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Formulation and In Vitro Evaluation of Floating Microspheres of Mefenamic Acid

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ABSTRACT

Floating microspheres are gastro retentive low density drug delivery systems based on non-effervescent approach. These systems have density less than gastric fluids and so remain buoyant in stomach without affecting gastric emptying rate for a prolonged period of time. The aim of the present work was to formulate and evaluate floating microspheres containing Mefenamic acid which is a non-steroidal anti-inflammatory drug in order to achieve an extended retention in the upper GIT, which may result in enhanced absorption and thereby improved bioavailability. Floating microspheres of Mefenamic acid were prepared by an emulsion solvent evaporation method by using different grades of polymers like HPMC K4M, HPMC K15M, Ethylcellulose, excipients and other solvents. FTIR studies confirmed that there were no incompatibilities between drug and polymer. SEM analysis of the microspheres revealed that all the prepared microspheres were discrete spherical in shape with satisfactory surface morphology. The prepared microspheres were evaluated for various parameters like percentage yield, particle size analysis, micromeritic studies, percentage drug entrapment efficiency floating test, in-vitro drug release studies, in-vitro kinetic studies and stability studies. Floating lag time and floating time duration were found to be in the range of 5 -10.7 mins, and 5- 9.7 hours respectively. All the prepared formulations showed good % buoyancy in the range 53.94 to 70.19%.The in-vitro kinetic studies of the optimized formulation F4 were carried out and found that it undergoes zero order kinetics based on regression coefficient (r2) values. The mechanism of drug release of optimized formulation was found to be case II transport. Among all formulations,F4 showed an appropriate balance between buoyancy and in vitro drug release rate (94.89% in 10 hours), hence it was considered as the best optimized formulation. The stability study was carried out on optimized formulation F4 as per ICH guidelines.

INTRODUCTION

Oral drug delivery has been known as the most widely used route of administration of pharmaceutical products for systemic drug delivery.[1] The oral route is increasingly being used for the delivery of therapeutic agents because the low cost of the therapy and ease of administration leads to high levels of patient compliance.[2] Conventional drug delivery system achieves as well as maintains the desired drug concentration within therapeutically effective range needed for treatment only when taken several times a day. Gastric emptying time of dosage forms is an extremely variable process and ability to prolong and control emptying time is a valuable asset for dosage forms, which resides in the stomach for a longer period of time.

An incomplete release of the drug and short residence time of the dosage form in the upper gastrointestinal tract will lead to lower its bioavailability. To avoid this problem, the oral controlled release formulations have been developed. Controlled release (CR) implies the predictability and reproducibility to control the drug release.[1] Gastro retentive dosage forms have potential for being used as controlled-release drug delivery systems.

Inflammation is a vital part of the body's immune response. Inflammation is often characterized by redness, swelling, warmth, pain and sometimes immobility. Non-steroidal Anti-inflammatory Drug (NSAID) is highly effective against inflammatory diseases and other inflammatory mediators like cytokines, histamine, thromboxanes, prostaglandins etc.[3]

NSAIDs are a group of drugs that together provide analgesic (pain-killing), antipyretic (fever-reducing) and anti-inflammatory effects in higher doses. The major side effect of NSAID is the gastric irritation. The adverse effects produced by the NSAIDS can be overcome by the development of controlled release formulations. Here the drug Mefenamic acid is chosen to formulate an oral controlled release dosage that is in the form of floating microspheres by ‘emulsion solvent evaporation technique’. Mefenamic acid is a highly effective non-steroidal anti-inflammatory drug that has analgesic and antipyretic activity. The biological half-life of Mefenamic acid is 2-4 hours. The recommended dose for the relief of acute pain in adults and adolescents (14 years) is 500 mg as an initial dose, followed by 250 mg every 6 hours as needed, usually not exceeding one week. The aim of the present work was to formulate and evaluate floating microspheres of Mefenamic acid, which belongs to the class NSAID by emulsion solvent evaporation technique using ethyl cellulose, HPMC K4M, HPMC K15M as polymers and also ethanol and dichloromethane as solvents.

MATERIALS AND METHODS:

MATERIALS:

Mefenamic acid and other polymers were received from Yarrow Chem. products Mumbai. All other excipients and solvents used were of analytical and pharmaceutical grade.

METHODS:

Compatibility studies

Drug - polymer compatibility study using FTIR:

FTIR spectroscopy of pure drug (Mefenamic acid) and physical mixture of drug and polymers was carried out to check the compatibility between drug and polymers. The FTIR spectra of the drug with polymers were compared with the standard FTIR spectrum of the pure drug. The samples were prepared by mixing the drug alone and the drug with polymers in 1:1 ratio. The physical mixtures of Mefenamic acid and polymers were scanned in the wavelength region between 400 -4000 cm^{-1} and the spectrum were recorded. The compatibility between the drug and polymer were evaluated using FTIR peak matching method.

Preparation of calibration curve of Mefenamic acid[18]

Accurately weighed 10 mg of Mefenamic acid was taken in 100 ml standard flask. Few ml of ethanol was added to dissolve the drug and made up the volume with 0.1N HCl to get a stock solution of concentration 100 µg /ml. From this stock solution, aliquots of 0.5, 1, 1.5, 2, 2.5 ml of solutions were transferred into separate 10 ml standard flasks and made up the volume with 0.1HCL to get a concentration of 5, 10, 15, 20, 25 µg /ml respectively. The absorbance of the resultant solution was measured at 285nm by using UV spectrophotometer. A graph of concentration vs absorbance was plotted.

Preparation of floating microspheres of Mefenamic acid by emulsion solvent evaporation technique[5]

Accurately weighed drug and polymers in different ratios were dissolved in the solvents like ethanol and dichloromethane in 1:1 ratio as shown in Table 1. The solution was poured into 100 mL of distilled water containing 0.01ml of tween 80 and 5 ml of n- hexane with stirring to form a homogeneous solution, which was maintained at 40°C temperature and at agitation

speed of 800 rpm for 1 and half hour to allow the volatile liquid to evaporate. The microspheres formed were filtered and air dried for 24 hours at room temperature.

Table 1: Formulation design of Mefenamic acid floating microspheres

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Mefenamic acid (gm)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ethyl Cellulose (gm)	0.4	0.8	1.5	2	0.4	0.8	1.5	2
HPMC K4M (gm)	0.3	0.5	0.3	0.5	-	-	-	-
HPMC K15M (gm)	-	-	-	-	0.3	0.5	0.3	0.5
Ethanol: DCM (mL)	10:10	10:10	10:10	10:10	10:10	10:10	10:10	10:10
Tween 80 (mL)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
n-hexane (mL)	5	5	5	5	5	5	5	5
Distilled water (mL)	100 mL							

Evaluation studies of prepared floating microspheres[5]

a) Determination of percentage yield: The prepared floating microspheres of all batches were accurately weighed. The weight of the prepared microspheres was calculated by total amount of all excipients and drug used in the preparation of the microspheres, which gives the percentage yield of the floating microspheres. It was calculating using the formula**:** [6,7]

$$\text{Percentage yield} = \frac{\text{Actual yield of the product} \times 100}{\text{Total weight of drug and polymer}}$$

b) Determination of Particle size analysis: All the prepared batches were analyzed for particle size by optical microscope. Triplicate readings were taken. One hundred particles from each batch were counted**.**[8]

c) Micromeritic studies[8]

The flow property of the prepared microspheres was studied by determining the parameters like angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio.

Determination of angle of repose: Angle of repose is defined as the maximum angle possible between the surface of the pile of the powder and the horizontal plane. Angle of repose (θ) of the microspheres, which measures the resistance to particle flow, was determined by the fixed funnel method. The height and diameter of the powder cone was measured and angle of repose was calculated using the following equation,

$$\tan \theta = h / r \quad \text{or} \quad \theta = \tan^{-1} (h / r)$$

where, h = height of pile, r = radius of the base of the pile, θ = angle of repose

Table 2: Relationship between angle of repose and flowability

Angle of repose	Flowability
25-30	Excellent
31-35	Good
36-40	Fair
41-45	Passable
46-55	Poor
56-65	Very poor

Determination of bulk density: Bulk density is the ratio of the weight of the powder and the volume it occupies. It is expressed in gm/mL. Bulk density is important in determining the size of the container needed for handling and processing. The bulk density was calculated using the formula**:**

$$\text{Bulk density} = \frac{\text{Weight of the microspheres (W)}}{\text{Initial volume occupied by the microspheres } (V_o)}$$

Determination of tapped density: Tapped density is the ratio of the weight of the powder and the volume occupied by it after a specified compaction process, usually involving vibration of the container. It is obtained by mechanically tapping a graduated cylinder containing the microspheres until little change from the initial volume is observed. It is expressed in gm/ml. Triplicate readings were taken.

$$\text{Tapped density} = \frac{\text{Weight of the microspheres (W)}}{\text{Final volume occupied by the microspheres } (V_f)}$$

Determination of Hausner's Ratio: It is another parameter for measuring flowability of the microspheres. Triplicate readings were taken. It was calculated using the formula,

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Lower Hausner's ratio (<1.25) indicates better flow properties than higher ones (>1.25)

Determination of compressibility Index[8]: It is an indirect measurement of bulk density, size and shape, surface area, moisture content and cohesiveness of materials since all of them can influence the consolidation index. It is also called as Carr's index. Triplicate readings were taken. It is denoted by Ci and is calculated using the formula below.

$$Ci = \frac{\text{Tapped density - Bulk density}}{\text{Tapped density}} \times 100$$

Table 3: Relationship between powder flowability and % compressibility

% Compressibility range (Carr's index)	Flow description
5-12	Excellent (free flowing granules)
12-16	Good (free flowing granules)
18-21	Fair and Passable (powdered granules)
23-35	Poor (very fluid powders)
33-38	Very poor
>40	Extremely poor

Shape and surface morphology[5,9]: The external and internal morphology of the microspheres were studied using scanning electron microscopy (SEM). The samples for SEM were prepared by lightly sprinkling on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with platinum to a thickness of about 10 Å under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The stub containing the coated samples was placed in the scanning electron microscope (JEOL JSM 6380LA, Japan) chamber. The samples were then randomly scanned, and photomicrographs were taken at the acceleration voltage of 20 Kv.

Percentage drug entrapment efficiency: Accurately weighed quantity of microspheres were taken and crushed with mortar and pestle. Then microspheres were extracted with 10 mL of ethanol. The extract was transferred to a 100 mL volumetric flask and made up volume with 0.1N HCl. The solution was filtered and dilutions were made and absorbance was measured against blank solution spectrophotometrically at 285 nm. The percentage drug entrapment efficiency of floating microspheres was calculated using the formula:

$$\% \text{ Drug Entrapment Efficiency} = \frac{\% \text{ experimental drug loading}}{\% \text{ theoretical drug loading}} \times 100$$

Floating lag time and floating time:[10,4] Floating lag time is the time between the introduction of microspheres into the dissolution medium and its rise to upper one third of the dissolution vessel which was measured by visual observation.

Floating time or duration of floating is the time for which the microspheres float in the dissolution medium.

These tests were performed in simulated gastric fluid 0.1N HCl (pH 1.2) maintained at 37°C in a 100 mL beaker.

***In-vitro* buoyancy studies**[5.12]**:** Prepared floating microspheres of 100 mg were spread over the surface of the dissolution medium of 500 ml simulated gastric fluid (pH1.2), which was placed in USP dissolution apparatus type II (rotating paddle). The medium temperature was maintained at 37±5°C and was agitated by paddle at 100 rpm for 12 hours. After agitation, the microspheres that floated over the surface of the medium and those that settled down at bottom of the flask were recovered separately and dried. All experiments were run in triplicate. The percentage buoyancy of the floating microspheres was calculated by using the formula:

$$\text{Buoyancy (\%)} = \frac{W_f}{W_f + W_s}$$

Where, Wf and Ws are the weight of the floating and settled microspheres respectively.

***In-vitro* drug release study**[5]**:** The drug release study from the floating microspheres was performed using USP type-II apparatus (rotating paddle) in 900 ml of simulated gastric fluid 0.1N HCl dissolution media (pH1.2) at 50 rpm at 37± 0.5°C. 5 ml of the sample was

withdrawn at different time intervals for 12 hours and the same volume of fresh buffer was replaced to maintain sink conditions. Withdrawn samples were analyzed spectrometrically at 285 nm by using UV visible spectrophotometer.

Kinetic modelling of dissolution profiles:[13,14] The results obtained from *in-vitro* release studies were attempted to be fitted into various mathematical models as follows:

1) Cumulative percentage drug released Vs. Time (Zero order kinetics)

2) Log cumulative percent drug remaining Vs. Time (First order kinetics)

3) Cumulative percentage drug released Vs. Square root of Time (Higuchi model)

4) Log cumulative percentage drug released Vs. Log Time (Korsmeyer- Peppas model)

Diffusion release mechanism based on diffusion or release exponent are shown in Table 4.

Table 4: Interpretation of diffusional release mechanism[13]

Diffusion or release exponent(n)	Diffusion release mechanism
< 0.45	Quasi- Fickian diffusion
0.45	Fickian diffusion
0.45 < 0.89	Anomalous (non-Fickian) diffusion
0.89- 1	Case- II transport (Zero order release)
>1.0	Super case- II transport

Stability Study of the Optimized Formulation:

In order to determine the change in evaluation parameters like physical appearance, drug entrapment efficiency, *in-vitro* buoyancy and *in-vitro* drug release profile on storage, the stability studies were carried out. Stability studies of optimized formulation F4 were carried out by packing in aluminum foil which was kept in a petridish at 40±2˚C and 75±5% RH in a humidity chamber for 1 month. Sample was withdrawn after 30 days and evaluated for changes in physical appearance, drug entrapment efficiency and in vitro drug release profile.

RESULTS AND DISCUSSION

1) Compatibility Studies

a) FTIR spectroscopy of Mefenamic acid: The FT-IR spectrum of Mefenamic acid is shown in Figure 1 and the corresponding IR frequencies are represented in Table 5 which complies with standard functional group frequencies.

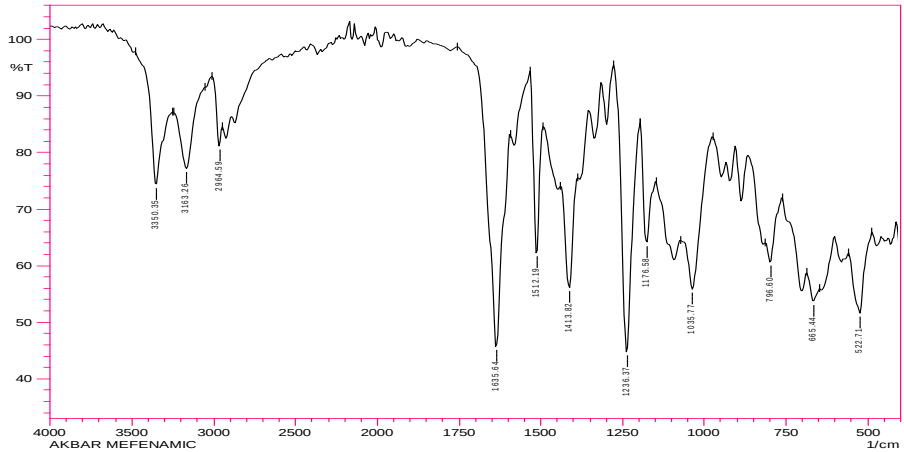

Figure 1: The FT-IR spectrum of Mefenamic acid

Table 5: IR frequencies of Mefenamic acid

Functional Group	Characteristic wavenumber or frequency cm^{-1}	Mefenamic acid observed wavenumber or frequency cm^{-1}
NH stretching	3300-3400	3350.35
NH bending	1500-1700	1512.19
C=O stretching	1650-1600	1635.64
C-CH_3 stretching	1450-1400	1413.82
OH bending	1200-1350	1236.37
C_6H_5 stretching	700-650	665.44

The FTIR spectrum for Mefenamic acid showed a weak peak at 3350 cm^{-1} due to the presence of a secondary amine. The broad band in the range of 3163-2900 cm^{-1} was due to the presence of –OH. The same also represents the intra- and intermolecular hydrogen bonding due to the –OH groups and also overlaps with the (–CH3) group. The peak at 1650-1600 cm⁻1 was due to the presence of a C=O group. The presence of a peak at 665.44 cm^{-1} indicates the presence of a phenyl group. The peaks analyzed in the table indicates that most

characteristic wave numbers of functional group like NH, C=O, C-CH_3, OH and C_6H_5 etc. were matched and compared to the observed frequencies.

b) Compatibility between drug and polymer: The FT-IR spectrum of combination of Mefenamic acid with excipients like ethyl cellulose and HPMC K4M are shown in Figure 2**,** and the corresponding IR frequencies are shown in Table **6.**

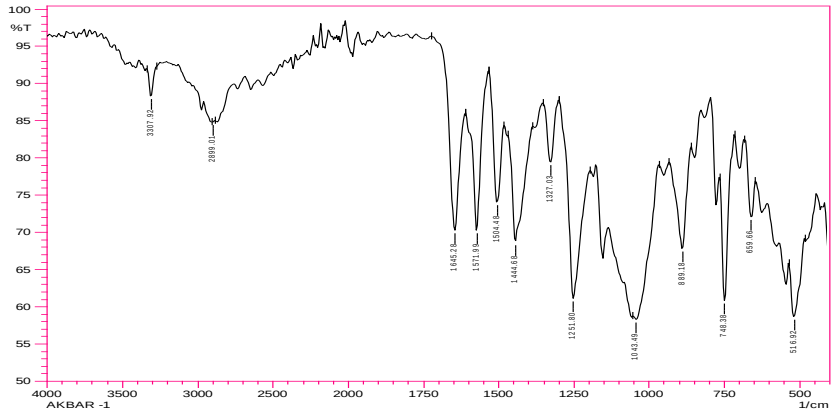

Figure 2: The FT-IR spectrum of combination of Mefenamic acid with excipients like ethyl cellulose and HPMC K4M

Table 6: IR frequencies of Mefenamic acid with ethyl cellulose and HPMC K4M

Functional group	Characteristic wavenumber or frequency cm^1	Mefenamic acid observed wavenumber or frequency cm^{-1}	Mefenamic acid + ethyl cellulose + HPMC K4M mixtures wave number cm^{-1}
NH stretching	3300-3400	3350.35	3307.92
NH bending	1500-1700	1512.19	1571.99
C=O stretching	1650-1600	1635.64	1645.28
C-CH_3 stretching	1450-1400	1413.82	1444.68
OH bending	1200-1350	1236.37	1251.80
C_6H_5 stretching	700-650	665.44	659.66

After the study of compatibility of drug with excipients, the IR spectra of pure drug and drug-excipients physical mixture were analyzed. The peaks analyzed in Table **6** indicates that most characteristic wave number or frequencies of functional group, which are NH Stretching, NH bending, C=O Stretching, C-CH_3 stretching, OH bending and C_6H_5 were

found unchanged. This showed that Mefenamic acid remained unaffected by the excipients used. No new complexes were observed as well. So it could be concluded that there was no major interaction between drug and excipients.

Preparation of standard calibration curve

The absorbance value remained linear and obeyed Beer's Lamberts Law in the range of 0-25 µg/ml with the R^2 value of 0.994. The standard plot is shown in Figure **3**

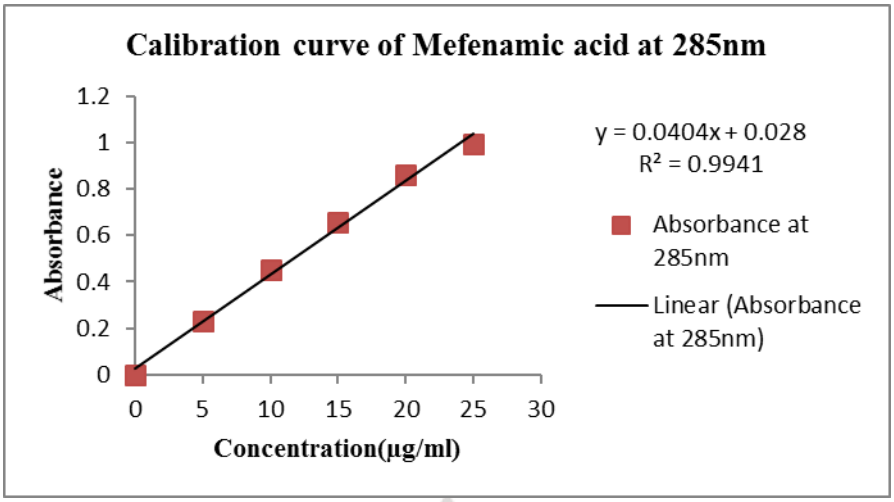

Figure 3: Standard calibration curve of Mefenamic acid in 0.1 N HCl at 285 nm

Formulation of floating microspheres of Mefenamic acid

Eight formulations of floating microspheres of Mefenamic acid were prepared by emulsion solvent evaporation method. F1-F4 formulations were prepared by using polymers such as ethyl cellulose and HPMC K4M in various proportions. The remaining formulations F5-F8 were prepared using ethyl cellulose and HPMC K15M and other solvents.

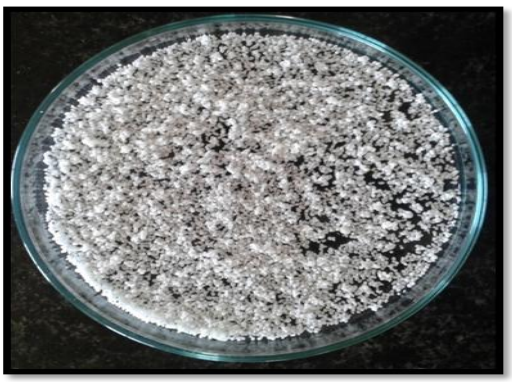

Figure 4: Prepared floating microspheres of Mefenamic acid

Evaluation Studies of Prepared Floating Microspheres

Percentage yield: The percentage yield of floating microspheres of Mefenamic acid was in the range of 44.82% to 85.48%. It was found that when the concentration of hydrophobic polymer, ethyl cellulose increased the percentage yield also increased and when the concentration of hydrophilic polymer, HPMC increased the percentage yield decreased due to migration of hydrophilic polymer into the aqueous phase.

Particle size analysis: Particle size of prepared floating microspheres was determined by optical microscope and mean particle size was calculated. The particle size ranged from 139µm - 225µm. The particle size increased when the concentration of ethyl cellulose was increased. Larger particles developed due to increased viscosity of the medium due to increased polymeric concentration. The size of microspheres was also significantly decreased with increasing agitation. This is because increasing rate of stirring produces higher energy that decreases the droplet sizes, thus producing smaller microspheres.[15]

Percentage drug entrapment efficiency: The drug entrapment efficiency of all formulations was found in the range of 37.83% to 86.29%. As the concentration of ethyl cellulose increased the drug entrapment efficiency also increased due to increase in viscosity of the solution and rapid hardening of the droplets that result in reduced drug diffusion into aqueous phase.

Table **7** represents the percentage yield, mean particle size and percentage drug entrapment efficiency of all formulations.

Table 7: Percentage yield and mean particle size and percentage drug entrapment efficiency of all formulation

Formulation code	Percentage yield mean ± s.d	Mean particle size (µm) ± s.d	Percentage drug entrapment efficiency mean ± sd
F1	44.82 ±0.214	139.15+1.73	37.83+0.48
F2	56.22 ±0.157	176.52+1.25	44.70+0.82
F3	69.33 ±0.360	185.50+0.96	59.66+0.74
F4	85.48 ±0.760	225.89+1.34	86.29+0.52
F5	48.56 ±0.120	145.50+0.86	35.40+0.35
F6	61.12 ±0.695	189.96+1.83	46.21+0.40
F7	77.88 ±0.612	198.32+1.72	61.13+0.38
F8	83.67 ±0.321	219.72+1.85	84.47+ 0.68

Micromeritic studies

Table 8: Micromeritic studies of all formulations

Formulation code	Angle of repose	Bulk density	Tapped density	Hausner's Ratio	Compressibility Index
F1	33.21±0.24	0.116 ±0.004	0.126±0.002	1.08±0.002	7.93±1.140
F2	29.08±0.16	0.122±0.006	0.142±0.007	1.16±0.003	14.08±1.08
F3	27.62±0.13	0.155±0.002	0.160±0.005	1.03±0.004	6.25±1.457
F4	24.89±0.08	0.325±0.016	0.391±0.008	1.20±0.006	16.87±0.478
F5	26.51±0.10	0.102±0.007	0.117±0.002	1.14±0.005	12.82±1.52
F6	28.64±0.07	0.132±0.005	0.138±0.001	1.04±0.007	4.34±0.985
F7	21.33±0.11	0.1730±0.010	0.185±0.003	1.03±0.010	6.48±1.15
F8	25.36±0.07	0.230±0.013	0.245±0.004	1.04±0.012	6.12±0.196

All the eight formulations were tested by various micromeritic studies including angle of repose, bulk density, tapped density, Hausner's ratio and compressibility index which was shown in Table **8**. The values of the angle of repose were in the range of 21.33° to 33.21°, which indicates good to passable flow properties, whereas the Carr's index for all formulations was in the range of 7.93 to 6.12%, which indicated good flow properties. This suggests that the microspheres can be easily handled during processing. The value of Hausner's ratio of all the formulation was below 1.25 which indicates good and better flow properties.

Shape and Surface Morphology

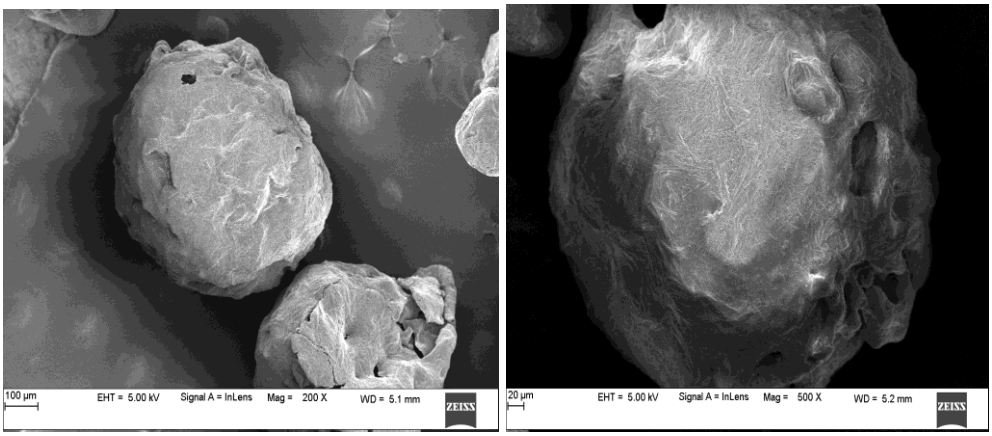

Figure 5: SEM Photographs of prepared floating microspheres of F4 at different magnifications

SEM photographs confirmed the spherical shape of the prepared microspheres with smooth perforated surface. The formation of pores was attributed to evaporation of solvents from microspheres. It was also observed that formation of irregular particles occurred with increased stirring rate. Scanning electron microscopy confirmed the hollow nature with pores on the surface which imparts floating properties of prepared floating microspheres.

Floating lag time and floating time

Table 9: Floating test for all formulations

Formulation code	Floating lag time (minutes) mean ± sd	Floating time (hours) mean±sd	*In-vitro* Buoyancy (%) mean ± sd
F1	10.7 ± 1.3	5.0 ± 0.03	53.94 ± 0.156
F2	8.4 ± 1.8	7.0 ± 0.12	56.84± 0.254
F3	7.8 ± 0.5	8.0 ± 0.8	59.77± 0.124
F4	5.8 ± 2.5	9.7 ± 0.12	70.19±0.205
F5	9.7 ± 0.8	5.5 ± 0.14	54.12±0.147
F6	9.3 ± 0.15	7.5 ± 0.4	55.76±0.524
F7	8.9 ± 0.2	7.8 ± 0.7	61.44±0.135
F8	6.7 ± 0.4	8.5 ± 0.5	69.52±0.265

When the concentration of hydrophobic polymer, ethyl cellulose increased a decrease in the floating lag time and increased or prolonged floating time duration. It was observed that the formulation–F4 had higher floating time duration (9.7 hours). When the concentration of hydrophilic polymer, HPMC increased, the floating time duration decreased due to increased wettability of HPMC. Floating lag time and floating time ranged from 5.8 mins to 10.7 mins and 5 hours to 9.7 hours respectively.

***In-vitro* buoyancy studies**

In-vitro buoyancy increased, as the ethyl cellulose concentration increased. When ethyl cellulose concentration is higher than HPMC it leads to increased buoyancy.[16] Average buoyancy of the microspheres was in the range of 53.94 - 69.52% at the end of 10 hours. The water permeable nature of HPMC and its tendency towards increased wettability causes increased amount of liquid medium to be absorbed, replacing the air inside the floating

microspheres, leads to microspheres to settle or sink down, thus rendering them less buoyant.[15] Results are shown in Table **9.**

Figure 6: In vitro buoyancy behaviour of F4 and F1 formulations

***In-vitro* Drug Release Study**

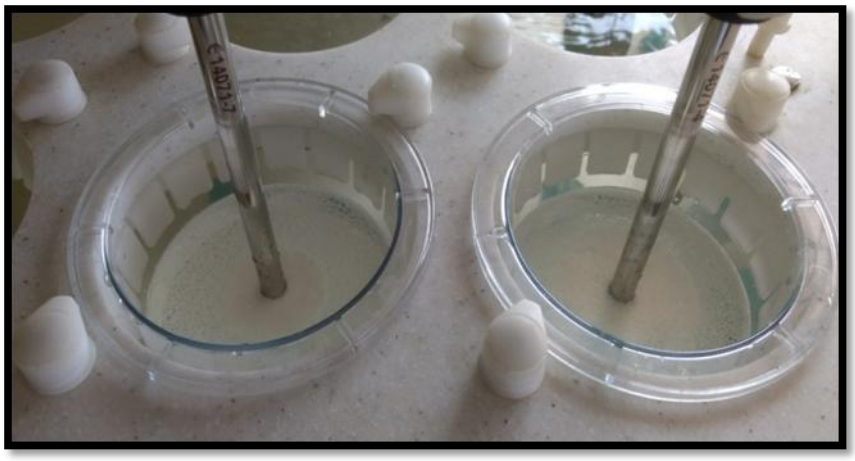

Figure 7: *In-vitro* dissolution studies using USP type-II apparatus (rotating paddle)

Table 10: *In-vitro* release studies of prepared floating microspheres

Sr. No:	Time (hours)	Percentage cumulative drug release							
		F1	F2	F3	F4	F5	F6	F7	F8
1.	0	0	0	0	0	0	0	0	0
2.	1	14.41	9.37	21.62	10.81	7.2	12.80	9.96	17.94
3.	2	20.18	10.09	36.04	19.82	11.5	14.60	15.47	21.92
4.	3	27.38	12.25	51.17	30.63	14.41	17.20	19.06	34.76
5.	4	32.43	14.05	57.66	37.84	15.85	18.02	25.30	47.84
6.	5	46.55	23.75	66.28	48.65	25.22	36.04	28.60	59.38
7.	6	56.22	30.27	69.91	59.46	36.04	41.44	31.64	64.88
8.	7	57.66	36.04	72.23	66.67	45.05	63.07	35.49	74.03
9.	8	63.42	45.41	72.08	79.28	49.04	75.08	39.44	82.02
10.	9	67.75	48.29	74.24	91.90	54.06	81.09	44.60	89.47
11.	10	73.15	54.21	81.96	94.89	62.25	86.14	57.17	90.02

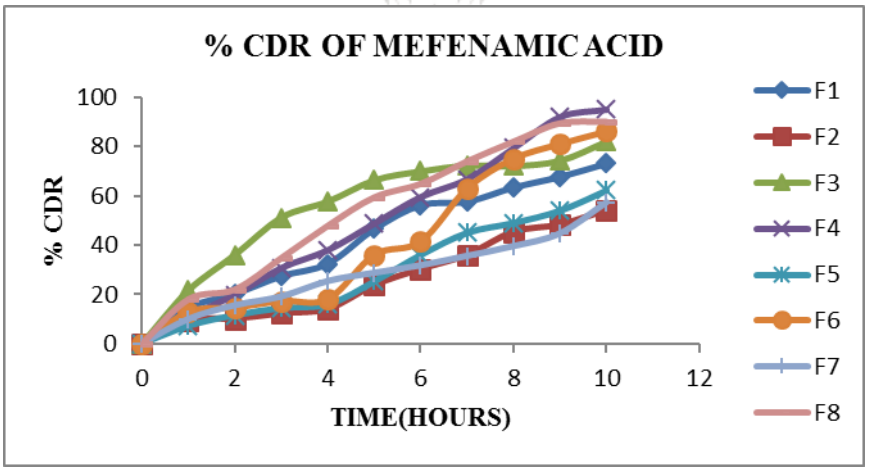

Figure 8: Percentage CDR release profile of Mefenamic acid formulations F1-F8

Microspheres prepared with combination of ethyl cellulose and HPMC showed good release. As the proportion of HPMC was increased, release rate also increased. It may be due to the aqueous solubility character of HPMC. The results were also clear that no burst effect was seen and the drug release was significantly sustained. The reason for retarded drug release may be the increased proportion of the hydrophobic polymer, ethyl cellulose that increases the polymer matrix density and thus results in increased diffusional path length, leading to a

decrease in drug release from the microspheres.[15] The results of the *in-vitro* dissolution studies shows the controlled and predictable manner in which, as the polymer concentration increases the drug release from the floating microsphere decreases. Thus the *in-vitro* performance of Mefenamic acid floating microspheres showed prolonged and controlled release.

Kinetic modeling of dissolution profiles

Table 11: Regression coefficient (r^2) values of all formulations (F1-F8)

Formulation code	Drug release kinetics				
	Zero order R^2	First order R^2	Higuchi R^2	Peppas	
				R^2	n
F1	0.975	0.987	0.953	0.979	0.756
F2	0.971	0.949	0.852	0.880	0.868
F3	0.849	0.950	0.971	0.951	0.550
F4	**0.996**	0.865	0.917	**0.997**	**0.964**
F5	0.977	0.950	0.858	0.947	0.991
F6	0.949	0.881	0.812	0.856	0.959
F7	0.972	0.942	0.926	0.981	0.981
F8	0.977	0.962	0.956	0.974	0.782

The release kinetics data of optimized formulation indicates that the release of drug best fits to zero order release kinetics because the regression coefficient (r^2) values of F4 (0.996) was higher in the case of zero order kinetics as compared to other formulations. Similarly, the r^2 value of Peppas model (0.997) was also higher in F4 than in other formulations and corresponding 'n' value is 0.964. So it was considered as the best optimized formulation. The release exponent 'n' values of all eight formulations were found to be in the range of 0.55 - 0.991.This is evident from the fact that the formulations- F1, F2, F3, F8 follow anomalous (non Fickian) release that is the drug release is controlled by more than one process, diffusion and erosion controlled, and all other formulations- F4, F5, F6, F7 follow case II transport, it involves polymer matrix relaxation mechanism. The results obtained after fitting into various kinetic models are summarized in Table **14**

Various kinetic models of optimized formulation F4 of zero-order, first order, Higuchi model and Korsmeyer - Peppas model are shown in the Figures **13 - 16** and their corresponding correlation coefficient (r^2) values were determined and summarized in Table **12.**

Table 12: Drug release kinetics data of optimized formulation F4

Formulation code	Zero order	First order	Higuchi	Peppas	
	r^2	**r^2**	**r^2**	**r^2**	**n**
F4	**0.996**	0.865	0.917	**0.997**	0.964

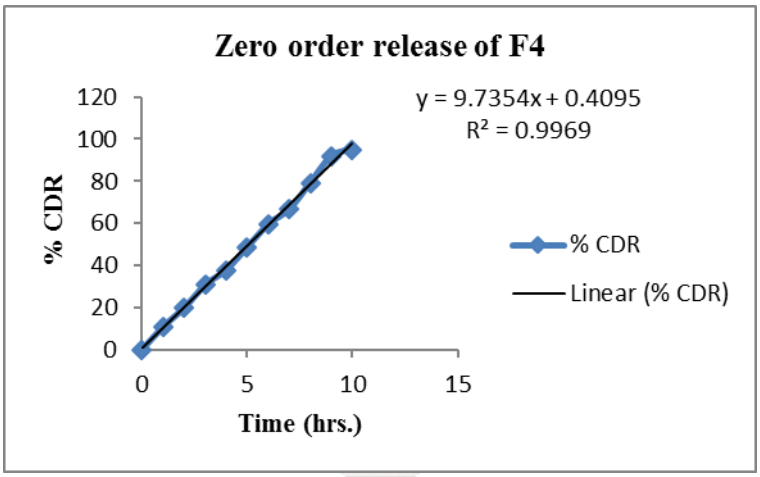

Figure 9: Zero Order Plot of F4

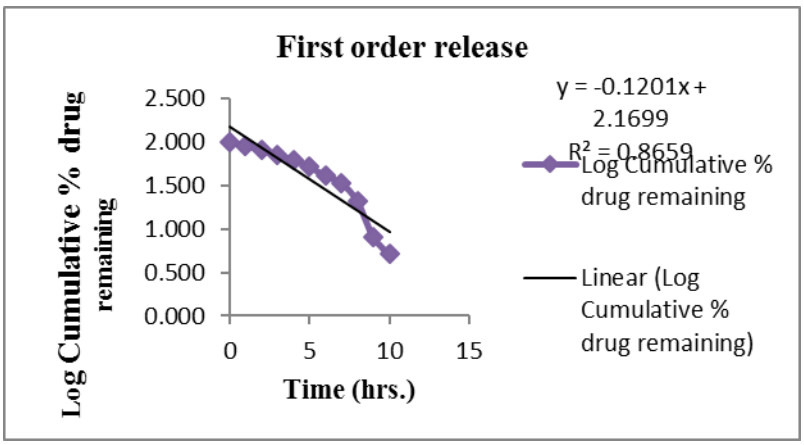

Figure 10: First order plot of F4

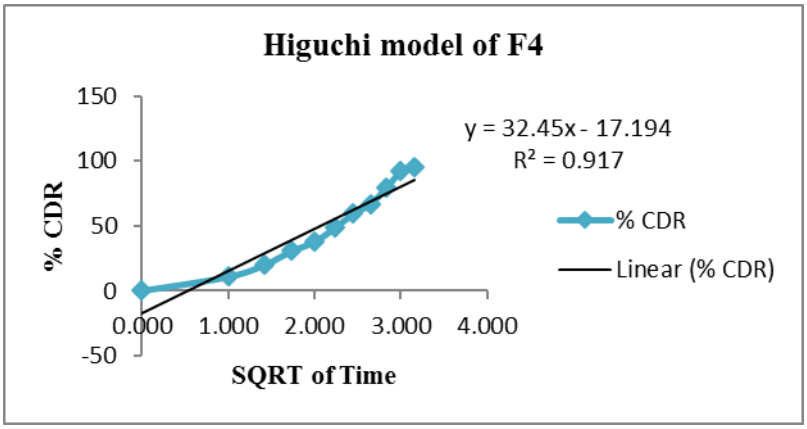

Figure 11: Higuchi plot of F4

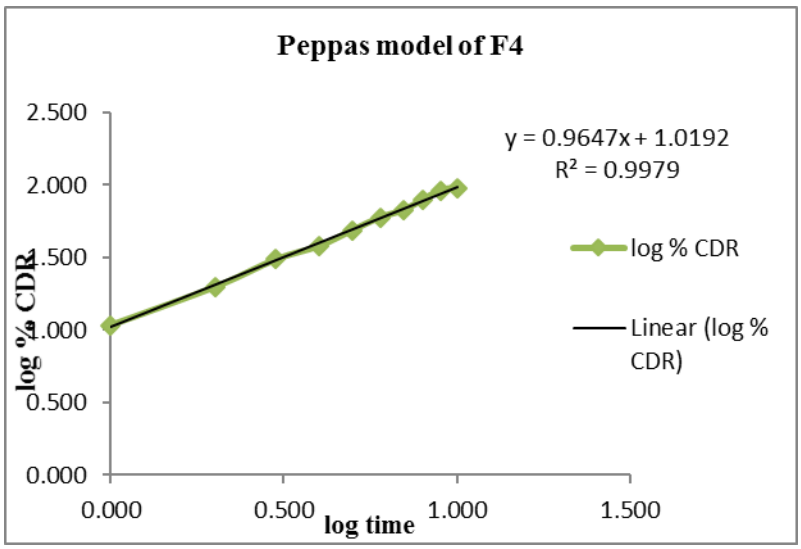

Figure 12: Peppas plot of F4

Therefore, it can be concluded from the results of each evaluation parameters like percentage drug entrapment efficiency, floating behavior, *in-vitro* drug release studies and kinetic modeling of the dissolution profile etc that the formulation F4 was found to be the best optimized formulation.

Stability studies of optimized formulation F4 (PERIOD – 30 DAYS)

a) Physical appearance

Table 13: Physical appearance of optimized formulation before and after stability studies

Formulation code	Physical properties	Physical properties before 30 days	Physical properties after 45 days at 40±2˚C and 75±5% RH
F4	Colour of microspheres	Pure white	Almost white
	Shape of microspheres	Spherical in shape	Almost Spherical

b) Drug Entrapment Efficiency and in vitro buoyancy

Table 14: Drug entrapment efficiency and % buoyancy of optimized formulation after stability studies

Formulation Code	% drug entrapment efficiency	% Buoyancy
F4	81.29%	69.19%

c) *In-vitro* drug release studies

Table 15: % CDR of optimized formulation before and after stability studies

Time (hours)	% CDR before stability study	% CDR after stability study
0	0	0
1	10.81	11.81
2	19.82	19.82
3	30.63	30.63
4	37.84	37.84
5	48.65	48.65
6	59.46	59.46
7	66.67	63.67
8	79.28	77.28
9	91.90	89.90
10	94.89	91.38

Stability studies were carried out for 30 days. Physical appearance, drug entrapment, *in-vitro* buoyancy and drug release of optimized formulation were performed. The *in-vitro* drug release values after stability were found to be exactly linear to that of values before stability.

There was no major change in the above specified parameters. Therefore the optimized formulation F4 was found to be stable.

SUMMARY AND CONCLUSION

Floating Microspheres of Mefenamic acid were prepared successfully by emulsion solvent evaporation method using different concentration of polymers like HPMC K4M, HPMC K15M, ethyl Cellulose and various solvents like ethanol, dichloromethane and other excipients. The properties of polymers and its concentration played a major role on particle size of microspheres, their floating time duration and release profile of drug molecule. The concept of formulating floating microspheres of Mefenamic acid offers a suitable, practical approach to achieve a prolonged therapeutic effect by continuously releasing the medication over an extended period of time by prolonging the gastric residence time, thus improving the oral bioavailability of the drug. So patient compliance can be achieved as compared to conventional dosage regimens. The developed floating microsphere system is a promising floating drug delivery system for oral controlled delivery of Mefenamic acid.

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