

FORMULATION, DEVELOPMENT & CHARACTERIZATION OF FLOATING MICROSPHERES OF SELECTED CALCIUM CHANNEL BLOCKER

ABSTRACT

The main aim of the present investigation is to study of Formulation, Development & Characterization of Floating Microspheres of Verapamil Hydrochloride. Floating microspheres with a central hollow cavity were prepared by using a modified Quasi-emulsion diffusion technique. Weighed quantities of Verapamil Hydrochloride, ethyl cellulose, polyethylene oxide and hydroxy propylmethyl cellulose (HPMC K15M) were dissolved in a mixture of ethanol and dichloromethane (1:1 solvent ratio) at room temperature in a magnetic stirrer at 50 rpm for 50 min. The samples were assayed for drug content using UV spectrophotometer at 228 nm after suitable dilution. No interference was found due to the other components of floating microspheres at 228 nm. The yield was determined by weighing the microspheres and then the percentage yield was calculated with respect to the weight of the input materials, i.e., weight of verapamil and polymers used. The polymers like ethyl cellulose, eudragit L 100, polyethylene oxide and HPMC were selected for hollow microspheres preparation. These formulations contained Ethyl cellulose (2%) and Polyethylene oxide (1%), HPMC K15M (1%) & Eudragit L100 (1%) respectively. The encapsulation efficiency ranged between 53 ± 2.2 to $89 \pm 1.9\%$, and was observed that the encapsulation efficiency increased with increasing amount of polymers in the hollow microspheres. The sphericity factors for all formulations were in the range of 1.01 ± 0.2 to 1.29 ± 0.6 and the sphericity values of best formulations F3, F7 and F9 were 1.05 ± 0.2 , 1.07 ± 0.1 and 1.16 ± 0.1 respectively. Quassi emulsion method used for preparation of hollow microspheres was suitable for poor water soluble drugs, because the drug was soluble in the internal organic phase.

KEYWORDS": Floating Microspheres, Gastro retentive formulation, Verapamil Hydrochloride, Entrapment Efficiency, Tapped Density

INTRODUCTION

More often, drug absorption is unsatisfactory and highly variable among and between

individuals, despite excellent in vitro release patterns. The reasons for this are essentially physiological and usually affected by the gastrointestinal (GI) transit of the form, especially its gastric residence time (GRT), which appears to be one of the major causes of the overall transit time variability [1,2].

Over the past three decades, the pursuit and exploration of devices designed to be retained in the upper part of the GI tract has advanced consistently in terms of technology and diversity, encompassing a variety of systems and devices such as floating systems, raft systems, expanding systems, swelling systems, bioadhesive systems and low-density systems [3,4].

The present study was aimed at the development of stomach specific drug delivery systems using various approaches like mucoadhesive or floating. Mucoadhesive microspheres of Verapamil hydrochloride, were prepared.

MATERIAL & METHODS

Formulation of hollow microspheres [5-7]

Floating microspheres with a central hollow cavity were prepared by using a modified Quasi-emulsion diffusion technique. Weighed quantities of Verapamil Hydrochloride, ethyl cellulose, polyethylene oxide and hydroxy propylmethyl cellulose (HPMC K15M) were dissolved in a mixture of ethanol and dichloromethane (1:1 solvent ratio) at room temperature in a magnetic stirrer at 50 rpm for 50 min. This solvent was poured drop wise into 100 ml distilled water containing 2 ml of Tween 80 maintained at a temperature of 50 ± 2 °C. The resultant solution was stirred with a pitched-blade-type impeller type agitator at 1100 rpm for 3 h to allow the volatile solvent to evaporate. This resulted in the formation of microspheres. Different ratios of polymers were used to prepare the microspheres. Eleven formulations were prepared by changing the amount of ingredients as shown in table.

Table 1: Formulation chart of Verapamil Hydrochloride hollow microspheres

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Verapamil Hydrochloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Ethyl cellulose (gm)	-	-	2	1	1	1	2	1	2	1	-
Polyethylene oxide (gm)	-	1	1	2	-	-	-	-	-	-	-

HPMC K15M (gm)	-	-	-	-	1	2	1	-	-	-	-
Eudragit S100 (gm)	-	-	-	-	-	-	-	1	1	2	1
Solvent ratio * (ml)	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1
Tween 80(ml)	2	2	2	2	2	2	2	2	2	2	2

* Ethanol and dichloromethane of 30 ml each.

EVALUATION

Percentage drug entrapment efficiency [8]

Floating microspheres equivalent to 4 mg of drug was dissolved in 10 ml ethanol. The samples were assayed for drug content using UV spectrophotometer at 228 nm after suitable dilution. No interference was found due to the other components of floating microspheres at 228 nm. The percentage drug entrapment efficiency and yield were calculated as follows.

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

Yield of floating microspheres

The yield was determined by weighing the microspheres and then the percentage yield was calculated with respect to the weight of the input materials, i.e., weight of verapamil and polymers used. The formula for calculation of percentage yield is as follows.

$$\% \text{ Yield} = \frac{\text{Total weight of floating microspheres}}{\text{Total weight of drug and polymer}} \times 100$$

Scanning electron microscopy (SEM)

The surface morphology of the microspheres was examined by scanning electron microscopy (SEM; JSM-5200, Jeol, Tokyo, Japan) operated at 15 KV on samples, gold-sputtered for 120 s at 10 mA, under argon at low pressure.

Sphericity of the microsphere [8]

The sphericity of the prepared microspheres can be confirmed using a camera lucida by taking the tracings of the microspheres on a black paper. The tracings help to calculate the circulatory factor and confirm the sphericity of microspheres if the obtained values are nearer to 1.

$$S = P^2 / 12.5 \times A$$

Where A is area (cm²) and, P is the perimeter of the circular tracing.

Micromeritic properties of microsphere [9-10]

The microspheres were characterized by their micromeritic properties, such as particle size, bulk density, compressibility index and angle of repose (values useful in prediction of flowability).

Floating Characteristics

***In vitro* buoyancy of microspheres [11]**

The floatation study was carried out to ascertain the floating behaviour of the microspheres prepared with various polymer combinations. Floating behaviour of hollow microspheres was studied using a USP dissolution test apparatus II by spreading the microspheres (100 mg) on 900 ml of 0.1 N HCl containing 0.02 % v/v tween 80 as surfactant. The medium was agitated with a paddle rotating at 100 rpm and maintained at $37^{\circ} \pm 0.5^{\circ} \text{C}$ for 12 h. Both the floating and the settled portions of microspheres were collected separately. The microspheres were dried and weighed. The percentage of floating microspheres was calculated using the following equation.

$$\% \text{ Floating capability} = \frac{\text{Weight of floating hollow microspheres}}{\text{Initial weight of hollow microspheres}} \times 100$$

Initial weight of hollow microspheres

***In vivo* floating behavior [12]**

Barium sulphate loaded microspheres were prepared by adopting the procedure as described earlier, except for using barium sulphate instead of drug. Healthy rabbit weighing approximately 2.3 Kg was used to assess *in vivo* floating behaviour. Ethical clearance for the handling of experimental animals was obtained from the institutional animal ethical committee (IAEC) of JSS College of Pharmacy, Mysore constituted for the purpose. The animal was fasted for 12 h and the first X-ray photographed to ensure absence of radio opaque material in the stomach. The rabbit were made to swallow barium sulphate loaded microspheres with 30 ml of water. During the experiment, rabbits were not allowed to eat but water was provided. At predetermined time intervals, the radiograph of abdomen was taken using an X-ray machine.

***In vitro* drug release study [13-14]**

The release rate of drug from formulations was determined using USP dissolution testing apparatus II (basket type). The dissolution test was performed using 900 ml of 0.1 N HCl, at $37 \pm 0.5^{\circ} \text{C}$ and 50 to 100 rpm. Aliquots (5mL) were withdrawn at regular intervals for 12 h, sample was replaced by its equivalent volume of fresh dissolution medium to maintain the sink condition. The samples were analyzed spectrophotometrically at wavelength corresponding to absorption maxima of the drugs. The release kinetics was fitted into various models using

PCP dissolution v2.08 software.

Stability studies [15]

A drug formulation is said to be stable if it fulfills the following requirements:

Formulations were packed in a screw capped bottle and studies were carried out for 12 months by keeping at $25 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ RH & $30 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH and for 6 months for accelerated storage condition at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH.

Samples were withdrawn on 0, 3, 6 and 12 months for long term storage condition and 0, 3 and 6 months for accelerated storage condition and checked for changes in physical appearance and drug content as per ICH Q1A (R₂) guidelines. Graphs were plotted using Sigmaplot 12.0 to determine the statistical significance.

RESULTS & DISCUSSION

Preparation of hollow microspheres

The hollow microsphere was prepared by removing water from the cavity of the microsphere with air drying. The presence of Tween 80 prevents aggregation of droplets, as it acts as an emulsifying agent, which gets adsorbed at the interface between droplets and the aqueous medium. It was observed that sudden addition of polymer solution to the water containing Tween 80 leads to the formation of large polymer precipitates, which caused decrease in the percentage yield of microspheres.



Figure 1: Prepared Rosiglitazone maleate hollow microspheres

Selection of polymers

The polymers like ethyl cellulose, eudragit L 100, polyethylene oxide and HPMC were selected for hollow microspheres preparation.

The effect of drug: polymer ratio

The entrapment efficiency of F3, F7 & F9 was higher compared to other formulations. These formulations contained Ethyl cellulose (2%) and Polyethylene oxide (1%), HPMC K15M (1%) & Eudragit L100 (1%) respectively.

The effect of stirring speed and mixing time

Average diameter of hollow microspheres was controlled by stirring speed. The ultimate mean diameter of hollow microspheres was determined by the particle size, which decreased with increasing rotational speed.

The effect of surfactant

These are substances which are added to the formulation to disperse a water- insoluble drug to form a colloidal dispersion. In other words, Tween 80 enhanced the solubility of the drug.

Percentage yield

During the process of microencapsulation, the mechanical variables cause loss of final product and hence process yield may not be 100%. Hollow microspheres were weighed after drying and the percentage yield was calculated.

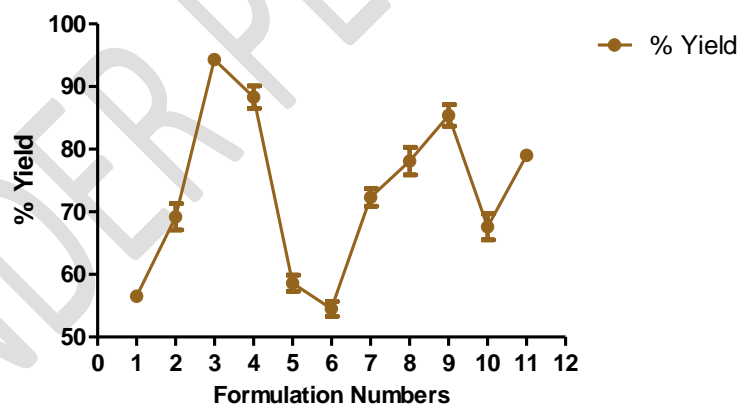


Figure 2: Effect on % yield of different formulation

Drug loading and encapsulation efficiency

The drug content test was carried out to ascertain that the drug is uniformly loaded in the formulation. Relatively high encapsulation efficiency was observed for all the formulations. The encapsulation efficiency ranged between 53 ± 2.2 to $89 \pm 1.9\%$, and was observed that the encapsulation efficiency increased with increasing amount of polymers in the hollow

microspheres. Formulations F3, F7, F9 showed relatively higher encapsulation efficiency as these formulations were composed of high concentration of polymer. When loading is high proportion of larger particle formed is also high. Among all formulations, F3, F7 and F9 showed maximum percentage yield and drug loading.

Table 2: Drug loading and encapsulation efficiency of prepared hollow microspheres of Verapamil Hydrochloride

Formulation	Theoretical Drug Loading* (%)	Actual Drug loading* (%)	Encapsulation Efficiency *(%)
F1	20.0	13.15±0.87	53±2.2
F2	30.9	20.16±0.44	72±2.1
F3	35.9	27.6± 0.71	89±1.9
F4	29.0	24.01± 0.71	69±1.4
F5	20.0	13.91± 0.34	58±1.3
F6	30.0	21.49± 0.95	56±2.2
F7	35.9	25.10± 0.52	71±1.4
F8	29.0	17.10± 0.43	74±2.1
F9	33.7	23.95± 0.88	89±1.3
F10	30.0	20.4± 0.62	65±2.3
F11	25.0	17.81± 0.51	58±1.7

Fourier transform infrared spectroscopy (FT-IR)

Verapamil Hydrochloride and the formulation F3 were subjected to FT-IR spectroscopic analysis for compatibility studies and to ascertain whether there is any interaction between the drug and the polymers used.

Table 3: FT-IR spectral data of Verapamil Hydrochloride and hollow microspheres (F3)

Groups	Peak positions in pure drug (cm ⁻¹)	Peak positions in F3(cm ⁻¹)
C=N stretch	1608	1556.61
C=O stretch	1703	1701.27
N-H stretch	3220	3294.53
C-N stretch	1260	1313.51

C-S stretch	773	663.53
Ar-C-H	3030	3007
Ali-C-H	2980	2929
C-O	1246	1246.06

Differential scanning calorimetry (DSC)

DSC is very useful in the investigation of the thermal properties of hollow microspheres, providing both qualitative and quantitative information about the physicochemical state of drug inside the hollow microspheres. There will be no detectable endotherm if the drug is present in a molecular dispersion or solid solution state in the polymeric hollow microspheres loaded with drug. In the present investigation, DSC thermograms of pure drug and drug loaded hollow microspheres were taken as shown in figure and data for the same is given in table. The thermal properties of the drug and the mixture of the drug and polymers are of importance as this ascertains the crystalline and amorphous status of the entrapped drug in the polymers thereby assessing the interaction among different components of the formulation during the fabrication process.

Table 4: DSC thermograms of Verapamil Hydrochloride and hollow microspheres (F3)

S. No.	Drug and formulation	T _o (°C)	T _m (°C)	T _c (°C)	Melting range (°C)
1	Verapamil Hydrochloride	118.60	122.80	126.79	8.19
2	Formulation F3	118.44	122.65	125.85	7.41

T_o - Onset of melt, T_m - Melting point, T_c - Completion of melt,

Scanning electron microscopy (SEM)

Porous structure was observed on the surface of microspheres shell due to the rapid diffusion of the solvent, there is also possibility of rupture of some microspheres. Microspheres floated more than 12 h because of presence of hollow cavity. SEM photographs are shown in figures.

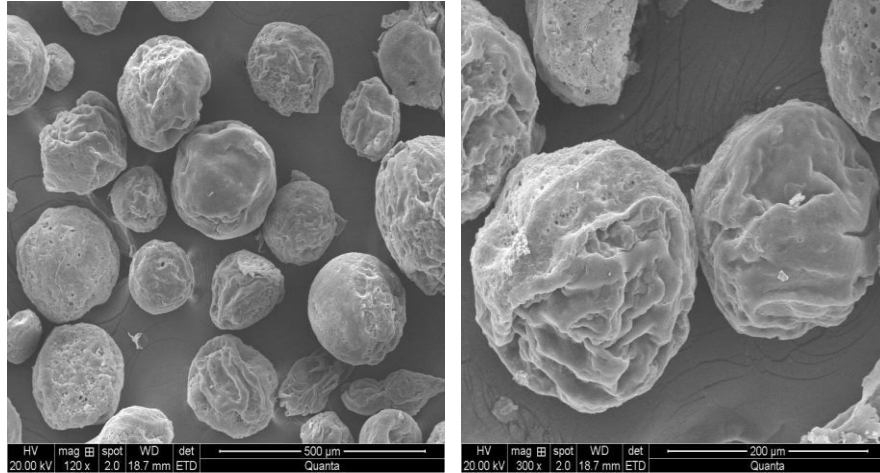


Figure 3: SEM photograph of microspheres at different magnifications

Sphericity of the microspheres

The sphericity of the prepared microspheres was confirmed and the calculated values were nearer to 1. The sphericity factors calculated for the microspheres are presented in table and shown in figure.

The sphericity factors for all formulations were in the range of 1.01 ± 0.2 to 1.29 ± 0.6 and the sphericity values of best formulations F3, F7 and F9 were 1.05 ± 0.2 , 1.07 ± 0.1 and 1.16 ± 0.1 respectively. The sphericity value nearer to 1 indicated that the prepared formulations were spherical in nature. A similar sphericity factor calculated for Indomethacin pellets was reported by Desay *et al.*

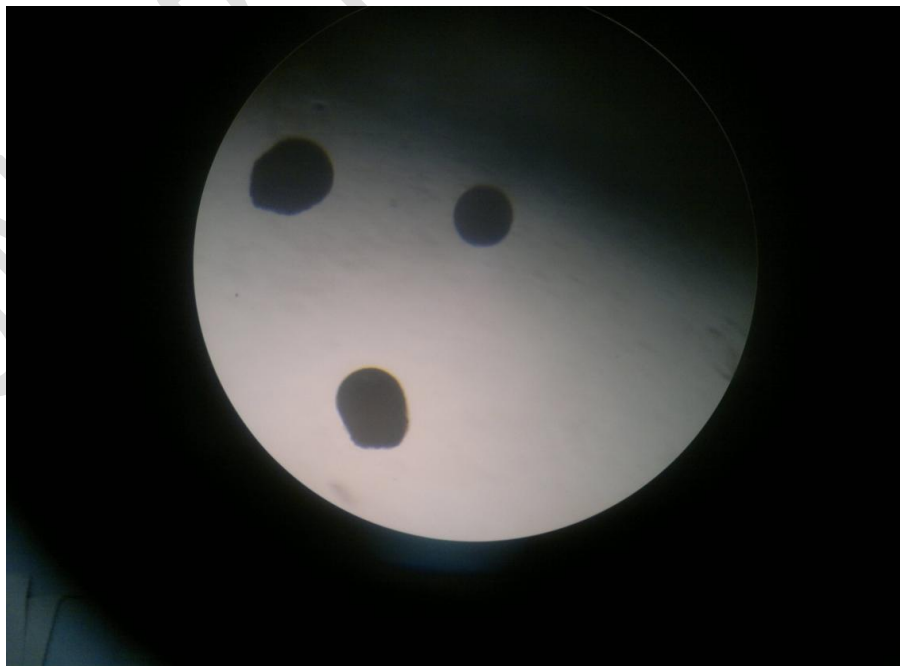


Figure 4: Image using camera lucida showing sphericity of hollow microsphere at 10x magnification

Micromeritics properties

The values of θ ranged from 25° to 29° indicating that the obtained values were well within the limits for powder to have good flow properties. This result clearly showed that the prepared hollow microspheres have reasonably good flow property. The value of CI was found to be in the range of 13.7 to 26.1 %. The values of tapped density ranged between 0.138 to 0.281 g / cm³. The values of compressibility index indicated fair to good flow properties. The size of particles depends on the concentration of polymer and stirring speed. In general, the size of particles ranged from 223 to 446 μm . The particle size increased with increasing concentration of polymer and particle size decreased with increase in stirring speed. The particle size range was between 223 \pm 2.6 to 446 \pm 5.2 and the mean particle size of the F3 microspheres was 312 \pm 4.1. The microspheres prepared with ethyl cellulose, HPMC and eudragit showed higher particle size as compared with ethyl cellulose and polyethylene oxide combination ($p < 0.05$).

Table 5: Micromeritic properties of Verapamil Hydrochloride hollow microspheres

Formulation	Mean size* (μm)	Θ°*	CI%*	Tapped density* (g/cm³)
F1	257 \pm 5.7	25 \pm 0.7	20.8 \pm 1.1	0.201 \pm 1.03
F2	306 \pm 2.3	28 \pm 1.2	16.2 \pm 1.6	0.197 \pm 1.2
F3	312 \pm 4.1	28 \pm 0.9	13.7 \pm 1.1	0.225 \pm 0.9
F4	308 \pm 3.7	28 \pm 2.1	18.6 \pm 2.0	0.166 \pm 1.3
F5	223 \pm 2.6	24 \pm 1.4	23.9 \pm 0.9	0.138 \pm 0.7

F6	334±3.4	29±1.1	26.1±1.5	0.210±1.3
F7	446±5.2	28±2.0	21.9±1.1	0.141±1.1
F8	347±4.1	28±1.5	25.8±1.3	0.228±1.12
F9	393±1.9	27±1.1	18.8±2.4	0.154±1.3
F10	377±2.8	26±2.3	21.7±1.7	0.174±1.0
F11	302±1.7	26±1.9	20.8±0.7	0.281±0.9

***In vitro* buoyancy of microspheres**

To assess the floating properties, the microspheres were placed in 0.1 N HCl containing 0.02 % v/v Tween 80 as surfactant to simulate gastric condition. 0.02 % v/v Tween 80 was used for the wetting of natural surface active agents, such as phospholipids in the gastrointestinal tract. Despite the solution being stirred for more than 8 h, the hollow microspheres still floated indicating that microspheres exhibited excellent buoyancy effect. The density of values of hollow microspheres ($<1.000 \text{ g/cm}^3$) was less than that of the gastric fluid ($<1.004 \text{ g/cm}^3$) further supporting floating nature. The *in vitro* floating test was conducted on the microspheres which showed floating capability of about 70 ± 1.1 %. All the formulations showed buoyancy of more than 8 h. As concentration of ethyl cellulose increased buoyancy increased. The floating behaviour was controlled by varying the concentration of ethyl cellulose (7 cps) in different formulations. Among all formulations, F1, F3, F7 and F9 showed maximum percentage floating ability. The result of *in vitro* % floating is depicted graphically in figure and data for the same is given in table.

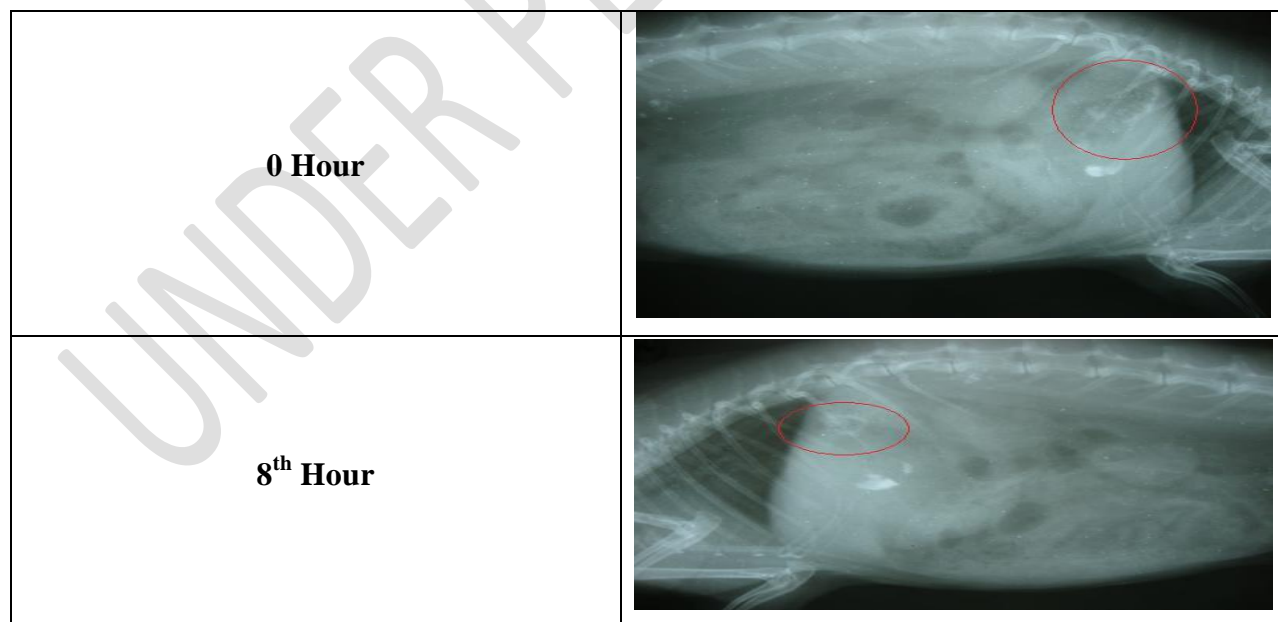
Table 6: *In vitro* % floating ability data of hollow microspheres

Formulation	% floating hollow microspheres
F1	83 ±0.15
F2	72 ±0.25
F3	84 ±0.56
F4	76 ±1.21

F5	78 ±1.62
F6	70 ±0.89
F7	80 ±0.2.1
F8	77 ±1.34
F9	84 ±1.21
F10	76 ±1.96
F11	65 ±1.73

***In vivo* floating behavior**

For investigating *in vivo* floating behavior of formulation F3 consisting of ethyl cellulose, polyethylene oxide (2:1) hollow microspheres loaded with barium sulphate, radiographic images (X-ray photographs) of rabbit's stomach at specific periods were taken. The amount of X-ray opaque material in these hollow microspheres was sufficient to ensure visibility by X-ray and also amount of Barium sulphate (100 mg) used was less but enough to enable the hollow microspheres to float. The hollow microspheres did not adhere to the gastric mucosa and floated on the gastric fluid for more than 12 h. This was evident by the X-ray photographs taken 4 h, 8 h & 12 h.



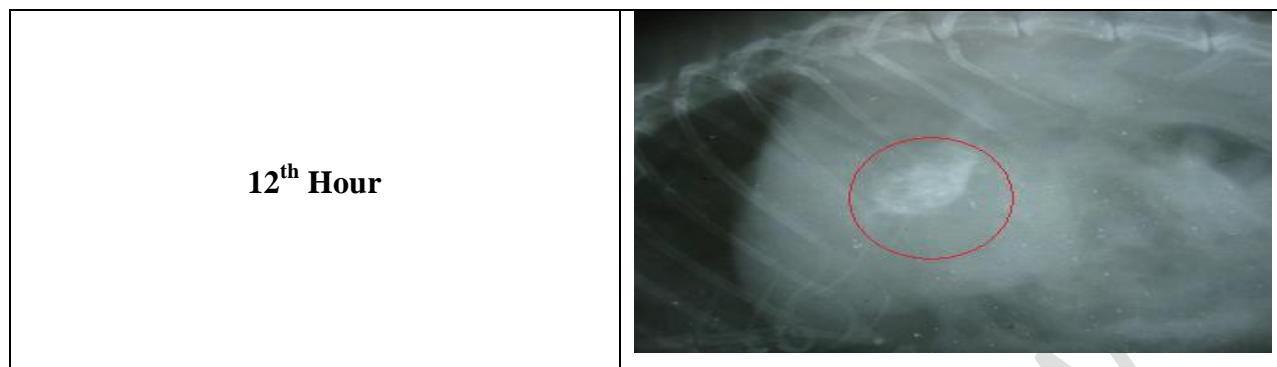


Figure 5: X-ray images showing floating ability of hollow microspheres

***In vitro* drug release studies**

Solubility of Verapamil hydrochloride is pH dependent and it gets easily absorbed in the stomach. Maximum absorption may be expected with increasing solubility in acidic environment. Hence the floating form was developed. It was assumed that better solubility of Verapamil hydrochloride in an acidic environment of the stomach may result in a greater absorption leading to higher plasma concentration. It is known that microspheres constitute multiple-unit dosage forms which have many advantages as compared to tablets. They spread uniformly in the stomach which leads to a decreased risk of high local concentration and exhibits less adverse effects. Moreover, these forms are characterized by a good reproducibility of drug release due to large surface area and short diffusion pattern of the drug. The *in vitro* release profiles of formulations are presented in figures. Due to their floating nature, the microspheres were forcibly immersed into the dissolution medium to avoid adherence to the surface of the jar, thus leading to nonparticipation in the dissolution process. The drug release was extended to 12 h. The formulations F3, F4 and F11 showed initial burst release. This may be attributed to the release of drug from the surface of microspheres as the drug might have migrated to the surface along with water during the drying process or presence of uncovered drug crystals on the surface of the microspheres. After 1 h, drug release slows down. In case of other formulations, burst release was not observed, which showed that the drug was sufficiently encapsulated in the polymer shell.⁷¹ *In vitro* dissolution studies of Verapamil hydrochloride from floating hollow microspheres of Verapamil hydrochloride were carried out for all formulations in pH 1.2 hydrochloric acid buffer for 12 h using electrolab dissolution test apparatus II. It was found that formulations F1-F11 showed 43.0% - 80.76 % of drug release

at 8 h and 75.3 % - 99 % of release at 12 h ($p < 0.05$).

Microspheres prepared with ethyl cellulose and HPMC (F5, F6 and F7) showed less release compared to other combinations. This may be probably due to the gelation property of HPMC, which forms gel matrix after contact with the dissolution medium. F1 and F11 showed more than 99 % drug release at the end of 12 h, which may be due to low polymer concentration resulting in smaller particle size with larger surface area.

Stability studies

The objective of stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and RH. The Verapamil hydrochloride containing optimized formulation F3 was subjected to stability studies carried out for 0, 3, 6 and 12 months for long term storage conditions and accelerated conditions. To assess stability, these samples were analyzed and checked for changes in physical appearance and drug content at regular intervals. The obtained data is presented in table. From the results, it is concluded that the formulation did not undergo any chemical change/interaction during the study period. The results obtained are shown in figures.

Table 7: Stability study for drug content of Verapamil hydrochloride hollow microspheres (F3)

Stability condition	Sampling interval (Months)	Physical appearance	% Drug content* F3	
25±2°C/60±5% RH	0	No change	89.98	0.24
	3	No change	89.27	0.18
	6	No change	87.69 ± 0.37	
	12	No change	87.23 ± 0.26	
30 ±2°C/65 ±5% RH	0	No change	89.98	0.24
	3	No change	87.69	0.29
	6	No change	87.03 ± 0.34	
	12	No change	86.84 ± 0.26	
40 ±2°C/75±5% RH	0	No change	89.98	0.24
	3	No change	85.60 ± 0.32	
	6	No change	84.72 ± 0.57	

CONCLUSION

Quassi emulsion method used for preparation of hollow microspheres was suitable for poor

water soluble drugs, because the drug was soluble in the internal organic phase. FT-IR and DSC studies indicated that there was no chemical interaction between the drug and the polymers used. The morphology of hollow microspheres was examined using SEM. The view of microspheres showed a hollow spherical structure with rough surface morphology. It was also evident that the hollow microspheres exhibited porous surfaces. Results of drug content determination from hollow microsphere inferred that there was proper and uniform distribution of drug. The percentage encapsulation efficiency of microspheres also showed that the drug loading was optimum and increased with increasing amount of polymers.

REFERENCES

1. Mishra AR, Mazumder MS. "Formulation and evaluation of floating microsphere of amlodipine besylate by using ethylcellulose and HPMC" *Pharmacopore* 2014, vol.5 (4), pg 602-609.
2. Swetha K, Madhavi H, Srimath K, Vasudha B. "Formulation and evaluation of gastro retentive floating microspheres of nimodipine", *Asian Journal of Pharmaceutics*, Oct–Dec 2016 (Suppl), 10 (4) pg 628-635.
3. Joselin J, Daisy PA, Boby JG, Praveenraj R, Noby T, Betty C. "formulation and evaluation of floating microspheres of pantoprazole sodium", *IJPPR*, 2015;4(4): 136-147.
4. Megha S, Seema K, Agnimitra D. "Floating microspheres of repaglinide: formulation, optimization, characterization and *in-vitro* evaluation", *Int. J. Chem. Sci.* 2014; 12(4): 1259-1272.
5. Bulgarelli E, Forni F, Bernabei MT. Effect of matrix composition and process conditions on casein-gelatin beads floating properties. *Int J Pharm.* 2000;198(2):157-65.
6. Umamaheswari RB, Jain S, Tripathi PK, Agrawal GP, Jain NK. Floating-bioadhesive microspheres containing acetohydroxamic acid for clearance of *Helicobacter pylori*. *Drug Deliv.* 2002; 9(4):223-31.
7. Streubel A, Siepmann J, Bodmeier R. Floating microparticles based on low density foam powder. *Int J Pharm.* 2002;241(2):279-92.

8. Ushimaru K, Nakamichi K, Saito H. Pharmaceutical preparations and a method of manufacturing them. US patent 4,702,918.1987 October 27.
9. Watanabe S, Kayano M, Ishino Y, Miyao K. Solid therapeutic preparation remaining in stomach. US patent 3,976,764.1976 August 24.
10. Ichikawa M, Watanabe S, Myake Y. Granule remaining in stomach. US Patent 4,844,905.1989 July 4.
11. Streubel A, Siepmann J, Bodmeier R. Floating microparticles based on low density foam powder. Int J Pharm. 2002; 241(2):279-92.
12. Dennis A, Timmins P, Lee K. Buoyant controlled release powder formulation. US Patent 5,169,638.1992 December 8.
13. Doshi MM, Joshi MD, Mehta .Pharmaceutical composition for controlled drug delivery system. Publication No. US 2003232081. 2003 December 18.
14. Sakkinen M, Tuononen T, Jurjenson H, Veski P, Marvola M. Evaluation of microcrystalline chitosans for gastro-retentive drug delivery. Eur J Pharm Sci. 2003;19(5):345-53.
15. Klausner EA, Eyal S, Lavy E, Friedman M, Hoffman A. Novel levodopa gastroretentive dosage form: *In vivo* evaluation in dogs. J. Controlled Release. 2003;88(1):117-26.