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Formulation and Evaluation of Colon Targeting Microspheres of an Anti-Bacterial Drug

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ABSTRACT

The aim of the present work was to prepare the colon targeting microspheres of anti-bacterial drugs. pH-sensitive polymer Eudragit S100, L100 separately and in combination was used to formulate the microspheres by the solvent evaporation method using various drug-polymer ratio. The prepared microspheres were evaluated for various physicochemical parameters such as particle size, percentage yield, drug entrapment efficiency, drug polymer compatibility, scanning electron microscopy and drug release of microspheres for 10hrs. The-formulated microspheres were spherical with relatively good flow properties. The release study was done in simulated gastrointestinal fluids for 2hrs and up to 8hrs in pH 7.4 and has shown that the drug was protected from being released in the physiological environment of the stomach and efficiently released in colon. It is concluded from the present study that eudragit microspheres are promising carriers for oral colon targeted delivery of anti-bacterial drug.

INTRODUCTION

The oral route of drug administration is the preferred route because it is patient friendly and no intervention by a health care professional is necessary to administer the drug, especially for chronic therapies when the repeated administration is required. Among the oral dosage forms, a tablet of various types is the most used one because it is a convenient and safe way of administration. In addition, it has advantages in terms of the chemical and physical stability as well as accurate dosing of the drug over liquid dosage form[1].

However, the oral route of drug administration using conventional tablets cannot be used for targeting the drug to lower gastrointestinal parts due to their almost complete release at upper gastrointestinal tract (GIT), which leads to their limited availability at the-lower-GIT. To overcome this difficulty, new approaches to drug delivery, have been developed. Among these approaches, colon targeted drug-delivery system has been extensively investigated[2].

From the last few decades, a great deal of research work has been devoted to the development of the site-specific drug delivery system which offers several benefits over the traditional drug treatments. The principal goal of the site-specific delivery is to deliver the drug to the specific-organs-of-the-body[1].

Targeted drug delivery into the colon is highly required for local treatment of a variety of bowel diseases such as ulcerative colitis, Crohn's disease, amebiasis, and colonic cancer. Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases of the colon like irritable bowel syndrome and constipation but also for the systemic delivery of proteins, therapeutic peptides, anti-asthmatic drugs, anti-diabetic agents and antihypertensive drugs[3]. A colon targeted drug delivery system (CTDDS) is preferred for drugs having instability in the acidic media, low solubility and short half-life, a large volume of distribution, poor absorption, and low therapeutic index. The most critical challenge in such drug delivery approaches is to protect the formulation during its passage through the stomach and about first six meters of the small intestine arriving at colon with no loss of active ingredient by preventing the dissolution and the release till it reaches the colon[2]. Microspheres are homogeneous, monolithic particles which improve the treatment by providing localization of the drug at the site of action and by prolonging the drug release. It suffers from the risk of early dissolution and release of the drug before reaching the colon due to its large surface area[4].

Norfloxacin is a synthetic Fluoroquinolone with broad-spectrum antibacterial activity against most gram-negative and gram-positive bacteria. Norfloxacin is widely distributed to body tissues and appears to cross the placenta. Protein binding is approximately 10-15 %. The plasma half-life of Norfloxacin is 2.3 to 4 hrs[5].

The aim of the present work is to formulate and evaluate colon targeting microspheres of Norfloxacin, which belongs to the class antibacterial agents by solvent evaporation technique using Eudragit S and L as polymers also glacial acetic acid as solvents.

The main objective of the proposed work is to evaluate the colon targeting microspheres of Norfloxacin for oral controlled delivery.

MATERIALS AND METHODS

MATERIALS

Norfloxacin was supplied from Yarrow Chem. Products, Mumbai. Eudragit S100 and L100 were also supplied from Yarrow Chem. Products, Mumbai. All other excipients and solvents used were of the analytical or pharmaceutical grade.

METHODS

❖ **Preformulation studies[6]**

Determination of organoleptic properties

The physical appearance of the drug was observed and compared with the pharmacopoeial specifications.

Determination of melting point

The melting point of Norfloxacin was determined by the capillary method.

Solubility

Small increments of Norfloxacin were added to 10ml of solvent (distilled water, acetone, ethanol, glacial acetic acid) in a 25ml stoppered standard flask with vigorous shaking. Visually observed the solution, if the solution was clear and no undissolved particles were

observed if it was insoluble again another increment of particular solvent was added and the procedure was continued until undissolved Norfloxacin was found.

❖ **Compatibility studies using FT-IR Spectroscopy**

The pure drug, drug, and polymer were prepared and scanned from 4000-400cm^{-1} in FTIR spectrophotometer. The FT-IR spectrum of the obtained sample of drug and drug + physical mixture were compared with the standard functional group frequencies of Norfloxacin, Eudragit S, and L100 respectively. The compatibility between the drug and polymer were evaluated using FTIR peak matching method.

Preparation of calibration curve of Norfloxacin at 278 nm[7]

Accurately weighed 100 mg of Norfloxacin was taken in 100 ml standard flask. And makeup to the volume with 0.1N HCl to get the stock solution of 1000 µg/ml. From this stock solution 1 ml was transferred into 10 ml standard flask and makeup to the volume with HCl we get the stock solution of 100 µg/ml. From this stock solution, aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 ml of solutions were transferred into separate 10 ml standard flasks and made up to the volume with 0.1N HCl to get a concentration of 1, 2, 3, 4, 5 µg/ml respectively. The absorbance of the resultant solutions was measured at 278 nm by using a UV spectrophotometer. A graph of concentration vs. absorbance was plotted.

Preparation of colon targeting microspheres of Norfloxacin by emulsion solvent evaporation technique[8]

Accurately weighed drug (Norfloxacin) and polymers (Eudragit S&L) in different ratios were dissolved in glacial acetic acid as shown in table **4.3**. The solution was poured in 100 ml of distilled water containing 0.1 ml of Tween 80 and 5 ml of n-hexane with stirring to form a homogeneous solution, which was maintained at 40 ^{0}C temperature and agitation speed of 800 rpm for 90 min to allow the volatile liquid to evaporate. The microspheres formed were filtered and air dried for 24 hrs at room temperature.

Table 1: Formulation design of Norfloxacin microspheres

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Norfloxacin (gm)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Eudragit S (gm)	0.8	1.4	1.6	1.8	2	*	*	0.93	0.7
Eudragit L (gm)	*	*	*	*	*	0.8	1.4	0.46	0.7
Glacial acetic acid (ml)	10	10	10	10	10	10	10	10	10
Tween 80 (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
n-hexane (ml)	5	5	5	5	5	5	5	5	5
Distilled water	100 mL								

EVALUATION STUDIES OF PREPARED MICROSPHERES

Determination of Percentage yield[9]

The prepared microspheres were collected and weighted. The actual weight of obtained microspheres divided by the total amount of all material that was used for the preparation of the microspheres using the following equation:

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

Determination of Particle size analysis: The particle size of the microspheres was determined by using optical microscopy method.

MICROMERITIC STUDIES[9,10]

Bulk density

The bulk density of a microsphere is the ratio of the mass of the powder sample to its volume including the contribution of the inter-particulate void volume. The bulk density is expressed in grams per milliliter (g/ml) or grams per cubic centimeter (g/cm^3). The bulk volume (V_b) and weight of the powder (M) were calculated using the formula.

$$\rho_b = M/V_b$$

Tapped density

The tapped density is an increased bulk density attained after mechanically tapping a container containing the microsphere. The minimum volume (V_t) occupied in the cylinder and the weight (M) of the blend was taken. The tapped density (ρ_t) was calculated by using formula.

$$\rho_t = M/V_t$$

Angle of repose

The angle of repose or critical angle of repose of a granular material is the steepest angle of descent or dip relative to the horizontal plane to which a material can be piled without slumping. The angle of repose (Θ) was calculated using the formula

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

where h = height of the heap

r = radius of the heap

Compressibility Index (I)

Compressibility Index is an indication of the compressibility of a powder. The Carr index is calculated by the formula

$$C = 100[(V_b - V_t)/V_b]$$

Where V_b is the volume that a given mass of powder would occupy if let settled freely

V_t is the volume of the same mass of powder would occupy after "tapping down".

Hausner ratio (H_R)

The Hausner ratio is a number that is correlated to the flowability of a powder or granular material. The Hausner ratio is calculated by the formula.

$$H_R = \rho_t/\rho_b$$

Where ρ_b is the freely settled bulk density of the powder

ρ_t is the tapped density of the powder.

SHAPE AND SURFACE MORPHOLOGY[11]

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 microsphere chamber. The samples were then randomly scanned, and photomicrographs were taken at the acceleration voltage of 20 Kv.

PERCENTAGE DRUG ENTRAPMENT EFFICIENCY[12]

Accurately Weighed Microspheres equivalent to 100 mg and suspended in 100 ml pH 7.4 and kept for 24 hrs. Next day, stirred for 5 min and filtered. After suitable dilution, drug content was analyzed spectrophotometrically at 278 nm. The drug entrapment efficiency (%) was determined through the formula.

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

In-vitro dissolution studies[13]

- **Preparation of 0.1N HCl**

Take 8.3 ml of conc. HCl in a 1000 ml beaker and makeup with distilled water.

- **Preparation of stimulated colonic fluid (pH 7.4)**

Dissolved 27.218 gm Potassium dihydrogen phosphate in 1000 ml and 8gm of NaOH in 1000 ml. Take 375 ml Potassium dihydrogen phosphate and 293.22 ml of NaOH in 1000 ml standard flask sufficient water to produce 1000 ml

- **Procedure for dissolution**

The in vitro drug release study of colon targeting microsphere was carried out in 0.1N HCl for first 2 hrs. Then the dissolution medium was replaced with pH7.4 and the study was continued till the end of release study.

The drug dissolution test of microsphere was performed by the paddle type method using USP XXIII paddle type dissolution apparatus at 50 rpm and 37 ^{0}C ± 0.5 ^{0}C. Microspheres were weighed and filled in tea bags. The tea bags were tied using a thread with a paddle and loaded into the basket of dissolution apparatus containing 900 ml of dissolution medium. The samples (5 ml) were withdrawn from the dissolution medium at a time interval of 1 hrs using a pipette and analyzed for the drug by UV spectrophotometer at 278 nm. Perfect sink condition was maintained during the drug dissolution study period with the addition of an equal volume of fresh release medium at the same temperature.

Kinetics of *In-vitro* drug release[14]

The results obtained from in-vitro release studies were attempted to fit into various mathematical models as follows:

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

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

3) Cumulative percent released Vs. The square root of Time (Higuchi model)

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

In the Peppas model, the value of ‘n' characterizes the release mechanism of the drug as described in Table 2.

Table 2: Interpretation of diffusional release mechanism

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

Evaluation of anti-bacterial activity[15,16]

Well Diffusion Method

Well diffusion method, antimicrobial susceptibility testing was done to detect the presence of anti-bacterial activities in the optimized formulation of microspheres. A sterile swab was used to evenly distribute bacterial culture over the Nutrient agar medium. The plates were

allowed to dry for 15 min before use in the test. Wells were then created and 100 µg dilution of the optimized sample was added into each well for positive and negative controls. The plates were incubated at 26 °C for 48hrs after which they were examined for inhibition zones. Norfloxacin used as positive control. The clear zones of inhibition around the test sample disc were shown for any antimicrobial activity.

Stability Studies[17]

In order to determine the change in evaluation parameters like physical appearance, drug entrapment efficiency, in vitro drug release profile on storage, the stability studies were carried out. Stability studies of optimized formulation F2 were carried out by packing in aluminum foil which was kept in Petri dish at 40 ± 2 °C and 75 ± 5 %RH in a humidity chamber for three months. The sample was withdrawn after 90 days and evaluated for changes in physical appearance, drug entrapment efficiency, and in vitro drug release profile.

RESULTS AND DISCUSSIONS

❖ **Preformulation studies**

Determination of Organoleptic properties

The organoleptic properties of Norfloxacin were found to be White to yellowish powder, odorless and crystalline state.

Determination of Melting point

The melting point of Norfloxacin was found to be 220ºC.

Solubility

The solubility of Norfloxacin in various solvents such as distilled water, ethanol, methanol, glacial acetic acid and was studied and found that it was freely Very slightly soluble in ethanol, methanol, and water, freely soluble in glacial acetic acid.

❖ **Compatibility studies**

- **FT-IR spectroscopy of Norfloxacin**

The FT-IR spectrum of Norfloxacin shown in figure 1 complies with standard functional group frequencies.

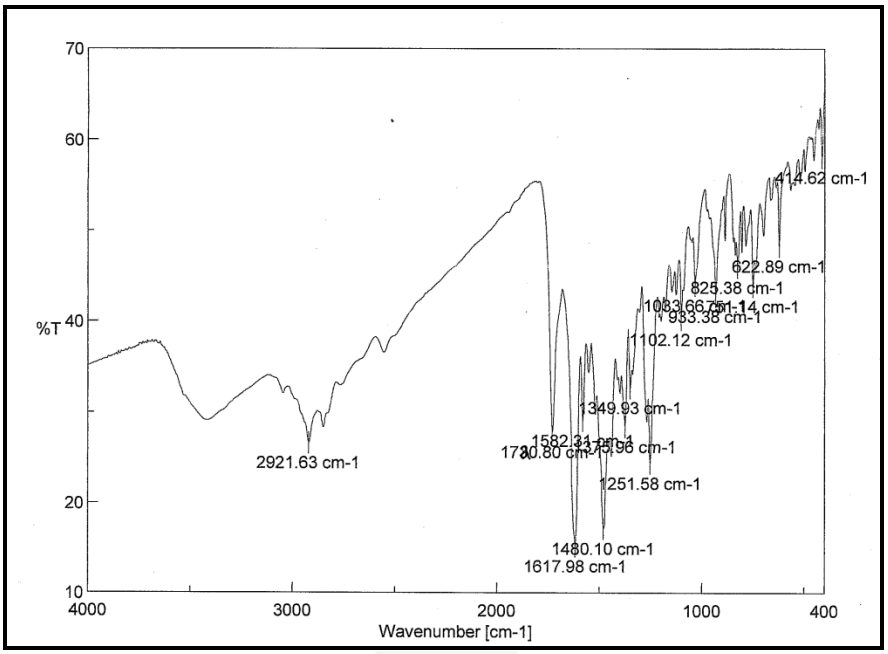

Figure 1: FT-IR spectrum of Norfloxacin

Table 3: IR frequencies of Norfloxacin

Functional group	**Characteristic wave number(cm^{-1})**	**Norfloxacin observed wave number (cm^{-1})**
NH stretching	3800-2800	2921.63
C=O stretching	1725-1705	1720.80
NH bending	1620-1560	1617.98
CN	1350-1280	1349.93
O-H bending	1300-1250	1251.58
CF	1150-1000	1102.12

- **Compatibility between drug and polymer**

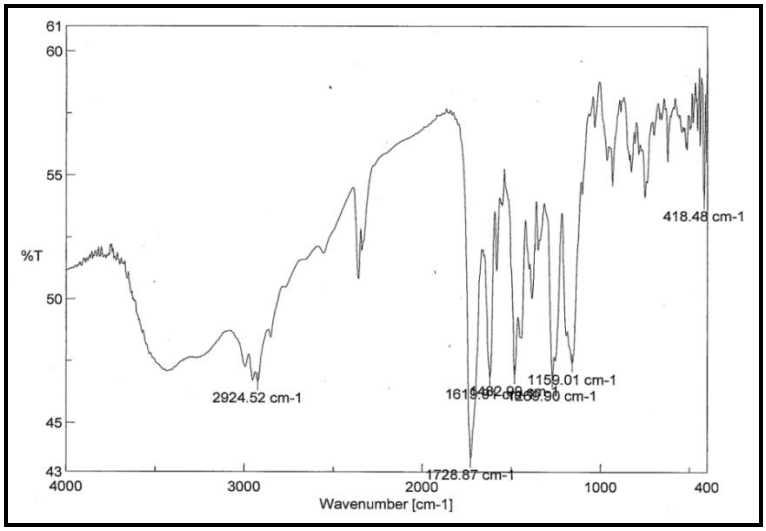

Figure 2: FT-IR spectrum of physical mixture Norfloxacin+ Eudragit S100 +Eudragit L100

Table 4: IR frequencies of physical mixture Norfloxacin+EudragitS100+EudragitL100

Functional group	**Characteristic wave number(cm^{-1})**	**Norfloxacin observed wave number (cm^{-1})**	**Norfloxacin + Polymer mixture (wave number) (cm^{-1})**
NH stretching	3800-2800	2921.63	2923.56
C=O stretching	1725-1705	1720.80	1729.83
NH bending	1620-1560	1617.98	1619.91
O-H bending	1300-1250	1251.58	1269.90

The compatibility between drug and polymer were carried out by using FT-IR peak matching method. All major peaks present in the spectrum of the pure drug were observed in the spectrum of the drug-polymer mixture. This suggests that the drug remains in its normal structure and hence this confirmed the absence of any chemical interaction or complexation between drug and polymers.

❖ **Preparation of a standard calibration curve of Norfloxacin**

Table 5: Calibration table of Norfloxacin at 278nm

Sl. NO.	Concentration (µg/ml)	Absorbance at 278 nm
1	0	0
2.	1	0.132
3.	2	0.261
4.	3	0.401
5.	4	0.560
6.	5	0.642

The calibration curve was found to be linear in the range of 1-5µg/ml at λmax 235nm

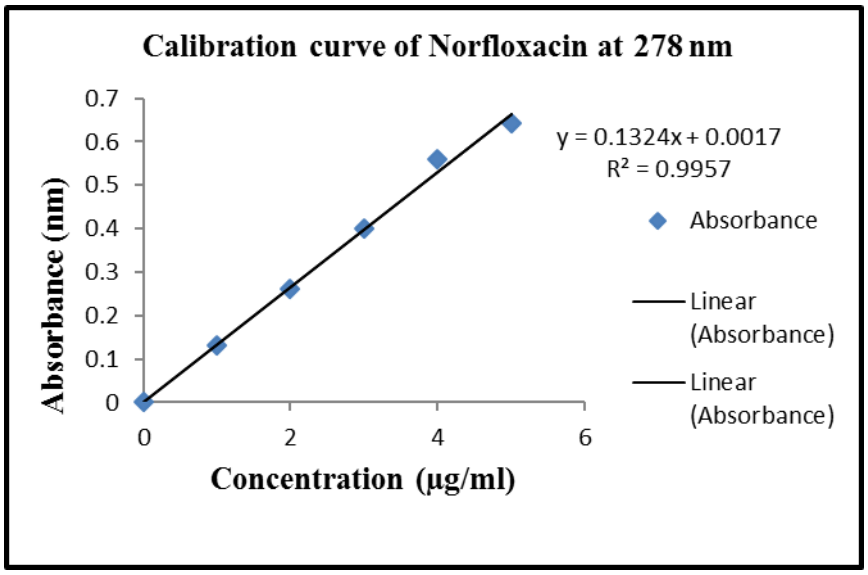

Figure 3: Calibration curve of Norfloxacin at 278nm

FORMULATION OF MICROSPHERE OF NORFLOXACIN

Nine formulations of colon targeting microspheres of Norfloxacin were prepared by an emulsion solvent evaporation method. F1-F5 formulations were prepared by using polymer Eudragit S in various proportions. The remaining formulations F6-F7 were prepared using Eudragit L and F8-9 were prepared using a combination of both polymers (Eudragit S & L).

EVALUATION STUDIES OF PREPARED MICROSPHERES

Table 6: Percentage yield and Particle Size analysis of formulations F1-F9

Formulation code	Percentage Yield (%±SD)	Particle size analysis (µm±SD)
F1	86.60 ± 0.214	27.1 ± 1.73
F2	94.71 ± 0.157	29.2 ± 1.25
F3	92.80 ± 0.360	26.0 ± 0.96
F4	90.95 ± 0.760	24.6 ± 1.34
F5	92.12 ± 0.120	23.2 ± 0.86
F6	91.00 ± 0.695	24.2 ± 1.83
F7	59.22 ± 0.612	28.2 ± 1.72
F8	67.14 ± 0.321	29.0 ± 1.85
F9	92.31 ± 0.412	28.5 ± 1.25

Micromeritic studies

Table 7: Micromeritic studies of formulations F1-F9.

Formulation code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle of repose	Compressibility index	Hausner's ratio
F1	0.11 ± 0.14	0.12 ± 0.14	30.91 ± 1.83	8.30 ± 0.33	1.09 ± 0.19
F2	0.12 ± 0.14	0.11 ± 0.25	26.50 ± 0.15	15.20 ± 0.90	1.18 ± 0.58
F3	0.13 ± 0.12	0.15 ± 0.06	32.15 ± 0.13	14.00 ± 1.80	1.16 ± 0.06
F4	0.12 ± 0.05	0.14 ± 0.07	30.89 ± 0.08	13.20 ± 0.60	1.15 ± 0.07
F5	0.16 ± 0.16	0.22 ± 0.21	30.51 ± 0.10	29.20 ± 0.31	1.40 ± 0.22
F6	0.17 ± 0.17	0.20 ± 0.19	30.64 ± 0.07	16.60 ± 0.52	1.20 ± 0.21
F7	0.21 ± 0.12	0.28 ± 0.20	32.33 ± 0.11	24.90 ± 0.25	1.33 ± 0.58
F8	0.11 ± 0.10	0.13 ± 0.04	27.36 ± 0.07	14.28 ± 1.20	0.93 ± 0.15
F9	0.21 ± 0.21	0.23 ± 0.12	29.45 ± 0.09	9.36 ± 0.35	1.16 ± 0.21

In-vitro dissolution studies

In-vitro dissolution studies of all formulations were carried out in dissolution test apparatus using 0.1N HCl for 3hrs and then in the stimulated colonic fluid as the dissolution medium for 7hr. Percentage cumulative drug release at each time interval as shown in the table and the data represented graphically.

Table 8: Percentage cumulative drug release data for Formulations F1-F5

Time(hr)	F1 %CDR	F2%CDR	F3%CDR	F4%CDR	F5%CDR
0	5.02	5.58	6.28	6.09	5.72
1	7.54	6.28	9.63	6.98	5.86
2	8.10	7.05	11.24	7.40	8.93
3	20.46	25.30	23.48	33.59	22.95
4	34.85	30.49	38.44	40.65	27.52
5	47.71	44.66	48.12	49.78	34.29
6	52.55	56.00	58.77	59.32	44.66
7	54.90	65.27	68.87	69.00	54.62
8	56.14	72.05	72.19	77.99	62.78
9	72.19	84.77	85.88	89.20	73.57
10	89.47	98.76	93.21	92.51	83.25

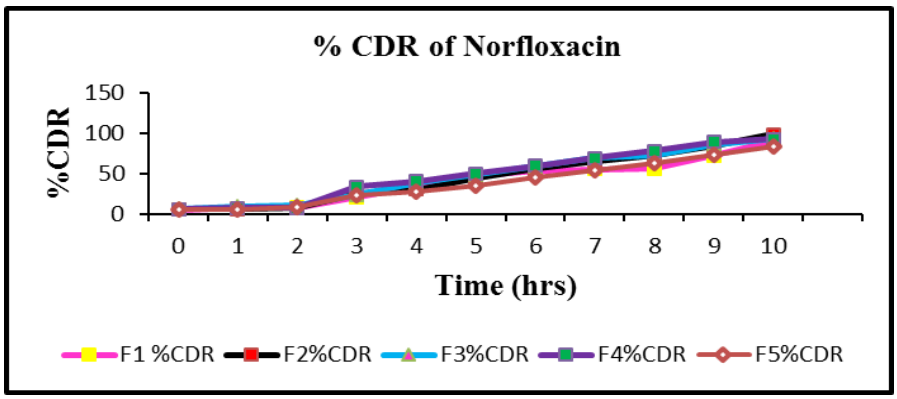

Figure 4: Percentage CDR release profile of Norfloxacin formulation F1-F5

Table 9: Percentage cumulative drug release data for Formulations F6-F7

Time (hrs)	F6 % CDR	F7 % CDR
0	5.93	6.14
1	7.19	7.68
2	7.47	7.96
3	17.14	25.72
4	22.68	27.38
5	30.97	30.42
6	44.80	32.49
7	53.79	40.65
8	60.57	44.94
9	68.04	53.24
10	74.95	58.22

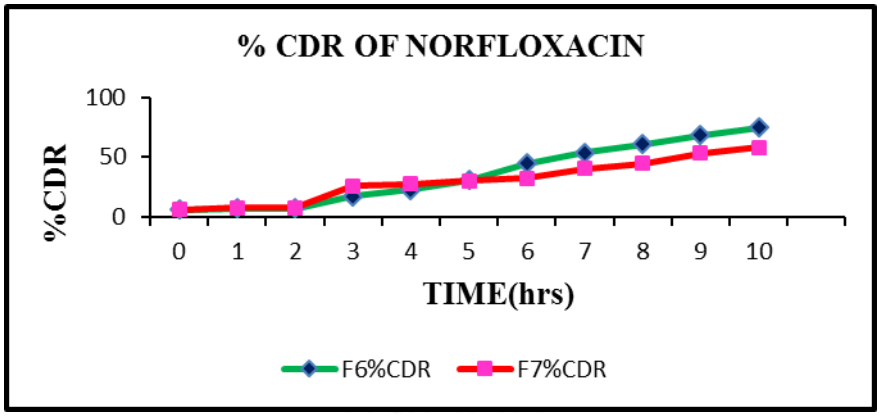

Figure 4.1: Percentage CDR release profile of Norfloxacin formulation F6-F7

Table 10: Percentage cumulative drug release data for Formulations F8-F9

Time (hr)	F8 % CDR	F9 % CDR
0	5.02	6.14
1	6.14	7.75
2	7.05	7.89
3	21.43	24.34
4	27.79	27.52
5	32.63	32.08
6	44.79	34.98
7	51.00	40.51
8	59.74	48.54
9	66.51	54.07
10	73.57	58.2

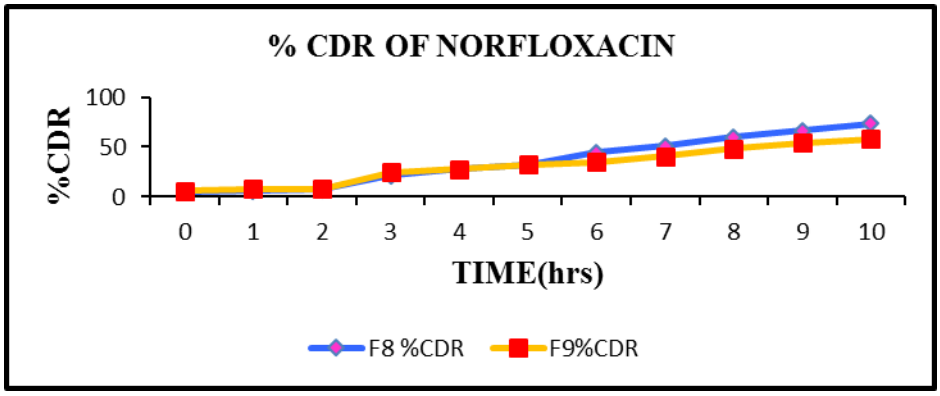

Figure 5: Percentage CDR release profile of Norfloxacin formulation F8-F9

The percentage drug release was found best in formulation F2 with 98.76 % release and least drug release was observed in formulation F7 and F9 with 58.22 %. The percentage drug release was in the range of 89.47 to 98.76 % at the end of the 10th hour for microspheres prepared with Eudragit S100. The formulations F6 & F7 prepared by Eudragit L100 showed drug release in the range of 58.22 % and 74.95 % and formulations F8 & F9 prepared by the combination of both the polymers showed drug release was in the range of 58.22 % and 73.57 %. The drug release for the formulation was found to be less than 10 % till the end of a 2nd hour even if the microsphere does not burst. After 4 hrs the release was increased this shows the pH sensitivity of the polymer releasing the drug only in the colon region. Thus the in vitro performance of Norfloxacin microspheres showed prolonged and controlled release.

Kinetic modeling of dissolution profiles

The kinetic models that fit the dissolution data were evaluated by comparing the regression coefficient (r^2) values obtained in various models. The release kinetics data of optimized formulation indicates that the release of drug best fits to zero-order release kinetics, the regression coefficient (r^2) values of F2(0.980). Similarly, the r^2 value of Peppas model (0.952) and corresponding n value is 1.313. In the formulation F1, F2, F3, F4, F5, F8 follow super case II transport. In F7 & F9 follow case-II transport. The regression coefficient values (r^2) obtained after fitting into various kinetic models.

Table 11: Regression coefficient (r^2) values of all formulations (F1-F9)

Formulation code	Drug release kinetics				
	Zero-order R^2	First order R^2	Higuchi R^2	Peppas	
				R^2	n
F1	0.958	0.812	0.844	0.939	1.155
F2	0.980	0.716	0.830	0.952	1.313
F3	0.985	0.879	0.800	0.957	1.013
F4	0.977	0.908	0.874	0.922	1.254
F5	0.981	0.895	0.834	0.982	1.204
F6	0.938	0.905	0.817	0.941	1.125
F7	0.967	0.956	0.867	0.919	0.939
F8	0.983	0.950	0.852	0.956	1.187
F9	0.975	0.970	0.876	0.948	0.972

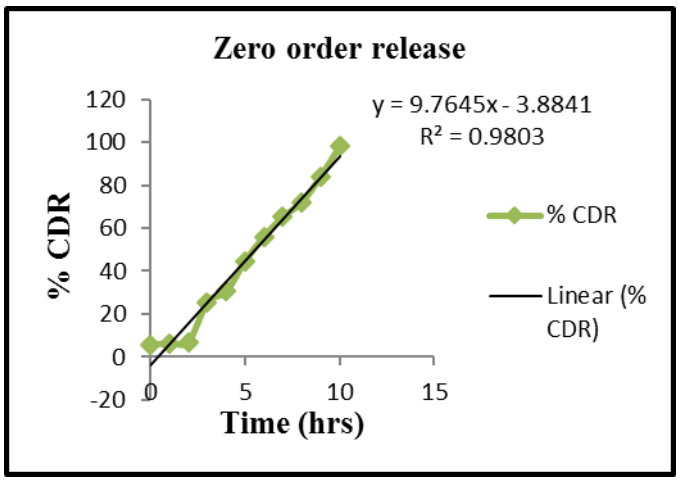

Figure 6: Zero order plot of F2

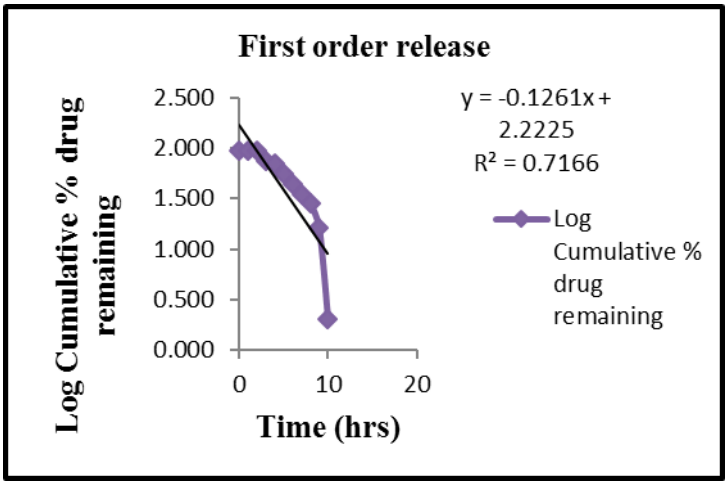

Figure 7: First order plot of F2

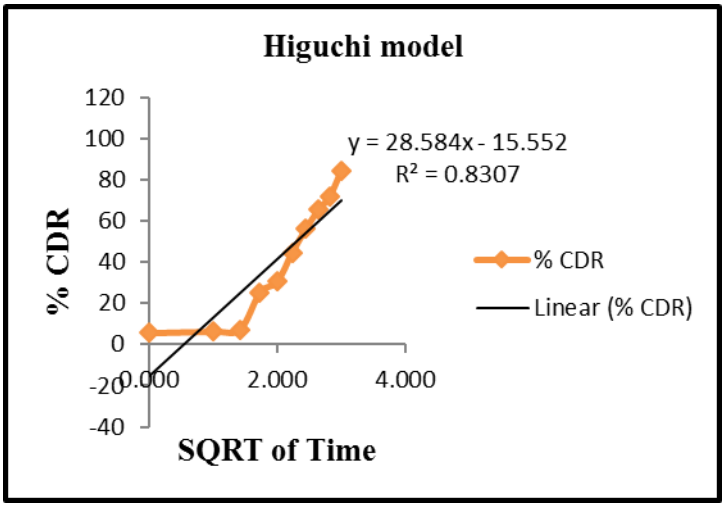

Figure 8: Higuchi model plot of F2

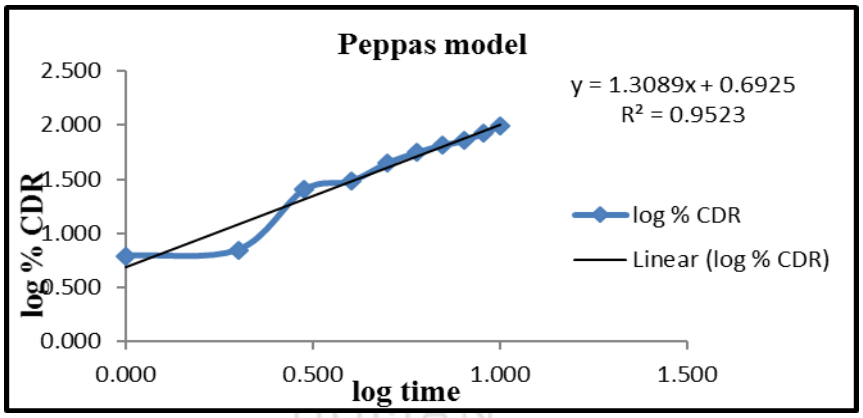

Figure 9: Peppas model plot of F2

Evaluation of antibacterial activity by a Well diffusion method

Figure 10: Clear zone of inhibition showed by formulation F2.

Stability studies:

Stability studies were carried out on formulation F9 for a period of 3 months and comparison of the parameters before and after stability studies was represented in table 12, 13and 14.

Table 12: Physical appearance of the optimized formulation before and after stability

Formulation code	Physical properties	Physical properties before 90dys	Physical properties after 30 days at 40±2 °C and 75 ± 5 % RH
F2	Color of microsphere	Pure white	white
	Shape of microsphere	Spherical	Almost spherical

Table 13: Drug entrapment efficiency of optimized formulation

Formulation code	Drug entrapment efficiency
F2	68.9

In vitro drug release studies

Table 14: % CDR of the optimized formulation before and after stability studies

Sl.No.	Time(hr)	% CDR before stability study	% CDR after stability studies
1.	0	5.58	5.55
2.	1	6.28	6.23
3.	2	7.05	7.10
4.	3	25.30	25.30
5.	4	30.49	30.42
6.	5	44.66	44.65
7.	6	56.00	56.21
8.	7	65.27	63.28
9.	8	72.05	70.25
10	9	84.77	82.85
11.	10	98.76	94.87

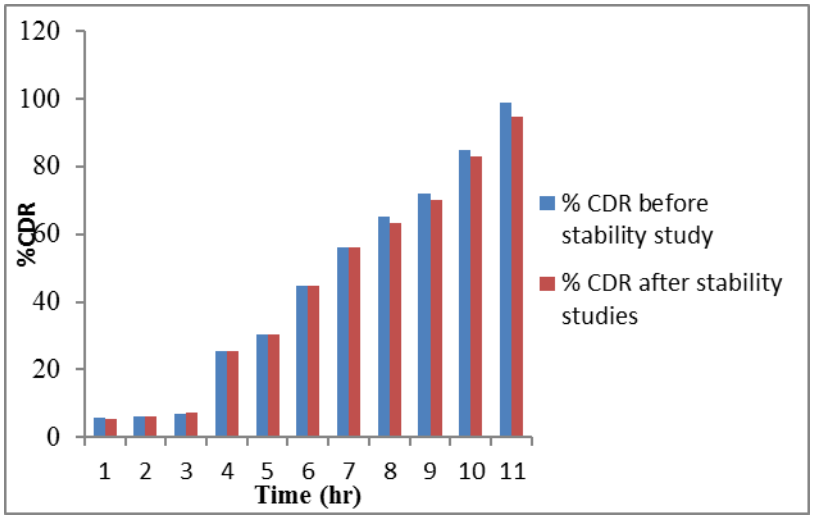

Figure 11: Release pattern of the optimized formulation before and after stability study

CONCLUSION

Colon targeting drug delivery systems are designed on the basis of site-specific delivery is to deliver the drug to the specific organs of the body. Preformulation studies like organoleptic properties, melting point, and solubility analysis were carried out and they comply with the standard. The FTIR spectral data indicates there was no incompatibility between the drug and the polymers. All the polymers are compatible with the drug.

Nine formulations were prepared by an emulsion solvent evaporation method and were evaluated. All the prepared microspheres were subjected to various evaluation parameters like percentage yield, particle size analysis, micromeritic studies, percentage drug entrapment efficiency, in vitro drug release studies, in vitro kinetic studies, anti-bacterial activity studies, and stability studies.

The percentage yield of the development formulation (F1-F9) of Norfloxacin microspheres was found to be in the range of 86.60 to 94.71% increased with polymer concentration. The mean particle size of the Norfloxacin microsphere was found to be in the range of 24.02-29.02µm. Micromeritic studies revealed that the prepared microspheres exhibited a good flow property.

Percentage drug entrapment efficiency of prepared microspheres was in the range of 43-71%. Formulation F2 with the drug: polymer ratio (1:3.5) having a better drug entrapment efficiency.SEM analysis of the microspheres revealed that all the prepared microspheres were spherical in shape. In vitro kinetics data revealed that the release of drug best fits to

zero order release kinetics the regression coefficient of F2 = 0.980**.** Similarly, the regression coefficient value of F2 is 0.952. In the formulation F1, F2, F3, F4, F5, F8 follow super case II transport. In F7 & F9 follow case- II transport

Stability studies of the optimized formulation indicate that there is no significant changes in the physical appearance, drug entrapment efficiency, in vitro dissolution studies after 90 days of storage conditions at temperature 40 ± 2^0C and 75 ± 5 % RH in a humidity chamber. From the evaluation of antibacterial activity, it was observed that the optimized formulation showed a clear zone of inhibition around the sample well compared to that of the standard.

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