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Design, Optimization and Characterization of Ofloxacin Nanoparticles

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ABSTRACT

The objective of this study is to prepare and evaluate nanoparticles of ofloxacin a fluoroquinolone derivative with a natural polymer like gelatin by coacervation phase separation method and chemical cross-linking method induced by the addition of glutaraldehyde a cross-linking agent. The Pre-formulation studies for the drug and polymer like compatibility studies by FT-IR spectroscopy, Thermal degradation studies and Solubility was performed. Five formulations (F1-F5) was prepared by changing the drug-polymer ratio and evaluated for various parameters like surface morphology by Scanning Electron Microscopy (SEM), particle size analysis, percentage yield, Drug entrapment efficiencies and *in-vitro* dissolution studies using USP dissolution apparatus. The effect of drug-polymer ratio on various parameters like particle size distribution, percentage yield, drug entrapment efficiency and *in-vitro* drug release was studied. Out of five formulations, F3 & F4 formulation showed drug release profile for an extended period of time and, mathematics the kinetic modelling of release data of ofloxacin from the gelatin nanoparticles exhibiting Korsmeyer-peppas model with n value 0.76 stating that the release of the drug from nanoparticles followed anomalous non fickian diffusion.

INTRODUCTION

During last two decades, considerable attention has been given to the development of Novel Drug Delivery System (NDDS). The rational for control drug delivery is to alter the pharmacokinetics and pharmacodynamics of drug substance in order to improve therapeutic efficacy and safety through the use of novel drug delivery system. Besides more traditional matrix or reservoir drug delivery system, colloidal drug delivery system has gained in popularity. The major colloidal drug delivery system includes liposome and polymeric nanoparticles. These systems have been investigated primarily for site specific drug delivery, for controlled drug delivery, and also for the enhancement of dissolution rate/bioavailability of poorly water-soluble drugs. The primary routes of administration under investigation are parenteral route, however, other routes such as the oral, ocular, or topical routes are also being investigated. In era of oral drug delivery system microsphere, microcapsule, nanoparticles, liposomes, and niosomes are better options to conventional dosage form. Nanoparticles are colloidal polymer particles of a size below 1mm and hold promise as drug delivery for parenteral, peroral and ocular administration as well as adjuvant for vaccines.(1,2)

Due to their greater stability and due to their easier manufacturing they offer advantages over other colloidal carriers such as liposomes and cell ghosts. They offer advantages like increased bioavailability, site specific drug delivery, sustained release of drug over longer period of time, retention of dosage form in entire length of gastrointestinal tract and convenient to patient due to reduction in frequent dosing. (3,4) Ciprofloxacin is a second generation fluoroquinolone antibiotic that is widely used in the therapy of mild-to-moderate urinary and respiratory tract infections caused by susceptible organisms. (5) Ciprofloxacin (sip" roe flox' a sin) is an oral fluoroquinolone that is used to treat mild-to-moderate urinary and respiratory tract infections. (6) Ciprofloxacin is also used for infectious diarrhea, typhoid fever, uncomplicated gonorrhea, treatment of Neisseria meningitides nasal carriage and prophylaxis against anthrax. (7,8) Like other fluoroquinolones, ciprofloxacin is active against a wide range of aerobic gram-positive and gram-negative organisms. The fluoroquinolones are believed to act by inhibition of type II DNA topoisomerase (gyrases) that are required for synthesis of bacterial mRNAs (transcription) and DNA replication. (9,10) Ciprofloxacin is available in multiple oral formulations of 100, 250, 500 and 750 mg tablets and extended release formulations of 500 and 1000 mg tablets. (11) Pharmacokinetics: The pharmacokinetic profile of ofloxacin tablets is comparable to the profile of ofloxacin administered i.v.(12) The bioavailability of ofloxacin

in the tablet formulation is approximately 98%. Ofloxacin is rapidly and completely absorbed from the upper small bowel following oral administration. Elimination is mainly by renal excretion. ofloxacin undergoes minimal biotransformation. Gelatin is a purified protein obtained by partial hydrolysis of animal collagen. Gelatin used in the manufacture of capsule shells or as a pharmaceutical aid in the manufacture of tablets may contain suitable antimicrobial agents. Description: Light amber to faintly yellow, translucent flakes, sheets, shreds, powder or granules; odour, slight. Stable in air but is subject to microbial decomposition when moist or in solution. (13-18)

MATERIALS AND METHODS

Ofloxacin was obtained as a gift sample, Gelatin was obtained from kemphasol, India, Acetone was procured form s-d fine Chem. Pvt. Ltd. and all the other used chemicals were of analytical grade.

Preparation of nanoparticle: Nanoparticles containing Ofloxacin were prepared by using Coacervation phase separation method.(19). Initially, Ofloxacin and gelatin are accurately weighed dissolved in little quantity of water (hot) thoroughly mixed and add span -20 with constant stirring added to the above mixture of Sunflower oil (10ml) then the clear solution become turbid and stirred for 2hrs and add 2ml of Glutaraldehyde and continued for 30min stirring then centrifuged at 5200 rpm and washed with acetone and then dried in room temperature for 24 hrs. By following the above mentioned procedure five other batches of nanoparticles ratio of 1:1, 1:2, 1:3, 1:4 and 1:5 were prepared and named F1, F2, F3, F4 and F5 respectively. (19-21)

Preformulation Studies: Pre formulation studies for the drug and the polymer were carried out by estimating the following parameters 1.Thermal degradation test by melting point apparatus (22) 2.solubilty studies (23) and 3. Determination of purity of the drug sample and also the compatibility studies for the drug and polymer by FTIR studies was carried out by using KBr disc method Compatibility studies for drug and polymer -FTIR Studies: Infra-red spectra of ofloxacin and physical mixture of the other ingredients were measured by KBr disc method from 400 to 2000cm^{-1} using the analytical model instrument. (24)

Physicochemical characterization of Nanoparticles:

Particle size and determination: The particle size and morphology of the prepared Nanoparticles was done by using the scanning electron microscopy at an accelerating voltage of 10 k V. 9.3mm * 100SE.(25)

Percentage yield of Nanoparticles: The prepared microspheres were collected and weighed. The measured weight was divided by the total amount of all ingredient components which were used for the preparation of the Nanoparticles.

$$\text{Percentage yield (\%)} = \frac{\text{Actual weight of the product}}{\text{Total weight of the drug and excipients}} \times 100$$

DEE (Drug Entrapment Efficiency): The drug content in the Ofloxacin loaded nanoparticles was determined by calculating the difference between the total and the free drug concentration in the suspension and the supernatant respectively supernatant was separated by centrifugation and the amount of free drug present in the supernatant (w) was assayed by UV-spectrophotometer at 263 nm. A standard calibration curve of concentration versus absorbance was plotted for this purpose the amount of drug in supernatant was then subtracted from the total amount of drug added (W). In effect, (W-w) will give the Amount of drug entrapped in the microspheres. The percentage drug entrapment was calculated by using formula.

$$\text{Percentage drug entrapment} = (W-w) \times 100$$

***In vitro* drug Release study:** The release rate of ofloxacin from Nanoparticles was determined using USP dissolution testing apparatus II (Paddle type). The dissolution test was performed using 900 ml of 0.1N HCl, at 37 ± 0.5°C and 100 rpm. Nanoparticles equivalent to 200 mg of Ofloxacin were used for the test. A 1 ml sample solution was withdrawn from the dissolution apparatus for every 1 h, and thereafter every 1 h up to 6 h. Samples were replaced by its equivalent volume of dissolution medium. The samples were filtered through Whatman filter paper and solutions after appropriate dilution were analyzed at 293 nm by UV Spectrophotometer. Cumulative percentage drug release was calculated with the help of standard calibration curve of pure ofloxacin drug.

Drug release kinetics The formulation which showed the best results of *in vitro* release profiles was fitted into four models of data treatment as follows:

1. Cumulative percent drug released versus time (zero-order kinetic model)
2. Log Cumulative percent drug released versus time (first-order kinetic model)
3. Cumulative percent drug released versus square root of time (Higuchi's model)
4. Log cumulative percent drug released versus log time (Korsmeyer-Peppas equation) (26)

RESULTS AND DISCUSSION

Ofloxacin nanoparticles were prepared by Coacervation Phase separation method, A number of trails of varying the proportions of the ingredients was carried out to formulate 5 formulations of varying proportions of the ingredients. The thermal degradation study of the drug revealed the melting point of the drug to be 250^0c. Solubility and purity of the drug were determined and it was found that the drug is soluble was water, methanol, and chloroform ethyl acetate and insoluble in ether and the purity was found to be 98.24%.

The compatibility studies for the drug and excipients interactions were carried out using FTIR studies. FTIR spectra of the individual drug, gelatine and the physical mixture are represented in Fig-1-3 and the interpretations of the interactions is mentioned in Table no 1. From the figures and data we conclude that there is no possible interactions with the ofloxacin and with other ingredients within the formulation and the presence of functional groups are within the range. So this may not affect the formulation stability during its shelf life. Particle size study for the formulated nanoparticles was carried out and the particle size range of the nanoparticles was found to be from 1-100nm, Table 2, further the SEM images of the plain and drug loaded nanoparticles revealed that plain nanoparticles are smooth, spherical with uniform size whereas the drug loaded nanoparticles are spherical to oval in shape with smooth surfaces., Fig-4-5. The percentage yields of the various nanoparticle formulations was carried out and it was found that the formulation F4 is having the highest percentage yield of 91.25%, Fig -6. When different formulations were prepared with a varying ratio of drug and polymer, there was no much difference in the percentage yield of the product. All the four formulations showed good percentage yields between 60.60-91.25%. The average percent drug entrapment efficiency of the Five formulations ranges from 61.23-70.43% where the formulation F_4 showed a maximum drug entrapment of 70.43%. Table 4. Drug release study for the formulation was carried out and the results are obtained are as follows. When five four formulations were carried for the *in-vitro* drug release studies, all the formulations showed good release profile where

formulations F1 and F2 showed complete release of drug with in 3-4 hrs and formulations F3 and F4 released the drug completely with in 5-6 hrs and F5 showed a drug release of about 4-5 hr. Out of all formulations, F3 & F4 released the drug for an extended period of time and were considered as optimised formulations and further all the formulations were studied for release kineteics. Table 5. After carrying out the drug release studies, all the formulations were studied for release kinetics by fitting in mathematical model. It ws found that the optimised formulations F3& F4 followed Korsmeyer-peppas release kinetics with N value of 0.73 & 0.76 stating that the release of the drug from nanoparticles followed anamolous non fickian diffusion. Table 6.

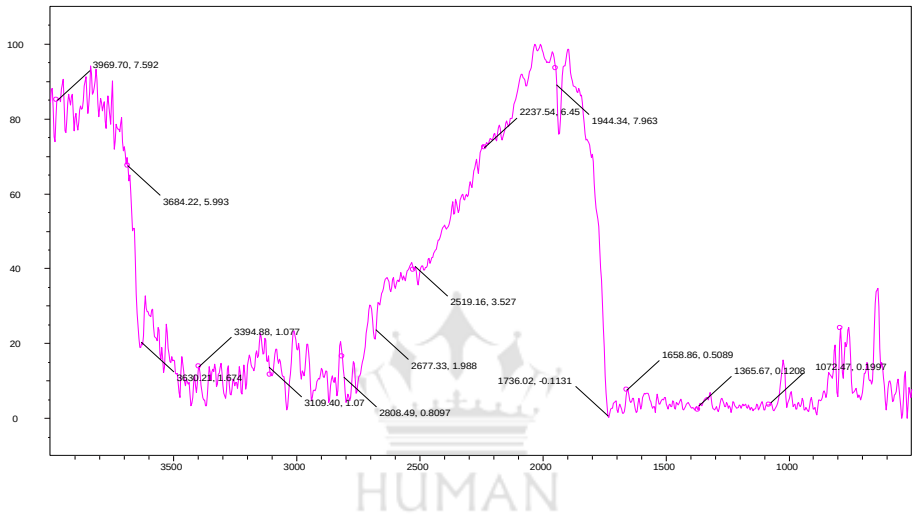

Figure No. 1: FTIR spectrum of ofloxacin

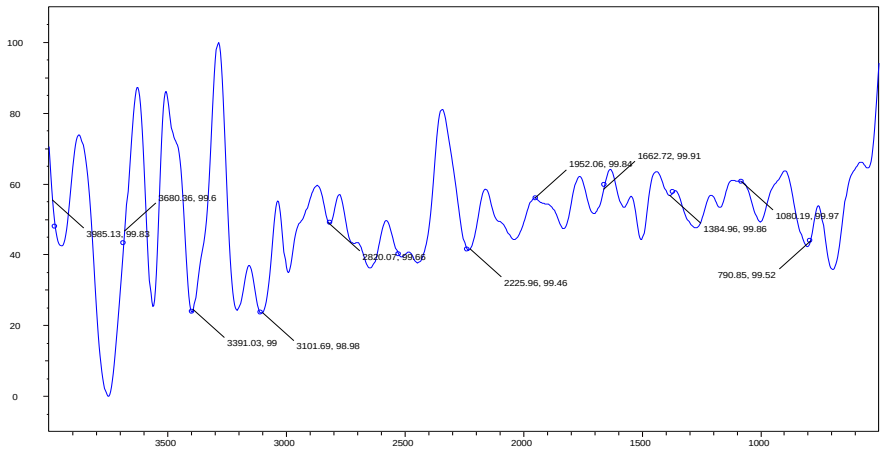

Figure No. 2: FT IR spectrum of gelatin

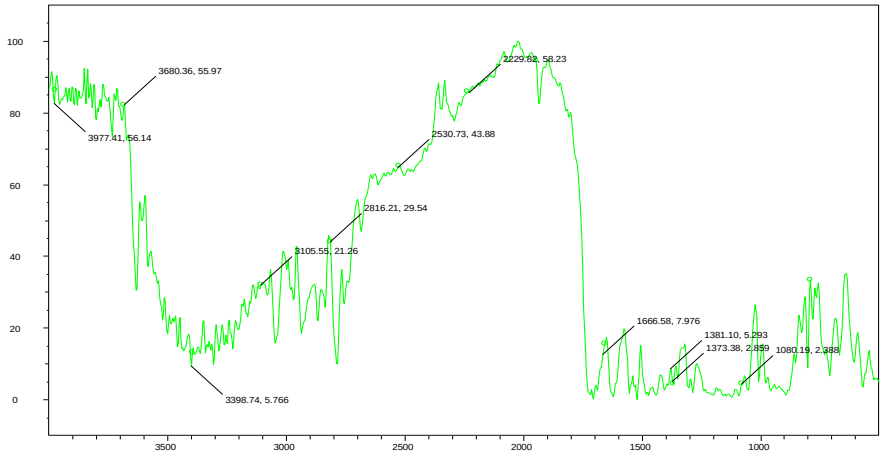

Figure No. 3: FT IR spectrum of physical mixture of ofloxacin and gelatin

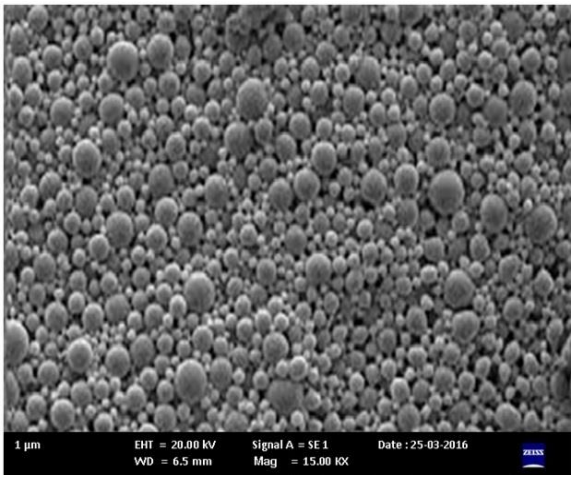

Figure No. 4: SEM images of the nanoparticles plain nanoparticles

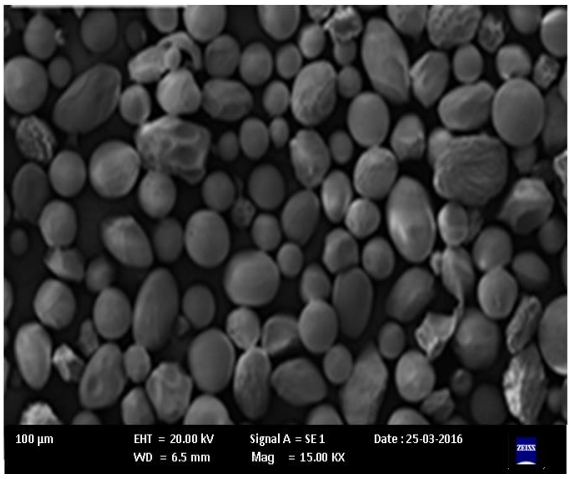

Figure No. 5: SEM images of the nanoparticles drug loaded nanoparticles

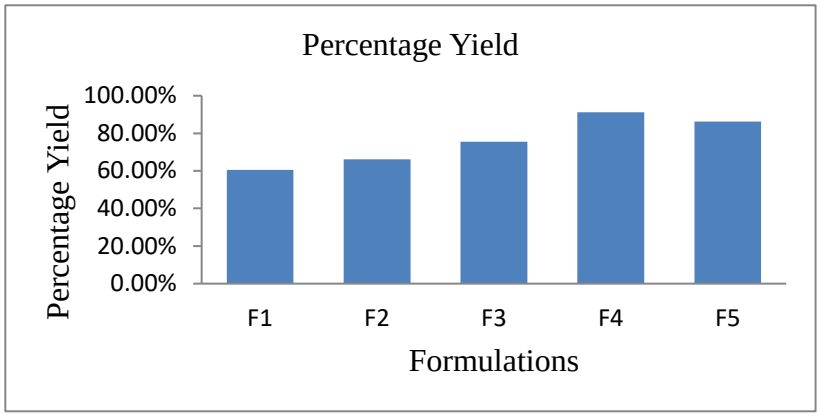

Figure No. 6: Percentage yields of formulations

Table No. 1: Drug – excipient interaction studies by FTIR:

S. No	Observed range cm^{-1}			Characteristic peak
	Ofloxacin	Gelatin	Physical mixture	
1.	1072.47	1080.19	1080	C-F (Bending), Aliphatic amines
2.	1365.67	1384.96	1381.10	C-H (Bending)
3.	1658.85	1662.72	1666.0	C=N (stretching)
4	2808.49	2880.07	2816.21	O-H (stretching)
5	3109.40	3101.69	3106.58	C-H (stretching)
6	3394.21	3391.03	3398.74	N-H (stretching)

Table No. 2: Particle size distribution of nanoparticles

Sr. No.	Formulations	Particle size
1	F1	252.8nm
2	F2	1.2nm
3	F3	3.0nm
4	F4	43.5nm
5	F5	10.1nm

Table No. 3: Percentage yields of various formulations

Sr. No.	Formulations	Percentage yield
1.	F_1	60.6%
2.	F_2	66.2%
3.	F_3	75.6%
4.	F_4	91.25%
5	F5	86.2%

Table No. 4: Drug Entrapment efficiencies of various formulations

Sr. No.	Formulations	% DEE
1.	F_1	61.23%
2.	F_2	63.21%
3.	F_3	68.52%
4.	F_4	70.43%
5	F5	66.56%

Table No. 5: Drug Release profile of Ofloxacin loaded nanoparticles

Time (min)	Cumulative Drug Release				
	F1	F2	F3	F4	F5
30	20.01	12.12	11.12	10.13	11.02
60	33.35	20.41	19.22	16.42	18.09
90	53.95	29.98	27.87	25.62	26.99
120	65.20	40.83	37.42	34.32	37.04
150	78.54	52.31	50.31	49.54	50.01
180	89.06	64.43	60.43	55.24	60.24
210	99.97	80.10	78.62	69.14	77.62
240		96.4	82.72	78.42	81.72
270			90.02	86.06	89.63
300			97.93	94.32	
330				98.78	

Table No. 6: Mathematical model fitting for drug release data

Formulation No	Zero order	First order	Matrix	Korsmeyer-peppas	
				R	N
F1	0.9596	0.8974	0.9914	0.9968	0.6
F2	0.9625	0.9648	0.9813	0.9895	0.65
F3	0.9876	0.9117	0.9644	0.9949	0.73
F4	0.9873	0.9677	0.9625	0.9951	0.76
F5	0.9723	0.9616	0.9824	0.9891	0.71

CONCLUSION

The present study has been a satisfactorily attempt to formulate nanoparticles of ofloxacin with gelatin by coacervation phase separation and chemical cross linking method. The effect of polymer with varying drug-polymer ratios on the characteristics of the nanoparticles was investigated. The natural, biocompatible and cost effective polymer like gelatin can be used to formulate an efficient nanoparticulate system with acceptable results like good entrapment efficiency of 61.23-70.43% and practical yield of 60.6-91.25%. The surface morphology of the particles was found to be round to oval in shape with porous nature when analyzed by SEM and the particle size analysis revealed that the particles were of the size range of 1.2-100 nm and showed good flow properties. Pertaining to *in-vitro* drug release studies all the five formulations showed good release within 3-6 hr and it was found that the change in polymer ratio is slightly known to alter drug release where the lower concentrations released the drug quickly when compared with the higher ratios. Among all the formulations the F3 &F4 formulation showed good release of 97-99 % within 5-6 hrs in a controlled manner. All the formulations were studied for release kinetics where the optimized formulation F3 &F4 exhibited Korsmeyer-peppas model with n value of 0.73 & 0.76 stating that the release of drug from nanoparticles followed anomalous non-fickian diffusion. Hence the present study was a successful attempt to formulate and extend the drug release of ofloxacin by nanoparticulate system with a view of conventional delivery of a drug.

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