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Development and evaluation gastro-retentive floating microspheres of ofloxacin

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ABSTRACT

Ofloxacin is a fluoroquinolones antibiotic with a broad spectrum activity against gram positive and negative bacteria. Conventional doses vary from 200-600 mg twice or thrice a day, as per severity of infection and its biological half-life is 5-6 hrs. Ofloxacin is absorbed in GIT and readily soluble in gastric pH of the stomach, so floating microspheres were developed for prolongation of gastric residence time with an aim to improve bioavailability. Eight formulations was prepared by different concentration of ethyl cellulose, HPMC K 4M and HPMC K 15M by non aqueous solvent evaporation technique. Depending upon the drug polymer ratios, the percentage yield is found between 86.75±0.96% to 95.93±0.94% and entrapment efficiency was 70.47± 0.96% to 91.28± 0.82% in all formulations. Microspheres showed a good specificity, spherical and uniform in shape with smooth surface and the mean particle size significantly increase with increasing polymer concentration and it was in the range between 182.41±0.54 to 229.43±0.48 µm. Percentage *in-vitro* buoyancy of the floating microspheres was in the range of 70.37± 0.68% to 86.07± 0.86%. *In-vitro* drug release studies was performed in simulated gastric fluid, it is revealed that formulation codes OF-III and OF-VII was found in 97.81±0.94% and 98.80±0.68% drug releases at the end of studies, when compared to all batches due to increases in polymer concentration. Stability studies of selected floating microspheres showed good in results. It could be concluding that the all the formulations were shown satisfactory results and suitable for potential therapeutic uses.

INTRODUCTION

Drugs having a short half-life are eliminated quickly from the blood circulation and incomplete release of the drug and a shorter residence time of dosage form in the upper GIT, a prominent site for absorption of many drugs, will lead to lower bioavailability through oral ingestion. One of the most feasible for achieving a prolonged and predictable drug delivery in the GI tract is to control the gastric residence time (GRT) by using gastro-retentive dosage forms (GRDFs), which prolong the residence time of the drugs in the stomach. It has several advantages including the minimization of fluctuation in drug concentration in plasma, reduce the side effect and total dose administered, reduction of administration frequency and improved the patient compliance. Floating Drug Delivery System (FDDS) is dynamically controlled / low-density systems based on non-effervescent approach is intended to float over the gastric content and remain buoyant in the stomach, the drug is released slowly at desired rate without affecting the gastric emptying rate resulting in prolonged period of time. These results in an increased gastric retention time and reduced fluctuations in plasma drug concentration and improve the bioavailability of medications that are characterized by a narrow absorption window [1-4].

Ofloxacin is a second generation fluoroquinolones antibiotic used in urinary and respiratory tract infections, gonorrhea and skin [5] and its having short half-life. As it requires frequent dosing to maintain its therapeutic concentration and the bioavailability in the GIT. Also its exhibit pH dependent solubility in the intestine, where the drug is readily soluble in the acidic environment of the stomach, however in the intestine slightly alkaline pH prevail precipitation of the active compounds occur, which adversely affect absorption in the lower section of

the intestine. Therefore this drug was found to be good candidate for the development of gastro retentive system aiming to increase their bioavailability and maintained the therapeutic level for an extended period of time, eliminating maxima in drug concentration associated with multiple doses [6-8].

The present work is to design controlled release formulation of floating microspheres using Ofloxacin that resides for maintain high concentrations of drug within gastric mucosa, remain buoyant over gastric contents for prolonged period, stabilize blood drug level and benefit for treatment, so as to decrease the necessity of multiple dosing especially in patients with several diseases.

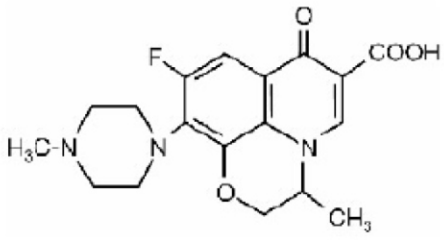


Fig 1 : Structure of Ofloxacin

MATERIALS AND METHODS

Materials

Ofloxacin was obtained as gift sample from Chetana Drugs and Chemical Pvt Ltd, Thrissur, Kerala. HPMC K 4M & HPMC K 15M from Balaji Drugs Pvt Ltd, Gujarat. Ethyl cellulose from Qualigens Fine Chem Pvt Ltd, Mumbai. All other chemicals and solvents used were of analytical grade.

Method

IR Spectral Analysis

The FT-IR spectrum of ofloxacin and polymers was recorded using KBr mixing method on the FT-IR instrument (Schimadzu FT-IR-8400 S). The drug alone, and in combination with polymers (mixed ratio of 1:1) was taken and subjected to FT-IR studies [9].

Preparation of Floating Microspheres of Ofloxacin

Microspheres were prepared by non-aqueous solvent evaporation method. Ethyl cellulose (EC), Hydroxy propyl methylcellulose (HPMC K 4M & HPMC K 15M), was mixed in the mixture of dichloromethane and ethanol at suitable ratio. Drug separately mixed in to small amount of glacial acetic acid and then added to above polymers solution. The slurry was slowly introduced by syringe in to 200 ml of liquid paraffin containing 0.01% Tween 80 while being stirred at 600 rpm using mechanical stirrer equipped with three bladed propellers at room temperature. The solution was stirred for 2 hrs and allowed the solvent to evaporate completely and filtered by using whatmann filter paper. The microspheres obtained were washed repeatedly with petroleum ether until free from oil. The collected microspheres were dried at room temperature and subsequently stored in desiccators. Same procedures were repeated for all other batches [10]. In this study, eight formulations were prepared by different ratios of drug and polymer as given in Table 1 and were evaluated for relevant parameters.

Characterization of Floating Microspheres

Percentage Yield

The prepared microspheres were collected, dried at room temperature and then weighed. The measured weight of prepared microspheres was divided by the total amount of all excipients and drug used in the preparation of microspheres which will give the total percentage yield of floating microspheres [11].

$$\text{Percentage yield (\%)} = \frac{\text{Amount of microspheres obtained (gm)}}{\text{Theoretical amount (gm)}} \times 100$$

Determination of Particle Size

Particle size of prepared microspheres was determined using an optical microscope (Olympus, India) method [12].

Micromeritic Properties

The floating microspheres were characterized by their Micromeritic properties such as bulk density, tapped density, carr's index, hausner's ratio and angle of repose [13-16].

Table 1 : Composition of various ofloxacin floating microspheres

Formulations Code	Drug: Polymer ratio	Liquid paraffin (ml)	Drug (%w/v)	EC (%w/v)	HPMC K 4M (%w/v)	HPMC K 15M (%w/v)
OF-I	1:1	200	1	0.5	0.5	-
OF-II	1:1	200	1	0.5	-	0.5
OF-III	1:1	200	1	0.5	0.25	0.25
OF-IV	1:1	200	1	1	-	-
OF-V	1:2	200	1	1	1	-
OF-VI	1:2	200	1	1	-	1
OF-VII	1:2	200	1	1	0.5	0.5
OF-VIII	1:2	200	1	2	-	-

Morphological Studies

The surface morphology of Ofloxacin floating microspheres were determined by Scanning Electron Microscopy (JEOL, JSM-6701 F, JAPAN) [17-19].

Entrapment Efficiency

The amount of drug entrapped was estimated by crushing the calculated quantity of microspheres and extracting with aliquots of 0.1N Hcl (pH 1.2) and it was transferred to a 100 ml volumetric flask and the volume was made up by same medium. The solution was filtered and the absorbance was measured by using UV spectrophotometer against appropriate blank. The amount of drug entrapped in the floating microspheres was calculated by the following equation [20].

$$\text{Entrapment efficiency (\%)} = \frac{\text{Estimated percent drug content}}{\text{Theoretical percent drug content}} \times 100$$

In-vitro Buoyancy Studies

An accurately weighed quantity of the floating microspheres containing drug were spread over 900 ml simulated gastric fluid (pH 1.2) containing 0.02% W/V Tween 80 in dissolution apparatus (USP, type-II) agitating at a speed of 100 rpm. After 10 hrs, the buoyant microspheres were pipette out and separated by filtration, particles in the sinking particulate layer were also separated and both particles were dried in desiccators. Both microspheres was weighed and buoyancy was determined as per the following equation [21].

$$\text{In-vitro buoyancy (\%)} = \frac{Wf \times 100}{Wf + Ws}$$

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

In-vitro Drug Release Studies

Accurately weighed quantity of the floating microspheres was introduced in to 900 ml of gastric pH (pH 1.2) maintained at 37±0.5^{0}C with paddle rotating at 100 rpm. Aliquots samples were withdrawn every 1 hr and replaced with the same volume of fresh dissolution medium. The concentration of drug released in the medium was assayed spectrophotometrically at 294 nm after suitable dilution. And in order to study the exact mechanism of drug release, *in-vitro* release data was analyzed using different kinetics models and mechanism of drug release is determined [22-24].

Stability Studies

Best formulations were placed in borosilicate screw capped glass containers and stored at different temperatures (27±2^{0}C, 60± 5% RH & 40±2^{0}C, 70± 5% RH) using stability chamber. At the end of specified days period, the samples was withdrawn and analyzed for their drug content [25].

RESULTS

Percentage yield of all formulations is between the range of 86.75% to 95.93%. The mean particle size of microspheres significantly increase with increasing polymer concentration and it was in the range between 182.41 to 229.43 µm. Entrapment efficiency of all formulations is between the range of 70.47% to 91.28% (Table-2).

From Table-3, bulk and tapped density values were lies in between 0.464 to 0.526 and 0.525 to 0.574 g/cm^3 respectively. Carr's index values in between 7.04 to 11.61% and Hausner's ratio were lies between 1.076 to 1.130 using different formulations. Angle of repose of all formulated microspheres is found to be less than 40° indicates acceptable flow properties.

From the results, prepared microspheres are floated for prolonged period of time over the surface of the dissolution medium. The percentage *in-vitro* buoyancy of floating microspheres is found in the range 70.37% to 86.07% after 12 hrs.

The results obtained from *in-vitro* release studies were analyzed in various kinetic models of data treatment as follows: Cumulative percentage drug release Vs Time (Zero order rate kinetics), Cumulative % drug retained Vs Time (First order rate kinetics), Cumulative percentage drug released Vs Square root of Time (Higuchi's classical diffusion), Log cumulative percentage drug release Vs log Time (Korsmeyer-Peppa's exponential). The kinetics data results is shown in Table-5.

The stability studies of best formulations (OF-VII) was stored

Table 2 : Percentage yield, Particle size and Drug entrapment of floating microspheres.

Formulations Code	Percentage Yield (%)	Average Particle Size (µm)	Drug Entrapment (%)
OF-I	89.64±0.72	189.03±0.68	75.93±0.36
OF-II	86.75±0.96	182.41±0.54	70.47±0.96
OF-III	92.72±0.86	201.18±0.73	84.83±0.54
OF-IV	92.12±0.67	200.89±0.61	83.13±0.71
OF-V	93.17±0.91	216.68±0.79	83.96±0.74
OF-VI	89.83±0.71	212.71±0.85	79.29±0.63
OF-VII	95.93±0.94	229.43±0.48	91.28±0.82
OF-VIII	94.18±0.63	226.09±0.91	88.93±0.41

Results are mean ± S.D of three trials (n=3)

Table 3 : Micromeritic properties data of floating microspheres

Formulations code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's index (%)	Hausner's ratio	Angle of repose (θ)
OF-I	0.497±0.023	0.549±0.013	9.31±0.87	1.102±0.38	$29^0.87'$ ±0.51
OF-II	0.502±0.031	0.540±0.019	7.04±0.63	1.076±0.33	$30^0.05'$±0.58
OF-III	0.489±0.029	0.529±0.021	7.56±0.73	1.080±0.47	$27^0.34'$±0.64
OF-IV	0.526±0.043	0.574±0.011	8.36±0.93	1.090±0.29	$31^0.23'$±0.31
OF-V	0.477±0.022	0.530±0.027	10.12±1.09	1.110±0.42	$28^0.46'$±0.47
OF-VI	0.480±0.047	0.539±0.016	10.94±0.68	1.123±0.46	$30^0.51'$±0.43
OF-VII	0.489±0.038	0.534±0.024	8.42±0.96	1.092±0.24	$26^0.48'$±0.36
OF-VIII	0.464±.026	0.525±0.014	11.61±1.18	1.130±0.39	$28^0.61'$±0.72

Results are mean ± S.D of three trials (n=3)

Table 4 : *In-vitro* buoyancy data of Ofloxacin floating microspheres

Formulations Code	Percentage Buoyancy (%)
OF-I	74.81±0.97
OF-II	70.37±0.68
OF-III	80.12±1.28
OF-IV	78.31±0.93
OF-V	81.24±1.49
OF-VI	79.57±1.72
OF-VII	86.07±0.86
OF-VIII	83.69±1.08

Results are mean ± S.D of three trials (n=3)

at different temperatures in stability chamber to access their stability. After 30, 60 and 90 days samples withdrawn and retested for drug content. The stability studies results is indicated in Table-6.

FT-IR spectra were taken in the wavelength region of 600-3800 cm^{-1} at ambient temperature and the resolution was 4 cm^{-1} and compared the position and relative intensity of absorption band of physical admixtures and pure drug is illustrated in Figure 2 to Figure 5. It has been observed that there is no chemical interaction between drug and the polymers used, also there was no considerable changes in comparison between the ratios of percent (%) transmittance.

Scanning electron microscopy for the best formulations was carried out and the scanned images are shown in Photomicrograph-1&2.

The release profile of all batches of microspheres using different ratios was studied in 0.1N Hcl (pH 1.2), for a period of 10 hours and it's indicated that the amount of drug release decreases with increases in polymer concentration. The comparative *in-vitro* drug release curve of all batches of floating microspheres was shown in Figure 6&7.

DISCUSSION

IR Spectral Analysis

In compatibility studies, IR spectrum of pure drug was found to be similar to the standard IR spectrum of which indicates that

Table 5 : Kinetics analysis data of Ofloxacin floating microspheres

Formulations Code	Release model							
	Zero order		First order		Higuchi's		Korsmeyer and peppa's	
	R	S	R	S	R	S	R	S
OF-I	0.9938	13.958	0.9341	-0.1152	0.9857	38.517	0.9907	0.8318
OF-II	0.9906	11.821	0.9445	-0.0865	0.9721	39.246	0.9807	0.8306
OF-III	0.9975	11.215	0.9428	-0.0987	0.9673	28.754	0.9958	0.8957
OF-IV	0.9987	11.503	0.9226	-0.0742	0.9814	35.104	0.9937	0.8519
OF-V	0.9959	11.414	0.9406	-0.1156	0.9657	33.919	0.9839	0.8807
OF-VI	0.9986	11.618	0.9217	-0.0623	0.9718	30.656	0.9896	0.8335
OF-VII	0.9976	12.320	0.9338	-0.3224	0.9719	32.184	0.9977	0.8526
OF-VIII	0.9935	12.714	0.9486	-0.0901	0.9927	37.702	0.9986	0.8758

Correlation coefficient (r), Slope(s)

Table 6 : Stability studies data of Ofloxacin Floating microspheres

At the end of period (in days)	Physical Appearance	Percentage Drug Content	
		At 27±2^{0}C, 60± 5% RH	At 40±2^{0}C, 70± 5% RH
30	No change	90.95±1.29	90.12±1.36
60	No change	90.08±1.47	89.86±1.49
90	No change	89.45±1.56	88.73±1.67

Results are mean ± S.D of three trials (n=3)

the obtained sample was pure ofloxacin. The IR spectra of all the pure samples and the ofloxacin physical admixtures of suitable proportion of polymers were subjected to the study and from the results, it was observed that there is no presence of interaction between drug and the polymers.

Percentage yield, Particle size and Drug entrapment

From the results of Table-2, the percentage yield of all formulations was found to be in the range between 86.75-95.93%, and it was observed that the concentration of polymer increased, the percentage yield of the floating microspheres was also slightly increased. The average particle size of all the formulations was observed in between 182.41 µm to 229.43 µm. As the Ofloxacin to polymer ratio was increased the mean particle size of Ofloxacin floating microspheres were also increased. The significant increase in particle size may be because of the increase in the viscosity of droplets of polymer solution. A high concentration of polymer produced a more viscous dispersion, which formed larger droplets and consequently larger microspheres. The particle size of 1:2 ratio microspheres are more than that of 1:1 microspheres. The entrapment efficiency of microspheres is increase with increasing the polymer concentration. The percentage entrapment efficiency of the microspheres was found to be in the range 70.47% to 91.28%. A maximum of 91.28% drug entrapped in Ofloxacin floating microspheres (OF-VII) prepared by EC: HPMC K 4M: HPMC K 15M.

Micromeritic Properties

The packing properties of the drug and their formulations widely depend upon bulk density. It has been stated that bulk density values less than 1.2gm/cm^3 indicate good flow and values greater than 1.5 gm/cm^3 indicate poor flow. From Table-3 results, it was observed that the bulk and tapped density values were lies in between 0.464-0.526 g/cm^3 and 0.525-0.574 g/cm^3 respectively indicates good packing. The Carr's index values and Hausner's ratio were lies in between 7.04-11.61% and 1.076 to 1.130 indicates good flow characteristics of the microspheres. The angle of repose for various batches of floating microspheres is found to be less than 40° which indicates good flow properties.

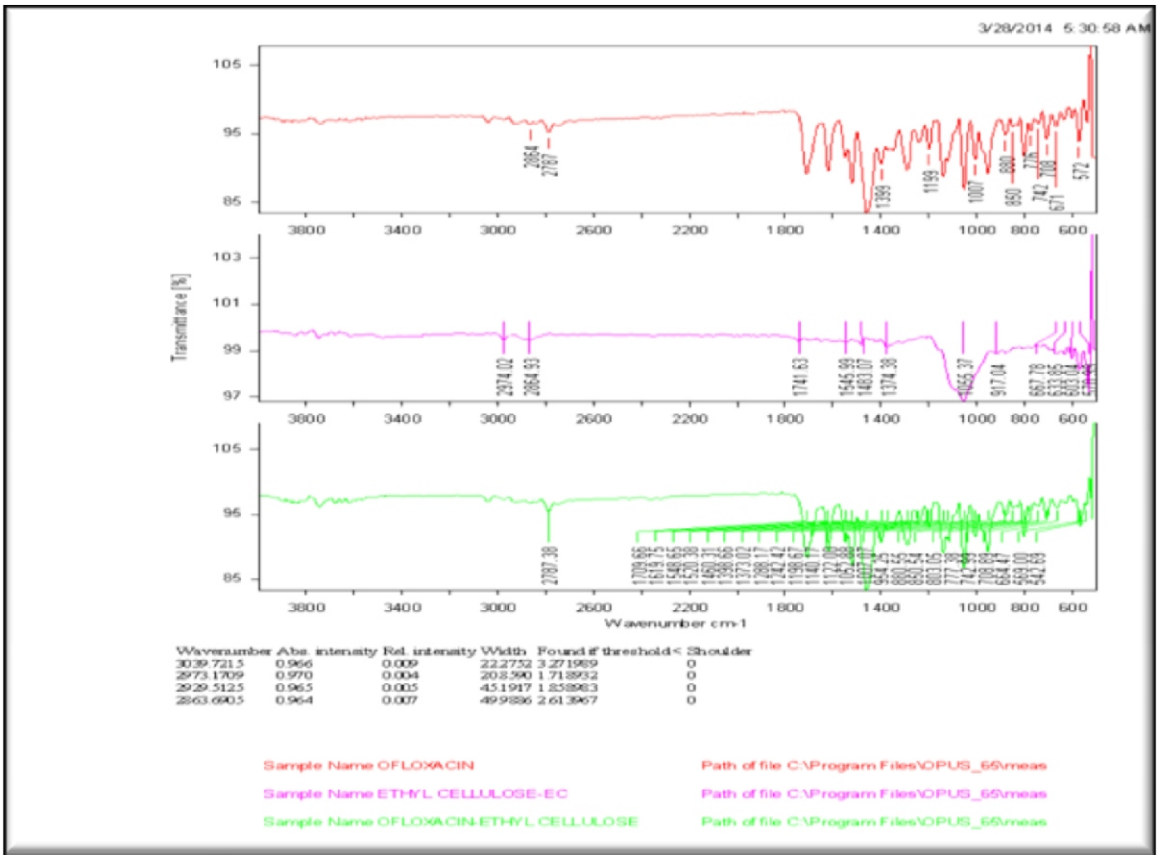


Fig. 2 : IR spectra studies of Pure Ofloxacin, Ethyl cellulose, Physical admixtures of Ofloxacin and ethyl cellulose

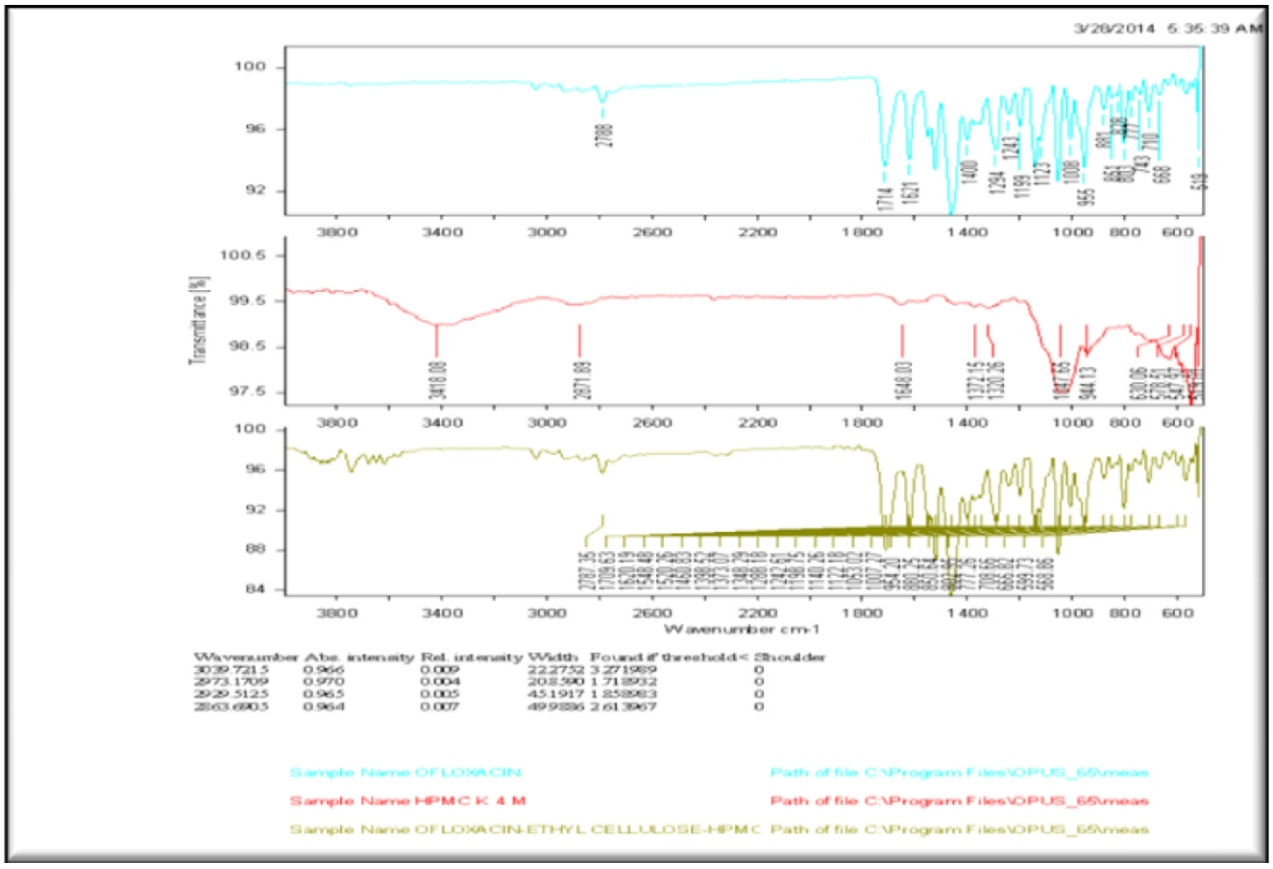


Fig. 3 : IR spectra studies of Pure Ofloxacin, HPMC K 4M, Physical admixtures of Ofloxacin, ethyl cellulose and HPMC K 4M

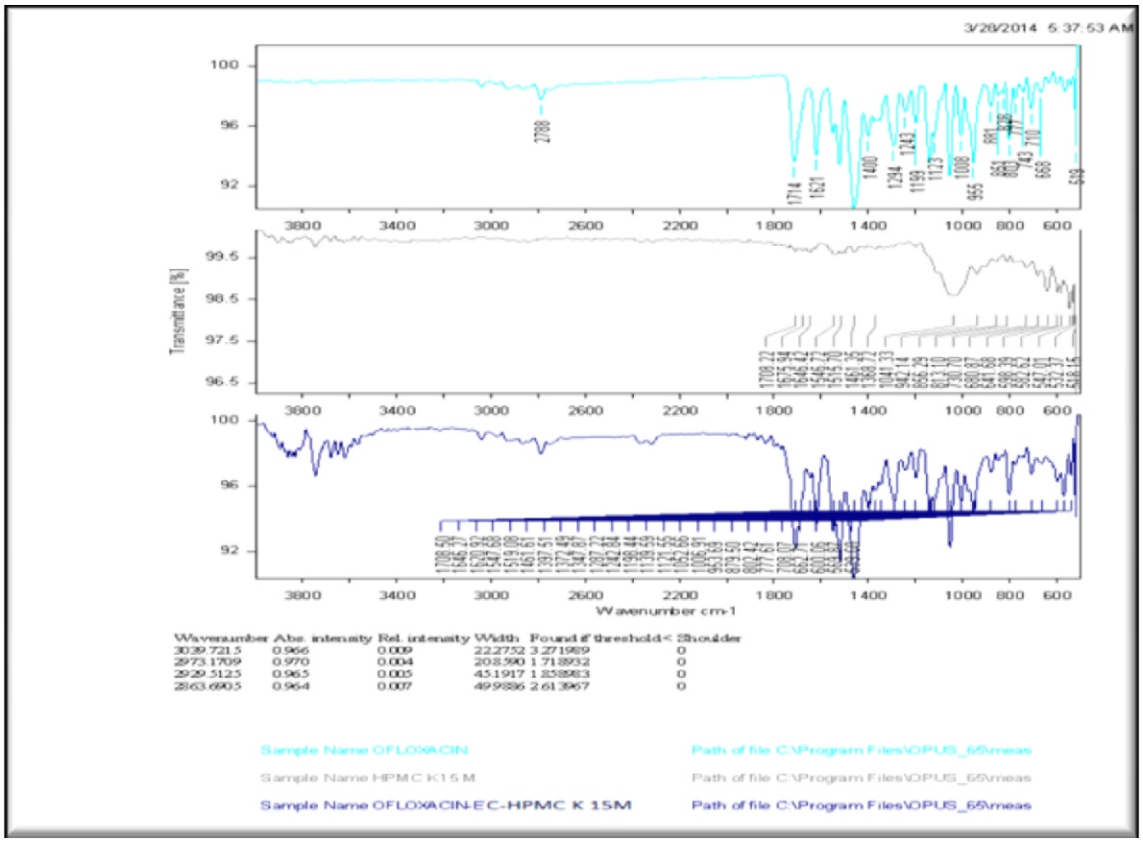


Fig. 4 : IR spectra studies of Pure Ofloxacin, HPMC K 15M, Physical admixtures of Ofloxacin, ethyl cellulose and HPMC K 15M

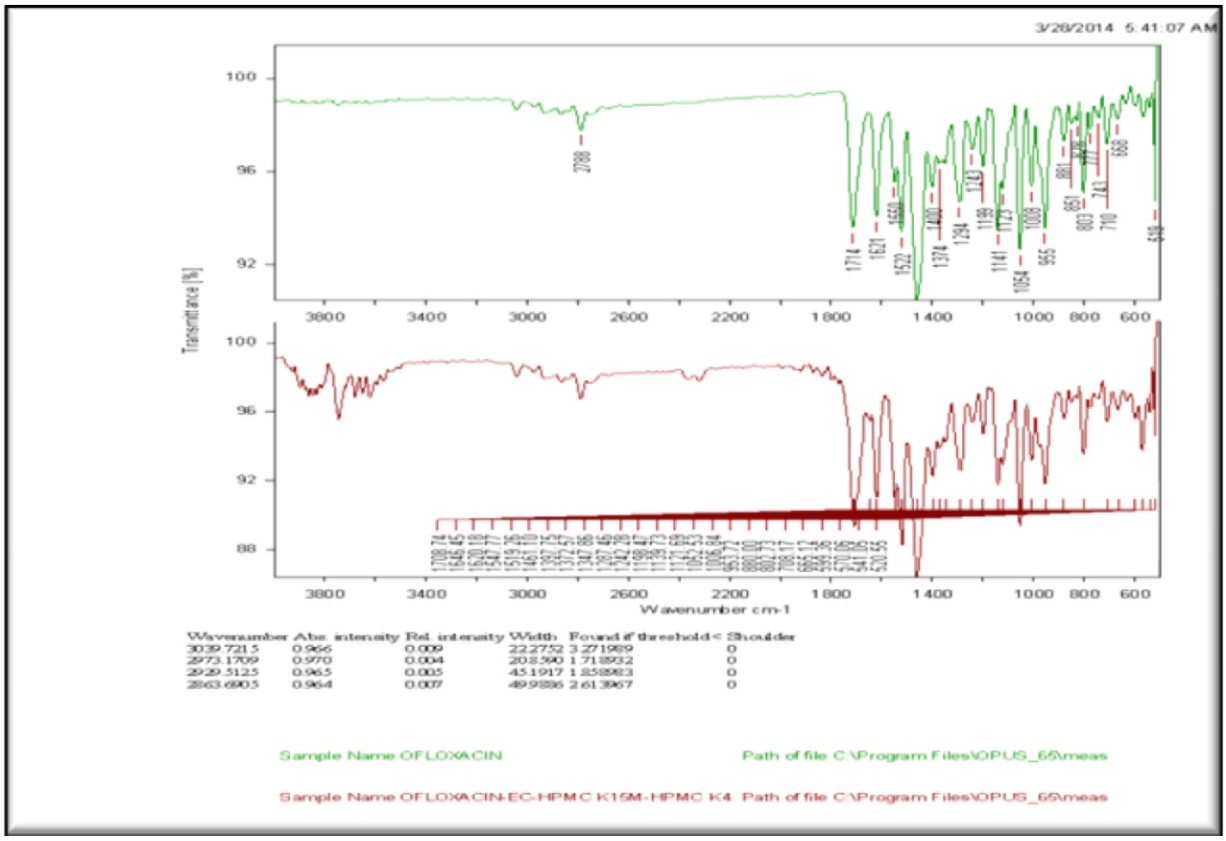
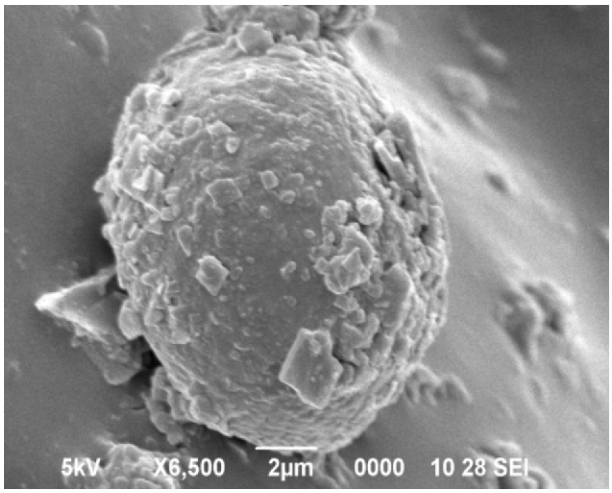
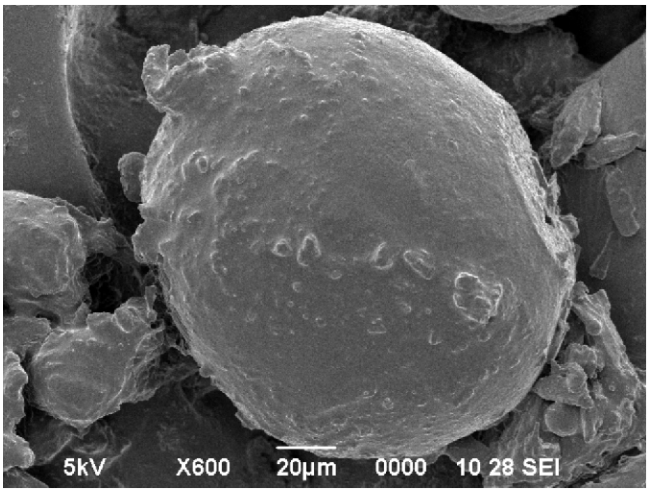


Fig. 5 : IR spectra of Pure Ofloxacin, Physical admixtures of Ofloxacin, Ethyl cellulose, HPMC K 4M, HPMC K 15M



Photomicrograph-1
SEM image of Ofloxacin floating microspheres (OF-III)



Photomicrograph-2
SEM image of Ofloxacin floating microspheres (OF-VII)

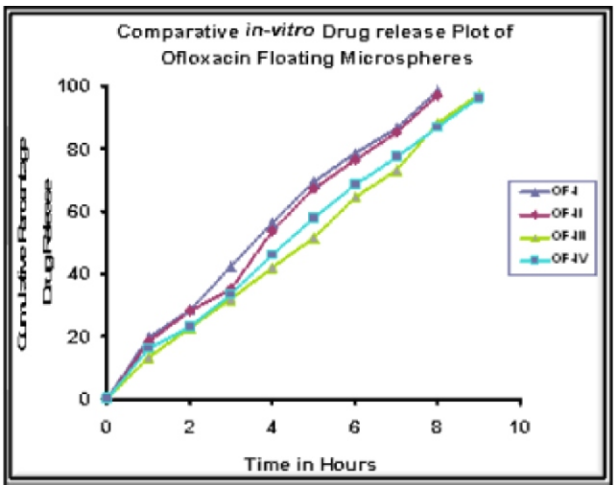


Figure 6 : Comparative *in-vitro* drug release plot of Ofloxacin floating microspheres (OF-I to OF-IV)

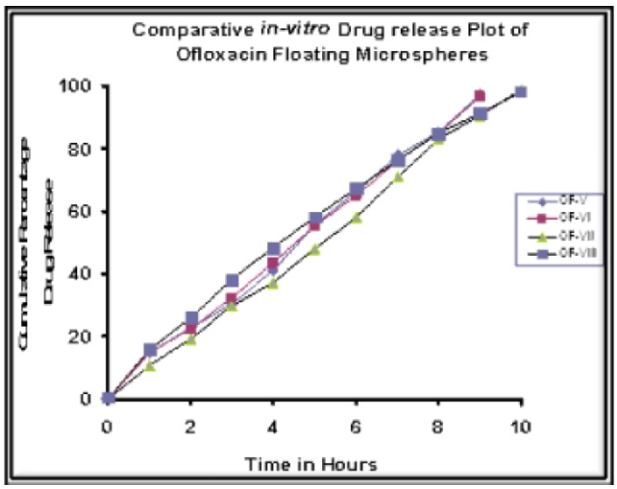


Figure 7 : Comparative *in-vitro* drug release plot of Ofloxacin floating microspheres (OF-V to OF-VIII)

Morphological Studies

The surface morphology of best formulations was determined by SEM for characterization of microspheres. Photomicrograph results showed that the prepared microspheres showed a good specificity, spherical and uniform in shape with smooth surface. And the surface smoothness of prepared microspheres was increased by increasing the polymer concentration, which is confirmed by SEM. At lower polymer concentration (OF-III) have rough and wrinkled surface and at higher polymer concentration (OF-VII) with smooth surface of floating microspheres was obtained.

In-vitro Buoyancy Studies

From the results, it was observed that the prepared microspheres floated at prolonged period of time (12 hrs) without any apparent gelation. As the polymer concentration increases the *in-vitro* buoyancy time increases. So percentage buoyancy of floating microspheres are high in OF-VII and OF-III batches.

In-vitro Drug Release Studies

The result of the *in-vitro* dissolution studies shows controlled and predictable manner as the polymer concentration increases, the percentage drug release and initial burst release from the floating microsphere decreases. The increased concentration of polymers leads to increased density of polymer matrix into the microsphere which results in increased diffusional path length and consequently retardation of drug release. From the results, it was observed that the drug release from OF-I, OF-II , OF-III and OF-IV (1:1 ratio) was found to be in the ranges of 98.31%, 97.93%, 97.81% and 97.15%, similarly in formulations OF-V, OF-VI, OF-VII and OF-VIII (1:2 ratio) was found in 97.18%, 96.94%, 98.80% and 98.01%, at the end dissolution studies respectively. From the results, the *in-vitro* drug release revealed that formulation code OF-III and OF-VII was found 97.81% and 98.80% of drug releases when compared to all batches due to increases in polymer concentration.

In order to understand the mechanism and kinetics of drug

release studies of all formulations were subjected to goodness of fit test by linear regression analysis according to various kinetics models. From the results, the correlation coefficient (r) values of OF-I, OF-II, OF-III, OF-IV were found to be 0.9938, 0.9906, 0.9975, 0.9987 and similarly in OF-V, OF-VI, OF-VII, OF-VIII were in the ranges of 0.9959, 0.9986, 0.9976, 0.9935 respectively. So the co-efficient of determination indicated that the release data was best fitted with zero order kinetics. When the drug release data was put in to Higuchi's equation, good correlation coefficient (r) values 0.9657 to 0.9927 were obtained, indicating the drug release was diffusion controlled release mechanism. The release data obtained were also put in Korsmeyer-Peppas model in order to find out n values, which describe the drug release mechanism. From the kinetics result data (Table-31), the diffusion exponent (n) values of microspheres were found in the range of 0.8306 to 0.8957 with correlation coefficient values from 0.9807 to 0.9986, indicating Non-Fickian diffusion mechanism.

Stability Studies

From the *in-vitro* results, best formulations (OF-VII) were taken and analyzed the stability studies. The results show that there is about 88.73% to 90.95% of drug is present in the formulations with no-observable physical changes after storage at different temperatures at the end of specified days period. This indicates a good stability of the ofloxacin microspheres.

CONCLUSION

The objective of the work was to develop the spherical ofloxacin floating microspheres by non aqueous solvent evaporation technique. The prepared formulations were free flowing, non sticky in nature and were evaluated for various parameters. All batches of microspheres showed prolonged and controlled release and compliance with Higuchi plot which reveals that the drug release follows Non-Fickian diffusion mechanism. All formulated microspheres results were found to be satisfactory and showed good stability and it might be a better, practical approach to achieve a prolonged therapeutic effect by continuously releasing the medication over extended period of time in the stomach. Hence, it is concluded that floating microspheres can be selected for the development of gastro retentive drug delivery system for potential therapeutic uses and these microspheres release the drug in the stomach and upper gastrointestinal tract and thereby improve the bioavailability.

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