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Formulation and Evaluation of Floating Microspheres of Norfloxacin

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ABSTRACT

The present study involves preparation and evaluation of floating microspheres with Norfloxacin as model drug for prolongation of gastric residence time. The microspheres were prepared by the Non Aqueous solvent diffusion method using polymers hydroxypropylmethyl cellulose and ethyl cellulose. The shape and surface morphology of prepared microspheres were characterized by optical and scanning electron microscopy, respectively. *In vitro* drug release studies were performed and drug release kinetics was evaluated using the linear regression method. Effects of the stirring rate during preparation, polymer concentration, solvent composition and dissolution medium on the size of microspheres and drug release were also observed. The prepared microspheres exhibited prolonged drug release (10 h) and remained buoyant for > 12 h. The mean particle size increased and the drug release rate decreased at higher polymer concentration. No significant effect of the stirring rate during preparation on drug release was observed. *In vitro* studies demonstrated super case II transport diffusion from the microspheres.

Keywords: Floating microspheres, Norfloxacin, *In-Vitro* drug release studies, Stability studies.

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INTRODUCTION

Since the last three decades many drug molecules formulated as Gastroretentive Drug Delivery System (GRDDS) have been patented keeping in view its commercial success. Oral controlled release (CR) dosage forms have been extensively used to improve therapy of many important medications [1]. The bioavailability of drugs with an absorption window in the upper small intestine is generally limited with conventional pharmaceutical dosage forms. The residence time of such systems and thus, of their drug release into the stomach and upper intestine is often short. To overcome this restriction and to increase the bioavailability of these drugs, controlled drug delivery systems with a prolonged residence time in the stomach can be used [2]. Incorporation of the drug into a CR-delivery system, which releases its payload in the stomach over a prolonged time period, can lead to significant therapeutic advantages owing to various pharmacokinetic and pharmacodynamic aspects. Gastroretentive dosage forms (GRDFs) are designed to be retained in the stomach for a prolonged time and release their active ingredients and thereby enable sustained and prolonged input of the drug to the upper part of the gastrointestinal (GI) tract [3].

Norfloxacin is a fluoroquinoline derivative used as an antibiotic requires multiple administration of drug, leading to fluctuation in plasma concentrations. Based on certain studies which highlight dose related renal toxicity, seizures, nausea and vomiting with Norfloxacin. Hence in make of recent development there is a lot of scope of preparing sustained release dosage for which reduces the need of repeated doses.

This technology has generated enormous attention over the last few decades owing to its potential application to improve the oral delivery of some important drugs for which prolonged retention in the upper GI tract can greatly improve their oral bioavailability and/or their therapeutic outcome. The challenge to developefficient gastroretentive dosage forms began near about 20 years ago, following the discovery of Helicobacter pylori by Warren and Marshall. Many attempts have been made to devise an extended release GRDDS where the dosage form is small enough to ingest and then retained in the GI area for a long enough time for the active agent to be dissolved and eventually absorbed. For example, many swelling and expanding systems have been attempted. There are dosage forms that swell and change their size thereby floating to the surface. Local delivery also increases the stomach wall receptor site bioavailability and increases the efficacy of drugs to reduce acid secretion. Gastric emptying of dosage forms is an extremely variable process and ability to prolong and control emptying time is a valuable asset for dosage forms, which reside in the stomach for a longer period of time than conventional dosage forms. Several difficulties are faced in designing controlled release systems for better absorption and enhanced bioavailability [4]. One of such difficulties is the inability to confine the dosage form in the desired area of the gastrointestinal tract. Drug absorption from the gastrointestinal tract is a complex procedure and is subject to many variables. It is widely acknowledged that the extent of gastrointestinal tract drug absorption is related to contact time with the small intestinal mucosa.

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MATERIALS AND METHODS

Norfloxacin was obtained as gift sample from Hlmack pharmaceuticals, Hyderabad. Ethyl Cellulose, HPMC K15 M and HPMC K100M were obtained from ONTOP Pharmaceuticals, Bangalore. All other chemicals and solvents used for analytical grade only.

Physical observation compatibility studies [5]

Physically accurately weighed quantities of drug and polymers with different ratio mixed well and stored in petri dishes. Correctly marked for each ratio of drug and polymer. Then the prepared petridishes was stored in different temperatures e.i 25 and 40°C. Then observe the any colourchanges is there in the physical mixture of the drug and polymers. The petridishes was observed in three times such first day, first week and second week .

Table No: 01 Physical observation test for drug polymer compatibility studies

S.NO	Polymers	D:P RATIO	Physical Observation					
			First Day		After One week		After two week	
			25°C	40°C	25°C	40°C	25°C	40°C
1	ETHYL CELLULOSE	1:2	NC	NC	NC	NC	NC	NC
2	HPMC K15M	1:1	NC	NC	NC	NC	NC	NC
3	HPMC100M	1:1	NC	NC	NC	NC	NC	NC
4	ETHYL CELLULOSE: HPMCK15M: HPMCK100M	1:1:1:1	NC	NC	NC	NC	NC	NC

Analytical compatibilitystudies

IR spectral analysis of pure drug and polymers was carried out and observation was made weather changes in chemical constitution of drug after combining it with the polymers occurred [6]. As shown in Table 2 & Figures 1-5.

Table No: 02 FTIR Interpretation results of drug and polymers

S.No	Name of the Compound	Wave Number (cm^{-1})
1	Norfloxacin	3461.04, 3041.73, 2969.62, 1687.20, 1251.83, 920.93, 735.19.
2	Ethyl Cellulose	3482.97, 2976.63, 2927.96, 1452.62, 1381.17, 1313.75, 1280.11, 880.44
3	HPMC K15 M	2931.25
4	HPMC K100 M	2927.91, 1380.62, 1119.77, 1064.07, 945.79
5	Drug and polymer combination	3465.31, 2969.62, 2970.61, 2931.18, 1687.40, 1483.70, 1395.99, 1282.14, 1143.63, 1120.72, 1075.51, 847.04

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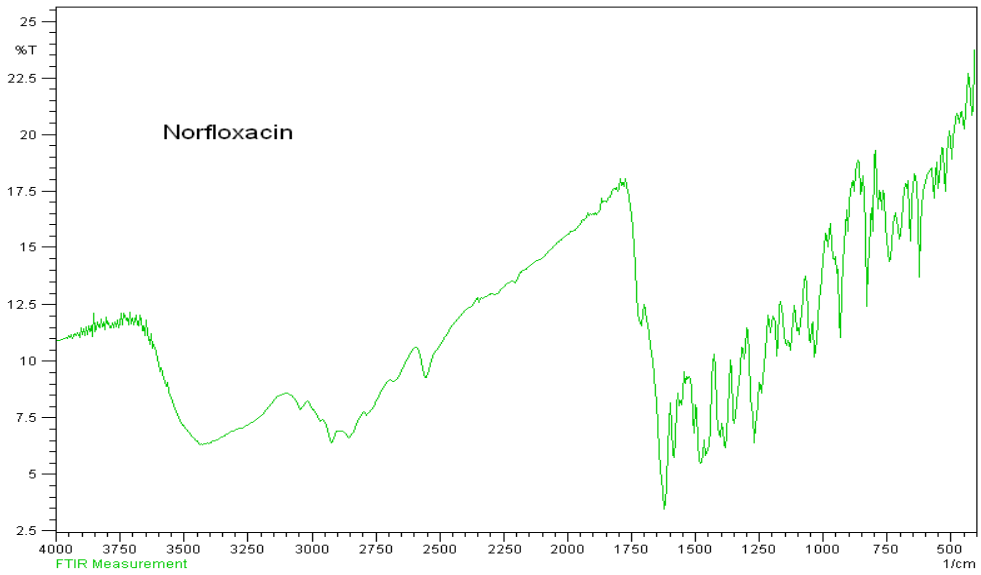

Figure No: 01 FTIR Spectral studies for Norfloxacin

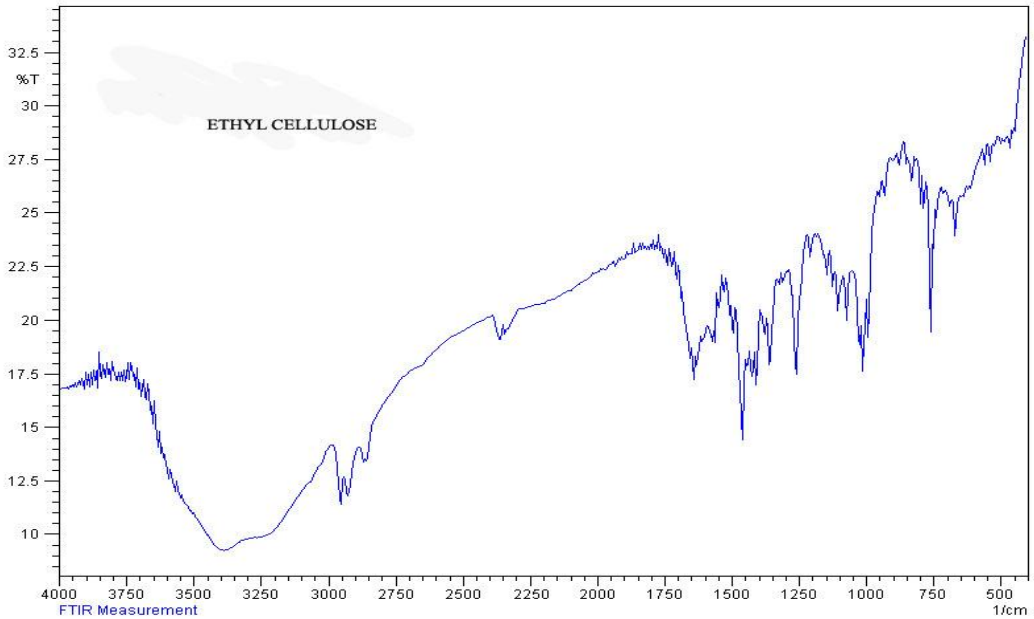

Figure No: 02 FTIR Spectral studies for ethyl cellulose

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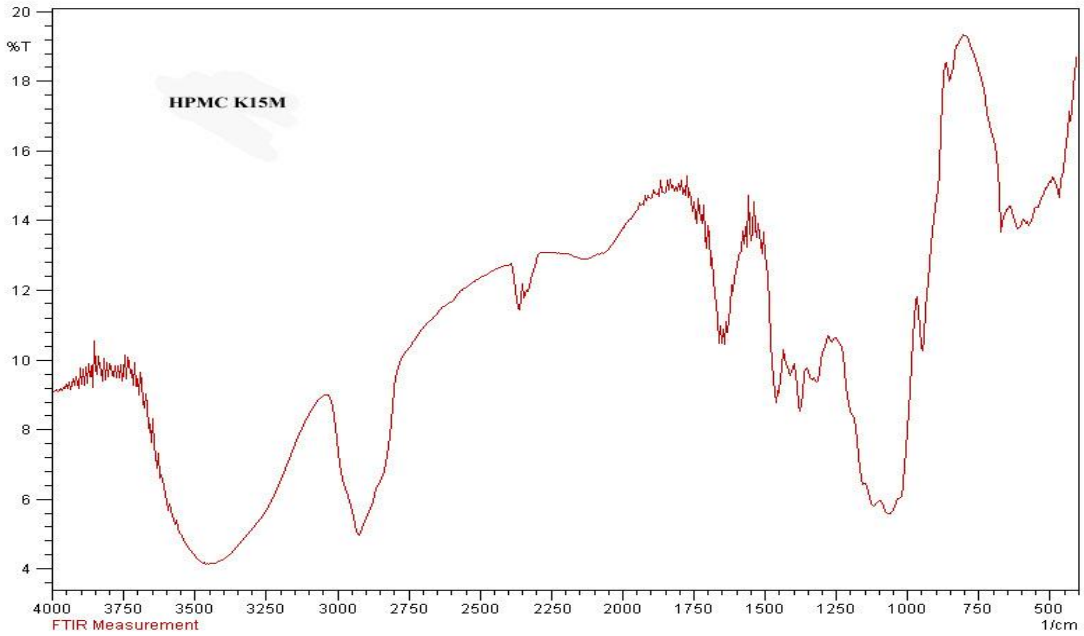

Figure No: 03 FTIR Spectral studies for HPMC K15 M

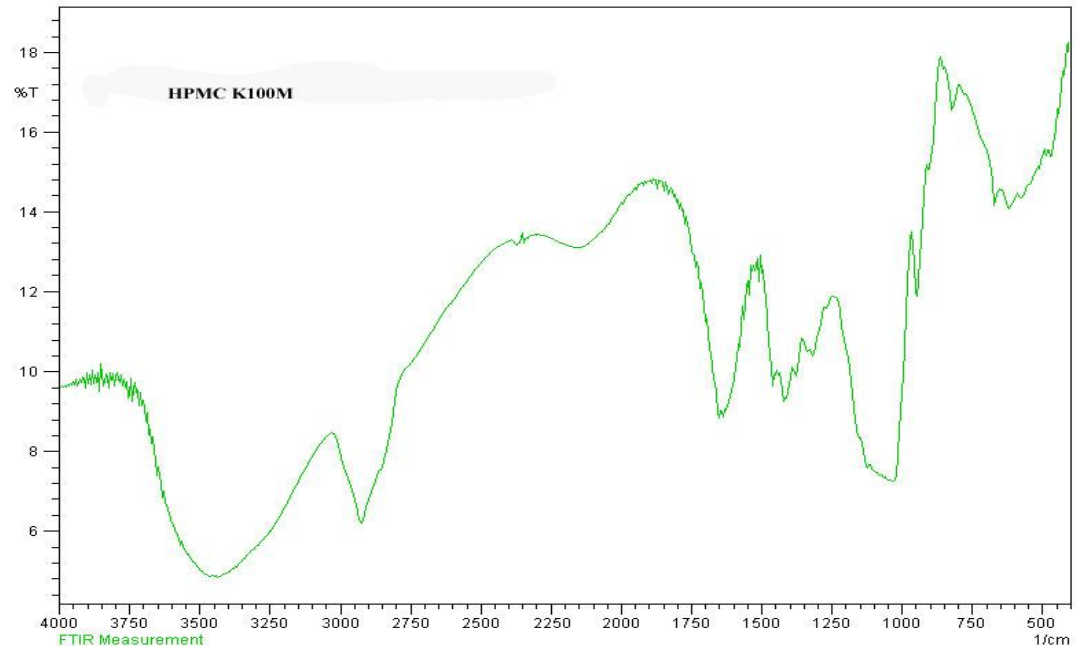

Figure No: 04 FTIR Spectral studies for HPMC K100M

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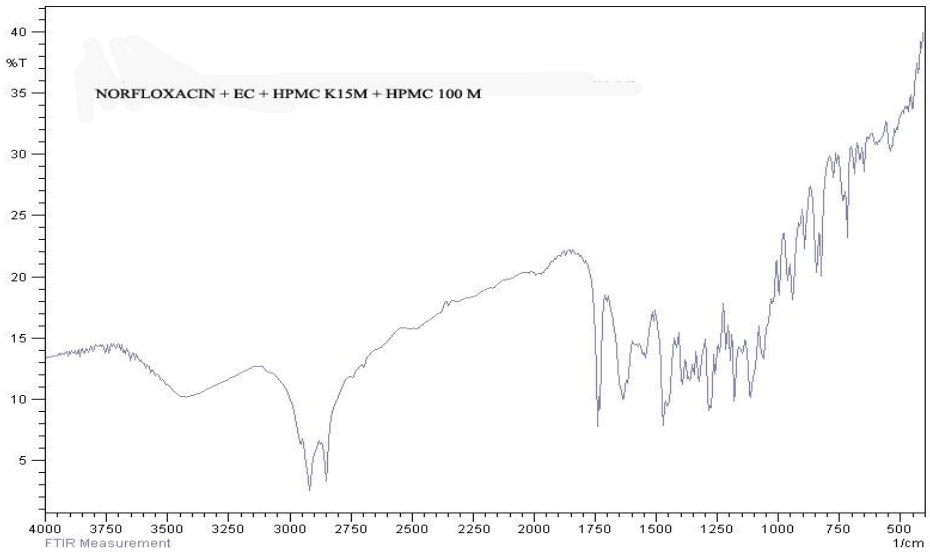

Figure No: 05 FTIR Spectral studies for combination of drug and polymer

Preparation of Floating Microspheres

The floating Microspheres were prepared by Non aqueous solvent evaporation method. Drug and polymers (Ethyl Cellulose, HPMC K 15M and HPMC K 100M) were mixed in acetone at various ratios by using blending solvent eg. Isopropyl alcohol [7]. The prepared slurry was introduced into 200ml of liquid paraffin while being stirred at 1200 rpm by homogenizer for 2 hours at room temperature. To allow for solvent evaporate completely and the microspheres were collected by filtration. The collected floating microspheres washed repeatedly with petroleum ether, until free from oil. The collected microspheres were dried for 2 days at room temperature. Different batches of drug and polymer ratios were shown in the Table no: 03.

Table No: 03 Formulation table for floating Microspheres of Norfloxacin

Formulation Code	Drug: polymer	Liquid paraffin in ml	Drug (gr)	Ethyl cellulose (gr)	HPMC K 15M (gr)	HPMC K 100M (gr)
NF – I	1:1	200	1	0.5	0.5	---
NF – II	1:1	200	1	0.5	---	0.5
NF – III	1:1	200	1	0.75	0.25	---
NF – IV	1:1	200	1	0.75	----	0.25
NF – V	1:1	200	1	1	---	---
NF – VI	1:1	200	1	0.5	0.5	1
NF – VII	1:2	200	1	0.5	1	0.5
NF – VIII	1:2	200	1	1	--	1
NF – IX	1:2	200	1	1	1	---
NF – X	1:2	200	1	1	0.5	0.5
NF –XI	1:2	200	1	1.5	0.5	---
NF – XII	1:2	200	1	1.5	---	0.5
NF – XIII	1:2	200	1	1.5	0.25	0.25
NF – XIV	1:2	200	1	2	---	---

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Determination of Percentage Yield [8]

Dried Microspheres were weighed and the percentage yield of microsphere of different formulations were calculated by using the formula

$$\% \text{ Yield} = \frac{\text{Practical yield (gm)}}{\text{Theoretical yield}} \times 100$$

Micromeritic Properties

Angle of repose

Angle of repose of different formulations was measured according to fixed funnel method. Completely dried Microspheres were weighed and passed through the funnel, which was kept at a height 'h' from horizontal surface [9]. The passed micropsheres formed a pile of the height 'h' above the horizontal surface and the diameter of the pile was measured and the angle of repose was determined for all the formulation using the formula,

$\tan \theta = h / r$

$$\text{Angle of repose } (\theta) = \tan^{-1} (h / r)$$

Where, h is the height of pile and r is the radius.

Bulk density and Tapped Density

The loose bulk density (LBD) and tapped bulk density (TBD) of microspheres were determined. The prepared microspheres was poured into calibrated measuring cylinder (10 ml) then noted initial volume. Then the cylinder was allowed to fall under its own weight onto the hard surface from the height of 2.5 cm at 2 seconds intervals. The tapping was the continued no further change in volume was noted. LBD and TBD were calculated using following equation,

LBD = weight of the powder / volume of the packing

TBD = weight of the powder / tapped volume of the packing

CompressibilityIndex [10]

The compressibility index (Carr's Index) of the all formulations were determined by using the below mentioned equation,

$$\text{Carr's Index (\%)} = [(TBD - LBD) \times 100] / TBD$$

Hausner's Ratio

Hausner's ratio is an indirect index of ease of powder flow. It is calculated by the following 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 Drug Content

An accurately weighed quantity of the floating microspheres equivalent to 50mg of the drug were taken for evaluation [11]. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1 N HCl repeatedly. The extract was transferred to a 100ml volumetric flask and the volume was made up using 0.1N HCl. The solution was filtered and the absorbance was measured after suitable dilution at 273 nm by using UV-visible spectrophotometer. The drug content was estimated in triplicate using a calibration curve constructed in the same solvent.

Determination of Drug Entrapment Efficiency

Amount of drug entraped in to the microspheres is determined by using the formula.

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

Determination of Mean Partical Size of Microspheres

Partical size determination of microspheres was carried out by sieve analysis method. A mixture quantity of dried microspheres was placed on the top side of the sieve. Then switch ON the instrument for specified time with specified rpm [12]. After the completion of sieving separate the individual sieve and weigh the microspheres. Then calculate the size of the microspheres.

Scanning Electron Microscopy

The surface morphology and particle size of microspheres were determined by Scanning Electron Microscopy using a JEOL JSM-T330A scanning microscope performed at I.I.T Hyderabad. Dry microspheres were placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of microspheres were taken by random scanning of the stub [13].

***In Vitro* Buoyancy Studies**

300 mg of Microspheres were spread over the surface of the dissolution medium (simulated gastric fluid, SGF, pH 1.2 containing 0.02%w/v of Tween 20) that was agitated by a paddle rotation speed at 100 rpm. After agitation for a predetermined time interval, the microspheres that floated over the surface of the medium and those settled at the bottom of the flask were recovered separately [14]. After drying, each fraction of the microparticles was weighed and their buoyancy was calculated by the following equation

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$$\text{Buoyancy \%} = \frac{Q_f}{(Q_f + Q_s)} \times 100$$

Where Qf and Qs are the weight of the floating and the settled microspheres, respectively.

***In- Vitro* Drug Release Studies**

The *In-Vitro* drug release studies were carried out using paddle type dissolution apparatus. Drug loaded microspheres were weighed equivalent to 100 mg of drug was introduced into the 900 ml of dissolution medium (1.2 pH HCl buffer) maintained at 37±0.5ºC with paddle rotating at 100 RPM. The samples were withdrawn with 1 h intervals upto 10 hours. Aliquots were withdrawn and the same volume of fresh medium was refilled for the maintenance of sink condition [15]. The prepared test solutions were measured at 273 nm by U.V.Spectrophotometer. The dissolution studies were carried out in triplicate and then mean values were plotted as percentage cumulative drug release against time.

Kinetics of Drug Release

To study the drug release kinetics, data obtained from *In-Vitro* release were plotted in various kinetic models such as Zero order equation, Higuchi kinetics and Korsmeyer – Peppas equation.If n value is 0.45 or less, the release mechanism follows "Fickian diffusion" and higher values of 0.45 to 0.89 for mass transfer follow a non-fickian model (anomalous transport). The drug release follows zero-order drug release and case II transport if the n value is 0.89. For the values of n higher than 0.89, the mechanism of drug release is regarded as super case II transport [16]. The model is used to analyze the release of pharmaceutical polymeric dosage forms when the release mechanism is not known or more than one type of release phenomenon was involved. The n value could be obtained from slop of the plot of log cumulative % of drug released Vs log time.

Stability Studies For NF – XIII

From the prepared floating microspheres which showed appropriate balance between the buoyancy and the percentage release was selected for stability studies. The prepared formulation NF – XIII were placed in borosilicate screw capped glass containers and stored at three different temperature (27±2°C, 65% RH), Oven temperature (40±2°C, 65% RH) and in freezing temperature (5 – 8°C, 65% RH) in stability chamber for a period of 90 days. The samples were evaluated for cumulative percentage drug release at regular intervals of two week [17].

RESULTS AND DISCUSSION

In the present study an attempt of was made to develop Norfloxacin floating microspheres using Ethyl cellulose, HPMC K15 M and HPMC K100 M polymers. The microspheres were prepared by using Non aqueous emulsion solvent evaporation technique,

with different ratios of the polymers. However, due to its short half-life (61.5 min) and low bioavailability (60%), traditional controlled release Norfloxacin solid dosage forms need to be administrated two times a day. The prepared floating microspheres were characterized for their % yield, drug loading, % entrapment, mean particle size, % buoyancy and *In Vitro* drug release. The microspheres were prepared by varying the polymeric ratio i.e., 1:1, 1:2 the microspheres shows more loading efficiency and more % drug entrapment in comparision with those prepared by 1:1, 1:2. The SEM photographs of the microspheres revealed that the microspheres were spherical with smooth surface and slightly aggregated and size range was 340 – 425 µm. The buoyancy result indicates that all formulation floated for more than 12 hours over the surface of the dissolution medium without any apparent gelation. The microsphere showing lower densities influence buoyancy and they were to be retained for longer than 12 hours, which helped in improving the bioavailability of Norfloxacin Percentage buoyancy of prepared microspheres are high and showing Combination of E.C and HPMC are good carrier for FDDS.

The *In-Vitro* drug release revealed that batch NF- XIII was having 98.8 cumulative releases at the end of 10^{th} hour when compared with all batches due to increase in polymer concentration as seen in formulations. The release kinetics of floating Norfloxacin followed Supercase II transport diffusion

Table No: 04 Percentage yield, Drug loading and Entrapment Efficiency

Formulation code	Percentage yield	Drug content In mg	Drug loading in microspheres in mg	Entrapment of efficiency (%)
NF-I	85.5	42.4	50	85.5
NF-II	87.6	43.6	50	89.6
NF-III	76.3	39.7	50	90.3
NF-IV	84.6	41.8	50	94.6
NF-V	85.9	42.8	50	92.8
NF-VI	92.6	31.5	33.3	93.3
NF-VII	90.7	30.2	33.3	91.9
NF-VIII	93.4	32.1	33.3	93.8
NF-IX	92.3	31.4	33.3	93.2
NF-X	90.5	30.2	33.3	91.9
NF-XI	94.7	32.9	33.3	94
NF-XII	91.8	30.9	33.3	92
NF-XIII	98.5	33	33.3	99
NF-IX	95.6	31.5	33.3	94.5

Table No: 05 Derived properties and flow properties

F. Code	Angle of repose	Bulk Density	Tapped Density	Carr's Indiex	Hausners Ratio
NF-I	25.7±0.23	0.41±0.011	0.49±0.015	16.326	1.195
NF-II	29.8±0.15	0.43±0.021	0.51±0.013	15.686	1.186
NF-III	27.5±0.09	0.47±0.016	O,54±0.08	12.962	1.148
NF-IV	25.5±0.05	0.45±0.017	0.49±0.021	8.163	1.088
NF-V	29.3±0.21	0.42±0.06	0.47±0.051	10.638	1.119

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NF-VI	26.7±0.021	0.51±0.042	0.55±0.022	7.272	1.078
NF-VII	25.6±0.08	0.54±0.07	0.61±0.012	11.475	1.129
NF-VIII	28.4±0.06	0.55±0.025	0.59±0.0112	6.779	1.072
NF-IX	25.8±0.15	0.49±0.012	0.56±0.012	12.5	1.142
NF-X	26.2±0.07	0.44±0.006	0.49±0.021	10.204	1.113
NF-XI	28.1±0.09	0.51±0.002	0.58±0.001	12.068	1.137
NF-XII	28.4±0.21	0.47±0.02	0.53±0.012	11.320	1.127
NF-XIII	24.8±0.32	0.49±0.013	0.55±0.014	10.909	1.122
NF-XIV	27.4±0.08	0.51±0.013	0.58±0.002	12.068	1.137

Table N0: 06 Size of the Microspheres and *In-Vitro* buoyancy

Formulation code	Size of the prepared floating microspheres	*In-Vitro* Buoyancy (%)
NF-I	406.51 ± 0.57	87.5
NF-II	407.41 ± 0.32	88.4
NF-III	418.712 ± 0.48	83.8
NF-IV	423.992 0.471	85.5
NF-V	393.911± 0.368	88.6
NF-VI	362.83± 0.98	88.7
NF-VII	418.42 ± 0.62	89.7
NF-VIII	408.53± 0.87	91.5
NF-IX	425.57± 0.91	94.2
NF-X	423.09± 0.25	97.3
NF-XI	351.11± 0.68	96.5
NF-XII	384.41± 0.41	93.6
NF-XIII	340.06 ± 0.76	97
NF-IX	425.57± 0.91	92.5

Table No: 07 Dissolution studies profile for first seven formulations

S.No	Time	Cumulative Percentage drug release						
		NF-I	**NF-II**	**NF-III**	**NF-IV**	**NF-V**	**NF-VI**	**NF-VII**
1	1	18.5	17.8	17.3	18.9	17.1	14.7	14.2
2	2	26.7	28.5	27.2	25.4	26.3	23.5	22.6
3	3	39.6	40.6	41.4	40.3	39.7	32.8	32.5
4	4	51.5	53.4	54.5	52.5	51.3	47.8	45.7
5	5	65.6	67.9	66.8	66.2	65.3	59.8	58.5
6	6	82.3	83.4	82.7	79.7	81.9	75.9	74.2
7	7	95.3	96.2	95.5	94.1	93.2	83.5	83.1
8	8	----	----	----	----	----	96.8	96.1

Table No: 08 Dissolution studies profile for second seven formulations

S.No	Time	Cumulative Percentage drug release						
		NF-VIII	**NF-IX**	**NF-X**	**NF-XI**	**NF-XII**	**NF-XIII**	**NF-XIV**
1	1	13.5	14.6	13.8	13.2	13.5	12.5	15.8
2	2	22.8	21.7	21.4	22.5	23.4	23.6	25.5
3	3	31.6	30.5	31.2	32.7	33.2	31.7	36.7
4	4	40.6	41.2	40.9	41.5	41.7	40.5	45.5
5	5	53.5	54.2	53.7	50.6	51.2	51.7	52.6

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6	6	61.2	62.7	62.8	64.9	64.3	63.7	68.9
7	7	73.9	71.2	70.5	78.4	79.5	79.7	79.4
8	8	86.8	85.7	84.1	82.6	83.1	83.6	82.6
9	9	96.7	95.7	95.9	89.4	91.2	91.4	90.4
10	10	----	----	----	97.5	97.9	98.8	96.5

Table No: 09 Drug release kinetics values of the different formulations with regression coefficient values

Formulation Code	Drug release kinetics			
	Zero order (r)	Higuchi's regression coefficient(r)	Peppa's release	
			(r)	(n)
NF-I	0.9952	0.9071	0.9792	0.8653
NF-II	O.9977	0.9152	0.9906	0.8809
NF-III	0.9983	0.9169	0.9916	0.8964
NF-IV	0.9959	0.9133	0.974	0.8572
NF-V	0.9969	0.9103	0.9877	0.8944
NF-VI	0.9963	0.906	0.9876	0.9398
NF-VII	0.9966	0.9007	0.9879	0.9527
NF-VIII	0.9973	0.9105	0.9928	0.9076
NF-IX	0.9968	0.9129	0.9856	0.8862
NF-X	0.9974	0.9138	0.9907	0.9021
NF-XI	0.9923	0.9327	0.9952	0.8988
NF-XII	0.9922	0.9329	0.9952	0.8889
NF-XIII	0.9928	0.9277	0.9953	0.9186
NF-XIV	0.9862	0.9510	0.9945	0.8146

Table No: 10 Stability studies of best formulation with cumulative percentage drug release

Time intervals in weeks	Cumulative Percentage Drug Release		
	8°C, 65% RH	**27°C,** 65% RH	**42°C,** 65% RH
2	98.6	98.8	98.8
4	98.5	98.7	98.7
6	98.5	98.7	98.7
8	98.2	98.3	98.2
10	97.5	97.6	97.3
12	96.7	96.4	96.8

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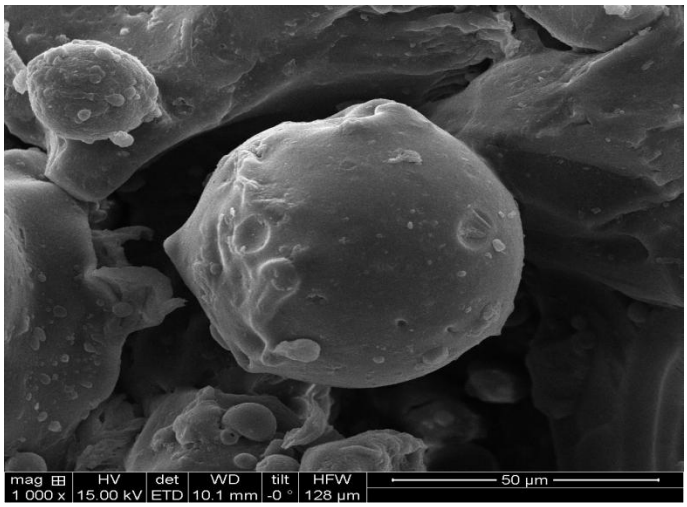

Figure No: 06 SEM Pictures of prepared best formulation microsphere (NF-XIII)

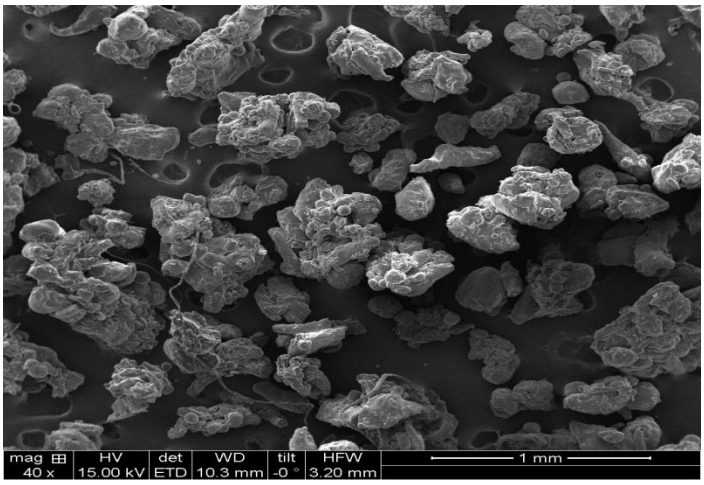

Figure No: 07, SEM Pictures of prepared best formulation microsphere (NF-XIII) internal structure

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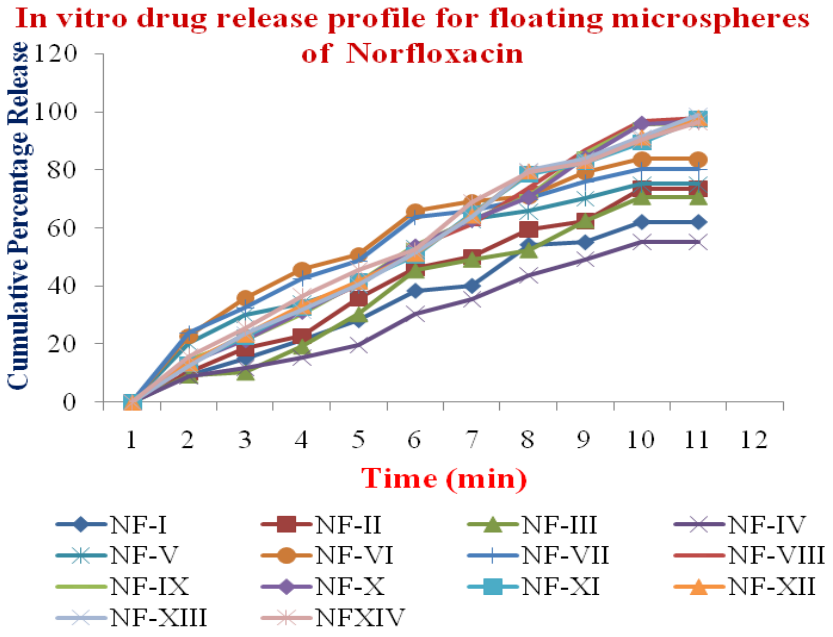

Figure No: 08 Dissolution studies profile for all formulations

CONCLUSION

The prepared all formulations of Norfloxacin revealed the fact that developed formulation (NF-XIII) showed comparable release characteristics, thus it may have fair clinical efficacy. Hence, the formulation NF-XIII has met the objectives of the present study.

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