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RESEARCH ARTICLE

Formulation, optimization and evaluation of gastro-retentive floating microspheres of norfloxacin

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ABSTRACT

The objective of the present study was to develop floating microsphere of norfloxacin in order to achieve an extended retention in the upper GIT which may enhance the absorption and improve the bioavailability. The microspheres were prepared by solvent evaporation method using different ratio of hydroxyl propyl methyl cellulose (HPMC K4M, HPMC K15M) and ethyl cellulose with drug in the mixture dichloromethane and ethanol at ratio of (1:1) with tween 80 (stabilizing agent). FTIR study shows that drug and other excipients are compatible with each other. The effects of polymer concentration on drug release profile were investigated. A 3^3 centre-composite design was applied to systemically optimize the drug release profile. Polymer to drug ratio; HPMC K4M (X_1), HPMC K15M (X_2) and stirring speed (X_3) were selected as independent variables. The floating microspheres were characterized by the results obtained as percentage yield, percentage buoyancy and *in-vitro* drug release was studied for 12 hour. The Surface morphology studied by scanning electron microscopy and Accelerated stability study was performed for three months indicated that optimized formulation was stable. The floating microspheres showed better result and it may be useful for prolong the drug release in stomach and improve the bioavailability.

Keywords: Floating microspheres, Norfloxacin, Gastroretentive, GIT, Non-aqueous solvent evaporation method, Microencapsulation, Hydroxyl Propyl Methyl Cellulose (HPMC), Optimization, Centre-composite design, *In-vitro* release studies and Bioavailability.

1. INTRODUCTION:

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). It remains in the gastric region for several hours and hence prolongs the GRT of the drug. It has several advantages over immediate release dosage form including the minimization of fluctuation in drug concentration in plasma and at the site of action over prolonged periods of time, resulting in optimized therapeutic efficiencies and reduce the side effect reduction of total dose administered and reduction of administration frequency leading to improved patient compliances.[1,2]

Floating microspheres are gastro-retentive drug delivery systems based on non-effervescent approach. These microspheres are characteristically free flowing powders having a size less than 200µm and remain buoyant over gastric contents and for prolonged period. As the system floats over gastric contents, the drug is released slowly at desired rate resulting in increased gastric retention with reduced fluctuation in plasma drug concentration.[3,4,5]

Norfloxacin is classified as an antibacterial belongs to Fluoroquinolone category. It has mean plasma half life of 3.5-4.5 hour and reduced bioavailability (30-40%). The fluoroquinolone antibacterial in general and norfloxacin in particular are bactericidal in their action. Carboxylic acid group and the ketone group are responsible for the antibacterial activity. Quinolones also inhibits the *in-vitro* activities of DNA topoisomerase-IV by interfering with separation of replicated chromosomal DNA into respective

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daughter cells during cell division. Norfloxacin has a short half life and low bioavailability in the upper GIT hence it is suitable for gastro-retentive system.[6]

The aim of the present work was preparation and evaluation of floating microspheres of norfloxacin using 3^3 centre-composite design. Layout by selecting independent variables like polymer (HPMC K4M, HPMC K15M) in different proportions and stirring speed at different rotations per minute.

2. MATERIALS AND METHODS

MATERIALS:

Norfloxacin is obtained as a free sample from Panacia pharma, Delhi and HPMC of both grades from Magus Pharmaceuticals, Mohali. All other polymers and solvents used were of pharmaceutical or analytical grade.

METHODS:

DRUG-EXCIPIENTS INTERACTION STUDIES:

Assessment of possible incompatibilities between an active drug substance and different excipients forms an important part of the preformulation stage during the development of solid dosage forms. Fourier Transform Inferred (FTIR) spectroscopy was carried out to check the compatibility between drug and polymer. The excipients used were HPMC K4M, HPMC K15M, and Ethyl cellulose. Norfloxacin and different excipients short listed for the preparation of microspheres were physically mixed in the ratio 1:1 by geometric mixing, filled in vials and kept at accelerated stability study conditions (40±2°C/75±5% RH). The samples were taken at predetermined time points (0, 2, 4, 7, 10, 12 and 14 days) and examined visually for any physical change in appearance.

The drug alone, and in combination with different excipients (mixed in the ratio of 1:1) was taken and subjected to FTIR studies. For proposed study the samples were properly diluted with dried KBr and IR spectra were acquired in the range of 400-4000 cm^{-1} with resolution of 4 cm^{-1} using Perking Elmer 1600.[7]

PREPARATION OF FLOATING MICROSPHERES:

Microspheres containing norfloxacin as a core material were prepared by non-aqueous solvent evaporation method. Ethyl cellulose, HPMC K4M and HPMC K15M were mixed in the mixture of dichloromethane and ethanol at 1:1 ratio. Drug separately mixed into small amount of glacial acetic acid and then added to above solution of polymers. The slurry was slowly introduced by syringe into 100ml of liquid paraffin containing 0.01% tween 80 while being stirred at 800 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 whatman 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 procedure was repeated for all the batches.[8]

CENTRE-COMPOSITE DESIGN:

A 3^3 centre-composite design was used in this study. In this design 3 factors were evaluated, each at 3 levels, and experimental trials are performed at all 20 possible combinations. The polymer HPMC K4M (X_1) HPMC K15M (X_2) and Stirring speed (X_3) were selected as independent variables. Percentage buoyancy, percent yield and percent drug release after 12hour were selected as dependent variables.[9]

EVALUATION OF MICROSPHERES:

Percentage Yield: The prepared microspheres were collected and weighed. The percentage yield of prepared microspheres was calculated[10] using Eq. 1.1.

$$\% \text{ Yield} = \frac{\text{Weight of microspheres obtained}}{\text{Total weight of drug and polymer used}} \times 100 \qquad \text{(Eq. 1.1)}$$

Particle Size: Particle size was measured using an optical microsphere, and the mean particle size was calculated by measuring 100 microspheres with the help of calibrated ocular micrometer.[11]

Buoyancy Percentage: 50 mg of floating microspheres were spread over simulated gastric fluid (pH 1.2, 900 ml) containing 0.02% w/v tween 80 in dissolution apparatus USP type-II agitating at the speed of 100rpm. After 12hrs the buoyant microspheres were pipette out and separated by filtration; particles in the sinking particulate layer were also separated by filtration. Particles of both types were dried in desiccators. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles as per the Eq 1.2.[12]

$$\text{Buoyancy } (\%) = \frac{Wf}{Wf + Ws} \times 100 \qquad \text{(Eq 1.2)}$$

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

Micromeritic Properties: The floating microspheres were characterized by their Micromeritic properties such as bulk density, tapped density, hausner's ratio carr's index and angle of repose.[10]

***In-Vitro* Drug Release Studies**: The *in-vitro* drug release studies of optimized formulation was carried out using Indian Pharmacopoeia (IP type-I) dissolution apparatus. Drug loaded microspheres equivalent to 100 mg of drug was introduced into 900 ml of HCl buffer (pH 1.2) maintained at 37 ± 0.5°C and stirred at 100 rpm. The samples were withdrawn after 1 hrs interval up to 12 hrs, and replaced with the same volume of fresh dissolution medium. The percentage drug release from optimized formulation was measured spectrophotometericaly at 279

nm. The graph was considered between cumulative percentage drug release and time for the optimized formulation.[10]

Scanning Electron Microscopy: The size and surface morphology of microspheres were studied using scanning electron microscopy (SEM). The sample for SEM was 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 100Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The stub containing the coated samples was placed in the (JSM 6100/Jeol/Japan) chamber.[13]

Stability Studies: The stability studies were carried out of the optimized formulation i.e., formulation A1. The formulation was stored at (40±2°C/75±5% RH) for a period of 3 months (Climatic zone IV condition for accelerated testing) to access their stability. The protocol of stability was in compliance with the WHO guidelines for the stability testing intended for the global market. After 7, 15, 30, 60 and 90 days samples withdrawn and retested for drug content, floating behavior and drug release.[13,14]

3. RESULTS AND DISCUSSION

FTIR STUDY: Drug excipients interactions play a vital role with respect to release of drug from the formulation amongst others. FTIR has been used here to study the physical and chemical interactions between the drug and excipients used; it has been observed that there is no chemical interaction between norfloxacin and the polymers used. The results of the FTIR study reported in fig 1.1, 1.2, 1.3 and 1.4.

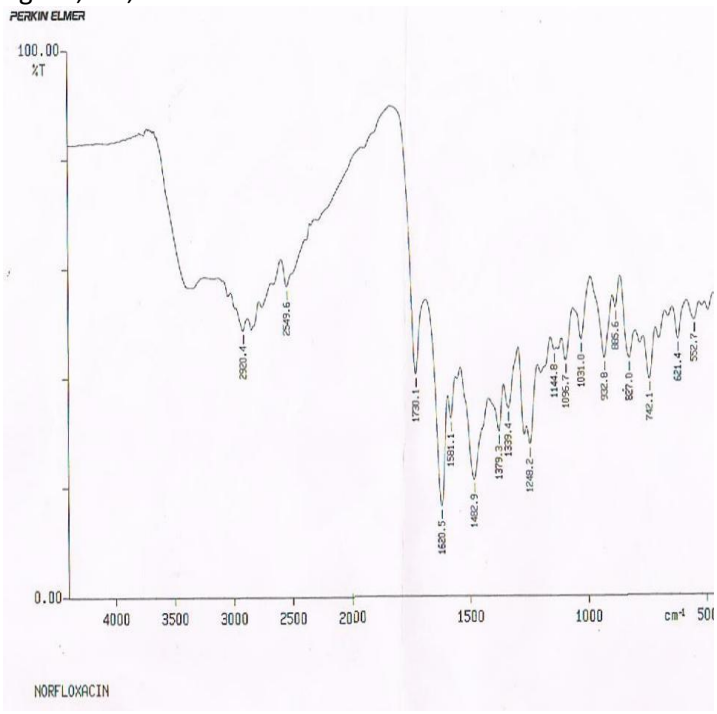

Fig 1.1: Scanned picture showing FTIR spectrum of Norfloxacin alone.

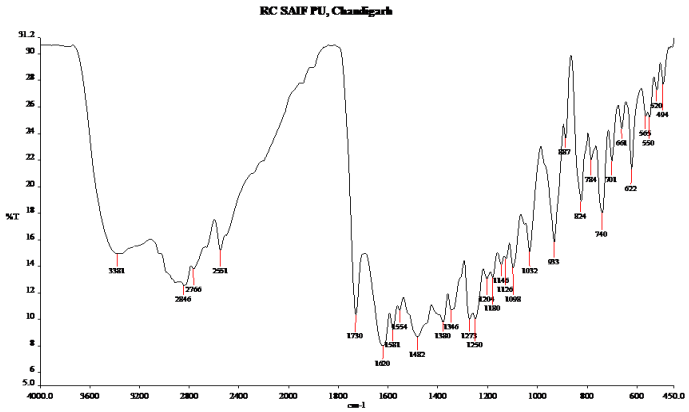

Fig 1.2: Scanned picture showing FTIR spectrum of physical mixture of Norfloxacin and HPMC K4M.

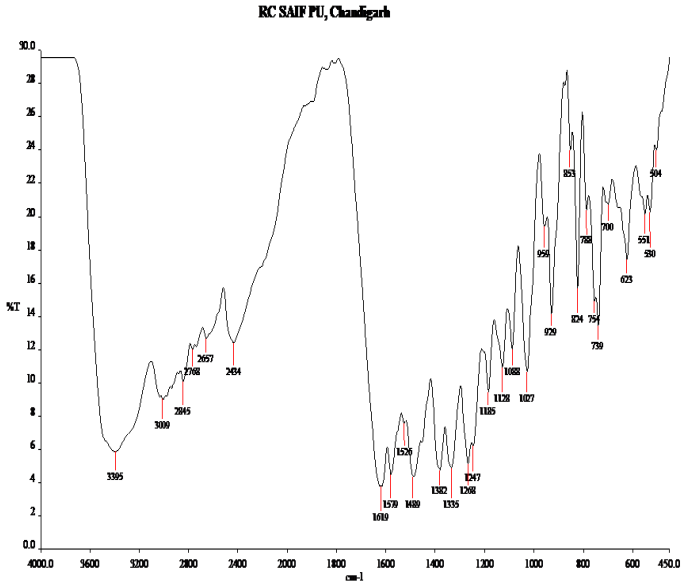

Fig 1.3: Scanned picture showing FTIR spectrum of physical mixture of Norfloxacin and HPMC K15M.

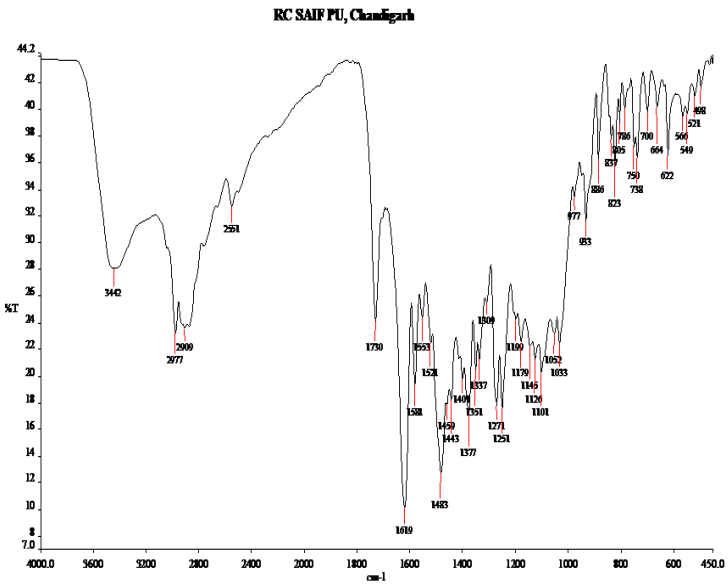

Fig 1.4: Scanned picture showing FTIR spectrum of physical mixture of Norfloxacin and Ethyl cellulose.

Formulation code	X_1(mg)	X_2(mg)	X_3(rpm)	Percent buoyancy	Percentage Yield	D_{12}
F1	1.00	1.00	800	76.2	74.5	92.3
F2	1.00	1.84	800	75	75	92.1
F3	0.16	1.00	800	74	75	90.3
F4	1.50	0.50	600	80.2	80	93.5
F5	1.84	1.00	800	79.3	79.3	88.3
F6	1.50	1.50	600	75	75	90.4
F7	1.00	1.00	800	75.8	74	92.4
F8	1.00	1.00	463.6	74	74	91.5
F9	1.50	0.50	1000	81.2	82.3	93.5
F10	0.50	0.50	600	75	75.5	91.4
F11	0.50	1.50	600	73.5	74	89.5
F12	1.00	1.00	800	76.8	75.1	92.1
F13	1.50	1.50	1000	80.7	80.7	92.1
F14	1.00	1.00	800	76.4	73.7	92.4
F15	1.00	1.00	800	76.5	75.6	92.4
F16	1.00	1.00	1136.4	75.5	73.4	91.6
F17	1.00	0.16	800	81	82	93.5
F18	1.00	1.00	800	76.1	74.6	91.3
F19	0.50	1.50	1000	76	76	92.8
F20	0.50	0.50	1000	78.5	79	92.1

Table 1.1: Factorial design based Norfloxacin floating microspheres.

Coded values	Actual values		
	X1	X2	X3
-1	0.5	0.5	600
0	1	1	800
1	1.5	1.5	1000

Table 1.2: Coded value and actual values used for optimization.

MICROMERITIC PROPERTY: The bulk density, tapped density, Hauser's ratio and angle of repose of formulation F1-F20 (Table 1.3) ranges from 0.234±0.02 to 0.743±0.02 gm/cm^3,

Parameters / Formulation	Bulk density (g/cc)	Tapped density (g/cc)	Carr's index (%)	Hausner ratio	Angle of repose (θ)
F1	0.436±0.02	0.480±0.01	9.17±0.17	1.101±0.01	29.50±0.08
F2	0.312±0.01	0.347±0.02	10.09±0.26	1.112±0.03	27.87±0.12
F3	0.234±0.02	0.275±0.02	14.91±0.43	1.175±0.02	31.42±0.06
F4	0.337±0.01	0.378±0.01	10.85±0.39	1.122±0.01	26.56±0.18
F5	0.478±0.01	0.527±0.01	9.30±0.22	1.103±0.05	28.98±0.20
F6	0.539±0.01	0.604±0.02	10.76±0.34	1.121±0.01	26.56±0.13
F7	0.463±0.02	0.524±0.02	11.64±0.28	1.132±0.04	29.07±0.17
F8	0.548±0.02	0.603±0.01	9.12±0.25	1.100±0.03	28.81±0.22
F9	0.677±0.01	0.733±0.02	7.64±0.34	1.083±0.03	27.56±0.15
F10	0.296±0.02	0.344±0.02	13.95±0.28	1.162±0.02	24.23±0.19
F11	0.584±0.01	0.651±0.02	10.29±0.23	1.115±0.01	21.05±0.33
F12	0.743±0.02	0.789±0.01	5.83±0.35	1.062±0.04	23.74±0.24
F13	0.646±0.01	0.694±0.01	6.92±0.38	1.074±0.02	29.98±0.21
F14	0.367±0.01	0.435±0.02	15.63±0.31	1.185±0.01	23.41±0.17
F15	0.445±0.02	0.504±0.02	11.71±0.24	1.133±0.02	23.22±0.25
F16	0.438±0.01	0.497±0.02	11.87±0.19	1.135±0.03	23.26±0.28
F17	0.582±0.01	0.645±0.01	9.77±0.27	1.108±0.01	23.94±0.24
F18	0.328±0.01	0.374±0.01	12.30±0.29	1.140±0.02	20.15±0.35
F19	0.460±0.02	0.512±0.01	10.16±0.38	1.113±0.04	24.80±0.18
F20	0.578±0.01	0.634±0.01	8.83±0.26	1.097±0.02	28.06±0.26

Table 1.3: Micromeritic evaluation of microspheres.

0.275±0.02 to 0.789±0.01 gm/cm^3, 1.062±0.04 to 1.185±0.01, 20.15±0.35 to 29.50±0.08 respectively. The value of Carr's index 5.83±0.35 to 15.63±0.31 shows excellent and angle of repose indicate good flow properties.

Coefficient	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_1^2	b_2^2	b_3^2
Percent buoyancy	76.27	1.69	-1.45	1.11	-0.21	0.088	0.46	0.34	0.82	-0.33
Percent yield	74.54	1.52	-1.67	0.91	-0.26	0.31	0.24	1.17	1.65	-0.049
D_{12} hr	92.13	0.025	-0.59	0.43	-0.41	-0.29	0.54	-0.85	0.39	-0.053

Table 1.4: Fitted equation relating the responses percentage buoyancy, percentage yield and drug release.

PRPERATION OF PREDICTED OPTIMUM FORMULATION:

The optimized formulation (A1) was prepared with chosen optimal composition and evaluated for buoyancy percentage, percentage yield and percentage drug release. The observed and predicted responses were critically compared.

Ingredients	Amount (g)
Ethyl cellulose	1.0
HPMC K4M	0.50
HPMC K15M	1.50
Stirring speed	986.74 rpm
Parameters	**Results**
Percentage Buoyancy	80.78
Percentage Yield	82.23
D_{12} hrs	91.8

Table 1.5: Optimized Norfloxacin floating microspheres formulation and evaluation results.

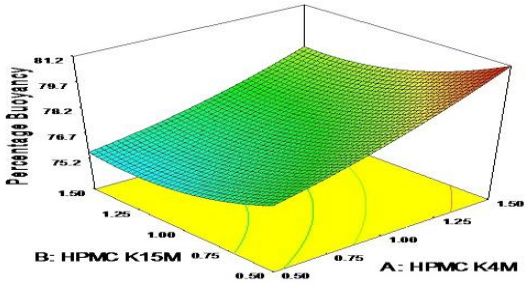

Fig. 1.5: Response surface (B) showing the effect of X_1 and X_2 on percent buoyancy (Y_1).

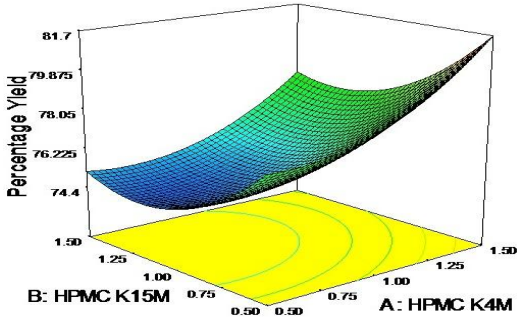

Fig. 1.6: Response surface showing the effect of X_1 and X_2 on percent yield (Y_2).

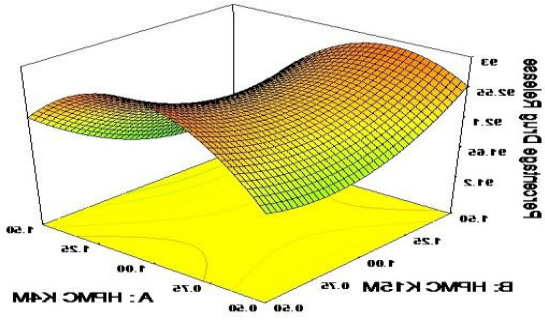

Fig. 1.7: Response surface showing the effect of X_1 and X_2 on percent percentage drug release (Y_3).

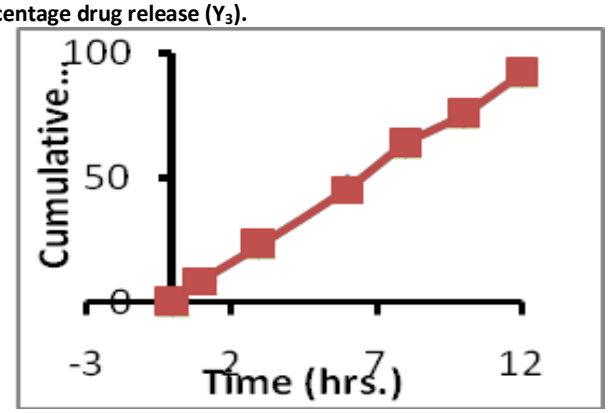

Fig. 1.8: *In vitro* drug release curve of optimized (A1) formulation.

Kinetics of Drug Release:

To study the drug release kinetics, data obtained from *in-vitro* release wre plotted in various kinetics models, the data reported tabulated in Table 1.6.

Formulation code	Zero order		First order		Higuchi		Hixon		Peppa's	
	R^2	K	R^2	K	R^2	K	R^2	K	R^2	N
A1	0.998	7.57	0.879	-0.086	0.973	33.71	0.947	-0.214	0.998	0.949

Table 1.6: Fit of various Kinetic Models for the microspheres of Norfloxacin.

SCANNING ELECTRON MICROSCOPY: The morphology of microspheres was examined using SEM. The view shows spherical structure with a smooth surface morphology (Fig 1.9) some of the microspheres shows a little ruff surface, but they showed good floating ability on the surface of the medium indicating intact surface.

Fig. 1.9: Scanning electron microscopy image of optimized formulation (A1).

STABILITY STUDIES:

No significant difference was observed in release profile of optimized formulation (A1) indicating that the fabrication process employed was reliable and reproducible. Further there was no change in physical appearance at the end of 90 days storage period at accelerated conditions (40±2°C/75 ± 5% RH). The optimized formulation was subjected for the estimation of weight variation, buoyancy percentage and in vitro release and there was no significant change in

Variation, buoyancy percentage and in vitro release as reported in Table 1.7

Time interval (Days)	Physical appearance	Weight variation	Buoyancy percentage	Percentage drug release (D_{12})
0	Creamy white	100±0.52	80.78±0.22	91.80±0.27
15	Creamy white	100±0.34	80.64±0.24	91.78±0.28
30	Creamy white	100±0.33	80.67±0.23	91.79±0.27
45	Creamy white	100±0.31	80.71±0.25	91.80±0.26
60	Creamy white	100±0.40	80.56±0.24	91.80±0.24
75	Creamy white	100±0.42	80.48±0.24	91.74±0.25
90	Creamy white	100±0.39	80.59±0.26	91.76±0.28

Table 1.7: Effect of storage conditions on optimized formulation at accelerated storage condition (40±2°C/75 ± 5% RH).

5. CONCLUSION

The results of the present study indicate that the floating microspheres of Norfloxacin exhibited well controlled and delayed release pattern. It was observed that the increase in polymer concentration, the entrapment efficacy as well as percentage yield increases. The *in-vitro* release studies showed better release profile with the formulation A1. This can be concluded that by formulating norfloxacin as floating microspheres can improve the low oral bioavailability by expended drug release in the upper part of stomach.

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Conflict of Interest: None Declared

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