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DEVELOPMENT AND *IN-VITRO/IN-VIVO* EVALUATION OF FLOATING MICROBALLOONS OF FLURBIPROFEN

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ABSTRACT
The aim of the present investigation was to develop and evaluate a sustained release floating microballoons of Flurbiprofen as a model drug, to enhance the residence time in the stomach by floatability in the gastric juice. Floating microballoons were developed by the emulsion solvent diffusion method using different ratio of acrylic polymers (Eudragit RS100 or Eudragit RL100 or both) as carriers. The acrylic polymers have different permeability characteristics at independent pH. Polymers were used separately and in combination to prepare floating microballoons using ethanol and dichloromethane as organic phase. The yield of microballoons was upto 88.30±3.30. It showed good flow properties. On the basis of optical microscopy, particle size was found in the range of 105.67±2.05 to184.00±8.83 µm. Scanning electron microscopy (SEM) confirmed their spherical shape and perforated smooth surface without core shell. It exhibited floating properties for more than 12 h. *In-vitro* drug studies were performed in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4). *In-vivo* studies were performed in male rabbits for plasma drug concentration. At last different drug release kinetic model were applied for selected batches.

Keywords: Floating Microballoons, Flurbiprofen, Eudragit RS 100 and Eudragit RL 100, Floating drug delivery system, emulsion solvent diffusion method.

1. INTRODUCTION
Conventional oral dosage forms such as tablets, capsules, provide specific drug concentration in systemic circulation without offering any control over drug delivery and also cause great fluctuations in plasma drug levels. The design of oral controlled drug delivery system should be primarily aimed to achieve more predictable and increased bioavailability because oral routes are very convenient routes for administration of therapeutic agents. It has also low cost therapy and ease of administration lead to higher levels of patient compliance[1].

Floating drug delivery systems (FDDS) have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system. After release of drug, the residual system is emptied from the stomach. These results in an increased gastric residence time (GRT) and a better control of fluctuations in plasma drug concentration. Floating microballoons are gastro-retentive drug delivery systems based on non-effervescent approach. These microballoons are in strict sense, spherical empty particles without core. These microballoons are characteristically free flowing powders consisting of proteins or synthetic polymers, ideally having a size less than 200 µm[2,3,4].

Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints. It can also cause inflammation of the tissue around the joints, as well as in other organs in the body. In some patients with rheumatoid arthritis, chronic inflammation leads to the destruction of the cartilage, bone, and ligaments, causing deformity of the joints. It is suspected that certain infections or factors in the

environment might trigger the immune system to attack the body's own tissues, resulting in inflammation in various organs of the body such as the lungs or eyes[5].
Flurbiprofen [1,1-biphenyl]-4acetic acid, 2-fluro-alphamethyl, is a non-steroidal anti-inflammatory drug (NSAID) and analgesic also with anti-pyretic properties. It is also used in Rheumatoid disorder to maintain therapeutic plasma level. The mechanism of action is the inhibition of prostaglandin synthesis. It is rapidly eliminated from the blood and its half-life is 3-6 h[6]. Drug which is easily absorbed from gastrointestinal tract (GIT) and having a short half-life is quickly eliminated from blood circulation. This problem can be resolved by formulation sustained floating microballoons. Thus controlled drug delivery system have been developed as the release the drug slowly into GIT and maintain a constant drug concentration in the serum for longer period of time.

2. MATERIALS AND METHODS

2.1 Materials

Flurbiprofen was obtained as a gift sample from F.D.C. Pvt. Ltd. Mumbai. Eudragit RS-100 and Eudragit RL-100 was obtained as a gift sample from Degussa India Pvt. Ltd., Mumbai (India) used as polymers. Polyvinyl alcohol (PVA) was obtained from Central Drug House, New Delhi. Dichloromethane (DCM) and Ethanol were procured from Merck Pvt. Ltd. Mumbai. All others chemicals and reagents used for this work were of analytical grade.

Table 1. Formulation table of the prepared floating microballoons

Formulation code	Drug : polymer ratio*	Solvent ratio** (EtOH:DCM)
FMB1	1:1:0	1:1
FMB2	1:0:1	1:1
FMB3	1:2:0	1:1
FMB4	1:0:2	1:1
FMB5	1:3:0	1:1
FMB6	1:0:3	1:1
FMB7	1:4:0	1:1
FMB8	1:0:4	1:1
FMB9	1:0.5:0.5	1:1
FMB10	1:1:1	1:1
FMB11	1:1.5:1.5	1:1
FMB12	1:2:2	1:1

*Flurbiprofen (model drug) : Eudragit RS 100 : Eudragit RL100,
** Ethanol : Dichloromethane (DCM)

2.2 Methods

2.2.1 Preparation method of floating microballoons

Floating microballoons were prepared by the emulsion solvent diffusion method**.** Weighted amount of flurbiprofen was dissolved into the mixture of ethanol and dichloromethane (DCM) containing Eudragit RS100 or Eudragit RL100 or both polymer as per table-1. The resulting drug –polymer solution was poured slowly to stirring 200 ml aqueous solution of 0.75% w/v Polyvinyl Alcohol (PVA) at room temperature. The stirring was done for 1 hrs at 500 rpm using mechanical stirrer to

evaporate organic phase. After evaporation of solvent, floating microballoons were collected by filtration, washed with distilled water and sieved between 40 and 25 mesh sizes. Then, it was dried at room temperature in desiccators for 24 hrs[7].

3. EVALUATION OF FLOATING MICROBALLOONS

3.1 Particle size analysis

The particle size was determined by using microscopic technique. In this method suspension of floating microspheres were prepared using castor oil. A drop of suspension was mounted on a slide and observed under optical microscope (Magnus MLX-DX, Olympus, India) about 200-300 particles by using a calibrated optical microscope were measured with the help of the eye piece micrometer. The mean particle size was then calculated[8].

Table 2. Micromeritic properties of Floating Microballoons of Flurbiprofen

Formulation Code	Mean particle size[1]	Bulk density (gm/cm^3)[2]	Tapped density (gm/cm^3)[2]	Carr's index[2]	Angle of repose[2]
FMB1	105.67±2.05	0.367±0.002	0.428±0.005	14.37±0.33	24.27±0.17
FMB2	124.67±3.68	0.461±0.003	0.554±0.004	16.60±0.29	20.26±0.18
FMB3	158.00±4.32	0.325±0.003	0.346±0.003	6.60±0.29	25.78±0.31
FMB4	154.00±4.55	0.348±0.003	0.373±0.005	7.60±0.29	24.72±0.21
FMB5	172.33±5.73	0.326±0.004	0.343±0.004	5.40±0.37	30.49±0.29
FMB6	161.33±5.73	0.322±0.004	0.338±0.006	5.57±0.29	29.49±0.26
FMB7	184.00±8.83	0.306±0.004	0.316±0.004	3.73±0.41	32.00±0.37
FMB8	175.00±10.2	0.295±0.005	0.305±0.005	4.33±0.34	34.67±0.37
FMB9	108.00±9.63	0.363±0.005	0.411±0.008	12.50±0.41	24.25±0.22
FMB10	147.67±8.99	0.334±0.004	0.383±0.005	21.33±0.25	19.61±0.27
FMB11	170.67±5.73	0.314±0.002	0.363±0.006	13.47±0.34	30.46±0.32
FMB12	171.67±7.76	0.320±0.003	0.331±0.006	3.60±0.49	30.98±0.45

1. Mean±SD, n=200-300 2. Mean±SD, n=3

3.2 Morphology

Scanning electron microscopy is performed to characterize the surface of prepared microballoons by sprinkling the powder on a both side adhesive tape to stubs. Gold palladium coatings on the prepared stubs were carried out by using sputter coater (POLARON Model SC-76430) in a high vacuum evaporator and the thickness of coating was 200^0A. The coated stubs were randomly scanned under Electron Microscope (LEO-430, UK) and acceleration voltage was set at 20 KV during scanning. Microphotographs were taken on different magnifications and higher magnification (2.3 KX) was used for surface morphology[9].

3.3 Yield of floating mircoballoons

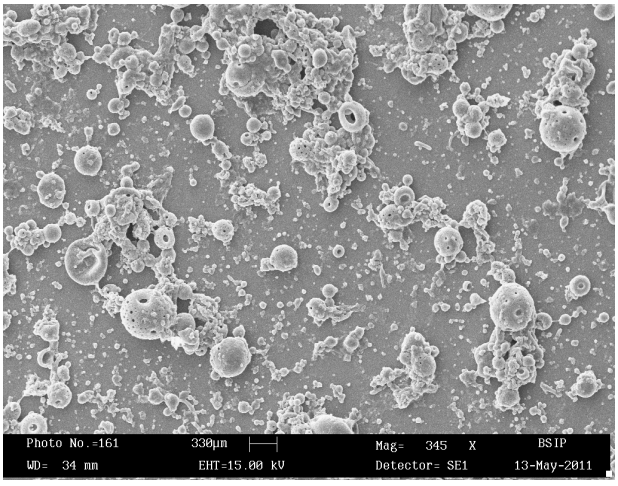
The percentage of floating microballoons were collected and weighted. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of microballoons[10,11].

3.4 Drug Entrapment Efficiency (DEE)

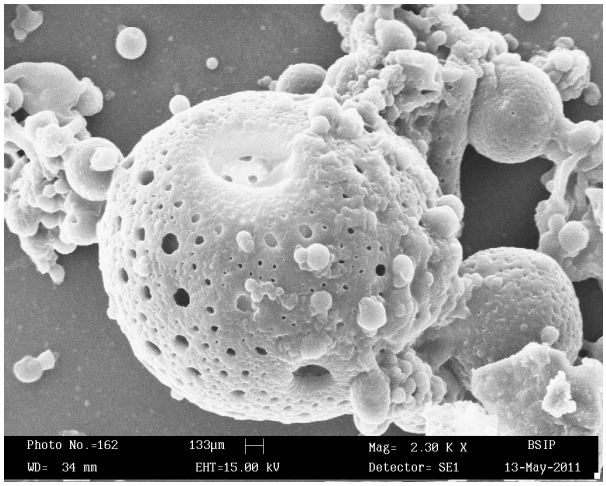
Floating Microballoons (20 mg) were weighted accurately and drug was extracted from floating microballoons for 24 hrs with 10 ml of 0.1N HCl (pH-1.2). During this period,

the mixture was agitated. After 24 hrs, the mixture was centrifuged at 2000 rpm for about 30 min. The supernatant obtained was analyzed with U.V. Spectrophotometer for drug contents after suitable dilutions at 247 nm. The percentage drug entrapped was calculated[12].

Figure 1. Scanning electron micrograph (SEM) of microballoons. (a. population of spherical microballoons, b. Internal view of the shell having porousness, c. Smoothness of the surface of spherical shaped floating microballoons)



a



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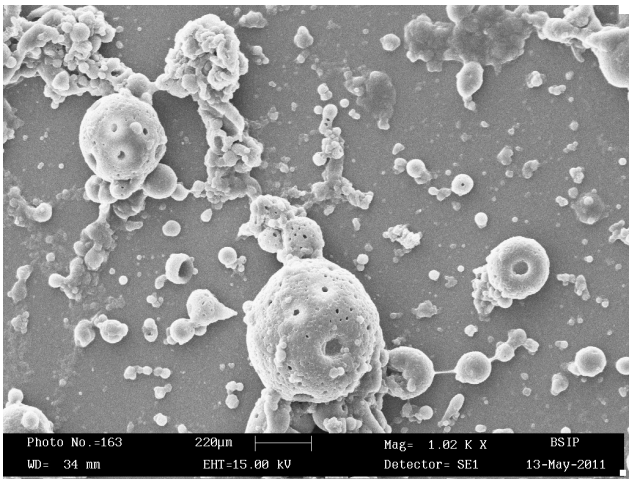

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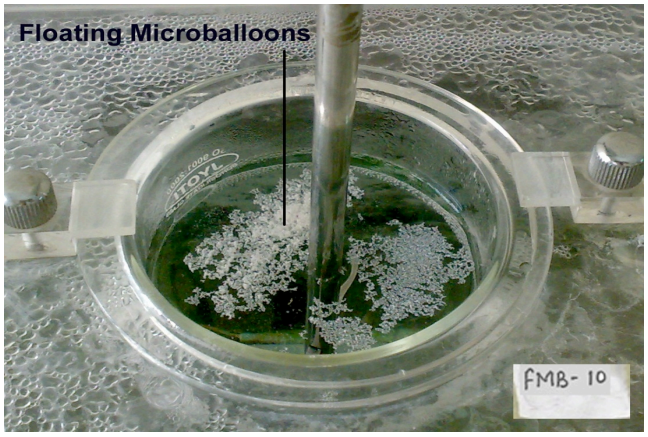


Fig.2: *In-vitro* buoyancy of floating microballoons

Table.3: Percentage yield, *in-vitro* buoyancy and incorporation efficiency of Floating microballoons of flurbiprofen.

Formulation code	Percentage yield	*In-vitro* buoyancy	Incorporation efficiency
FMB1	63.7±2.87	63.00±1.63	64.34±2.05
FMB2	68.7±5.31	60.00±1.63	61.45±2.04
FMB3	81.0±3.27	73.67±4.11	83.43±1.02
FMB4	75.3±4.50	64.33±2.05	73.63±2.05
FMB5	77.3±4.50	76.67±2.87	72.66±2.05
FMB6	72.0±4.08	69.33±2.05	68.35±2.05
FMB7	79.0±5.72	74.67±2.05	70.79±2.47
FMB8	69.3±4.11	73.00±1.63	69.37±2.05
FMB9	71.7±3.68	64.33±7.73	83.70±2.49
FMB10	81.0±5.10	77.67±4.11	89.68±2.49
FMB11	85.3±4.11	75.33±2.05	72.08±3.27
FMB12	88.3±3.30	72.00±5.72	68.52±2.86

Mean±SD, n=3

3.5 In-vitro floating behaviour (Buoyancy)

In-vitro floating behaviour studies on floating microballoons were carried out using USP XXIII Peddle Type Dissolution Apparatus. The microballoons (100 mg) were place in simulated gastric fluid (0.1 N HCl at pH 1.2, 900 ml) at 37^{o} C containing 0.02% w/v Tween 80 (mimic the effect of natural surfactant in the stomach). The mixture was agitated by a paddle rotating at 100 rpm for 12 hrs (Fig-2). After 12 hrs, the layer of buoyant microballoons was pipetted and separated by filtration and collected sample was kept in desiccators for drying[13,14].

3.6 In-vitro drug release studies

The USP (XXIII) Basket Type Dissolution Apparatus was used for determining the drug release rate from floating microballoons. The quantity of microballoons equivalent to 50 mg flurbiprofen from each batch was placed in a non-reacting muslin cloth that had a smaller mesh size than that of the microballoons. The mesh was tied with a nylon thread to avoid the escape of any microballoons and glass bead was used in the mesh to induce the sinking of microballoons. The dissolution test was performed in 900 ml of 0.1N HCl (pH 1.2). At specific time intervals, 10 ml aliquots were withdrawn, filtered through whatmann filter paper No. 44, appropriately diluted with the same medium and analysed at 247 nm for Flurbiprofen using U.V. Spectrophotometer (Shimadzu UV-1700)[15,16].

3.7 In-vivo studies[17]

The *in-vivo* study was conducted in healthy New Zealand six rabbits weighing 2.2-2.5 kg and provided by the Institutional animal ethical committee (IAEC). These were divided into two groups and fasted for 24 hrs. One batch was fed with 15 mg Flurbiprofen (pure drug) in a gelatin capsule and second batch was given floating microballoons formulation equivalent to 15 mg of pure drug in gelatin capsule. Water was given ad-libitum during fasting and throughout the experiment.

Blood sample, 1 ml each were collected from the marginal ear vein of the rabbits, into centrifuge tubes just before dosing and at 1, 2, 4, 6, 8, 10, 12 and 24 hrs during the study. Blood sample was centrifuged at 1500 rev/min for 15 min and the plasma was separated.

To 0.5 ml each of the other plasma sample, 5 ml of acetonitrile was added. The centrifuged tubes were then centrifuged at 2500 rev/min for 15 min, 4 ml of the supernatant was pipetted out to which 0.2 ml of 1.47 M perchloric acid was added and drug concentration was determined by U.V. spectroscopy at 247 nm.

The blank sample was prepared of 0.5 ml of Plasma, 4 ml of acctonilrile and perchloric acid .

The calibration curve for flurbiprofen was prepared by using undosed animal plasma, acetonitrile and perchloric acid. First of all, prepared 1-10 µg/ml Flurbiprofen solution, 1 ml of this solution was made upto 5 ml using acetonitrile. Then add the 0.5 ml of undosed plasma into 1-10 µg/ml solution then supernatant 4 ml was then pipette out to which 0.2 ml of 1.47 M perchloric acid was added and absorbance was measured by U.V. spectroscopy at 247 nm.

The calibration curve for Flurbiprofen was obtained as absorbance at 247 nm verse concentration . It was linear over the range of 1-10 µg/ml with the correlation coefficient of 0.9963.

RESULT AND DISCUSSION

4.1 Particle Size Analysis

The particle size of floating microspheres varied somewhat among the formulation due to variation in the composition of formulations. The effects of stirring speed and polymer to polymer ratio on the particle size of microspheres are shown in Table 1. Formulation FMB-7 showed relatively higher percentage of large size and formulation FMB-1 showed relatively small size floating microspheres because as viscosity of the medium increases at a higher polymer concentration resulting in enhanced interfacial tension. Shearing efficiency is also diminished at higher viscosities. This results in the

formation of larger particles. The ethanol diffused out from emulsion droplets and the dichloromethane became a major constituent of internal organic phase.

The size and surface morphology of floating microspheres were confirmed by scanning electron microscopy (SEM) as shown in figures 1 illustrating the microphotographs of formulation FMB-10. The floating microspheres were spherical with no visible major surface irregularity. The view of microballoons showed a hollow spherical structure with a smooth surface morphology (fig. 1c) and exhibited a range of sizes with the batch (fig. 1 a). The outer surface of the microballoons was smooth and dense; while the internal surface was porous. The shell of the microballoons also showed some porous structure (fig. 1 b). It may be caused by rapid evaporation solvent entrapped within the shell of microballoons after forming a smooth surface and covered with coat polymer.

The percentage yield was found in the range of 63.7±2.87% to 88.3±3.30% in the Table-3. The percentage yield was increased in combination of the Eudragit RS100 and Eudragit RL100 was used, instead of single use of Eudragit RS100 or Eudragit RL100 in formulations (Table-1). This was due to the combination of the polymers reduces the probability of formation of aggregates by reducing the viscosity of system. Therefore, the percentage yield depends on the properties of the polymers and their different ratio with the other polymers. The decrease in the percentage yield in some formulation might be due to loss of the polymer at surface of stirrer surface.

4.2 Incorporation efficiency

The drug incorporation efficiency of microballoons was found to be good in the range of 61.45±2.04% to 89.68±2.4% in the Table-3. Among all formulation FMB-3, FMB-4, FMB-9, and FMB-10 was found to be highest incorporation efficiency of 83.43±1.03%, 73.63±2.05%, 83.70±2.49%, and 89.68±2.4% respectively. This was observed in the combination of polymers had greater drug loading compare to single polymers. This may be due to the higher solubility of eudragit RS100 or eudragit RL100 in the organic phase and better encapsulation of drug but at the higher concentration of eudragit RS100, the incorporation efficiency decreased because of the enhanced permeability of walls of microballoons. Thus the combination of the polymers shows the more incorporation efficiency instead of the single polymer. Another cause of good incorporation efficiency may be the poor solubility of drug in water. Results demonstrated that increase in concentration of acrylic polymer increased the entrapment of the drug. The drug entrapment efficiency was found to be good in all the formulations.

***4.3 In-vitro* floating behavior (buoyancy)**

The purpose of preparing floating microballoons was to extent the gastric residence time of a drug. The buoyancy test was carried out to investigate the floatability of the prepared microballoons. All formulation was found good floating ability and its range was 60±1.63% to 77.67±4.11% in the Table-3. Such floating performance was due to insolubility of polymers in the gastric fluid and this was observed that floating behaviour of microballoon is due to use of Dichloromethane (DCM), which made pores at surface of microballoons. The results also showed a tendency that the larger the particle size, the longer floating time upto 12 h. (Fig.2).

***4.4 In-vitro* Drug Release**

The release of Flubriprofen from floating microballoons was studied in 0.1N HCl (pH-1.2) and phosphate buffer solution (pH-7.4). The drug release of microballoons were found to be in the range of 62.10±0.83 to 78.03±1.63 in 0.1N HCl and of 89.44±1.05 to

98.51±0.41 in phosphate buffer solution. Among all formulation, FMB-10 was found to be the best formulation as it drug release 98.51±0.41% in a sustained manner with constant fashion over extended period of time (after 24 hr) in fig-3,4. This may be due to drug was soluble in phosphate buffer (pH-7.4). Since the acrylic polymers are insoluble in acidic medium.

It was observed as the concentration of eudragit was increased percent release of Flurbiprofen decreases. The increase in eudragit concentration leads to the increased density of polymer matrix into the microballoons which result in an increased diffusional pathlengh. This may decrease the overall drug release from polymer matrix. Furthermore smaller microballoons are formed at lower polymer concentration and have larger surface area exposed to dissolution medium.

Fig.3: *In-vitro* drug release profile in 0.1 N HCL at pH-1.2

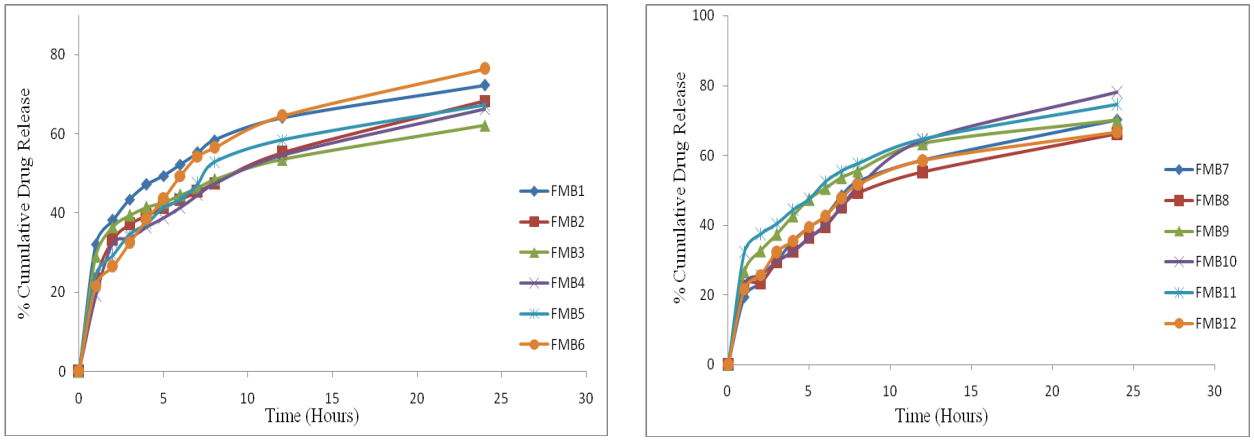
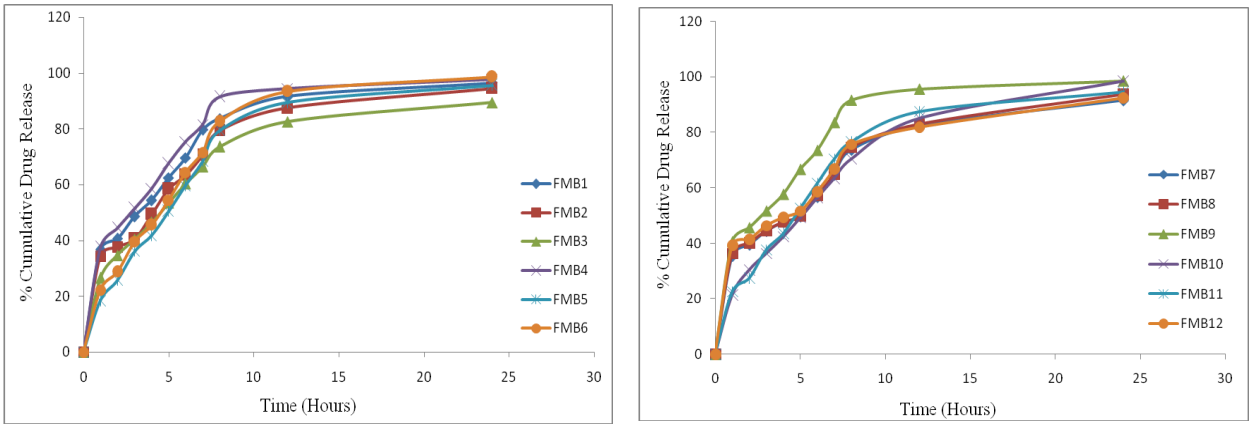


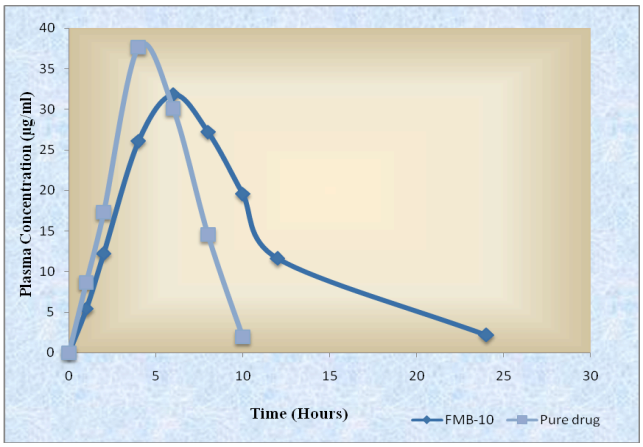
Fig.4: *In-vitro* drug release profile in Phosphate buffer solution (pH-7.4)



4.5 In-vivo evaluation

The *in-vivo* evaluation of the floating microballoons of Flurbiprofen was conducted in two groups of New Zealand rabbits. The rabbits have been chosen as the model for study. Figure-5 showed the graph of blood plasma concentration of the drug verse time for flurbiprofen and formulation FMB-10 of floating microballoons. The maximum plasma drug concentration was found at the fourth hours and then the plasma drug concentration decreases rapidly for the pure drug and shows a "peak and valley" profile of pure drug, but the formulation FMB-10 of floating microballoons showed controlled release kinetic profile. The observed C_{max}, t_{max} and AUC for the pure drug was 37.67 µg/ml, 4 hours and 201.24 µg h ml^{-1}, the C_{max}, t_{max} and AUC for the FMB-10 was 31.84 µg/ml, 6 hours and 327.71 µg h ml^{-1}. Thus the release of the drug from the formulation FMB-10 showed the controlled release. The results of the estimation of the area under curve showed that the bioavailability was lesseer for the pure drug which was increased with the formulation FMB-10.

Fig.5: *In-vivo* bioavailability study of pure drug and formulation FMB-10



4.6 Mechanism of drug release

The mechanism of release was determined by fitting the release data to the various kinetic equations such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas. The best-fit model was found to be Higuchi, Korsmeyer-Peppas, and First-order.

CONCLUSION

Floating microballoons of Flurbiprofen can be successful prepared using the eudragit RS-100/RL-100 as polymers by emulsion solvent diffusition method.

The percent yield of all floating microballoons formulation was more than 60% suggesting that the methods used for encapsulation was effective. The percentage yield was significantly increased as the amount of polymer was increased in each preparation method.

Hence, finally it was concluded that the prepared floating microballoons of Flurbiprofen of eudragit RS-100 and eudragit RL-100. The drug:polymers (ERS100:ERL100) ratio (1:1:1) was found satisfactory formulation because the polymer have low and high permeability characteristics thus the formulation FMB-10 may prove to be potential candidate for safe and effective sustained drug delivery over

an extended period of time which can reduce dosing frequency and may be used for effective management of Rheumatoid Arthritis.

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REFERENCES

1. Banker GS, Anderson NR. Tablets: the theory and practice of industrial pharmacy. 3rd . Bombay: *Varghese Pub. House*, 2003; 1(1): 74-79.
2. Vyas SP, Khar RK. Gastro-retentive system in Controlled Drug Delivery System: Concept & Advances. Ist Ed. New Delhi: *Vallabh Prakashan*, 2002; 1(2): 417-441.
3. Shivkumar HG, Vishakanta GD, PramodKumar TM. Formulation and evaluation of mucoadhesive drug delivery system for some anti-asthmatic drugs. *IJPE*, 2004 Oct-Dex. ; 38(4): 22-26.
4. Arora S, Ali J, Ahuja A, Khar RK, Baboota S. Floating drug delivery system. A review. *AAPS Pharm Sci Tech*, 2005; 6(3): 372-390.
5. www.google.com/wikipedia/rheumatoid.
6. www.drugbank.com.
7. Sato Y, Kawashima Y, Takeuchi H, *In-vivo* evaluation of riboflavin-containing microballoons for floating controlled drug delivery system in healthy human volunteers. *Journal of Controlled Release*, 2003; 93: 39-47.
8. Parasuram RR, Moidutty lugman, Chetan HB. Preparation and evaluation of delayed release aceclofenac microspheres. *Asian Journal of Pharmaceutics,* 2008;Oct-Dec:252-254.
9. Hemangi SP, Moreshwar PP, Bharat WT, Formulation and *in-vitro* evaluation of floating microspheres of Acyclovir. *Arch Pharm Sci & Res*, 2009; 1: 194-198.
10. Bharate SM, Yogesh SR, Rupesh MS, Pawar KR, Rahane RD. Formulation and evaluation of floating microspheres of Ketrolac trometamol. *Int. Journal of Pharmaceutical research and development-online*, 2005;25(2):150-178.
11. Jung HL, Tae GP, Hoo KC. Effect of formulation and processing variables on the characteristic microspheres for water soluble drugs prepared by pharmaceutics, 2000;196:75-83.
12. Patel A, Ray S, Thakur RS. *In-vitro* evaluation and optimization of controlled release floating delivery system of metformin HCL. *DARU*, 2006; 55:277-285.
13. Nakagawa T, Ichikondo S, Sasai YK. Preparation of floating drug delivery system by plasma technique. *Chem Pharm. Bull*, 2006; 54(4): 514-518.
14. Nathalie RA, Eric AM, Marianne GF, Luc BB, Ewart T. Cole C. Comparative pharmacokinetic study of a floating multiple-unit capsule, a high-density multiple-unit capsule and an immediate-release tablet containing 25 mg atenolol. *Pharmaceutica Acta Helvetiae*, 1998;73:81-87.
15. Parauvathanahalli SR, Brahmeshwar Mishra. Preparation and *in-vitro* characterization of gellan based floating beads of acetohydroxamic acid for eradication of helicobactor pylori. *Acta pharm.,* 2007;57:413-427.
16. Nakagawa T, Ichikondo S, Sasai YK. Preparation of floating drug delivery system by plasma technique. *Chem Pharm. Bull*, 2006; 54(4): 514-518.
17. Joseph NJ, Lakshmi S, Jayakrishan A, A floating-type oral dosage form for piroxicam based on hollow polycarbonate microspheres: *in-vitro* and i*n-vivo* evaluation in rabbits. *Journal of Controlled Release*, 2002; 79: 71-79.