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FORMULATION AND EVALUATION OF FLOATING DRUG DELIVERY SYSTEM OF PENTOXIFYLLINE

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ABSTRACT

A sustained release system for pentoxifylline designed to increase its residence time in the stomach without contact with the mucosa was achieved through the preparation of floating microspheres by the emulsion solvent diffusion technique. Four different ratios of either Eudragit RS 100 (ES) alone or with HPMC were used to prepare the floating microspheres. The drug retained in the floating microspheres decrease with increase in HPMC content. All formulation show good flow properties. The microspheres were found to be regular by SEM. FT-IR study confirmed the drug-polymer compatibility. Although pentoxifylline release rate from Eudragit RS floating microspheres was very slow and incomplete but when HPMC was added, release rate increased, at the same time floating ability was decreasing. Formulation F_2 containing ES:HPMC (9:1) which showed appropriate balance between release rate and buoyancy could be advantageous in terms of increased bioavailability of pentoxifylline. Formulation F_2 was subjected for *in vivo* anti-inflammatory activity in rats and showed better efficacy compare to standard preparation.

Keywords: Pentoxifylline, floating microspheres, Eudragit, emulsion solvent diffusion.

INTRODUCTION

Drugs that are easily absorbed from the gastrointestinal tract (GIT) and have a short half–life are eliminated quickly from the blood circulation, require frequent dosing. To avoid this problem, the oral controlled release (CR) formulations have been developed in an attempt to release the drug slowly in to the GIT and maintain a constant drug concentration in the serum for longer period of time. Such oral drug delivery devices have a restriction due to the gastric retention time (GRT), a physiological limitation[1, 2]. Therefore, prolonged gastric retention is important in achieving control over the GRT because this helps to retain the CR system in the stomach for a longer time in a predictable manner[3,4]. Thus the real issue in the development of oral controlled release dosage form is not just to prolong the delivery of the drugs, but to prolong the presence of the dosage forms in the stomach or somewhere in the upper small intestine until all the drug is released for the desired period of time. It may improve bioavailability and reduce drug waste.

Over the last three decades, various approaches have been pursued to increase the retention of an oral dosage form in the stomach, including floatation system[5,6], bioadhesive systems, which adhere to mucosal surface[7,8], density controlled system, which either float or sink in gastric fluid[9], swellable delivery system, which increase in size after swelling and retard the passage through the pylorus[10], modified shape systems[11], magnetic systems[12], superporous hydrogel system[13] and other delayed gastric emptying devices.

In fact, the buoyant dosage unit enhances gastric residence time (GRT) without affecting the intrinsic

rate of emptying[14]. Unfortunately, floating devices administered in a single-unit form such as hydrodyanamically balanced system (HBS)[15] are unreliable in prolonging the GRT owing to their 'all-or-nothing' empty process[16] and, thus, they may cause high variability in bioavailability and local irritation due to a large amount of drug delivered at a particular site of the gastrointestinal tract (GIT). In constract, multiple unit particulate dosage form (e.g. microspheres) have the advantages that they pass uniformly through the GIT to avoid the vagaries of gastric emptying and provide an adjustable release, thereby reducing the intersubject variability in absorption and risk of local irritation[17].

Pentoxifylline is a xanthine derivative improves the flow properties of blood by decreasing its viscosity. It was chosen as a model drug since it has a very short half life (1.6 h) and low oral availability (19 ± 13 %)[18]. The objective of the present study was to develop floating microspheres of pentoxifylline in order to achieve an extended retention in the upper GIT, which may result in enhanced absorption and thereby improved bioavailability.

MATERIAL AND METHODS

Materials: Pentoxifylline was received as a gift sample from Shreya Life Science Ltd (Aurangabad, India). Eudragit RS 100 was purchased from Rohm Pharma (Darmstadt, Germany), Hydroxypropylmethylcellulose was purchased from Loba Chemie Pvt. Ltd, (Mumbai, India), Ethanol was obtained from Sakthi Sugar Pvt Ltd (Erode, India) Dichloromethan and sodium lauryl sulphate were purchased from S.D. Fine Chem. Ltd (Mumbai, India). All other chemicals were of analytical grade.

Preparation of floating microspheres of pentoxifylline: Floating microspheres containing pentoxifylline were prepared using emulsion- solvent diffusion technique[19,20]. The drug to polymer ratio used to prepare the different formulations was 1:1. The polymer content was a mixture of Eudragit RS 100 (ES 100) Hydroxypropylmethylcellulose (HPMC), 10:0 (F_1), 9:1 (F_2), 8:2 (F_3), and 7:3 (F_4). The drug polymer mixture dissolved in a mixture of ethanol (8 mL) and dichloromethane (8 mL) was dropped in to 0.2 % sodium lauryl sulfate solution (300 ml). The solution was stirred with a propeller-type agitator at room temperature for 1 hour at 150 rpm. The formed floating microspheres were filtered, washed with water and dried at room temperature in a desicator.

Micromeritic properties: The microspheres were characterized by their micrometric properties, such as particle size, true density, tapped density, compressibility index (% CI: a value useful in prediction of flowabiltiy), porosity and flow properties. The particle size was measured using an optical microscope and the mean particle size was calculated by measuring around 200 particles with the help of a calibrated ocular micrometer. The tapping method was used to determine the tapped density and percentage compressibility index as follows.

Tapped density = Mass of floating microspheres / Volume of floating microspheres after tapping

% Compressibility index = $[1-V/V_O] \times 100$

Where V and V_0 are the volumes of the sample after and before the standard tapping respectively. The true density of floating microspheres was determined by liquid displacement method using n-hexane as solvent[21]. Porosity (ε) of the floating microspheres was calculated using the following equation[22]:

$\varepsilon = [1-P_P/P_T] \times 100$

Where P_t and P_p are the true density and tapped density respectively. Angle of repose (θ) of the floating microspheres, which measures the resistance to particle flow, was determined by a fixed funnel method and calculated as

$\tan \theta = 2\ H/D$

where 2 H/D is the surface area of the free standing height of the microshperes heap that is formed on a graph paper after making the microspheres flow from the glass funnel.

Scanning electron microscope: The floating microsheres were examined for surface morphology and shape using scanning electron microscope (Jeol JSM – 5610, Japan). Samples were fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 20KV during scanning. Microphotographs were taken on different magnification.

Drug content and yield of floating microspheres: The floating microspheres containing drug were dissolved in a mixture of dichloromethane and ethanol (1:1 v/v) by ultrasonication. The dissolved drug amount was measured by UV-spectrophotometer (UV-1601 Shimadzu, Japan) at 274 nm after suitable dilution. No interference was found due to the other components at 274 nm. Drug content and yield were calculated as follows.

Drug content (%) = [Calculated drug content / Theoretical drug content] x100

% Yield = [Total weight of floating microspheres / Total weight of drug and polymer] x 100

Fourier transform infra-red spectroscopy (FT-IR) analysis: The FT–IR analysis was conducted for the analysis of drug polymer interaction and stability of drug during microencapsulation process. Fourier transform infra-red spectrum of pure pentoxifylline, Eudragit RS 100, HPMC and floating microspheres were recorded using FT–IR spectrophotometer (IR-470, Shimadzu, Japan).

In vitro evaluation of floating ability: Fifty milligrams of the floating microspheres were placed in 0.1 N HCl (100 mL) containing 0.02% Tween 20. The mixture was stirred at 100 rpm in a magnetic stirrer. The layer of buoyant microspheres was pipetted and separated by filtration at 1, 2, 4 and 8 hours. The collected microspheres were dried in a desiccator over night.

The percentage of floating microspheres was calculated by the following equation:

%Floating microspheres = (Weight of floating microspheres / Initial weight of floating microspheres) x100

In vitro release studies: The drug release rate from floating microspheres was carried out using the USP dissolution paddle assembly (Model DT-06, Erweka, Germany). A weighed amount of floating microspheres equivalent to 100 mg drug were dispersed in 900 mL of 0.1 N HCl (pH 1.2) maintained at 37 ± 0.5°C and stirred at 100 rpm. At preselected time intervals one ml sample was withdrawn. The collected samples were suitably diluted with 0.1 N HCl and analysed spectrophotometrically at 274 nm. The initial volume of the dissolution fluid was maintained by adding same volume of fresh dissolution fluid after each withdrawal. The dissolution studies were repeated using phosphate buffer pH 6.8 as dissolution medium.
All the previous experiment were done in triplicate.

Kinetic modeling of drug release: The dissolution profile of all the batches was fitted to zero-order, first order, Higuchi[23] and Korsemeyer-pepas models to ascertain the kinetic modeling of drug release.

Stability study: The stability study was conducted for best formulation F_2. The prepared floating microspheres (F_2) were placed in borosilicate screw capped glass containers and stored at room temperature (27 ± 2°C), oven temperature (40 ± 2°C) and in refrigerator (5-8°C) for a period of 6 weeks. The samples were assayed for drug content at regular intervals of two week.

In vivo anti–inflammatory study: It has been reported in the literatures that pentoxifylline may have therapeutic potential as anti-inflammatory agent either alone or in combination with non–steroidal anti–inflammatory drugs[24] Therefore the *in vivo* release behaviour of the best formulation F_2 was studied by measuring anti–inflammatory activity in adult male wistar rats using cotton pellet granuloma method The study was approved by institutional animal ethical committee. The male wistar rats were divided in to three groups, each group consisting of 6 animals. One group served as control, second group served as standard, received 50 mg/kg of pentoxifylline as solution in water in two divided dose orally. While third group received floating microspheres containing pentoxifylline orally (188.28 mg of formulation require to release about 50 mg/kg of pentoxifylline) once daily during the experiment. The rats with an average weight of 250 g were anaesthetized with ether. The back skin was shaved and disinfected with 70% ethanol. The cotton pellets each weighing 20 ± 1 mg were prepared and sterilized in hot air oven at 120°C for 3 hours. Then 4 sterilized cotton pellets one each in to both the axillae and groin region were subcutaneously implanted under aseptic conditions. The animals were treated for 7 days. All animals had free access to drinking water and food *ad libitum*. The cotton pellets were removed after the period of treatment, under ether anesthesia and the wound was closed by suturing. These pellets were dried in hot air oven over night at 70°C and dry weight was determined. The weight of granuloma was determined by calculating the difference. Data was analysed by unpaired student's t-test.

RESULTS AND DISCUSSION

Micromeritic properties: The particle size of floating microspheres varied somewhat among the formulations due to variation in the composition of formulations. Formulation F_1 showed a relatively high percentage of the big size floating microspheres and formulation F_4 showed relatively small size floating microspheres. The tapped density values ranged from 0.363 to 0.416 g/cm^3, while their true density ranged between 0.814 to 1.000 g/cm^3 of all the formulations obviously, the density values of the floating microspheres were less than that of the gastric fluid (~1.004 g/cm^3) thereby, employing that these floating microspheres will have the propensity to exhibit an good buoyancy effect *in vivo*. The porosity of all the formulations was found to be in the range of 55.40 – 58.35%. All formulation showed excellent flowability as represented in terms of angle of repose (<40°) except F_4 (41.02°), probably due to

its high content of HPMC. However, percentage compressibility value ranged between 12.23 to 20.01% suggested excellent flowability of floating microspheres (Table 1).

Morphology: Scanning electron microscopic photographs of floating microspheres are shown in Figure 1. Floating microspheres were spherical with no visible major surface irregularity. Few wrinkles and inward dents were appeared at the surface. It may be due to the collapse of the floating microspheres during the in situ drying process. Formulation F_1 formed entirely of ES 100 were observed to be fairly spherical, sphericity was decreasing when the content of HPMC increased. The surface morphology of floating microspheres was examined at higher magnification, which illustrate the smooth surface of floating microspheres. Some small pores and cavities were present on the surface of floating microspheres, probably arising as a trace of solvent evaporation during the process.

Drug content and yield of floating microspheres : Drug content slightly decreased with the increase in HPMC content of floating microspheres. This could be due to the permeation characteristics of HPMC that could facilitate the diffusion of a part of entrapped drug to the surrounding medium during preparation of floating microspheres. On the other hand the percentage yield for all formulations was almost similar. The yield of floating microspheres appeared unchanged by changing HPMC ratio (Table 2).

Fourier transform infrared spectroscopy (FT-IR) analysis: The drug, pentoxifylline present in the formulation F_2 was confirmed by FT-IR spectra. The characteristic peaks due to pure pentoxifylline at 615, 757, 1222, 1454, 1548, 1660, 1702, 2864, 2943 and 2951 for aromatic ring, N-H bending, C=N stretching, C=C stretching, C=O stretching and C-H stretching have appeared in floating microspheres spectra, without any markable change in their position after successful encapsulation, indicating no chemical interaction between pentoxifylline, Eudragit RS 100 and HPMC. It also confirmed the stability of drug during microencapsulation process (Figure. 2).

In vitro evaluation of floating ability: The floating test was carried out to investigate the floatability of the prepared microspheres in 0.1 N HCl (pH 1.2) containing 0.02% Tween 20. Tween 20 was added to counteract the downward pulling at the liquid surface by lowering surface tension. The floating ability differed according to the formulation tested. Formulation F_1 gave the best floating ability as evidenced by the percentage of particles floated at different time intervals (table 3). The floating ability of floating microspheres decreased on increasing the HPMC ratio. These results were attributable to conversion of less spherical form of floating microspheres on adding HPMC. In addition 0.1 N HCl (pH 1.2) can readily penetrate floating microspheres due to the dissolution of HPMC in solution.

In vitro drug release study: Release of pentoxifylline from floating microspheres was evaluated in 0.1 N HCl (pH 1.2) and phosphate buffer pH 6.8. Eudragit RS 100 polymer, which is present in all formulations, is of low permeability and insoluble in acid medium. It is an anionic copolymer of methacrylic acid, methyl methacrylate containing free carboxylic and ester groups. It's very low permeability results from high intermolecular attraction between its molecules. Hydrogen bonding between the hydroxyl groups of the carboxylic moiety and the carbonyl oxygen of ester groups increase the degree of compactness of the polymer and decrease its porosity and permeability. Because of these characters, drug release rate from floating microspheres prepared only by Eudragit RS 100 (Formulation 1, F_1) was very slow and incomplete at both pH values. Thus, in order to modulate the drug release rate from the floating microspheres, they were prepared by mixing with a water-soluble polymer, HPMC and it was found that the greater the content of HPMC in formulations, the greater was the rate of drug release. Since the acrylic polymer used is not soluble in acidic pH, the release of pentoxifylline in 0.1 N HCl (pH 1.2) was generally low compared to that in phosphate buffer pH 6.8 for all formulations and in pH 6.8 release rate was high because of high dissolution rate of Eudragit RS 100 (Figure 3, A and B)

The ideal properties of floating microspheres are a high buoyancy and sufficient release of drug in pH 6.8. There was an inverse relationship between the buoyancy of floating microspheres and the level of pentoxifylline release from floating microshperes. In developing a desired intragastric floatation system employing these floating microshperes, it was necessary to select an appropriate balance between buoyancy and drug release rate and formulation F_2 found to be best due to it's desired drug release and floatable properties.

Kinetic modeling: The *in-vitro* release data was applied to various kinetic models to predict the drug release kinetics and mechanism. The release constant was calculated from the slop of appropriate plots, and the regression coefficient (r^2) was determined. It was

found that the *in-vitro* drug release of floating microspheres was best explained by first order kinetic as the plots showed the highest linearity. The correlation coefficient (r^2) was in the range of 0.979-0.995 for various formulations in 0.1N HCl. However, drug release was also found to be very close to zero order kinetic for formulation F_2 (r^2 = 0.989), indicating that the concentration was nearly independent of drug release. Formulation F_2 also showed first order kinetic (r^2 = 0.998) in phosphate buffer pH 6.8 followed by Higuchi (r^2 = 0.983) and zero order (r^2 = 0.907) (Table 4, A and B)

The mode of drug release from floating microspheres was evaluated using Korsmeyer - Peppas model. The corresponding plot for Korsmeyer- Peppas equation indicated a good linearity in both dissolution medium (r^2 > 0.96). The value of n was 0.551 and 0.643 in 0.1N HCl and phosphate buffer pH 6.8, respectively suggested a coupling of the diffusion and erosion mechanism so called anomalous diffusion and may indicated that drug release was controlled by more than one process.

Stability study: In stability study there was no remarkable change in the drug content of floating microspheres. This indicated that the formulation F_2 was stable at the above-mentioned temperatures.

In vivo anti-inflammatory activity: The release behaviour of the best formulation F_2 *in vivo* was studied by measuring anti-inflammatory activity in adult male wistar rats using cotton pellet granuloma method, in which inflammation and granuloma developed during a period of 7 days. The floating microspheres formulation F_2 showed 47.2% decrease in the weight of granuloma, whereas, the standard showed a decrease of 32.4% compare to control. This indicates that the prepared microspheres exhibited a better efficacy than the standard preparation. It can be considered as a proof of constant release of the drug from floating microspheres in good correlation with the *in-vitro* release pattern. When data was analyzed by unpaired student's t-test, the statistical analysis showed significant difference ($P<0.001$) compare to control (Table 5).

CONCLUSION

Gastro-retentive floating microspheres have emerged as an efficient means of enhancing the bioavailability and controlled delivery of many drugs. The present formulation study of pentoxifylline was performed in an attempt to prepare floating drug delivery system of floating multiple unit system using emulsion-solvent diffusion technique and the performance of these formulations was evaluated. The release rate from the formulation F_1 was very slow and incomplete at both pH value. In order to increase the release rate, microspheres were prepared by mixing various HPMC ratio and it was found that the greater the content of HPMC in formulations, the greater is the rate of drug release but at the same time, floating ability of floating microspheres decreased on increasing the HPMC ratio. The ideal properties of floating microspheres are a high buoyancy and sufficient release of drugs in pH 6.8 solution. In developing a desired intragastric floating system employing these microspheres, it is necessary to select an appropriate balance between buoyancy and drug releasing rate and formulation F_2 showed the best appropriate balance between buoyancy and drug release rate. The designed system F_2 combining high buoyant ability and suitable drug release rate, could possibilily be advantageous in terms of increased bioavailability of pentoxifylline. The designed system F_2 might be able to float in the stomach. This phenomenon could prolong the gastric residence time (GRT) and delay drug arrival at the absorption site; consequently, the sustained action provided, in addition, floating microspheres enabled increased drug absorption rate of drug as the floating microspheres in the stomach gradually sank and arrived at the absorption site. Therefore, multiple unit floating system, i.e, floating microspheres should be possibility beneficial with subject to sustain action. The developed formulation overcomes and alleviates the drawbacks and limitations of sustained release preparations.

Major advantages of the prepared formulations include.

- Easy of Preparation
- Good Buoyancy
- Sustained Release Over Several Hours.

Table 1 : Micromeritic properties of different floating microspheres

Formulation code	Mean particle size* (μm)	True density* (g/cm^3)	Tapped density* (g/cm^3)	Compressibility index* %	Porosity*	Angle of repose* (°)
F_1	377.02 ± 17.91	0.819 ± 0.074	0.363 ± 0.006	12.23 ± 0.83	55.40 ± 4.41	29°98' ± 0.41'
F_2	327.11 ± 23.82	0.944 ± 0.023	0.387 ± 0.013	13.87 ± 0.59	59.28 ± 1.46	32°83' ± 0.84'
F_3	309.2 ± 32.13	0.993 ± 0.023	0.400 ± 0.008	16.63 ± 1.11	59.7 ± 1.10	34°9 '± 0.54'
F_4	288.63 ± 23.42	1.000 ± 0.026	0.416 ± 0.016	20.01 ± 0.66	58.35 ± 0.59	41°02' ± 1°22'

* Average of three preparation ± S.D.

Table 2 : Percentage yield and entrapment drug content of floating microspheres

Formulation code	Percentage yield* (%)	Drug content* (% w/w)
F_1	66.6 ± 3.46	55.85 ± 2.22
F_2	64.2 ± 4.49	53.11 ± 2.07
F_3	61.7 ± 2.88	46.74 ± 2.05
F_4	63.85 ± 3.42	41.91 ± 1.79

* Average of three preparation ± S.D.

Table 3 : Percentage floating of different formulations of floating microspheres in 0.1 n HCl (ph 1.2) containing 0.02 % Tween 20

Formulation code	1 hr*	2 hrs*	4 hrs*	8 hrs*
F_1	94.62 ± 1.81	90.46 ± 2.86	80.71 ± 1.44	69.99 ± 0.429
F_2	88.31 ± 1.13	80.37 ± 1.32	64.99 ± 1.24	59.65 ± 0.815
F_3	74.84 ± 1.11	70.07 ± 0.757	49.59 ± 1.197	42.92 ± 0.484
F_4	70.04 ± 0.798	60.34 ± 0.923	36.02 ± 0.844	20.11 ± 0.538

* Average of three preparation ± S.D.

Table 4 (A) : Release kinetics of floating microspheres in 0.1 n HCl (ph 1.2)

Formulation	Zero order		First order		Higuchi		Korsmeyar – Peppas	
	r^2	K_o	r^2	K_1	r^2	K_H	r^2	n
F_1	0.985	1.795	0.995	0.020	0.982	7.304	0.977	0.502
F_2	0.989	2.834	0.992	0.037	0.978	11.484	0.979	0.551
F_3	0.977	4.216	0.994	0.067	0.991	17.31	0.976	0.451
F_4	0.938	5.016	0.979	0.107	0.977	20.864	0.944	0.365

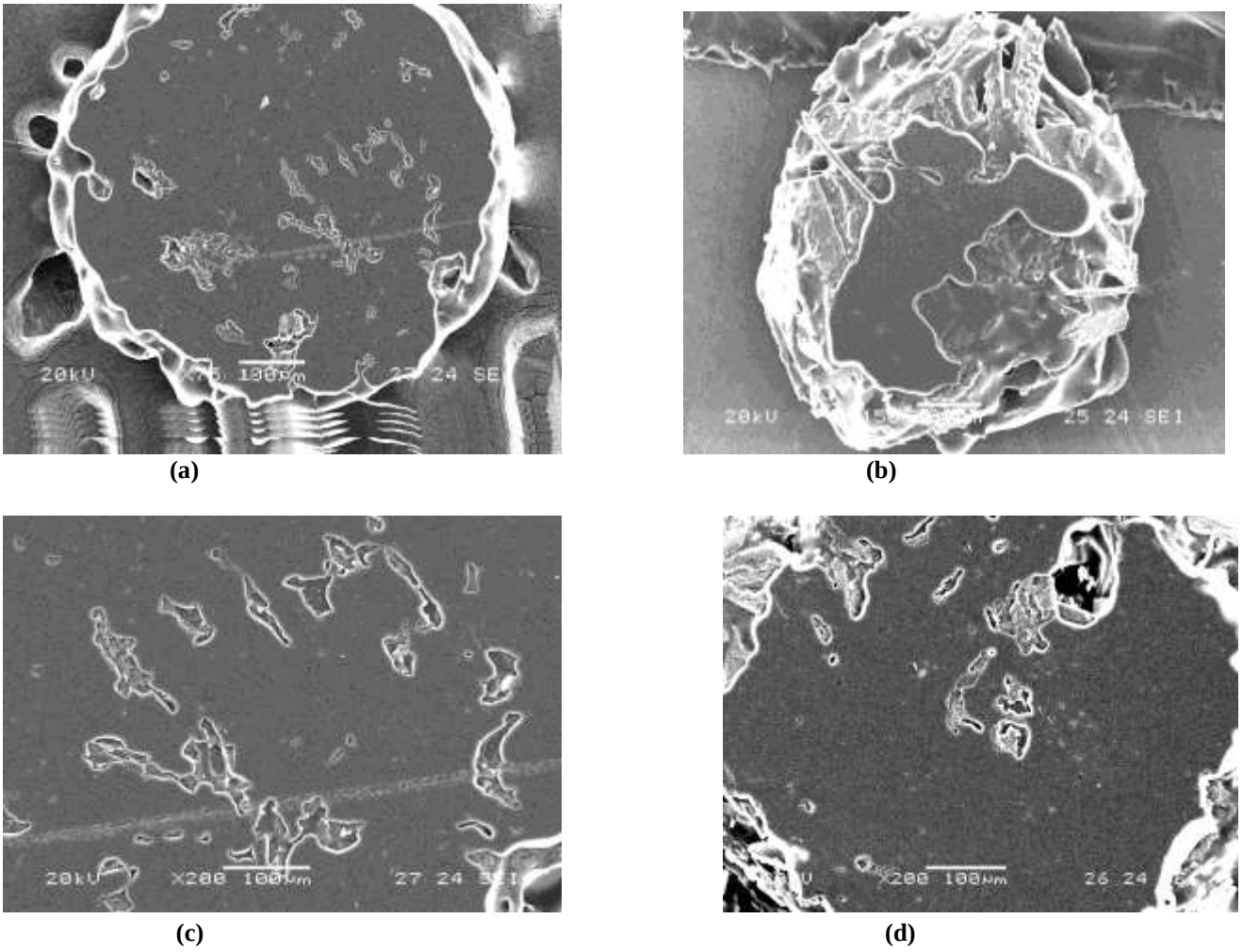
Table 4 (B) : **Release kinetics of floating microspheres in phosphate buffer pH 6.8**

Formulation	Zero order		First order		Higuchi		Korsmeyar – Peppas	
	r^2	K_o	r^2	K_1	r^2	K_H	r^2	n
F_1	0.911	2.516	0.944	0.033	0.971	10.587	0.961	0.34
F_2	0.907	7.427	0.998	0.221	0.983	31.507	0.967	0.643
F_3	0.740	6.616	0.980	0.382	0.891	29.565	0.900	0.366
F_4	0.690	5.363	0.974	0.375	0.853	24.292	0.894	0.243

Table 5 : Result of anti-inflammatory activity measurement

Treatment	Dose mg/kg	Weight of Cotton Pellet (mg)		Weight of granuloma (mg)	Percentage decrease in granuloma (%)
		Before	After		
Control	-	20.2±0.081	65.5±0.204	45.3±0.230	-
Standard	50	20.1±0.115	50.7±0.198	30.6±0.236*	32.4
Floating microspheres formulation F_2	Equivalent to 50 mg/kg (188.28)	20.1±0.09	44.1±0.107	23.9±0.098*	47.2

values are mean ± standard error of mean . Number of data points are 24 (6 animals) *P<0.001 when compared to control.



(a) **(b)**

(c) **(d)**

Figure 1 : **Scanning electron microphotographs of (a) formulation f_1 (b) formulation f_2 (c) surface morphology of f_1 (d) surface morphology of f_2**

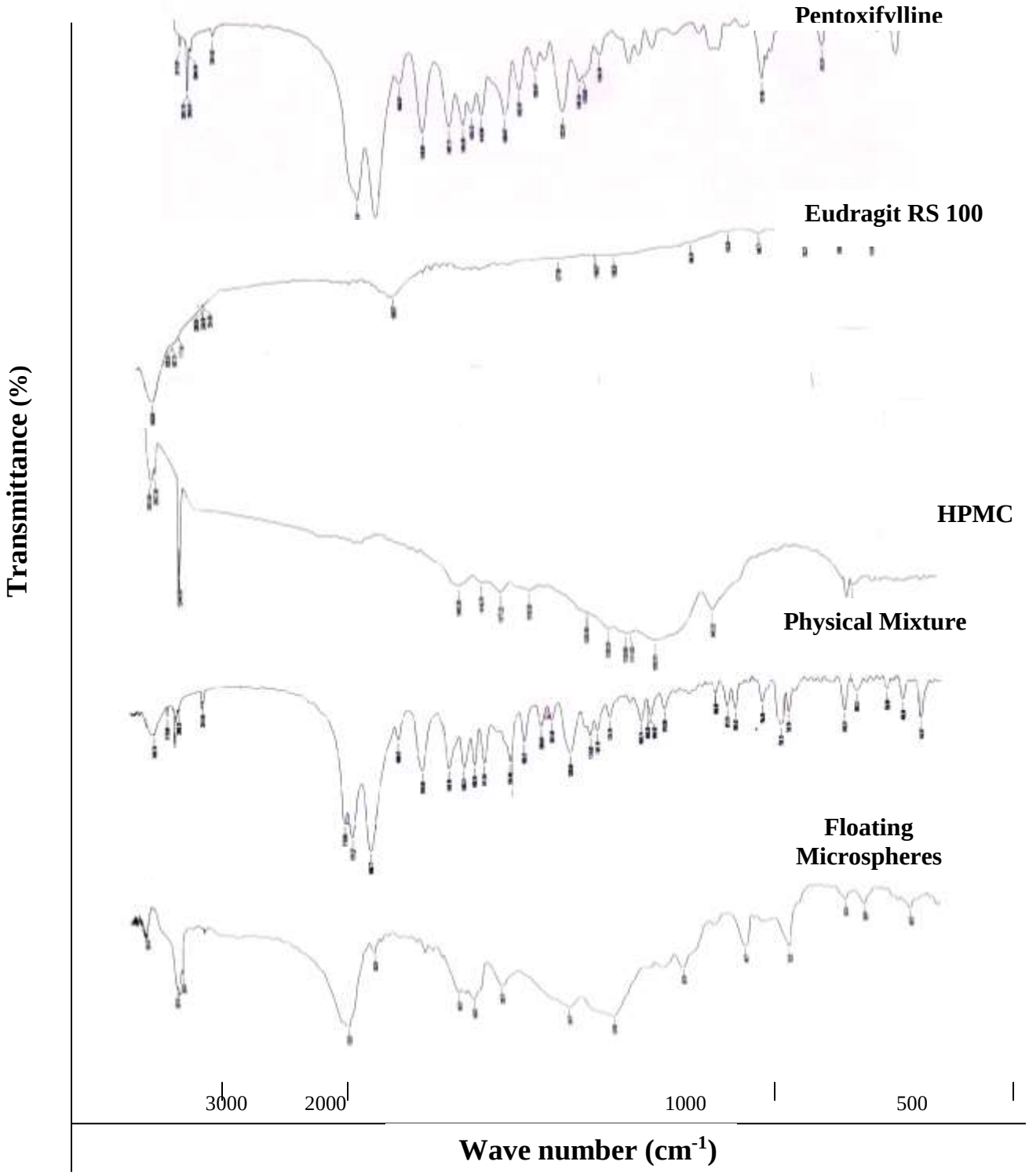


Figure 2 : FT-IR spectrum of pentoxifylline, Eudragit RS 100, HPMC, physical mixture of drug-polymer and floating microsphere

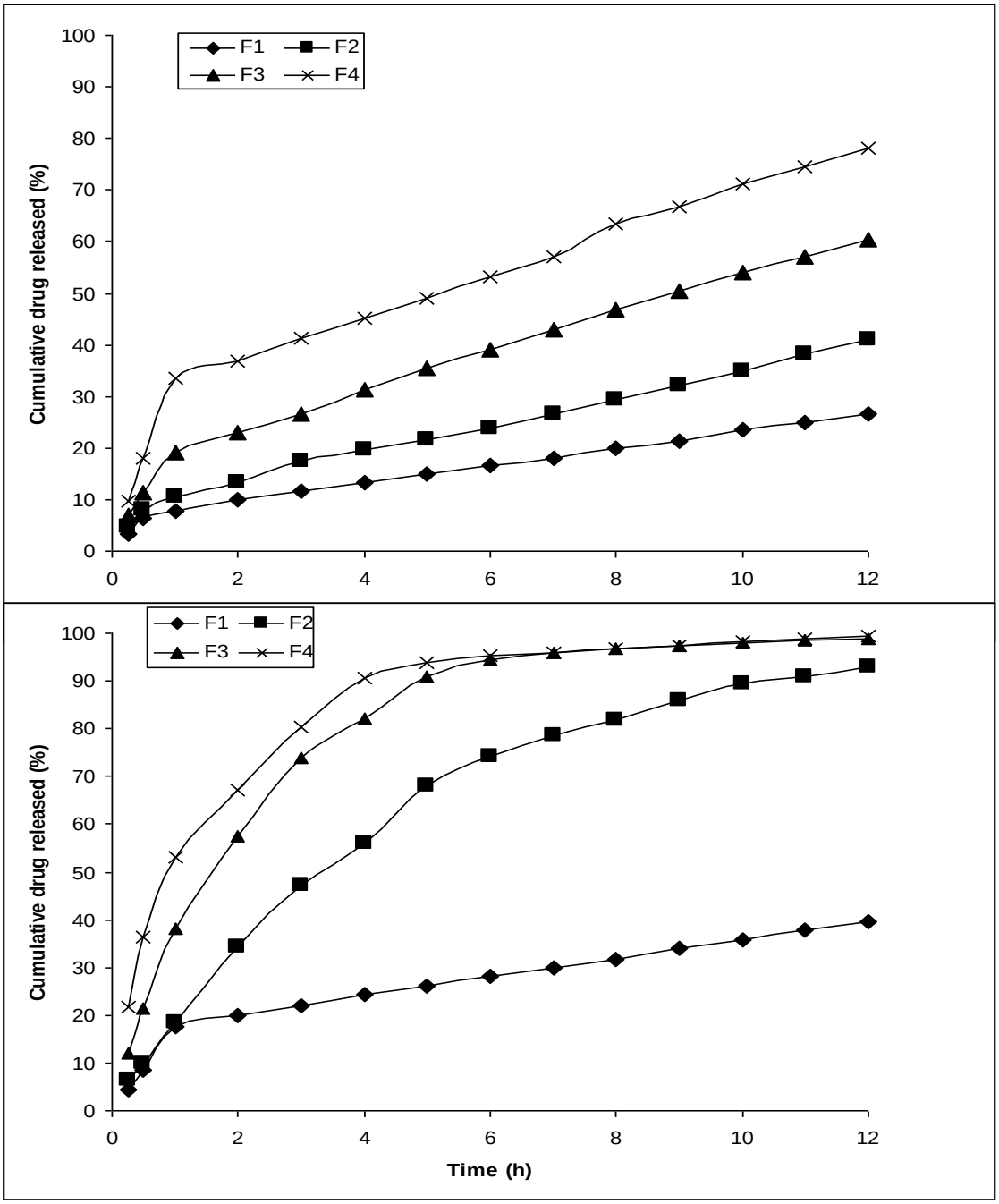


Fig. 3 : ***In vitro*** **drug release profile of different floating microspheres in (A) 0.1 N HCl (B) phosphate buffer pH 6.8**

REFERENCES

1. Arora S, Ali J, Ahuja A, Khar RK, Baboota S. AAPS PharmSciTech, 2005; 06: E 372-90.
2. Singh BN, Kim KH. J Control Release, 2000; 63: 235-59.
3. El-Gibaly I. Int J Pharm, 2002; 249: 7-21.
4. Sato Y, Kawashima Y, Takeuchi H, Yamamoto H, Fujibayashi Y. J Control Release. 2004; 98: 75-85.
5. Rouge N, Cole ET, Doelker E, and Buri P. Pharm Dev Technol. 1998; 3: 73-84.
6. Lee JH, Park TG and Choi HK. J Microencapsul. 1999; 16: 715-29.
7. Akiyama Y, Nagahara N, Kashihara T, Hirai S, Toguchi H. Pharm Res, 1995; 12: 397-405.

8. Deshpande AA, Rodes,CT, Shah NH, Malick AW. Drug Dev Ind Pharm, 1996; 22 : 531-539.
9. Whitehead L, Fell JT, Collett JH, Sharma HL, Smith AM. J Control Release, 1998; 55: 3-12.
10. Fix JA, Cargill R, Engle K. Pharm Res, 1993; 10: 1087-89.
11. Kedzierwicz F, Houvenot P, Lemut J, Etienne A, Hoffman M. J Control Release, 1999; 58: 195-205.
12. Groning R, Bertgen M and Georgarakis M. Eur J Pharm, 1998; 46: 285-91.
13. Park K. Biomaterials, 1988; 9: 435-41.
14. Stithit S, Chin W, Price JC. J Microencapsulation, 1998; 15: 725-37.
15. Sheth PR and Tossounian J. Drug Dev Ind Pharm, 1984; 10: 313-39.
16. Kawashima Y, Niwa T, Takeuchi H, Hino T and Ito Y. J Control Release, 1991; 16: 279-90.
17. Rouge N, Leroux JC, Cole ET, Doelker E, Buri P. Eur J Pharm Biopharm, 1997; 43 : 165-171.
18. Baumgartner S, Kristl J, Vricer F, Vodopivec P and Zorko B. Int J Pharm, 2000; 195 : 125-35.
19. Kawashima Y, Niwa T, Takeuchi H, Hino T, Itoh Y. J Pharm Sci, 1992; 81: 135-40.
20. El-Kamal AH, Sokar MS, Al Gamal SS, Naggar VF. Int J Pharm, 2001; 220: 13-21.
21. Soppimath KS, Kulkarni AR and Aminabhavi TM. Drug Dev Ind Pharm, 2001; 27: 507-515.
22. Sato Y, Kawashima Y, Takeuchi H and Yamamoto H. Eur J Pharm Biopharm, 2003; 55: 297-304.
23. Higuchi T. J Pharm Sci, 1963; 52: 1145-49.
24. Abdel-Salam OM, Baiuomy AR, El-Shenawy SM and Arbid MS. Pharmacol Res, 2003; 47: 331-40.