ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

DEVELOPMENT AND EVALUATION OF FLOATING MICROSPHERES OF ANTICONVULSANT DRUG BY 3^2 FULL FACTORIAL DESIGN

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ABSTRACT

Aim of the present study was to develop and evaluate gastroretentive carbamazepine microspheres by solvent evaporation technique. From the preliminary study, the batch FMC2 was selected for factorial study. Factorial batches showed the mean particle size (FFMC1- FFMC9) in the range of 245.32±0.21 to 405.14±0.14µm. The percentage yields were found to be in the range from 72.09±0.21 to 96.83±0.23. The buoyancy percentage was calculated and found that all formulations were able to float on dissolution medium for 12 h. The swelling study was found to be 799.0±0.14 to 876.4±1.67. Batch FFMC2 showed better drug release *i.e.* 99.25%. The optimized formulation FFMC2 showed n value of 0.8601 and R^2 value of 0.9984 respectively. The optimized formulation obeys Korsmeyer-Peppas release. SEM images of microspheres were spherical, discrete, and freely flowing. ANOVA for the formulations showed P-value less than 0.0500. The stability study indicated no significant change in the microspheres. In radiographic images floating microspheres were retained in stomach of rabbit for 12 hours.

Keywords: Gastroretentive, Anticonvulsant, Radiographic, Microspheres, Rabbit.

1. INTRODUCTION

Microspheres are small spherical particles, with diameters in the micrometer range (typically 1µm to 1000µm). Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Solid and hollow microspheres vary widely in density and, therefore, are used for different applications. Recent advances have resulted in the development of various types of microspheres (such as floating, mucoadhesive, radio-active, double-walled, and magnetic) to serve different purposes [1]. For example, floating/mucoadhesive microspheres have been developed as gastro-retentive delivery systems [1]. The current study focus on the development of the microspheres with the intention to increase the gastric residence time and improve the effect of drug. The study aim was to develop and evaluate gastroretentive carbamazepine microspheres by solvent evaporation technique.

2. MATERIAL AND METHODS

2.1. Materials

Carbamazepine was obtained as a gift sample from Alkem Laboratories, Mumbai. All other Excipients used were of analytical grade.

2.2. Preparation of microspheres

Carbamazepine loaded floating microspheres were prepared by solvent evaporation technique [2]. Ethyl cellulose and Eudragit S 100 were dissolved in a mixture of ethanol and dichloromethane at various ratios at room temperature. Carbamazepine was added to above solutions and then it was stirred on a magnetic stirrer to form a homogenous solution. The above solution of carbamazepine was then poured into 100 ml of water containing 0.01% Tween 80 maintained at room temperature. The mixture was stirred for three hour. The microspheres were separated by filtration and then dried at room temperature. Formulation plan is described in tables 1 and 2.

2.3. Characterization of floating gastroretentive microspheres

The floating microspheres were characterized by numerous tests to detect their properties that obey USP standards.

Table 1: Formulation composition of floating microspheres of carbamazepine preliminary batches

Formulation code	FMC1	FMC2	FMC3	FMC4	FMC5	FMC6
Carbamazepine	200	200	200	200	200	200
Ethyl Cellulose: Eudragit S 100	0.5:0.50	0.5:0.75	0.5: 1.00	0.5:0.25	0.75:0.5	1.00 : 0.25
Solvent ratio Ethanol: Dichloromethane (%v/v)	1:1	1.5:1	2:1	1:1	1: 1.5	1: 2

Table 2: Formulation composition of floating microspheres of carbamazepine factorial batches

Formulation code	FFMC1	FFMC2	FFMC3	FFMC4	FFMC5	FFMC6	FFMC7	FFMC8	FFMC9
Carbamazepine	200	200	200	200	200	200	200	200	200
Ethyl Cellulose : Eudragit S 100	0.5:0.5	0.37:0.75	0.25:0.75	0.25:0.25	0.5:0.25	0.5:0.75	0.37:0.50	0.37:0.25	0.25: 0.50
Solvent ratio Ethanol : Dichloromethane (%v/v)	1:1	1.5:1	2:1	1:1	1: 1.5	1: 2	1:1	1.5:1	2:1

2.3.1. Particle size analysis

The floating microspheres were separated into different size fractions by sieving for 10 min through a series of standard sieves, #40, #60, #80, and #100, and the particle size of 50 floating microspheres was calculated using an optical microscope and the mean particle size was calculated [2, 3].

2.3.2. Bulk density (BD):

Bulk density=Mass/Bulk volume

2.3.3. Tapped density (TD):

Tapped density=Mass/Tapped volume

2.3.4. Carr's (compressibility) index (CI):

% Compressibility index= $\frac{Tapped\ density - Bulk\ density}{Tapped\ density} X100$

2.3.5. Hausner ratio(HR):

Hausner ratio = Tapped density/Bulk density

2.3.6. Angle of repose (AR):

$\theta = Tan^{-1}(h/r)$

Where θ =angle of repose, h=height of pile, and r=radius of the pile.

2.3.7. Particle size (PS)

The particle size of the carbamazepine loaded microsphere was determined by optical microscopy method using a compound microscope (Olympus India) equipped with ocular and calibrated stage micrometers [4].

2.3.8. Percentage yield (PY)

Collected dried microspheres were weighed to determine the recovery of microspheres [5].

% Yield= $\frac{Mass\ of\ microspheres\ obtained\ (g)}{Theorotical\ mass\ of\ microspheres\ (g)} X100$

2.3.9. Measurement of microspheres hydration

Microspheres recovered weighed immediately at the end of each microencapsulation process and are represented as (M1). When the microspheres are dried to constant weight, they were weighed again and represented as (M2). It is represented by the following equation [6]:

% Microspheres hydration = $\frac{M1}{M2} X100$

2.3.10. Determination of Drug loading of microspheres

The 20 mg of carbamazepine loaded hollow microspheres samples were dissolved in 50 ml of ethanol at room temperature by ultra-sonication. The liquid was then filtered through a millipore filter (0.45µm). The drug concentration was determined with the UV-visible detector (UV1700-1800; Shanghai Phoenix Optical Instrument Co., Ltd., Shanghai, China) at 284 nm. The drug loading of microspheres was calculated as by following equation [7]:

Drug loading amount = $\frac{\text{Amount of FD in hollow microspheres}}{\text{Amount of hollow microspheres containing}} X100$

2.3.11. Drug entrapment efficiency (DEE)

Dried microsphere (50 mg), were crushed in a mortar and pestle, and the fine microspheres dissolved in a few ml of ethanol and dilute with 50 ml of 0.1N HCl for 24h. After 24h, the solution was passed through a 0.45µm filter and the concentration of the carbamazepine present in the filtrate was evaluated spectrophotometrically at 284 nm using UV-visible spectrophotometer (Shimadzu, UV-1800, Japan) with respect to 0.1N HCL as blank [8, 9].

Drug entrapment efficiency = $\frac{\text{Weight of drug in microspheres}}{\text{weight of fed drug}} X100$

2.3.12. swelling measurement (SM)

The swelling study was conducted using the dissolution test apparatus II. An accurately weighed amount of carbamazepine microspheres was placed in the vessels containing SGF and allowed to swell. Rotation speed was set at 50 rpm. The microspheres were withdrawn at predetermined time intervals and blotted with filter paper to remove the excess amount of water. The changes in weight were measured at different time intervals until the maximum weight was gained. The swelling index was then calculated using the following equation: [10]

$$\text{Swelling index (S):} \frac{Wm - Wt}{Wt} X100$$

Where, Wt denotes the initial weight of the microspheres, and Wm denotes the weight at equilibrium.

2.3.13. In vitro buoyancy (IV-B)

In vitro floating properties of the carbamazepine loaded microspheres were evaluated in a USP dissolution apparatus II (paddle type). Individual microspheres from each formulation were immersed into the vessel filled with 500mL of SGF. The paddles were rotated at 50 RPM and the temperature was maintained at 37±0.5°C. The number of floating microspheres was counted at hourly intervals up to 8 hours. *In vitro* buoyancy was expressed as a percentage and was calculated from the following equation [11]:

$$F\% = \frac{\text{Weight of floating microspheres}}{\text{WtWeight of initial microspheres}} X100$$

2.3.14. *In-vitro* drug release

Dose equivalent to 100 mg of floating microspheres of carbamazepine was accurately weighed and dissolution studies were carried out using simulated gastric fluid (enzyme free) 900 ml at temperature 37±0.5°C using USP type II apparatus. The speed of rotation was maintained at 100. Aliquot of 5 ml of dissolution medium was withdrawn at predetermined time interval up to a period of 12 h and replaced with a fresh medium. The content of carbamazepine microspheres was determined by using a UV spectrophotometer (Spectro UV 2080, double beam, analytical technologies, India) at 284 nm against SGF as blank, dissolution studies were conducted in triplicate [12].

2.3.15. Scanning electron microscopy

Dry microspheres of carbamazepine were placed on an electron microscope brass stub coated with gold in an ion sputter. Then a picture of microsphere was taken by random scanning of a stub. The SEM analysis of the microspheres was carried out by using JEOL, JSM-670F Japan (Sastra University, Tanjavur. The microspheres were viewed at an accelerating voltage of 3.0 [13].

2.3.16. Drug release kinetics

Four kinetic models are more frequently applied to determine the drug release from different controlled release preparations. The *in vitro* drug release data obtained was assessed by the five models to find the best fitting equation. Zero-order release kinetics is a system in which drug release is not dependent on the concentration of the drug. Equation for zero order release is [6],

Zero-order kinetics: $F\ t = K0t$

Where, F indicates the fraction of drug release in time t and K0 is the zero-order release constant.

First-order kinetics: $\text{Ln}\ (1 - F) = -K_1 t$

Where, F shows the fraction of drug release in time t and $K_1$is the first-order release constant

Higuchi model: $F = K2\ t1/2$

Where, F represents the fraction of drug release in time t and K2 is the Higuchi constant.

Korsmeyer-Peppas model: $M\ t/M\infty = K_3 t^n$

Here, Mt is the amount of drug released in time *t*, $M\propto$ is the amount of drug release at time infinity, K3 is the kinetic constant and n is the exponent describing the swelling mechanism.

2.3.17. Stability study [14]

On the basis of the previous *in vitro* release study, the optimized formulation was chosen for the stability study. Microspheres equivalent to 100 mg of carbamazepine, were filled in tightly closed glass vials and stored at 5°C and 40°C with the relative humidity of 75% in thermostatically controlled ovens. The samples stored at different temperatures, were taken at the time intervals of 0, 7, 15, 30, 45, 60, 90, 120, 150, and 180 days, and were assayed for their drug content.

2.3.18. In-vivo study

The experiment conducted as per CPCSEA guidelines (registration number 1336/AC/10/CPCSEA.) were approved by the Institutional Animal Ethics Committee (IAEC). The final formulation floating microspheres ware studied *in vivo* using an X-ray imaging approach on the New Zealand albino rabbit. For the *in vivo* study, floating microspheres with barium sulphate was

administered orally to the rabbits, followed by 50mL of water. Before study, the animals were acclimatized to the experimental setting for seven days (at 22°C with humidity regulation and a 12 h dark/light cycle). They had unlimited access to the regular rabbit diet as well as drinking water *ad libitum*. During the experiment, the rabbits were fasted overnight with free access to water. The rabbits were placed in a supine position and an X-ray machine (WiproGEDX300 with the horizontal X-ray system, model SI01463128 capacity 300 MA100 KVP, Pune, India) was used to check the position of the pill in the gastrointestinal region at 0 h, 3 h, 6 h, 9 h, and 12h. The radiographs were taken just before the floating pill was administered to check that there was no radio-opaque material in the stomach [15].

3. RESULTS AND DISCUSSION

3.1. Micromeritic studies of carbamazepine microspheres preliminary batches

The mean particle size of the floating microsphere formulations (FMC1-FMC6) was in the range of 357.4±0.21 to 448.4±0.14 µm. Rheological studies included bulk density, tapped density, compressibility index or Carr's index (*Ci*), Hausner's ratio (*Hr*) and angle of repose. All the nine formulations were studied for all the properties. *Ci* values lies between 0.24±0.17 and 10.22±0.33 which showed an excellent flow of microspheres. *Hr* values of all seven formulations were below 1.25 indicating good flow properties. Values of angle of repose of all formulations were below 21° also indicating free flow properties of microspheres.

3.2. Evaluation of carbamazepine microspheres

Good *in-vitro* percentage buoyancy was observed for all the microspheres formulations. Microspheres formulation FMC2 showed the best floating ability (82.95±0.41 buoyancy) in SGF as compared with other formulations. The floating ability of micro balloons for 8 h may be considered a satisfactory performance of the prepared formulations. The percentage entrapment of drug was found to be good for all loading. The highest drug loading was found in batch FMC2 was 10.84±0.35. The highest swelling index was found in batch FMC 2 was 875.5±0.06.

3.3. *In-vitro* drug release

It was shown that the rate of drug release from microspheres was retarded, as the concentration of polymers increased. Batch FMC2 showed the better drug release 97.46% as shown in fig. 1.

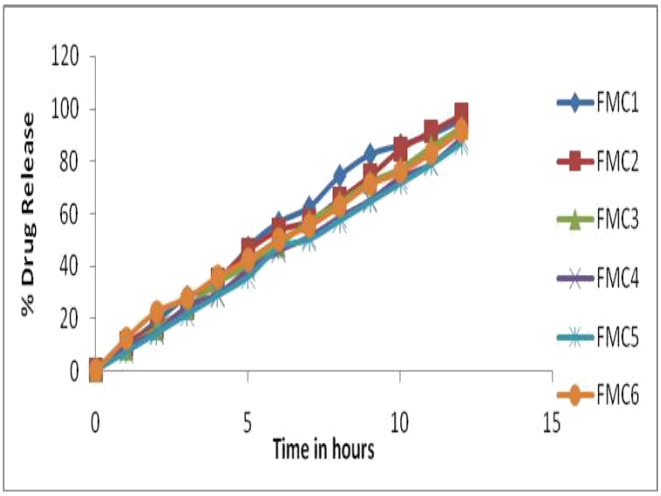


Fig. 1: Percent Drug release for carbamazepine microspheres for preliminary batches

Table 3: Evaluation of carbamazepine microspheres

Formulation Code	Percentage yield	drug entrapment efficiency	*in vitro* buoyancy	Drug loading of microspheres	Swelling measurement
FMC1	68.8±0.21	72.15±0.08	71.43±0.55	9.632±0.20	812.5±2.93
FMC2	85.4±0.27	83.5±0.03	82.95±0.41	10.84±0.35	875.5±0.06
FMC3	82.7± 0.35	72.8±0.12	74.10±0.34	8.598±0.56	837.5±0.15
FMC4	72.38±0.23	80.5±0.17	50.92±0.74	9.93±0.48	800.0±0.37
FMC5	75.9± 0.23	78.2±0.06	78.54±0.82	8.998±0.58	850.0±1.23
FMC6	81.4±0.20	79.5±0.12	79.54±0.79	9.978±0.69	850.0±1.23

3.4. Micromeritic studies

The mean particle size of the floating microsphere formulations (FFMC1- FFMC9) were found to be in the range of 245.32±0.21 to 405.14±0.14µm.

The values of Carr's index *(Ci)* of all seven formulations lies between 0.23 ± 0.08 and 16.18 ± 0.04 which showed an excellent flow of microspheres. Hausner's ratio *(Hr)* values of all seven formulations were below 1.25 indicating good flow properties. Values of angle of repose of all formulations were below 30° also indicating free flow properties of microspheres.

3.5. Evaluation of Carbamazepine Factorial batches

The percentage yield of floating microsphere was examined and found to be in the range of 72.09±0.21 to 96.83±0.23. Drug entrapment was found to be in the range of 88.48±0.12 to 94.30±0.06. In buoyancy study, it was found that all formulations were able to float on the dissolution medium (0.1 N HCl, pH 1.2) for 12 h. The buoyancy percentage of the microspheres was found to decrease with an increase in ethyl cellulose concentration. The results are shown in table 4. The drug loading for carbamazepine microspheres was found to be 8.960±0.48 to 11.43±0.10, this variation was found due to change in concentration of polymers used. Swelling study indicates that more gastric retention was achieved due to enormous swelling of polymers used in the study. The swelling for various batches was found to be 799.0±0.14 to 876.4±1.67

3.6. *In-vitro* drug release for carbamazepine factorial batches

The *in-vitro* release studies of the floating microspheres were studied for all the formulations (fig. 2). Drug release from the floating microspheres was investigated using the USP dissolution apparatus I (basket type) where FFMC2 batch showed the highest drug release 99.25%.

Table 4: Evaluation of Carbamazepine Factorial batches

Formulation Code	Percentage yield	drug entrapment efficiency	in vitro buoyancy	Drug loading of microspheres	Swelling measurement
FFMC1	72.09±0.21	89.87±0.08	85.89±0.38	9.532 ± 0.15	825.2 ± 1.45
FFMC2	96.83± 0.23	94.30±0.06	92.34±0.5	8.960 ± 0.48	876.4 ± 1.67
FFMC3	94.94±0.45	88.48±0.12	90.56±0.29	11.43 ± 0.10	850.1 ± 0.72
FFMC4	88.09±0.23	92.36±0.17	88.90±0.74	10.93 ± 0.29	799.0 ± 0.14
FFMC5	93.99±0.27	92.10±0.03	89.69±0.34	9.883 ± 0.49	822.2 ± 0.16
FFMC6	94.34±0.20	89.09±0.12	85.85±0.65	9.589 ± 0.64	847.2 ± 1.18
FFMC7	95.87±0.44	93.11±0.20	88.77±0.18	9.749 ± 0.20	814.1 ± 1.87
FFMC8	92.26±0.20	94.01±0.23	90.72±0.20	9.320 ± 0.50	849.0 ± 1.18
FFMC9	91.90±0.19	90.99±0.45	92.10±0.12	10.37 ± 0.45	825.8 ± 0.13

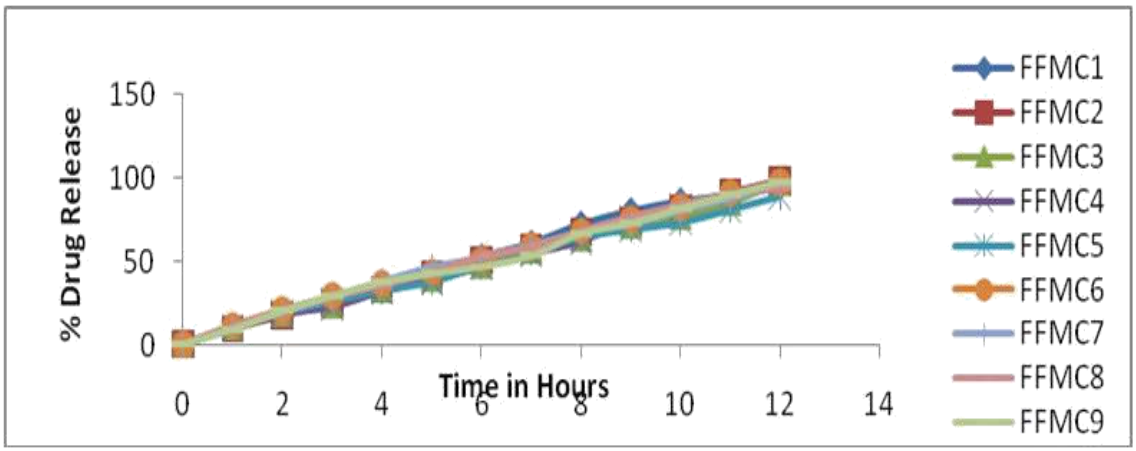


Fig. 2: Percent Drug release for carbamazepine microspheres for factorial batches

3.7. Scanning electron microscopy

The microsphere seems to be more spherical in shape with smooth surfaces *i.e.* Fig. 3(c) while some smaller particles showed wrinkled morphology *i.e.* Fig. 3(a). The surface of the drug-loaded microspheres showed the presence of drug particles on their surface as shown in Fig. 3(a), (b) and (d). All the microspheres also showed small pores on their surfaces that as shown in Fig. 3(c). These pores believed to facilitate the diffusion of solvent into the shell of microparticles as well as release of the drug out of the particle matrix.

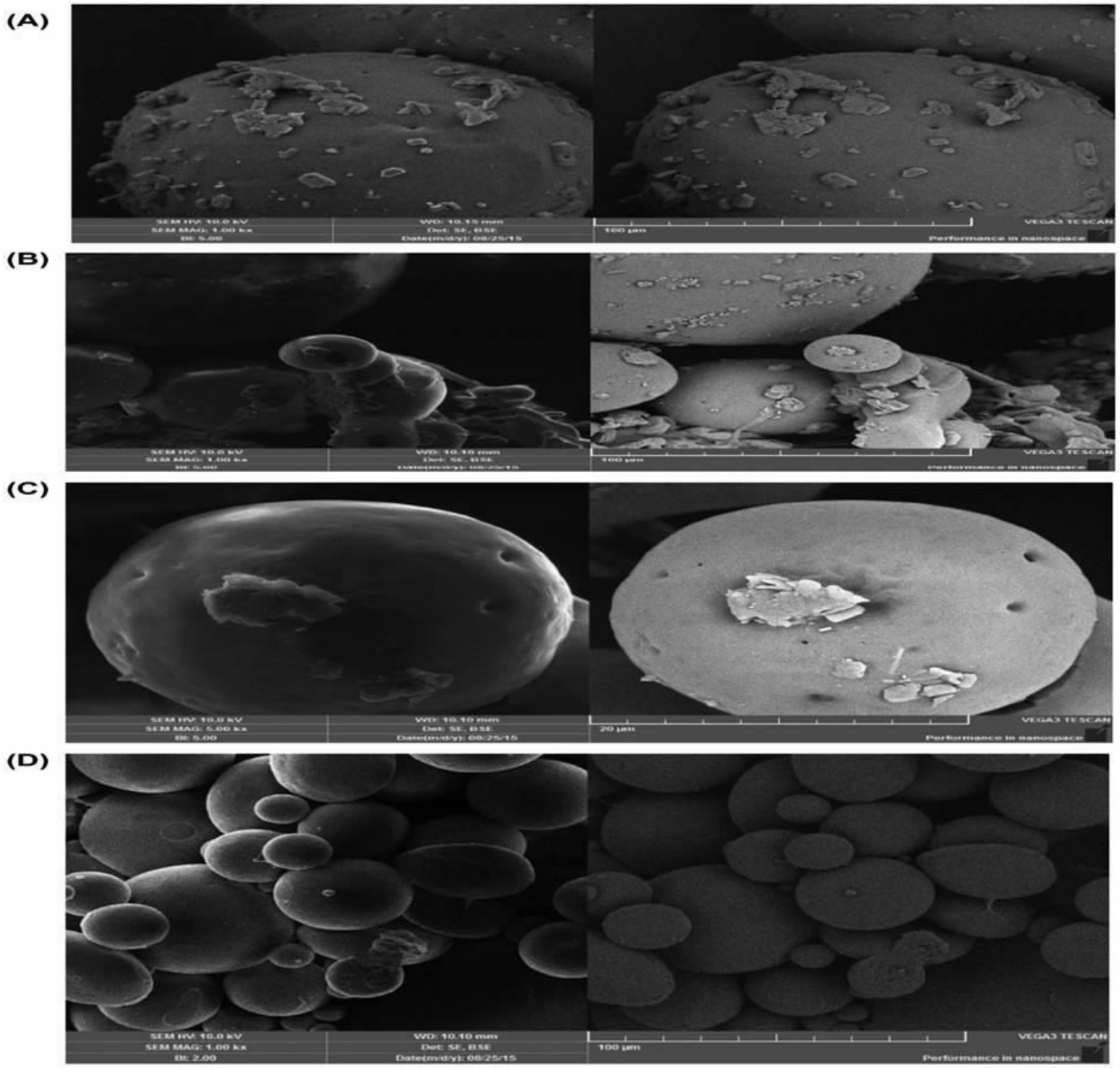


Fig. 3: Surface analysis for carbamazepine microspheres

3.8. Drug release kinetics

To analyze the release mechanism of carbamazepine, *in vitro* release data was fitted into various release equations and kinetic models (Zero order, First order, Higuchi and Korsmeyer-Peppas) for all the selected batches. When the release profile was plotted versus square root of time, a linear relationship was observed with the regression coefficient close to one. The equation was used to determine the value of release exponent, *n*; the value of n is indicative of mechanism of drug release. When n takes the value of 0.5 it indicates diffusion controlled release and for the value 1 it indicates swelling controlled drug release. A value of *n* in between 0.5-1 represents the release mechanism by diffusion as well as swelling (anomalous transport). The optimized formulation FFMC2 shows n value of 0.8601 and R^2 value of 0.9984. Hence it can be concluded that the optimized formulation obeys Korsmeyer-Peppas release kinetic model. The results obtained are shown in fig. 4.

3.9. Carbamazepine

ANOVA for 2FI model

Final Equation in Terms of Actual Factors

Drug Release = +96.18889 -1.96833 HPMC K 100+ 2.24333 Cellulose Acetate Phthalate+2.16000 HPMC K 100 * Cellulose acetate phthalate

Final Equation in Terms of Actual Factors

Floating lag time = +57.54778 +2.12500 HPMC K 100+10.77000 Cellulose acetate phthalate -2.55500 HPMC K 100 * Cellulose acetate phthalate + 0.738333 HPMC K 100²+17.13333 Cellulose acetate phthalate

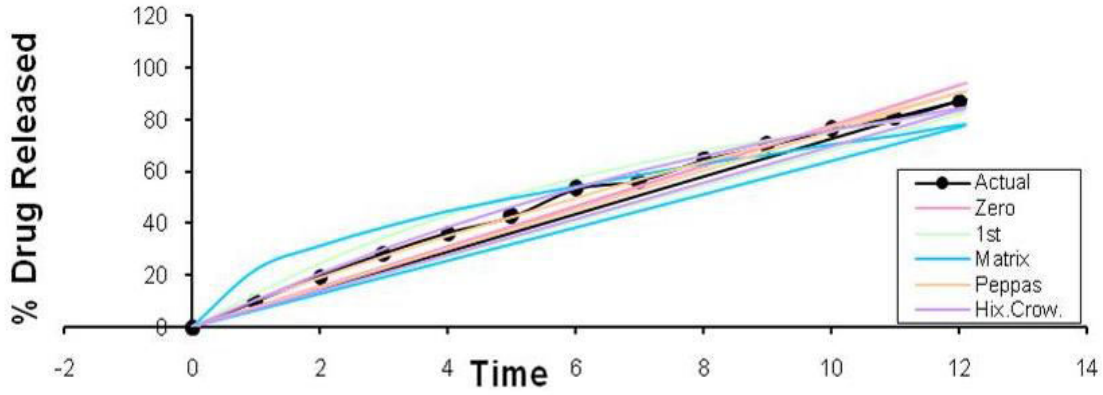


Fig. 4: Drug release kinetics for Carbamazepine microspheres for batch FFMC2

Table 5: Response 1: Drug release

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	72.10	3	24.03	53.58	0.0003	significant
A-HPMC K 100	23.25	1	23.25	51.82	0.0008	
B-Cellulose Acetate Phthalate	30.20	1	30.20	67.31	0.0004	
AB	18.66	1	18.66	41.60	0.0013	
Residual	2.24	5	0.4486			
Cor Total	74.35	8				

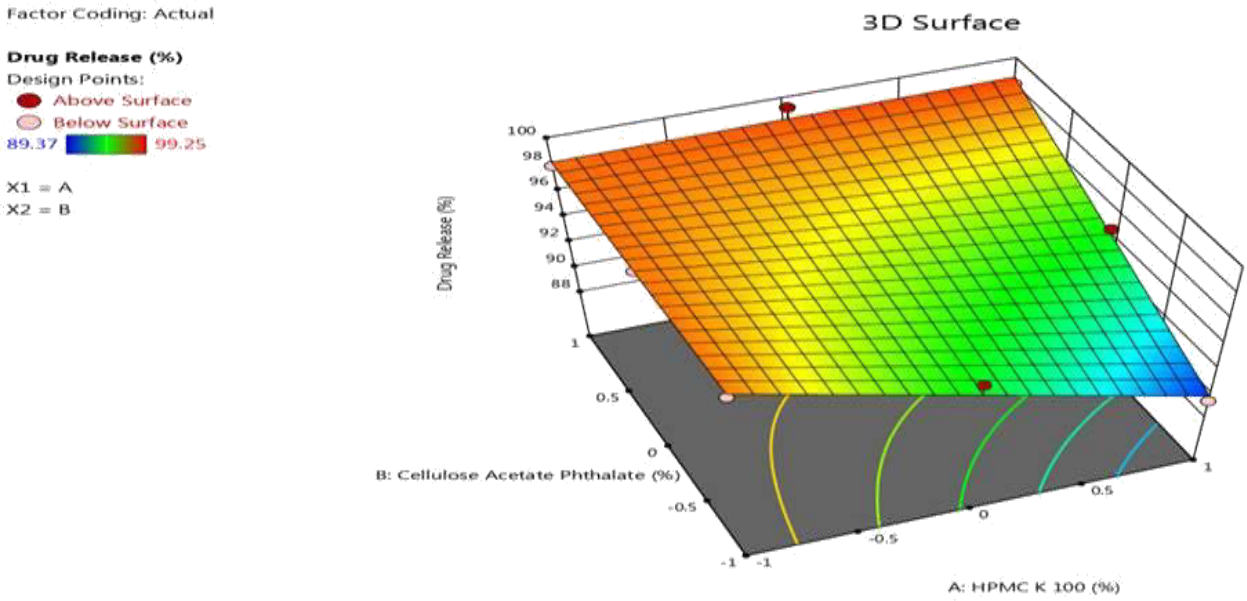


Fig. 5: 3D plot for % drug release for factorial batches of carbamazepine microspheres ANOVA for Quadratic model

3.10. *In-vivo* study

The hard gelatin capsule containing $BaSO_4$ loaded hollow floating microspheres was clearly visible in the stomach after oral administration of dosage form. In the radiographic image taken after 1hour, all microspheres were found to be scattered in the stomach. Dense images of microspheres were seen at initial hours, but, as time passed on, the images of microspheres became lighter. It may be because of the distribution and scattering of microspheres within GI region. The radiographic images indicted that these hollow floating microspheres were retained successfully in the stomach up to 12 hours.

Table 6: Response 2: Floating lag time

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1337.36	5	267.47	67.68	0.0028	significant
A-HPMC K 100	27.09	1	27.09	6.86	0.0791	
B-Cellulose Acetate Phthalate	695.96	1	695.96	176.11	0.0009	
AB	26.11	1	26.11	6.61	0.0825	
A^2	1.09	1	1.09	0.2759	0.6358	
B^2	587.10	1	587.10	148.56	0.0012	
Residual	11.86	3	3.95			
Cor Total	1349.21	8				

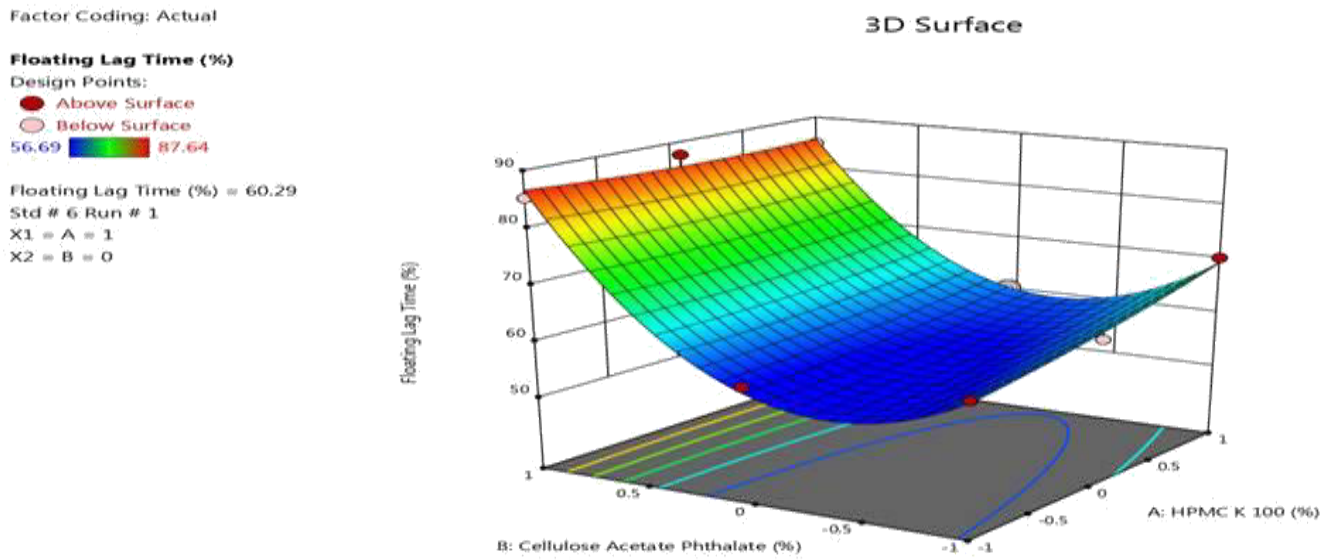
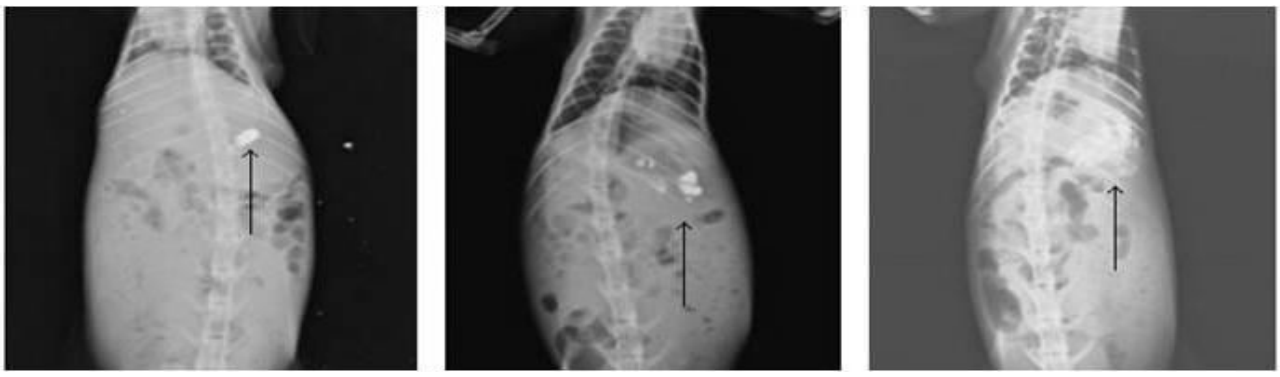
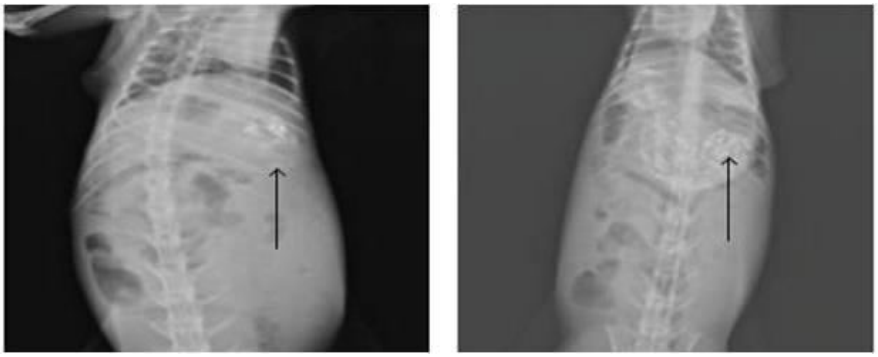


Fig. 6: 3D plot for floating lag time for factorial batches of carbamazepine microspheres



(a) 0 hrs **(b) 3 hrs** **(c) 6 hrs**



(d) 6 hrs (e) 12 hrs

Fig. 7: X-ray images of in-vivo gastric microspheres retention for carbamazepine floating microspheres for 12 hours

4. CONCLUSION

The better flow of microspheres indicated that the floating microspheres produced were non-aggregated. In swelling study due to more swelling and hydration of microspheres gastric residence time was also increased to greater extent. Drug release study was simulated in acidic medium since the drug has an absorption window in stomach. The optimized formulation FFMC2 shows n value of 0.8601 and R^2 value of 0.9984. It was concluded that the optimized formulation obeys Korsmeyer-Peppas release kinetic model. It was noted in the SEM images that the microspheres were spherical, discrete, and freely flowing. The ANOVA study showed the probablity value *i.e.* P-value found was also less than 0.0500. The results indicated no significant changes in the microspheres properties. Hence it can be concluded that the formulated floating gastroretentive micro-spheres are stable under appropriate storage conditions. The radiographic images indicted that these hollow floating microspheres were retained successfully in the stomach up to 12 hours. The study can be further extending by using various other methods of formulation and fortunate to get promising and comprehensive effects.

Conflict of interest

Author declares no conflict of interest.

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