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FORMULATION AND EVALUATION OF OFLOXACIN MICROSPHERES BY USING ETHYL CELLULOSE AS A POLYMER AT DIFFERENT RATIO

S. Harish Kumar*, A.Sheikalisha, Dr.S.Chandra, R.Suresh, S.Sangeetha, Tamilselvan A

*Department of Pharmaceutics, JKKMMRF's College of Pharmacy, Komarapalayam, Namakkal - 638183, Tamil Nadu, India.

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Corresponding author address

S. Harish Kumar
Department of Pharmaceutics, JKKMMRF's College of Pharmacy, Komarapalayam, Namakkal - 638183, Tamil Nadu, India.

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ABSTRACT

Ofloxacin is preferably absorbed from the upper part of the gastrointestinal tract and is readily soluble in the acidic environment of the stomach, the floating microspheres of ofloxacin were formulated to develop By Using Ethyl Cellulose as a Polymer at Different Ratio. These floating microspheres release the drug in the stomach and upper gastrointestinal tract and thereby improve the bioavailability. In the present study, three formulations of ofloxacin were prepared as floating microspheres by solvent diffusion technique using polymers such as ethyl cellulose, ethanol in different ratios. The prepared microspheres were evaluated for different physicochemical tests such as particle size, Percent Encapsulation Efficiency, Particle Size , Angle of Repose , drug content uniformity, In Vitro drug release studies and stability studies. The results of all the physicochemical tests of all formulations were found to be satisfactory. The formulation F1 showed better entrapment efficiency than other formulations. In-vitro drug release studies were carried out with formulation F1 to F3. All formulations showed the slow drug released initially, which may be ascribed to the low permeability of Ethyl cellulose. At the end of 8hrs, drug release from the Microspheres prepared with drugs: Ethyl cellulose ratios of 1:1, 1:1.5 and 1:2 were 61.71, 60.34 and 57.85 respectively. concluded that the ratio of 1:1 of Ofloxacin and ethyl cellulose produced the Microspheres with all desired characteristics and sustained release of drug for an extended period of 8 hours.

Keywords: Ofloxacin Microspheres, Ethyl Cellulose , Polymer

Introduction

Oral drug delivery is the most used and preferred route of administration with the obvious advantage of ease of administration and patient acceptance. To develop a drug delivery system for oral administration, it is necessary to optimize not only the release rate of an active ingredient from the system but also the residence time of the system in the gastrointestinal tract.

The term Microsphere is defined as a spherical particle with size varying with diameters in the micrometer range (typically 1μm to 1000μm (1mm), containing a core substance. The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 200 micrometer. Microsphere have been extensively studied for use as drug delivery systems, where they have been shown to protect sensitive macromolecules from enzymatic and acid degradation, and allow controlled release and tissue targeting of the formulated drug. Drug carriers one can name soluble polymers, microparticles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes, and micelles. The carriers can be made slowly degradable, stimuli-reactive (e.g. pH or temperature-sensitive), and even targeted (e.g. by conjugating them with specific antibodies against certain characteristic components of the area of interest).

The Novel Drug Delivery System started the alternative means of delivering the drug in the form of microspheres. A unique pharmaceutical delivery system comprising a physiologically acceptable nonaqueous liquid such as an edible oil and a thickening agent such as colloidal silicon dioxide which together form a semi-solid having the consistency of a pudding is provided. Microspheres prepared with gelatin as the polymer have been found to be highly mucoadhesive and have been used for the controlled release of many drugs. Microspheres prepared from admixtures of gelatin and crosslinked chitosan demonstrated some advantage over that prepared from gelatin alone in terms of better controlled release rate of cemetidine. Nanoparticles (including nanospheres and nanocapsules of size 10-200nm) are in the solid state and are either amorphous or crystalline. They are able to absorb and/or encapsulate a drug, thus protecting it against chemical and enzymatic degradation. Novel drug delivery system development is largely based on promoting the therapeutic effects of a drug and

minimizing its toxic effects by increasing the amount and persistence of a drug in the vicinity of a target cell and reducing the drug exposure of nontarget cells.

Classification

There are different types of Microspheres.

1. Glass microspheres
    - · Hollow glass microspheres
    - · Solid glass microspheres
2. Polymer microspheres
    - · Polyethylene microspheres
    - · Polystyrene microspheres
    - · Fluorescent microspheres
3. Starch microspheres
    - · Cross-linked starch microspheres
4. Ceramic microspheres
5. Albumin microspheres
6. Gelatin microspheres
7. Dextran microspheres
8. Poly lactide and polyglycolide microspheres
9. Poly anhydride microspheres
10. Poly phosphane microspheres
11. Chitosan microspheres
12. Lipid cross linked chitosan microspheres
13. Carrageenan microspheres
14. Alginate microspheres
15. Poly (alkyl cynoacrylate) microspheres
16. Poly acrolein microspheres

Advantages

(1) Improved antigenicity by adjuvant action.

(2) Modulation of antigen release.

(3) Stabilization of antigen.

(4) Microspheres are very small particles that can be loaded easily.

Prerequisites

The material utilized for the preparation of microspheres should ideally fulfil the following prerequisites: -

(1) Longer duration of action

(2) Control of content release

(3) Increase of content of therapeutic efficiency

(4) Protection of drug

(5) Reduction of toxicity

(6) Biocompatibility

(7) Serializability

(8) Relative stability

(9) Water solubility or dispersibility

(10) Bioresorbability

(11) Targetability

(12) Polyvalent

Governing Factors

In the preparation of microspheres, the governing factors are:-

1. The particle size requirements
2. The drug or protein should not be affected by the process.
3. Reproducibility of the release profile and the method.
4. No stability problems.
5. No toxic products produced as the final product.

Ofloxacin hydrochloride is an anti-infective drug, used mainly in the treatment of lower respiratory infections, skin infection, urinary tract infections, and sexually transmitted diseases (except syphilis). Ofloxacin has broad activity against bacterial (Helicobacter pylori) infections and is used in combination with other drugs to treat tuberculosis. The bioavailability of ofloxacin is strongly dependent on the local physiology in the GI tract. Ofloxacin is preferably absorbed from the upper part of the gastrointestinal tract. Ofloxacin is readily soluble in the acidic environment of the stomach. In the intestine, where neutral to slightly alkaline pH conditions prevail, precipitation of the active compound occurs, which adversely affects absorption in the lower sections of the intestine. Therefore there is a need for systems that reside in the stomach over a relatively long time and release the drug there in a sustained manner . This can be achieved by the design and development of sustained release gastroretentive floating drug delivery system for ofloxacin (using suitable polymers) which would float and deliver the drug in the upper part of GIT in a sustained manner. Earlier gastroretentive drug delivery system for ofloxacin had been formulated as floating tablets. The objective of this study was to develop and evaluate Ofloxacin Microspheres By Using Ethyl Cellulose as a Polymer at Different Ratio.

Materials and Methods

Materials

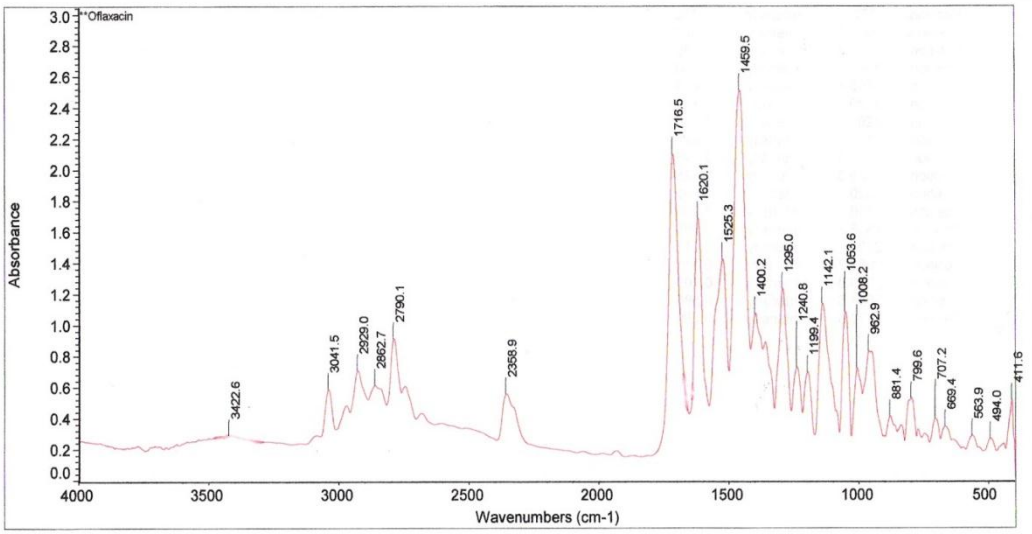
1. Ofloxacin (Gifted sample from Vertex Pharma Chemicals, Pondicherry.
2. Ethyl Cellulose (Nice chemicals, Mumbai)
3. n-Hexane (Merck chemicals, Mumbai)
4. Acetone (Nice chemicals, Cochin)
5. Distilled water
6. Sterile water
7. Ethanol (Loba chemicals, Mumbai)
8. Liquid Paraffin (Loba chemicals, Mumbai)
9. Methanol (Loba chemicals, Mumbai)
10. Chloroform (Nice chemicals, Mumbai)
11. Phosphate buffer (pH 7.4)

Preformulation Studies

Identification of Ofloxacin pure drug

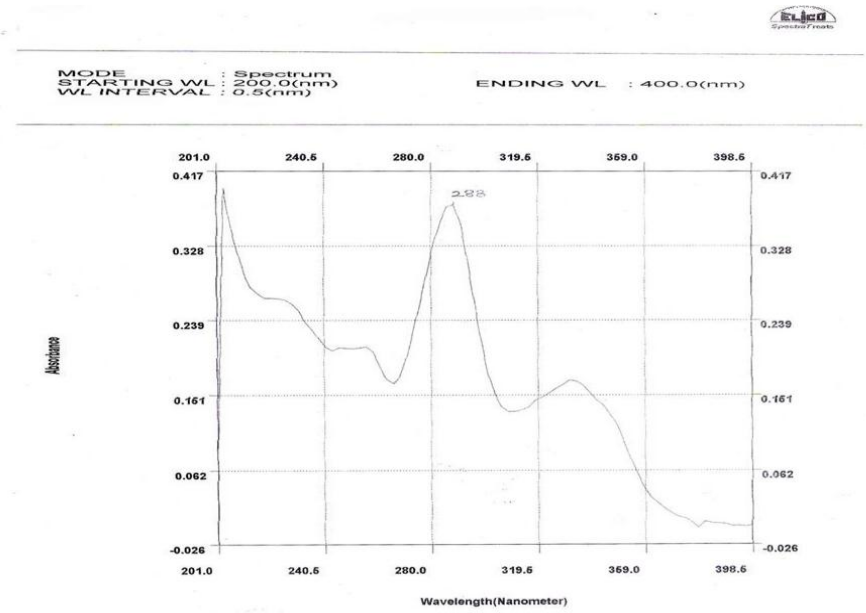
It complies the B.P/I.P limits and **Fig.1** show the I. R. spectrum of Ofloxacin.

Fig 1 A U.V spectrum of Ofloxacin



It gives 3 peaks at 288,331 and 333nm respectively using phosphate buffer saline pH 7.4 as a solvent.

Fig.2 U.V. Spectrum of Ofloxacin



Sample : **Ofloxacin** [CONC.4µG ML $^{-1}$]

Reference : **Phosphate Buffer**, Ph 7.4 [Pbs]

Remarks : **peak observed at 288,331 and 333 nm**.

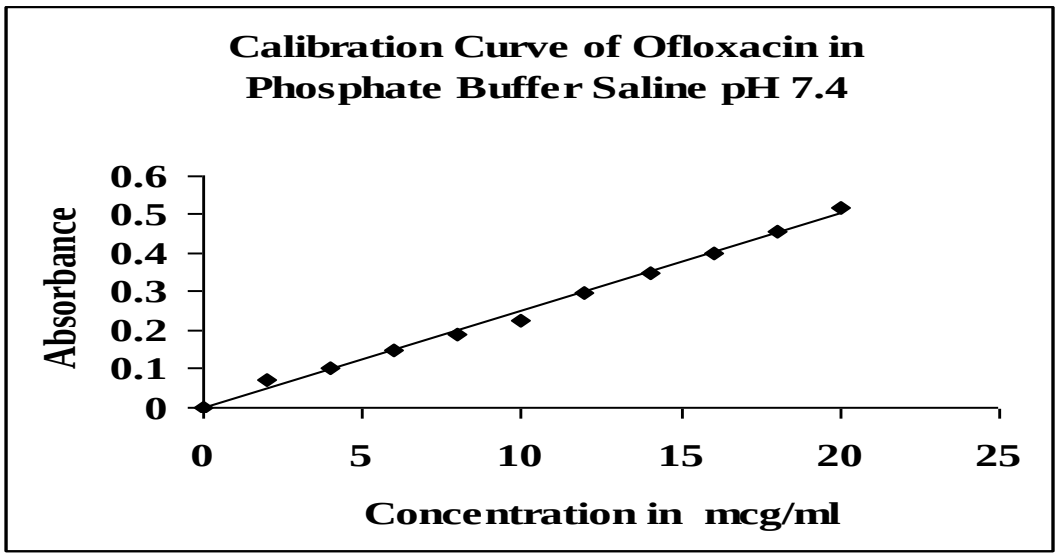
Construction of Standard Curve

Calibration curve for Ofloxacin in phosphate buffer. (pH 7.4)

Table No 1

Concentration (mcg/ml)	0	2	4	6	8	10	12	14	16	18	20
Absorbance at 288 nm	0	0.074	0.103	0.150	0.188	0.227	0.298	0.347	0.402	0.457	0.518

Fig -3. Standard Curve for Ofloxacin in pH 7.4 Phosphate Buffer Saline



Slope = 0.0251 Regression = 0.9968

Preparation of Microspheres

Technique used: Solvent Evaporation

Preparation method

The drug and polymer in different proportions (1:2, 1:3, 1:4, 1:5 and 1:6) were weighed & dissolved at room temperature into Methanol with vigorous agitation to form uniform drug polymer dispersion. This was slowly poured into the dispersion medium consisting of light liquid paraffin (200 ml) containing 0.1% Span 80.

The system was stirred using Magnetic stirrer at 500 rpm, at room temperature over a period of 2-3 hours, to ensure complete evaporation of the solvent. The liquid paraffin was then decanted & the microspheres were separated by filtration through a Whatmann filter paper, washed thrice with 180 ml of n- hexane and air dried for 24 hours.

Table 2: Preparation Of Microspheres

S.No	Formulation	Name of the Drug & Amount taken (in gm)	Name of the Polymer & Amount taken (in gm)	Drug: Polymer Ratio
1	Formulation I	Ofloxacin (1gm)	Ethyl cellulose (1gm)	1:1
2	Formulation II	Ofloxacin (1gm)	Ethyl cellulose (1.5gm)	1:1.5
3	Formulation III	Ofloxacin (1gm)	Ethyl cellulose (2gm)	1:2

Morphological Study

The morphology of ethyl cellulose microspheres was observed by scanning electron microscopy (SEM). The microspheres were mounted directly onto the SEM sample stub, using double sided sticking tape and coated with gold film under reduced pressure (001 Torr). It's shown in (figure 3,4.5)

Figure 3: Ethyl cellulose microspheres SEM

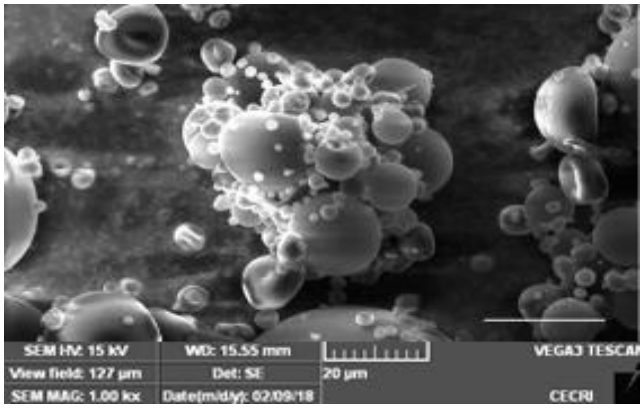


Figure 4: Ethyl cellulose microspheres SEM

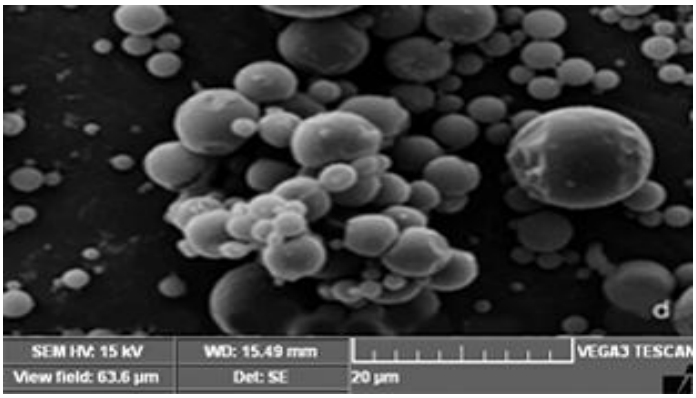
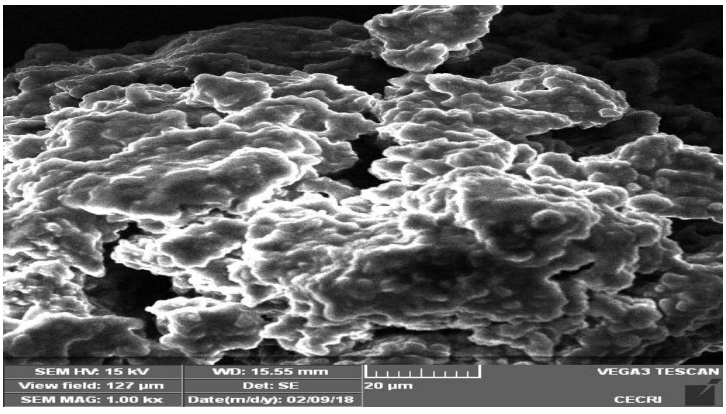


Figure 5: Ethyl cellulose microspheres SEM



Bulk Density

Since particles may be hard and smooth in one case and rough and spongy in another, one must express densities with great care. Density is universally defined as weight per unit volume; the difficulty arises when one attempts to determine the volume of particles containing microscopic cracks, internal pores and capillary spaces.

For convenience, three types of densities may be defined (a) true density (b) Granule density (c) Bulk density as determined from the bulk volume and the weight of a dry powder in a graduated cylinder. [08)]

Method

A weighed amount (about 10 gm) was introduced in to a 100 ml measuring graduated cylinder. The cylinder was fixed on the bulk density apparatus and the timer knob was set for 100 tappings. Then noted the volume continued another 50 tappings and noted the final volume. This volume was noted as bulk volume.

$$\textbf{Tapped bulk density} = \frac{\text{Mass of a powder}}{\text{Tapped Bulk volume}}\ g / cm^3$$

$$\textbf{Loose Bulk density} = \frac{\text{Mass of a powder}}{\text{Loose Bulk Volume}}\ g/ cm^3$$

Table 3 Data Showing Bulk Densities of Microspheres

S.No	Formulation	Tapped Bulk density ±SD	Loose Bulk density ±SD
1.	Formulation I	0.712 g / cm^3±0.06	0.687 g / cm^3±0.05
2.	Formulation II	0.697 g / cm^3±0.07	0.573 g / cm^3±0.04
3.	Formulation III	0.654 g / cm^3±0.05	0.563 g / cm^3±0.03

Percent Encapsulation Efficiency

Microspheres equivalent to 50mg of ofloxacin were weighed and crushed, then dissolved in 50ml of phosphate buffer solution (pH 7.4), filtered. This solution (1ml) was diluted to 100ml with phosphate buffer solution (pH 7.4) to obtained of final solution.[11,14]

Table 4: Percent Encapsulation Efficiency

S.No	Formulation	Encapsulation Efficiency
1.	Formulation I	72.20 %
2.	Formulation II	69.10 %
3.	Formulation III	64.80%

Particle Size Analysis

Particle Size

The particle size of the prepared Microspheres was estimated by sieving method. Sieving method directly gives weight distribution. The sieving method finds application in dosage form development of formulations.

Method

Seives were arranged in a nest with the coarest at the top. A sample (15 gm) of the Microspheres was placed on the top sieve. The sieve set was fixed and shaken for a certain period of time (20 minutes). The Microspheres retained on each sieve was weighed. Frequently, the Microspheres were assigned the mesh number of the screen through which it passed or on which it was retained. It was expressed in terms of the arithmetic mean of the two sieves. [9-10)]

E.g. Microspheres passing through a 36 mesh and retrained on 44 mesh sieve was assigned an Arithmetic mean diameter of (425 + 355) /2 or 390 µm. Data processing for a granules were shown in table No : 5-7.

Table 5 Particle Size Analysis For Formulation – I

S.No	Sieve number passed/Retained	Arithmetic Mean size of opening (µm) X_i	Weight retained on a sieve (gm)	Percent weight Retained(%) F_i	Weight size Xi Fi
1.	16/25	800.0	1.273	8.48	6789.33
2.	25/36	562.5	1.678	11.18	6292.50
3.	36/44	390.0	2.573	17.15	6689.79
4.	44/60	302.5	2.732	18.21	5509.53
5.	60/100	200.0	2.982	19.88	3976.00
6.	100/120	137.5	3.582	23.88	3283.50

$$\text{Mean particle size} = \frac{\Sigma Xi\ Fi}{\Sigma Fi}$$

$$= \frac{32540.65}{98.78}$$

=329.42 µm or 0.329 mm

Table 6 Particle Size Analysis For Formulation – II

S.No	Seive Number Passed/Retained	Arithmetic Mean Size of opening (µm) X_i	Weight Retained on a sieve (gm)	Percent weight retained (%) F_i	Weight Size $X_i F_i$
1.	16/25	800.0	1.120	7.46	5973.33
2.	25/36	562.5	1.855	12.36	6956.25
3.	36/44	390.0	2.390	15.93	6213.99
4.	44/60	302.5	2.750	18.33	5545.83
5.	60/100	200.0	2.960	19.73	3946.66
6.	100/120	137.5	3.725	24.83	3414.88

$$\text{Mean particle size} = \frac{\Sigma Xi\ Fi}{\Sigma Fi} = \frac{32050.94}{98.64}$$

= 324.92 µm or 0.324 mm.

Table 7 Particle Size Analysis For Formulation – III

S.No	Sieve Number Passed/ Retained	Arithmetic Mean size of opening (µm) X_i	Weight retained on a sieve (gm)	Percent weight Retained (%) F_i	Weight size $X_i F_i$
1.	16/25	800.0	1.892	12.61	10090.66
2.	25/36	562.5	2.008	13.38	7530.00
3.	36/44	390.0	2.505	16.70	6513.00
4.	44/60	302.5	2.888	19.25	5824.13
5.	60/100	200.0	1.732	11.54	2309.33
6.	100/120	137.5	3.805	25.36	3487.91

$$\text{Mean particle size} = \frac{35755.03}{98.84}$$

$$= 361.74 \text{ µm or } 0.361 \text{ mm}$$

ANGLE OF REPOSE

The flow characteristics are measured by the angle of repose. Improper flow of powder is due to frictional forces between the particles. These frictional forces are quantified by the angle of Repose. Angle of Repose is defined as the maximum angle possible between the surface of a pile of the powder and the horizontal plane. The angle of repose is given by the equation,

The following table shows the relationship between angle of Repose (θ) and powder flow.

Table 8: Angle of Repose

Angle of Repose (θ)	Flow
< 25	Excellent
25 – 30	Good
30 – 40	Passable
> 40	Very Poor

Method

A glass funnels was held in place with a clamp on a ring support over a glass plate. 15 gm of granules were transferred into a funnel kept the orifice of the funnel blocked by the thump. As the thumb is removed, the Microspheres were emptied from funnel, then measured the height of the pile (h) and the Radius of the base (r) with the ruler. [9-10)]

The angle of repose was calculated by using a formula.

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

Table 9: Data Showing Angle of Repose Of Microspheres

S.No	Formulation	Angle of repose(θ)
1.	Formulation I	22º2’
2.	Formulation II	21º3’
3.	Formulation III	25º7’

Drug Content

Drug content was determined by using the following procedure.

Weighed accurately a quantity of the microspheres equivalent to 50 mg of Ofloxacin, shaken with 60ml of methanol in a 200 ml volumetric flask and diluted to volume with methanol. Diluted 5 ml of this solution to 100 ml with methanol and measured the absorbance of the resulting solution at 333 nm. From the absorbance, The drug content was calculated. [12)]

Table 10: DRUG CONTENT

Formulation	Amount present (mg)	Drug content (%)
Formulation I	47.02	94.04
Formulation II	47.55	95.10
Formulation III	48.32	96.64

In-Vitro Release Study

In-vitro release of Ofloxacin from the microspheres formulation was carried out by the paddle method of dissolution described in U.S.P on LABINDIA DS 8000 eight spindle Dissolution Apparatus.

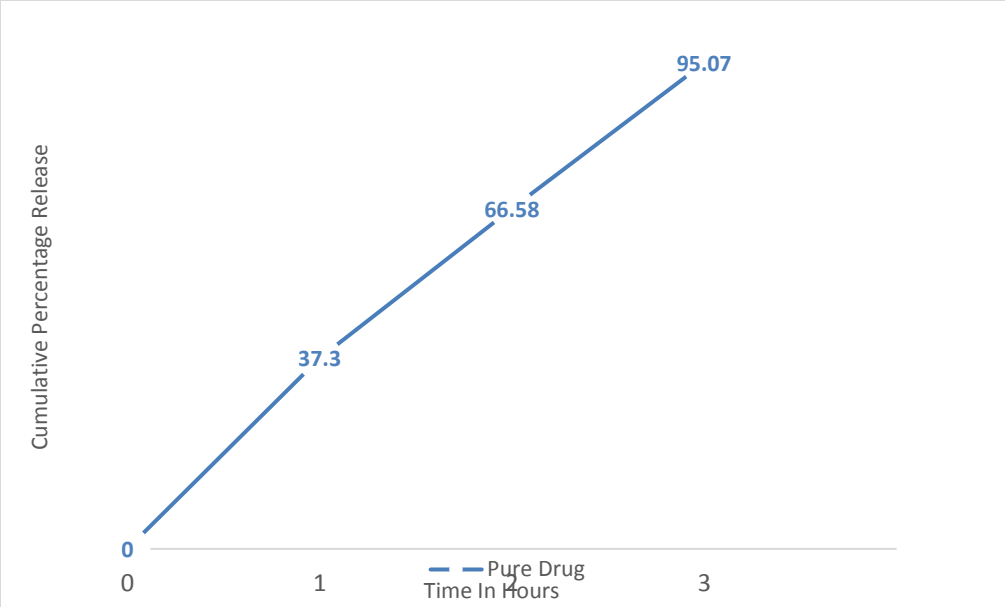
The Ofloxacin microspheres were placed in a bowl and immersed in 900 ml of phosphate buffer (pH 7.4) at a temperature of 37^{o}C ± 1^{o}C and paddle rotation was set for 50 RPM. 5ml of the sample was withdrawn from the dissolution media at predetermined time interval (every 1 hour) and the same volume of fresh medium was replaced immediately. Collected samples were filtered and diluted with phosphate buffer pH7.4 blank made up to 10 ml (2 fold), and the absorbance was measured at 276 ηm by using shimadzu double beam spectrophotometer. The amount of Ofloxacin released at respective time intervals were calculated. Cumulative percentage release of the drug was also calculated. The study was performed in triplicate. The standard error of the means of the 3 replicates point was determined[13).]

Table – 11: In vitro Release of Pure Drug

Time (in minutes)	Absorbance	Concentration mcg/ml	Amount released in mg	Cumulative amount released in mg	Cumulative percentage release ±S.D
10	0.075	2.988	1.494	1.494	7.47±0.055
20	0.078	3.107	1.553	3.047	15.23±0.125
30	0.072	2.286	1.434	4.481	22.40±0.085
60	0.150	5.976	2.980	7.461	37.30±0.079
120	0.294	11.713	5.856	13.317	66.58±0.202
180	0.286	11.394	5.697	19.014	95.07±0.212

Standard Deviation = Mean of three values (n=3).

Figure-6 Invitro Release of Pure Drug-Ofloxacin



In-Vitro Release Study – Formulation I

Medium : Phosphate buffer pH 7.4

Method : Paddle

RPM : 50

Table 12: In-Vitro Release Study – Formulation I

S. No	Time (hrs)	Concentration (mcg/ml)	Amount Released in 900ml (mg)	Amount Released in 5 ml (mg)	Cumulative amount Released (mg)	Cumulative percentage released	Standard Deviation
1.	1	2.47	4.42	0.041	4.42	8.84	+ 0.54
2.	2	3.98	7.12	0.0710	7.17	14.34	+ 0.48
3.	3	7.08	12.67	0.126	12.74	25.49	+ 0.38
4.	4	8.28	14.67	0.146	14.80	29.60	+ 0.66
5.	5	11.68	20.90	0.209	21.05	42.11	+ 0.44
6.	6	12.97	23.21	0.232	23.42	46.85	+ 0.56
7.	7	13.88	24.84	0.248	25.07	50.15	+ 0.57
8.	8	17.10	30.60	0.306	30.85	61.71	+ 0.60

In-Vitro Release Study – Formulation II

Medium : Phosphate buffer pH 7.4

Method : Paddle

RPM : 50

Table 13: In-Vitro Release Study – Formulation II

S. No	Time (hrs)	Concentration (mcg/ml)	Amount Released in 900ml (mg)	Amount Released in 5 ml (mg)	Cumulative amount Released (mg)	Cumulative percentage released	Standard Deviation
1.	1	2.12	3.96	0.039	3.96	7.91	+ 0.58
2.	2	3.72	6.66	0.066	6.70	13.40	+ 0.64
3.	3	5.87	10.51	0.105	10.57	21.15	+ 0.54
4.	4	7.98	14.28	0.142	14.39	28.78	+ 0.78
5.	5	10.12	18.11	0.181	18.26	36.51	+ 0.82
6.	6	12.68	22.69	0.226	22.88	45.76	+ 0.34
7.	7	13.56	24.27	0.242	24.92	49.85	+ 0.54
8.	8	16.72	29.93	0.299	30.17	60.34	+ 0.68

In-Vitro Release Study – Formulation III

Medium : Phosphate buffer pH 7.4

Method : Paddle

RPM : 50

Table 14: In-Vitro Release Study – Formulation III

S. No	Time (hrs)	Concentration (mcg/ml)	Amount Released in 900ml (mg)	Amount Released in 5 ml (mg)	Cumulative amount Released (mg)	Cumulative percentage released	Standard Deviation
1.	1	1.98	3.56	0.035	3.56	7.12	+ 0.52
2.	2	3.57	6.39	0.063	6.42	12.85	+ 0.64
3.	3	5.47	9.79	0.097	9.85	19.71	+ 0.72
4.	4	7.89	14.12	0.142	14.22	28.44	+ 0.68
5.	5	9.78	17.56	0.175	17.71	35.41	+ 0.76
6.	6	12.13	21.72	0.217	21.88	43.77	+ 0.64
7.	7	13.26	23.73	0.237	23.95	47.91	+ 0.58
8.	8	16.31	29.19	0.291	29.43	57.85	+ 0.64

In-Vitro Release Study

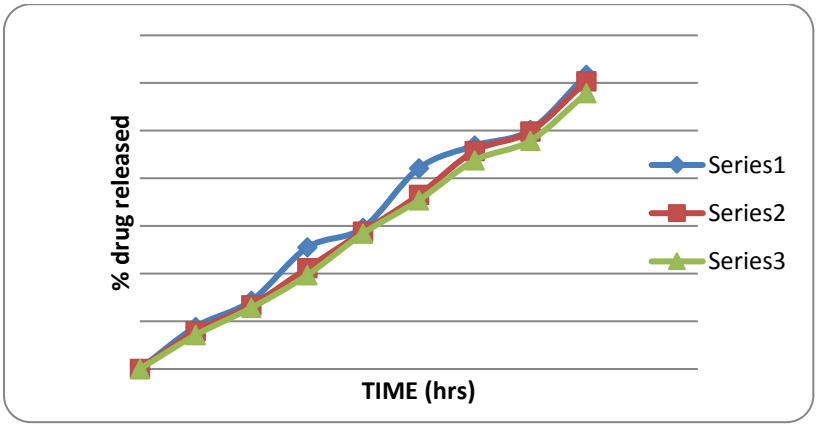


Figure: 7 SERIES 1 - F1 SERIES 2 - F2 SERIES 3 - F3

Pharmacokinetics

Determination of Order of Drug Release

In order to understand the order of drug release, the drug release data of the invitro dissolution study were analyzed using Zero order models and coefficient of correlation (r) values were calculated for the linear curves obtained by regression analysis of the plots.

The preparations followed the zero order release which is indicated by the linearity of the cumulative concentration Vs time curve as shown in above Figure-8 to 9 and Figure-10.

The zero order release curve of Microspheres using Ethyl cellulose 1gm, 1.5 gm and 2 gms (1:1, 1:1.5 and 1:2 ratio in each series) prepared by solvent evaporation method was shown in Figure-8 to Figure-9, and Ethyl cellulose (1:1) , Ethyl cellulose (1:1.5) & Ethyl cellulose (1:2) prepared by solvent evaporation technique respectively was shown in Figure-10.

And zero order invitro release data of Microspheres using various Ethyl cellulose 1gm, 1.5 gm and 2 gms (1:1, 1:1.5 and 1:2 ratio in each series) as shown in Table-10.

Figure-8: Zero order Release Plot of Microspheres prepared by Solvent Evaporation Method using Ethyl cellulose (1:1 ratio)

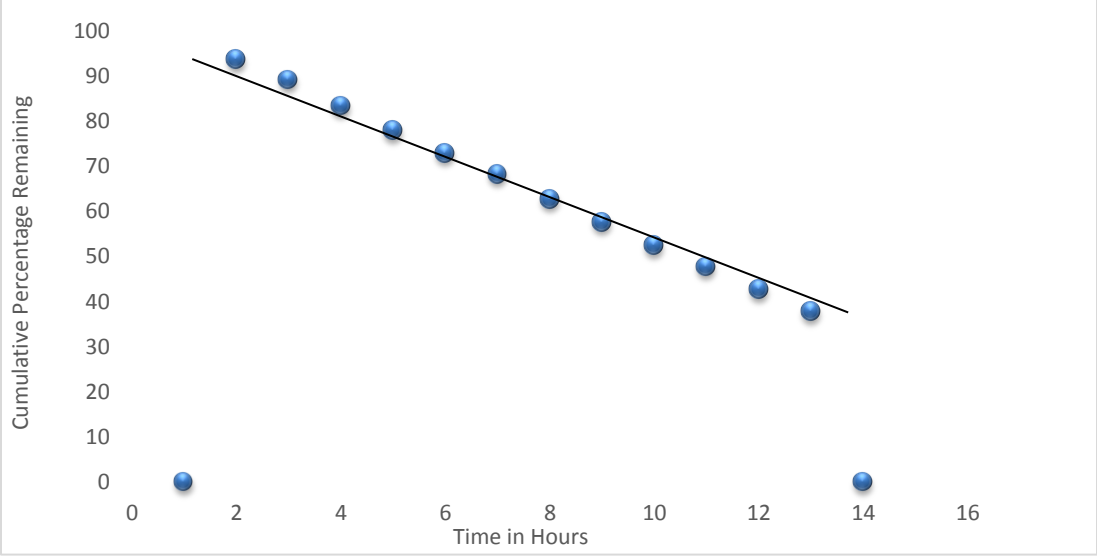


Figure-9:Zero order Release Plot of Microspheres prepared by Solvent Evaporation Method using Ethyl cellulose (1:1.5 ratio)

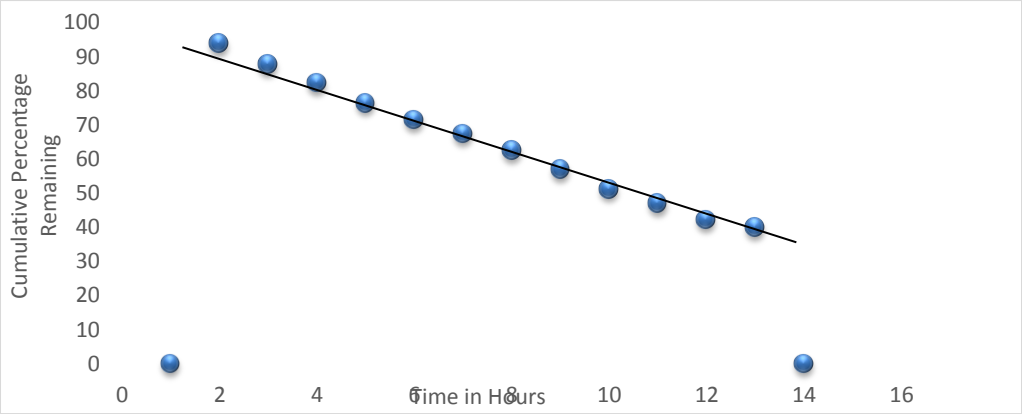
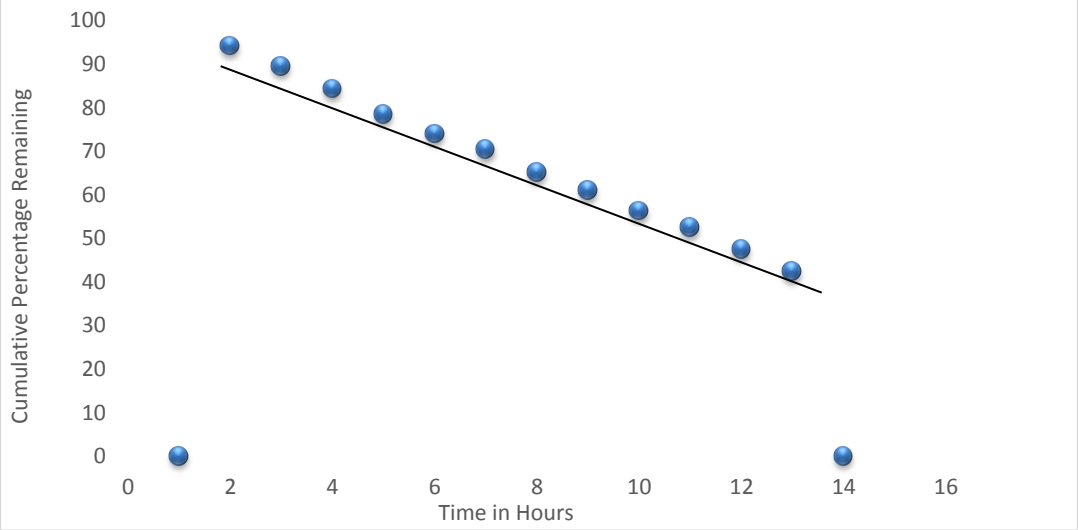


Figure-10: Zero order Release Plot of Microspheres prepared by Solvent Evaporation Method using Ethyl cellulose (1:2 ratio)



Stability Studies of Prepared Microspheres

The formulated Microspheres were divided into 3 groups and each one was stored in amber-colored vials, one at 4ºC, the second portion at room temperature27±2ºC and the third at 37ºC.The amount of drug leaked from the Microspheres present in the dialysate was estimated spectrophotometrically and determined the percentage drug retained in Microspheres as shown in **Table-14**

Table - 15: Data of Percentage Retention of Ofloxacin in Microspheres prepared by solvent evaporation method at Various Temperatures [Ethyl Cellulose Formulation]

Storage temperature At	Ratio	Amount of drug retained (%) after—week				
		1st	2nd	3rd	4th	5th
4ºC	**1:1**	**98.04**±0.091	**97.29**±0.146	**94.43**±0.192	**92.04**±0.247	**92.24**±0.113
	1:1.5	98.19±0.176	96.85±0.093	93.74±0.158	92.23±0.122	91.80±0.125
	1:2	98.21±0.182	96.89±0.087	93.74±0.158	92.45±0.104	90.73±0.133
27±2ºC	**1:1**	**91.14**±0.079	**90.26**±0.171	**87.85**±0.219	**70.31**±0.239	**65.59**±0.155
	1:1.5	91.68±0.120	90.12±0.174	87.08±0.191	65.68±0.162	63.68±0.142
	1:2	91.22±0.185	89.20±0.176	88.32±0.187	63.35±0.094	60.83±0.135
37ºC	**1:1**	**91.60**±0.125	**89.38**±0.110	**86.26**±0.130	**67.38**±0.168	**53.55**±0.111
	1:1.5	92.38±0.107	88.22±0.121	86.26±0.098	63.22±0.149	48.38±0.106
	1:2	92.31±0.098	88.19±0.133	86.26±0.055	59.15±0.130	45.70±0.096

Standard Deviation = Mean of three values (n=3).

In **solvent evaporation method**, at the end of 5th week 92.24% of drug was retained at 4ºC using ethyl cellulose Microspheres formulation which is nearly similar value compared than 91.80% drug retained in (1:1.5).And 90.73% of drug was retained in ethyl cellulose **(1:2)**.But in other temperatures, the drug retainment in Microspheres are totally reduced at 4th and 5th weeks respectively.So the loss of entrapped drug at various conditions of storage may be due to the leakage of drug during the fusion of Microspheres and diffusion of the drug across the bilayer due to residual hydrating medium.

Results and Discussion

The solvent evaporation technique was used for preparation of Ofloxacin Microspheres with Ethyl cellulose. The delayed release Microspheres of all batches were found to be discrete,

spherical and free flowing (fig 4) the size range of different batches of Microspheres was in the range of 324.92 µm to 361.74 µm (table 5-7). Drug content analysis showed that the distribution of drug within IP limits.

The packing properties of the drug and the formulation 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 1.5gm/cm^3 indicate poor flow characteristic. It is seen from Table 4 that the bulk density values are less than 1.2gm/cm^3 indicating good flow characteristics of the Microspheres. Angle of repose less than or equal to 40^0 indicate free flowing properties of the Microspheres. The Angle of repose for all the formulations table 9 is seen to be between $21^0 3'$ to $25^0 7'$ indicating good flow property.The formulation F1 showed better entrapment efficiency than other formulations. In-vitro drug release studies were carried out with formulation F1 to F3. All formulations showed the slow drug released initially, which may be ascribed to the low permeability of Ethyl cellulose. At the end of 8hrs, drug release from the Microspheres prepared with drugs: Ethyl cellulose ratios of 1:1, 1:1.5 and 1:2 were 61.71, 60.34 and 57.85 respectively. This stated the drug release retardation was directly proportional to the Ethyl cellulose content of the particle. It has been documented that the most types of cellulosic membranes including Ethyl cellulose swells in aqueous environments and thus in high concentration these retards the drug release by forming more barriers of thick gel around the drug particle. Furthermore, in this study the higher concentration of Ethyl cellulose in formulation produced the large Microspheres.

Conclusion

The solvent evaporation method was found suitable to produce spherical Ethyl cellulose Microspheres with smooth surface and better drug content. The formulation F1 was found to be the best formulation as is shown by the In-vitro studies and the evaluation of other formulations.From the result, it can be concluded that the ratio of 1:1 of Ofloxacin and ethyl cellulose produced the Microspheres with all desired characteristics like sieve analysis, bulk density, and sustained release of drug for an extended period of 8 hours. The Ethyl cellulose formed the semi permeable membrane over the Ofloxacin to give sustained delivery of the Ofloxacin.

References

1. Mona Semalty*, Shikha Yadav and Ajay Semalty "Preparation and Characterization of Gastroretentive Floating Microspheres of Ofloxacin Hydrochloride" International Journal of Pharmaceutical Sciences and Nanotechnology. Volume 3 • Issue 1 • April – June 2010.
2. Vidhyaa Kumaria , Vignesh Muruganandhamb* "Formulation, Development & Characterization of Ofloxacin Microspheres" Indo Global Journal of Pharmaceutical Sciences, 2012; 2(2): 130-141.
3. Kawashima Y, Niwa T, Takeuchi H, Hino T, Ito Y. Preparation of multiple unit hollow microspheres (microballoons) with acrylic resin containing tranilast and their drug release characteristics (in vitro) and floating behavior (in vivo). J. Controll. Release. 16; 279-290 (1991).
4. Lee J-H, Park TG, Choi H-K. Development of oral drug delivery system using foating microspheres. J Microencapsulation. 16: 715-29 (1999).
5. Semalty A, Semalty M. Preparation and characterization of mucoadhesive microspheres of ciprofloxacin hydrochloride. Indian Drugs. 44: 368-72 (2007).
6. Semalty M, Semalty A. Gastroretentive floating microspheres of diltiazem hydrochloride, Pharma Buzz. 3(12): 24-28 (2008).
7. Sen H, Kshirsagar RS. Pharmaceutical composition for a controlled release of active ingredient. (2002) WO024187.
8. Soppimath KS, Kulkarni AR, Aminabhavi TM. Development of hollow microspheres as floating controlled release system for cardiovascular drugs. Drug Dev. Ind. Pharm. 27(6):507- 15 (2001).
9. Streubel A, Siepmann J, Bodmeier R. Floating matrix tablets based on low density foam powder: effects of formulation and processing parameters on drug release. Eur. J. Pharm. Sci. 18: 37–45 (2003).
10. J.M.Covino, M. Cummings, B .Smith, S.Benes , K.Draft , M.William. Comparison of Ofloxacin and Ceftriaxone in the Treatment of Uncomplicated Gonorrhea Caused by Penicillinase-Producing and Non-Penicillinase-Producing Strains. Antimicrobial Agent Chemotherapy, 1990, 34: 148–149

11. J.H. Yuk, C.H. Nightingale R., Quintiliani and K.R.Sweeney .Bio-vailability and pharmacokinetics of ofloxacin in healthy volunteers. Antimicrobial Agent Chemotherapy., 1991, 35: 384-386
12. A.Arunachalam. B.Stephen Rathinaraj, Subramanian, Prasanta Kumar Choudhury, Kishore A Reddy, Md.Fareedullah. Preparation and evaluation of ofloxacin microspheres using Natural gelatin polymer. International Journal of Applied Biology and Pharmaceutical Technology.,2010,1(1):61-6
13. G.Angela, Hausberge, P.Patrick , DeLuca .Characterization of biodegradable poly (D,L-lactide-co-glycolide) polymers and microspheres. Journal of Pharmaceutical and Biological Analysis. 1995, 13(60):747-760
14. A.K. Anal, D Bhopatkar, S. Tokura, H. Tamura, W.F. Steven. Chitosan–alginate multilayer beads for gastric passage and controlled intestinal release of protein. Drug Developement. Industrial Pharmaceutical. 2003, 29, 713–724.
15. S.Takka, F. Acarturk. Calcium alginate microparticles for oral administration. I.Effect of sodium alginate type on drug release and drug entrapment efficiency. Journal of Microencapsulation.,1999,16:275-290
16. K. Upadhye, S Bakhle, G. Dixit.Preparation and Evaluation of Gelatin Microspheres Containing Ciprofloxacin Hydrochloride. Indian Drugs., 2004,41(11): 665-668
17. S. Shiraishi, T. Imai, M. Otagiri. Controlled-release preparation of indomethacin using calcium alginate gel. Biology and Pharmaceutical Bulletin., 1993, 16 (11): 1164–1168
18. A. Semalty & M. Semalty. Preparation and Characterization of Mucoadhesive Microspheres of Ciprofloxacin Hydrochloride. Indian Drugs.,2007,44(5): 368-373
19. S.P.Vyas & R.K.Khar .Targeted and Controlled drug delivery. 1st edition.,2006, 325-326: 417-457
20. M.Alagusundaram, C. Madhu Sudana Chetty, K. Umashankari, Attuluri Venkata Badarinath,C. Lavanya , S. Ramkanth. Microspheres As A Novel Drug Delivery System - A Review International Journal of ChemTech and Research.,2009,1(3): 526-534.
21. F. Coi, D. Cun , A. Tao, M.Yang , Y.K.Shi and Y.Guan. Preparation and characterization of melittin- loaded poly (DL-lactic acid) or poly (DL-lactic –co- glycolic acid

)microspheres made by double emulsion method, Journal of Controlled release. 2005,107 (2): 310-319.

22. G.T.Kulkarni, K.Gowthamarajan, B.Suresh. Stability testing of Pharmaceutical Products: An overview. Indian Journal of Pharmaceutics Education and Research. 2004, 38(11): 194-202.