating microspheres were formulated using different ratios of HPMC K4M and ethyl cellulose polymers.

Compatibility studies

FTIR spectroscopy was carried out to study the compatibility of pure drug Lafutidine with the polymer HPMC K4M, Sodium alginate and Ethyl cellulose used in the formulation of floating microspheres. All important functional group frequencies for Lafutidine showed no significant shifts in combination spectra indicating no interaction between Lafutidine and polymers (Figure 1). It shows that there was no significant change in the chemical integrity of the drug.

Percentage yield

Percentage yield of all the formulations ranged from 75%-83.7%. As shown in result, microspheres containing HPMC K4M exhibited higher percentage yield than ethyl cellulose microspheres (Table 2).

Percentage entrapment efficiency

Entrapment efficiency of all the formulations ranged from 61.5% to 79%. As shown in result, microspheres formed from HPMC K4M exhibited good encapsulation efficiency than ethyl cellulose (Table 2).

In vitro buoyancy

The floating ability of the prepared beads were evaluated in acidic buffer (pH 1.2). The floating ability of the beads is directly related to the amount of gas generating agent added, in order to make the beads to float onto the surface of the media. Floating capacity of all the formulations ranged from 67.3% to 87% (Table 2). All the formulations showed a total floating time of for more than 10 h.

Particle size and surface morphology

The mean particle sizes of the Lafutidine microsphere formulations ranged from 85 μm-329 μm. The microspheres prepared with HPMC K4M showed smaller particle size than those prepared with ethyl cellulose which showed larger particle size. The microscopy image of the optimized formulation is shown in (Figure 2). The prepared microspheres were spherical in shape with slight distinct pores of the slightly rough surface of microspheres.

In vitro release study

The *in vitro* drug releases of all the formulations were found to be in the range of 57.15% to 87.43% at the end of 10 h. Drug release from microspheres prepared with HPMC K4M showed higher release compared to that prepared with ethyl cellulose. The comparative *in vitro* release profile of the formulations is shown in the (Figure 3).

By considering the percentage yield, drug entrapment efficiency and the *in vitro* drug release studies, the formulation F4 was selected as the optimized formulation.

Release kinetics

The release data was fitted to various kinetic models in order to determine the release constant and regression coefficient (R^2). The drug release profiles for formulations (F1 to F8) were best fitted with Korsmeyer Peppas model based on regression coefficients of 0.9973, 0.9979, 0.9965, 0.9963, 0.9979, 0.9958, 0.9974, and 0.9939 respectively. The diffusion exponent (n) values for all the formulations were greater than 0.5, indicating the drug release by non-fickian diffusion mechanism.

In-vivo floating behaviour

The *in vivo* gastric residence was studied by radiological studies (X-rays) of radio labeled microspheres using rabbit as animal model. Radiographic images (Figure 4) shows X-rays scans taken on the rabbit during radiological studies. It can be interpreted from the images that the microspheres were clumped together, intact and remained floating for [F4] 8 h.

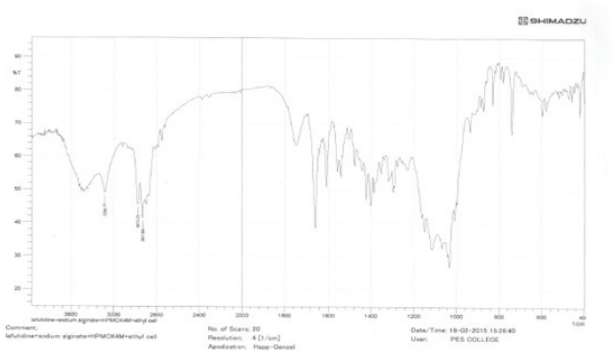


Figure 1: FTIR Spectra of Lafutidine and physical mixture(HPMC K4M+Ethyl cellulose + sodium alginate)

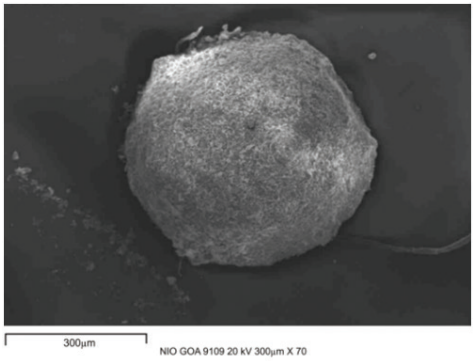


Figure 2: SEM photograph of Optimized Formulation F4

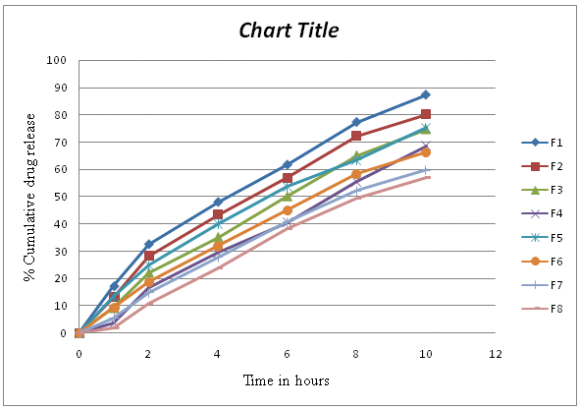


Figure 3: *In vitro* release profile of Lafutidine floating microspheres for formulations F1-F8 in 0.1N HCl

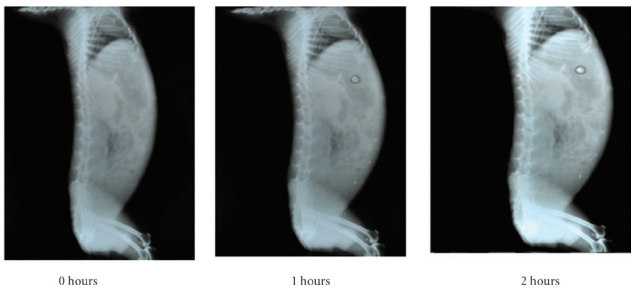


Figure 4: *In vivo* X-ray floating study of optimized formulation F4

Table 1: Composition of Lafutidine floating microspheres

Formulations	F1	F2	F3	F4	F5	F6	F7	F8
Lafutidine (mg)	20	20	20	20	20	20	20	20
Sodium alginate (%w/v)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
HPMC K4M (mg)	20	40	60	80	-	-	-	-
Ethyl cellulose (mg)	-	-	-	-	20	40	60	80
Sodium bicarbonate (mg)	2500	2500	2500	2500	2500	2500	2500	2500
Calcium chloride (%w/v)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Table 2: Results of yield, Floating buoyancy study and Drug Entrapment Efficiency of Lafutidine floating microspheres

Formulation code	Percentage yield* (%)	*In vitro* buoyancy* (%)	Drug entrapment Efficiency* (%)
F1	77.3 ± 0.816	87.0 ± 6.00	69.3 ± 0.06
F2	80.2 ± 1.632	82.3 ± 7.54	72.3 ± 0.03
F3	82.4 ± 2.160	77.3 ± 6.55	75.7 ± 0.06
F4	83.7 ± 2.054	70.0 ± 8.18	79.0 ± 0.05
F5	75.0 ± 1.247	83.6 ± 6.08	61.5 ± 0.04
F6	77.9 ± 1.632	78.0 ± 7.54	64.4 ± 0.06
F7	79.9 ± 1.414	73.0 ± 3.0	67.0 ± 0.08
F8	81.3 ± 1.632	67.3 ± 13.07	70.2 ± 0.06

*n=3.

Stability study

There was no significant change observed in the buoyancy %, entrapment efficiency and *in vitro* drug release as conducted at an interval of 10 days after 2 months at 40 ± 2ºC.

DISCUSSION

Lafutidine floating microspheres were successfully prepared by Ionotropic gelation method using Sodium alginate, HPMC K4M and Ethyl cellulose as polymers, sodium bicarbonate as gas generating agent and calcium chloride as cross linking agent. FTIR spectra of the physical mixture revealed that the drug and the polymers used were compatible. The Flow properties of all formulations were within the acceptable range. The particle size of floating microspheres were found to increase with increase in polymer concentration i.e. the formulations with HPMC K4M gave particles in the range of 85-312 µm and that of Ethyl cellulose exhibited particles in the range of 167-329 µm. The surface topography study of floating microspheres revealed that the microspheres were spherical in shape with slightly rough surface having small distinct pores on the surface which may be responsible for drug release. The percentage yield obtained in all the batches was good and in the range of 75% to 83.7%. The drug release decreased with the increase in polymer concentrations in floating microspheres. Formulations F1, F2, F3, F4, F5, F6, F7 and F8 followed Peppas model with non fickian drug release mechanism. Radiological studies revealed that the optimized microspheres remained intact floating in stomach for more than 10 h.

CONCLUSION

Formulation F4 showed good results with respect to the various evaluation parameters, so it was selected as the optimized formulation. The particle size increased with increase in polymer concentration. The drug entrapment efficiency was increased with increase in concentration of polymers. *In-vitro* buoyancy and the *in vitro* drug release decreased with respect to increase in concentration of polymers. The optimized formulation showed good floating for 8 h in stomach of rabbit. The formulation was stable at the end of 60 days with stability study.

ACKNOWLEDGEMENT

The authors are thankful to ENALTEC LABS PVT LTD, Nashik, Maharashtra, India for providing the free gift sample of drug Lafutidine. Authors wish to thank the Principal of KLE University College of Pharmacy, Belagavi for providing necessary facilities to carry out this work.

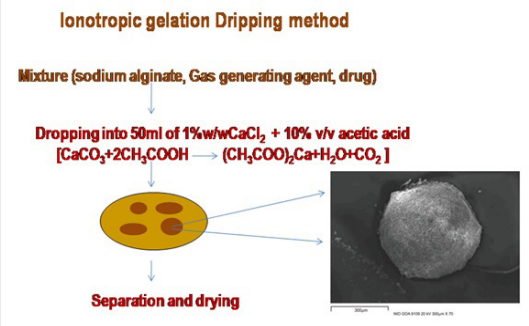
CONFLICT OF INTEREST

The research carried out at the institution don't have any conflicts among the authors. The animal study is carried out with the permission of Institutional Animal Ethics committee.

REFERENCES

1. Sharma S, Pawar AP. Low density multiparticulate system for pulsatile release of meloxicam. Int J Pharm. 2006;313(1):150-8.
2. Soppimath KS, Kulkarni AR, Aminabhavi TM. Development of hollow microspheres as floating controlled-release systems for cardiovascular drugs: preparation and release characteristics. Drug Dev Ind Pharm. 2001;27(6):507-15.
3. Kumar KR. Floating Microspheres: A Novel Approach in Drug Delivery. J Drug Delivery Res. 2012;1(4):1-20.
4. Rouge N, Buri P, Doelker E. Drug absorption sites in the gastrointestinal tract and dosage forms for site specific delivery. Int J Pharma. 1996;136(1):117-39.
5. Kapoor D, Vyas RB, Lad C, Patel M, Sharma S. Formulation and evaluation of stomach specific Floating tablet of anti-ulcer drug. World J Pharm and Pharm Sci. 2014;3(5):1534-45.
6. Lohithasu D, Midhun kD, Hemasundara R. Design and evaluation of Lafutidine floating tablets for controlled release by using semi-synthetic and natural polymer. J. Drug Disc Ther. 2014;2(24):01-08.
7. Gadad AP, Patil MB, Naduvinamani SN, Mastiholimath VS, Dandagi PM, Kulkarni AR. Sodium alginate polymeric floating beads for the delivery of Cefpodoxime proxetil. J Applied Polymer Sci. 2009;114(3):1919-22.
8. Lachman L, Liberman HA, Kang JL. The theory and practice of industrial pharmacy. 3rd ed. Varghese publication house. 1991;296-302.
9. Manivannan R, Baig MA, Purushothaman M, Kumar NS. Formulation and Evaluation of Eletriptan Hydrobromide Microspheres by using Natural Polymers. Int J Pharm Drug Analysis. 2014;2(3):347-53.
10. Patel B, Patel J, Thakor R. improvement of solubility of cinnarizine by using solid dispersion technique. Int Res J Pharm. 2010;1(1):127-31.
11. Revathi S, Madhulatha V, Dhanaraju MD. Formulation and Evaluation of Stavudine loaded Sodium Alginate Beads by Ionotropic Gelation Method. Int Res J Pharm. 2014;5(9):706-12.
12. Gadad A, Naval C, Patel K, Dandagi P. Formulation and evaluation of floating microspheres of captopril for prolonged gastric residence time: Indian J novel drug delivery. 2011;3(1):17-23.
13. Kapoor D, Vyas RB, Chaitali L, Patel M. Formulation Development, Optimization and Characterization of Floating Microspheres of Rabeprazole Sodium. World J Pharm. Pharm. Sci. 2013;2(6):6235-45.
14. Costa P, Lobo SJM. Modellingand comparison of dissolution profiles. Eur J Pharm Sci. 2001;13(2):123-33.
15. Gangadharappa HV, Biswas S, Getyala A, Gupta NV, PramodKumar TM. Development, *in vitro* and *in vivo* evaluation of novel floating hollow microspheres of rosiglitazone maleate. Der Pharmacia Lettre. 2011;3(4):299-316.

PICTORIAL ABSTRACT



ABBREVIATIONS USED

HPMC K4M: Hydroxy Propyl Methyl Cellulose Grade K4M; **ICH:** International Conference of Harmonization; **FTIR:** Fourier Transform Infrared Spectroscopy.

SUMMARY

- The particle size of floating microspheres were found to increase with increase in polymer concentration i.e. the formulations with HPMC K4M gave particles in the range of 85-312 μm and that of Ethyl cellulose exhibited particles in the range of 167-329 μm.
- The surface topography study of floating microspheres revealed that the microspheres were spherical in shape with slightly rough surface having small distinct pores on the surface which may be responsible for drug release.
- The percentage yield obtained in all the batches was good and in the range of 75%-83.7%. The drug release decreased with the increase in polymer concentrations in floating microspheres. Formulations F1, F2, F3, F4, F5, F6, F7 and F8 followed Peppas model with non fickian drug release mechanism.
- Radiological studies revealed that the optimized microspheres remained intact floating in stomach for more than 10 h.

About Authors

Anand P. Gadad: Is a Professor and Head, Department of Pharmaceutics, Faculty of Pharmacy, KLE University, Belagavi. He is working on areas of targeted drug delivery system viz., Gastroretentive drug delivery system, Polymeric nanoparticles, enhancing solubility of poorly soluble drugs, etc.

Panchaxari M. Dandagi: Is a Professor, Department of Pharmaceutics, Faculty of Pharmacy, KLE University, Belagavi. He is working on areas of targeted drug delivery system viz., Colon drug delivery system using pH dependent polymers and also Ocular drug delivery system through Nano and microspheres.

Sneha S. Naik: Obtained her post-graduate in 2015 from Dept. of Pharmaceutics, Faculty of Pharmacy, KLE University, Belagavi. She worked in the area of Gastroretentive floating microsphere to enhance the drug bioavailability.

U. B. Bolmal: Is an Assistant Professor, Department of Pharmaceutics, Faculty of Pharmacy, KLE University, Belagavi. He is working on biosynthesis of Biosurfactants from the bacteria, solubility enhancement techniques using Biosurfactants.