



Formulation, Development & Characterization of Ofloxacin Microspheres

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ABSTRACT: Ofloxacin is anti bacterial agent that has a wide range of activity against gram (-ve) and gram (+ve) microorganisms. Multiple doses of Ofloxacin are required to attain steady state concentration. The main objective of this study was to formulate, develop and characterize Ofloxacin microspheres to prolong the release rate so as to decrease the necessity of multiple dosings especially in patients with renal impairment. The Ofloxacin microspheres were prepared using natural polymers by non-ionic crosslinking technique. Five different formulations were prepared with respective quantities of the polymer (Chitosan) with co-polymer (Gelatin and sodium alginate) with drug in different drug-polymer ratio of 1:0.5, 1:0.75 and 1:1. The prepared microspheres were evaluated for percentage drug loading, entrapment efficiency, surface morphology, and *in-vitro* release characteristics to identify the effect of addition of these polymers. Cumulative release data were fitted into kinetic models. The Scanning Electron Microscope analysis revealed a smooth and spherical surface morphology with mean particle size of the microspheres ranging from 7 to 14 μ m. The drug-loaded microspheres of F5 showed 80-90% of entrapment efficiency and the drug release was found to be 94.32%. Drug loading was found to increase with the increase in the concentration of encapsulating polymer, chitosan, sodium alginate and gelatin concentration. Drug release obeyed the first order kinetics. The Ofloxacin microspheres stability study was observed for a month with periodical intervals with 93% of drug content and no observable changes. © 2011 IGJPS. All rights reserved.

KEYWORDS: Ofloxacin; Microspheres; Chitosan; Gelatin; Sodium Alginate; Kinetic Models.

INTRODUCTION

The usual goal of sustain release dosage form is to enhance the performance of a particular drug and its chemical entity, and increase patient compliance. A therapeutically controlled effective systemic level of a drug can be sustained over a prolonged period of time without the need of multiple daily drug administration. Sustain release dosage forms also serves as better options as to avoid the side effects connected with increased concentration and the decreased activity connected with low concentrations in providing better

overall therapy. Many drugs require controlled administration into certain conditioned patients such as patient who are undergoing dialysis for renal impairment or for patient who has are hepatically insufficient. This is because the uncontrolled release can render the drug to be toxic and further harmful to other organs. Ofloxacin is a synthetic fluorinated carboxyquinolone that has a broad spectrum of activity against both gram-negative and gram-positive bacteria [1]. It is indicated for uncomplicated skin infections, complicated urinary tract infection, respiratory tract infections and some sexually transmitted diseases [2]. Normal dosage regimen varies from 200-600 mg administered twice or thrice a day, depending on severity of infection. Biological half-life of drug is from 5-6 hrs [3]. As it requires multiple dosing to obtain the required therapeutic doses and due to the established warnings of its dangerous adverse effects, this drug has been chosen to be the model of our study. The formulation of sustained release dosage form through the design of Ofloxacin microspheres could also potentiate the drug's ability to reduce the development of drug resistant bacteria. The coat material can be of various types ranging from natural polymers, such as albumin, gelatin [4] chitosan and synthetics such poly (vinyl alcohol), poly (lactide-co-glycolide) and a combination of two polymers such as chitosan-sodium CMC, alginate-chitosan [5] or alginate-gelatin. The current method that is being practiced in this study is done with natural polymers. The main advantages of natural polymers are that they are biocompatible, biodegradable and produce no systemic toxicity on administration [6]. The method is also easier, convenient, and cost effective, less time consuming and expected to produces an efficient release rate. The polymer that is being used in this study is chitosan and co-polymers are gelatin [7] and sodium alginate [8].

MATERIALS & METHODS

Materials

Ofloxacin was obtained as gift sample from Reachem Laboratory chemicals, pvt ltd, Chennai, India. Chitosan, sodium alginate and gelatin was purchased from R &M Chemicals, Essex, UK. All other reagents used were of analytical grade.

Preparation of Ofloxacin Microspheres

Chitosan-coated alginate-gelatin microspheres of Ofloxacin were prepared by non-ionic crosslinking method. As presented in Table 4.1, five different formulations were prepared with respective quantities of the polymer (Chitosan) with co polymer (Gelatin) and sodium alginate with drug in different drug-polymer ratio of 1:0.5, 1:0.75 and 1:1. Sodium alginate was dissolved in a 25 ml of preheated distilled water of about 55°C. Chitosan dissolved in 1-2% of acetic acid solution was taken and to that, 100 mg of powdered Ofloxacin was added and were placed on the sonicator (Elma S80 H Elmasonic) for approximately 15 min with a light stirring occasionally. This was to ensure that the drug is equally dispersed on the aqueous medium and to remove any air bubbles the resulted from the stirring by glass rod. While the solution was still on the sonicator, 2-2.5% of gelatin was added and continuously sonicated until the gelatin forms a mixture of uniform dispersion. By using a 20 gauge sized needle, the drug-polymer mixture was slowly added drop wise into 1% of Calcium Chloride solution. The microspheres formed are let stand for 10 min before filtering it with Whatmann filter paper using a vacuum pump. The filtered microspheres were dried at room temperature for 48 hrs as in **Table 1**.

Evaluation and Characterization of Microspheres

Drug Content

To prepare a standard solution, 50 mg of the Ofloxacin drug was dissolved in 50 ml of phosphate buffer solution of pH 7.4. From the solution, 1 ml was withdrawn and diluted with 100 ml of phosphate buffer.

To prepare a test solution, 500 mg of Ofloxacin microspheres was weighed and crushed with the aid of mortar and pestle. From that, 50 mg equivalent of microspheres was weighed and placed in the standard flask. To this, 2 ml of 0.1N HCl was added and volume was made up to 50 ml with phosphate buffer pH 7.4. The resulting solution was filtered through 0.45 µm filter membrane. From the solution 1 ml was diluted with 100 ml of phosphate buffer pH 7.4. The above solution was analyzed by UV spectrometer at 294 nm. The percentage of content uniformity was estimated using following formula:

$$\text{Percentage of Ofloxacin content} = \frac{\text{Sample Abs}}{\text{Standard Abs}} \times \frac{\text{Standard dilution}}{\text{Sample dilution}} \times 100 \quad \dots\dots \text{Eq 1}$$

Percentage Drug Loading

Ofloxacin content in the microspheres was estimated by UV Spectrophotometric based on the measurement of absorbance at 294 nm [9]. Microspheres equivalent to 100 mg were weighed and added in 100 ml of 0.1N HCl which was dissolved in a volumetric flask. The volume was made up to 100 ml with 0.1N of HCl. The sample was withdrawn, diluted suitably and measured spectrophotometrically at 294 nm for the drug content [10]. It was performed in triplicates. The percentage of drug loading in microspheres was estimated using the formula below:

$$\text{Percentage drug loading} = \frac{\text{Amount of drug in bead}}{\text{Amount of bead taken}} \times 100 \quad \dots\dots \text{Eq 2}$$

Entrapment Efficiency

The entrapment efficiency of the prepared microspheres was calculated by the formula [11]:

$$\text{Percentage entrapment efficiency} = \frac{\text{Practical drug loading}}{\text{Theoretical drug loading}} \times 100 \quad \dots\dots \text{Eq 3}$$

Particle Size Analysis

The size distribution and average size particle of microspheres were studied by using optical microscope (Olympus CX21, ME00001947) fitted with eye piece micrometer which was then calibrated with stage micrometer. The average size was calculated by retrieving the size of about 100 microspheres from each batch [12].

The particle size was calculated by using equation:

$$X_g = 10 \times [(n_i \times \log X_i) / N] \quad \dots\dots \text{Eq 4}$$

X_g is geometric mean diameter, 'n_i' is number of particle in range, 'x_i' is the midpoint of range and 'N' is the total number of particles. All the experimental units were analyzed in triplicate (n=3)

Surface Morphology

The surface morphology of the microspheres was carried out by using Scanning electron microscope. Samples were fixed on an aluminium SEM sample holder, coated with a thin layer of gold and then observed with a SEM microscope at 10 kV [13].

In-vitro Drug Release Studies

Accurately weighed samples of the microspheres were added to dissolution medium of pH 7.4 buffer [Shaharyar M. *et. al.*, 2006] for 24 hrs using dissolution apparatus (USP II TDT-081 Electrolab). Temperature was maintained at 37°C ± 2°C and fluid was agitated at 100 rpm. One ml of dissolution media was drawn at periodic intervals (2, 6, 8, 12 & 24 hrs) and the withdrawn volumes were replaced with equal quantity of the buffer and the constant volume maintained. After suitable dilution, the samples withdrawn were analyzed spectrophotometrically at 294 nm (Henry A. *et. al.*, 2008) according to Indian Pharmacopoeia 2007 using UV spectrophotometer (JASCO-V500, Japan). The *in-vitro* dissolution studies were performed in triplicates.

Formulation Code	Drug : Chitosan	Sodium Alginate (%)	Gelatin (%)	CaCl ₂ (%)	Citric acid (ml)
F1	1 : 0.5	1	-	1	2
F2	1 : 0.5	2	2	1	-
F3	1 : 0.75	2.5	2.5	1	1
F4	1 : 1	3	-	1	1
F5	1 : 1	3	2.5	1	2

Table 1: Composition of microspheres with different ratios of drug and polymers

Formulation Code	Mean particle size (µm)	Entrapment efficiency (%) mean± SD	Surface Morphology
F1	7.12±0.89	64.88±3.22	Spherical & smooth
F2	9.58±0.61	69.50±0.63	Spherical & smooth
F3	11.09±1.22	72.09±1.97	Spherical & smooth
F4	10.17±0.87	77.20±2.01	Spherical & smooth
F5	13.76±0.43	89.46±0.91	Spherical & smooth

Table 2 .Mean Particle size distribution and entrapment efficacy

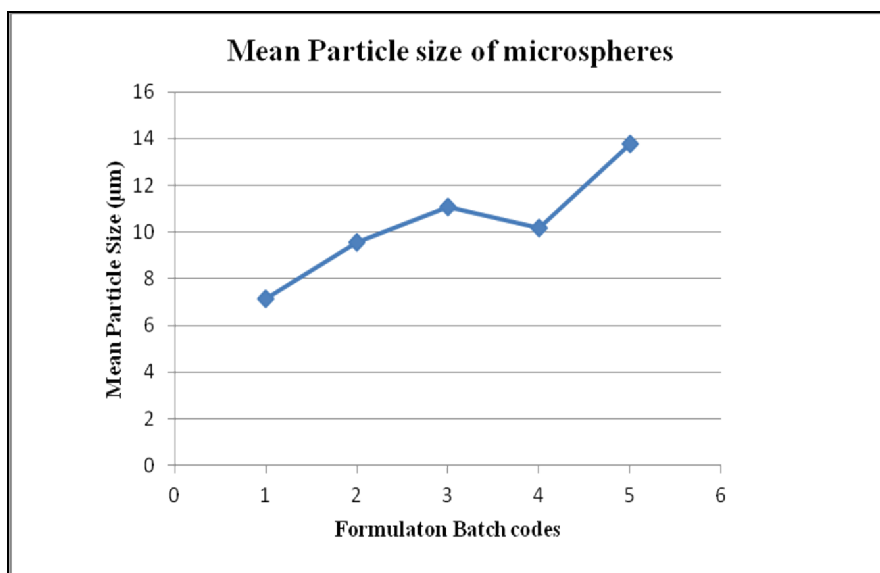


Figure 1: Mean Particle size of Ofloxacin microspheres (µm)

Kinetics of Drug Release

To examine the drug release kinetics and mechanism, the cumulative release data were fitted into kinetic models. The zero order rate Eq. (5) describes the systems where the drug release rate is independent of its concentration [16]. The first order Eq. (6) describes the release from system where release rate is concentration dependent. Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion Eq. (7). The Hixson-Crowell cube root law Eq. (8) describes the release from systems where there is a change in surface area and diameter of particles or tablets [17].

$$C = k_0 t \quad \dots\dots\dots \text{Eq 5}$$

Where, K_0 is zero-order rate constant expressed in units of concentration/time and t is the time.

$$\text{Log} C = \text{Log} C_0 - K t / 2.303 \quad \dots\dots\dots \text{Eq 6}$$

Where, C_0 is the initial concentration of drug and K is first order constant.

$$Q = K t^{1/2} \quad \dots\dots\dots \text{Eq 7}$$

Where, K is the constant reflecting the design variables of the system.

$$Q_0^{1/3} - Q t^{1/3} = K_{HC} t \quad \dots\dots\dots \text{Eq 8}$$

Where, Q_t is the amount of drug released in time t, Q_0 is the initial amount of the drug in tablet and K_{HC} is the rate constant for Hixson-Crowell rate equation.

Time (hrs)	Percentage of drug release (%)				
	F1	F2	F3	F4	F5
2	37.55	34.12	28.98	17.34	12.48
6	68.12	61.98	49.27	32.76	34.03
8	-	79.78	63.23	49.23	46.60
12	-	-	89.12	72.34	64.90
24	-	-	98.12	89.26	94.32

Table 3: Percentage of drug release from microspheres

Time (hrs)	\sqrt{t}	$\log t$	Amount released (mg)	% drug released	% drug to be released	$\log \% \text{ drug released}$	$\log \% \text{ drug to be released}$	(D) ^{1/3}
2	1.414	0.301	12.48	12.48	87.52	1.096	1.942	4.439
6	2.449	0.778	34.03	34.03	65.97	1.532	1.819	4.040
8	2.828	0.903	46.60	46.60	53.40	1.668	1.727	3.765
12	3.464	1.079	64.90	64.90	35.10	1.812	1.545	3.274
24	4.898	1.380	94.32	94.32	05.68	1.974	0.754	1.784

Table 4: Calculated drug release kinetics values of the formulated Ofloxacin Microspheres (F5)

Where,

D – Percentage drug to be released

t - Time

Korsmeyer–Peppas model describe the mechanism of drug release [18].

The following plots were made: *cumulative % drug release vs. time* (zero order kinetic model); *log cumulative of % drug remaining vs. time* (first order kinetic model); *cumulative % drug release vs. square root of time* (higuchi model) *log cumulative % drug release vs. log time* (korsmeyer-peppas model) and *cube root of drug % remaining in matrix vs. time* (hixson-crowell cube root law).

S. No	Storage condition	Time (Days)	Drug content (%)	Physical stability (Visual observation)
1	RT	Initial	94.32	NOC
2	RT	7 th day	94.16	NOC
3	RT	14 th day	93.90	NOC
4	RT	21 st day	93.47	NOC
5	RT	28 th day	93.28	NOC

Table 5. Stability studies of Ofloxacin Microspheres[NOC – No observable changes; RT – Room temperature (25⁰C ± 2⁰C)]

Stability studies

The finalized formulation (F5) of microspheres were taken in a crucible and stored at room temperature (25⁰C ± 2⁰C) for 28 days .The readings were taken at 7 days at periodical intervals (0th, 7th, 14th, 21th, and 28th day). The microspheres were analyzed spectrophotometrically at 294 nm after proper dilutions to evaluate the drug content. The physical stability was also observed periodically [3].

RESULTS & DISCUSSION

In this study, attempts have been made to develop and formulate chitosan-coated microspheres by non-ionic cross linking technique. The concentration of calcium chloride was retained in all batches. This was to prevent instant gelling of sodium alginate on addition of calcium chloride and squeezing out of the aqueous phase from gel lattice. The influence of alginate concentration was evaluated with three different concentrations (1.0; 2; 2.5; 3.0%).

The SEM micrographs and typical surface morphology of the microspheres were shown in Figure 4A and Figure 4B. The microspheres prepared were spherical with smooth surface. The smooth surface was due to the use of optimum concentration ratio of calcium chloride as 1% in all batches. As expected, decreasing the alginate concentration decreased the microsphere size. Therefore, an alginate concentration of 3% was used in the further preparations.

The average particle size of microspheres was between 7 to 14 μm which was carried out by using optical microscope fitted with eye piece micrometer which was then calibrated with stage micrometer.The average size of microsphere particles increased with increasing polymer (chitosan).

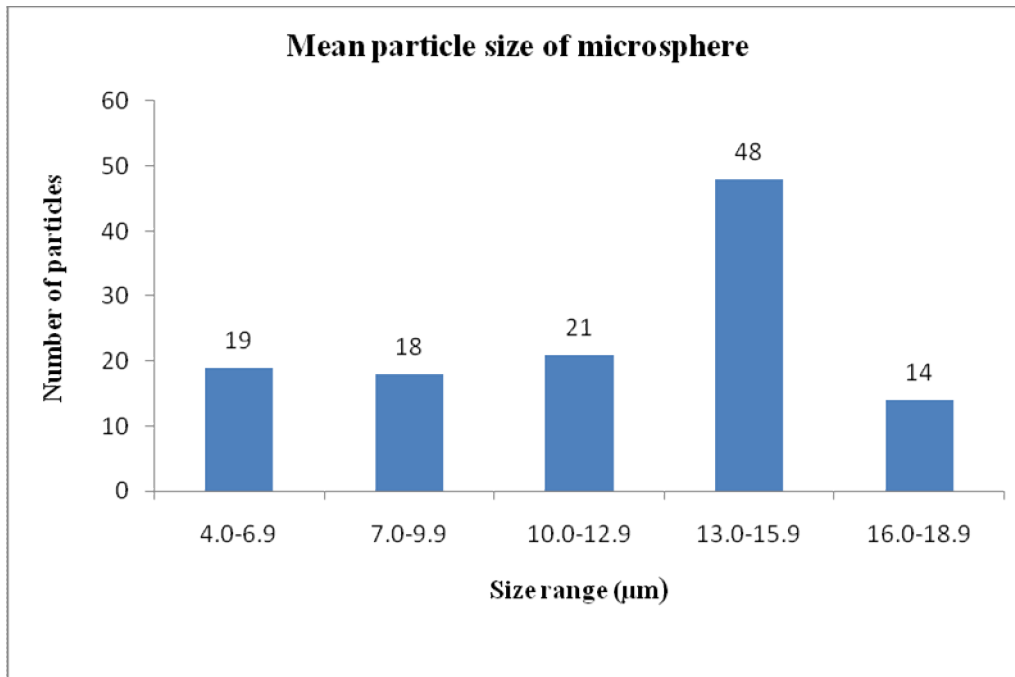


Figure 2: Mean particle size range of 100 Ofloxacin microspheres (F5)

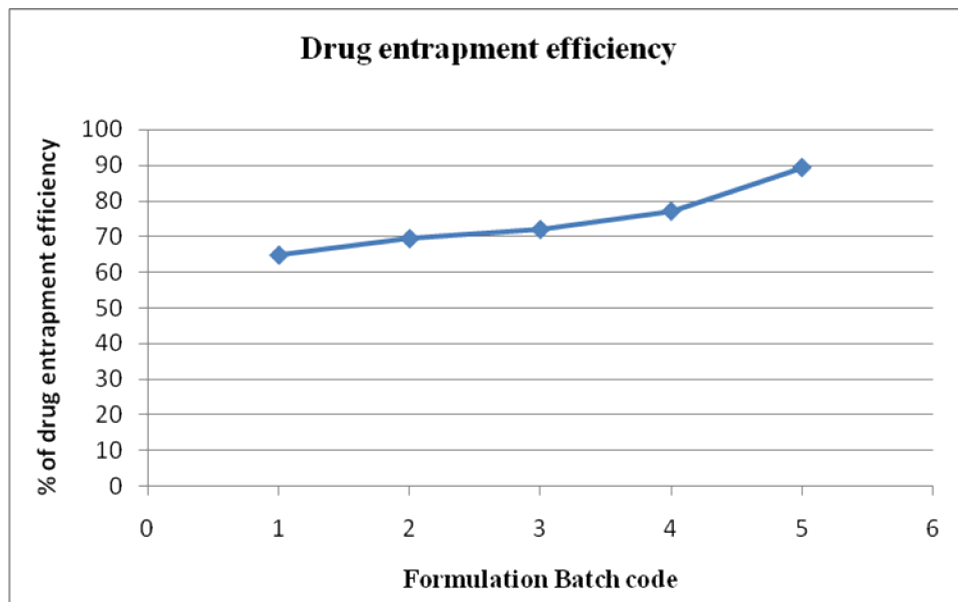


Figure 3: Percentage of drug entrapment efficiency

The percent encapsulation efficiency was increased up to $89.46 \pm 0.91\%$ for F5 with increasing polymer concentration. This can be attributed to the increased availability of the polymer for encapsulating the drug, Ofloxacin. The high levels of sodium alginate lead to increased encapsulation efficiency whereas percentage encapsulation efficiency also increases with the increase in ratio of drug-chitosan (1:1).

Accurately weighed samples of the microspheres were placed into the Visking tube and 1 ml of dissolution medium was allowed to disperse the microspheres inside the tube. Both ends were properly tied with thread and were placed in to the dissolution medium of pH 7.4 buffer for 24 hrs using dissolution apparatus. The F1 the drug release was found to be improper and has faster

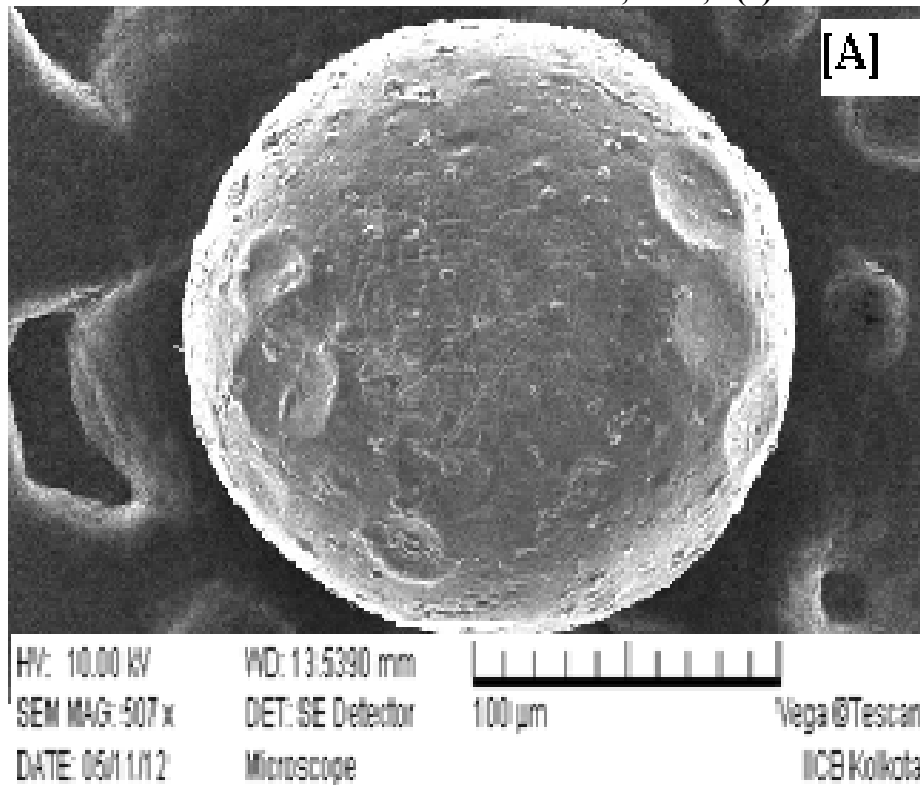


Figure 4A: SEM photograph of Ofloxacin microspheres

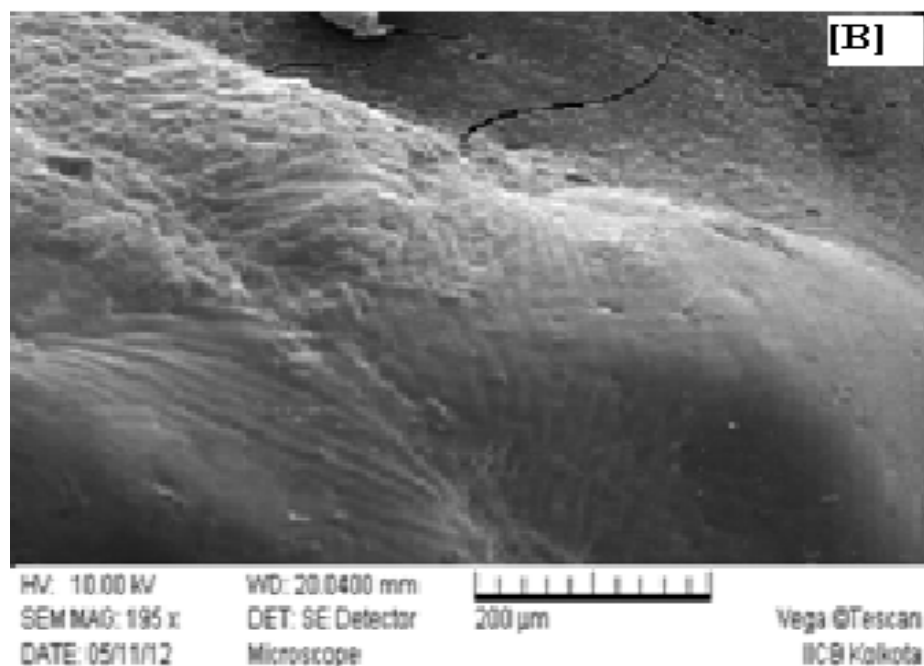


Figure 4B: Surface of Ofloxacin loaded microsphere (F5) at 80x

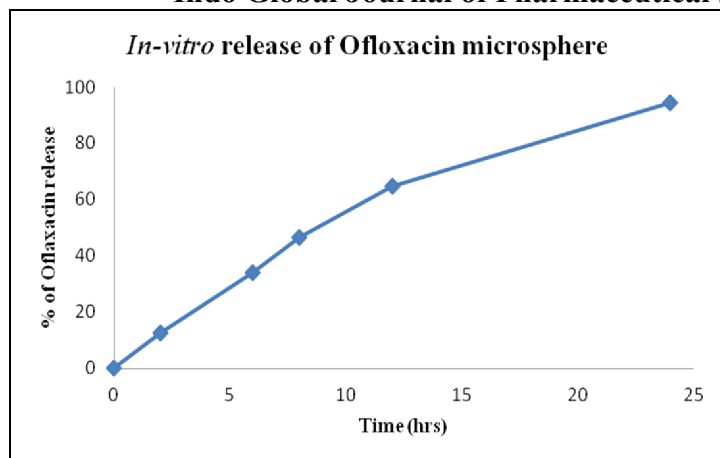


Figure 5: Percentage of Ofloxacin release from microspheres (F5)

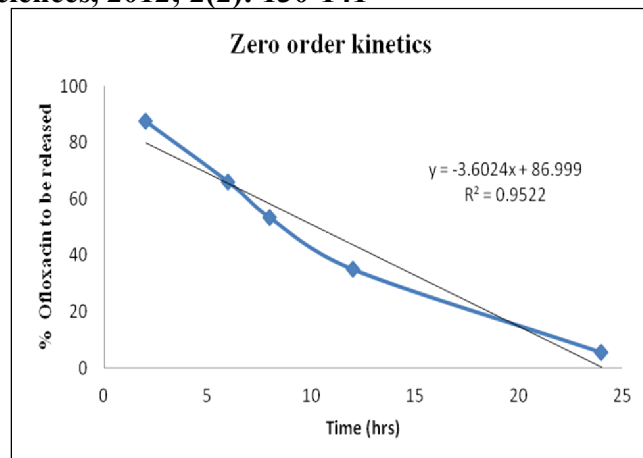


Figure 6: Zero order Release Model of Ofloxacin Sustained Release formulation (F5)

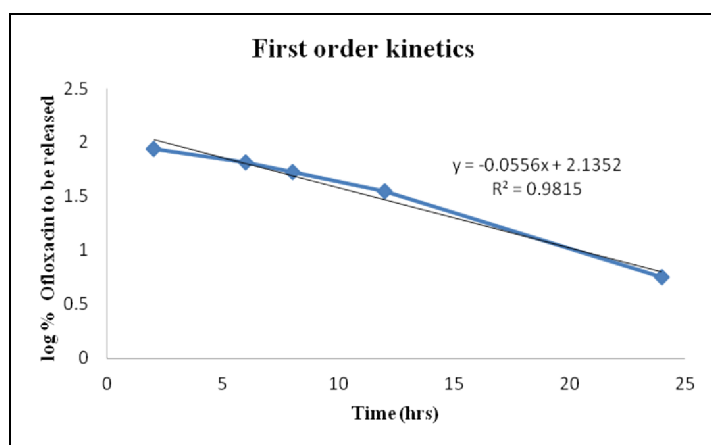


Figure 7: First order Release Model of Ofloxacin Microspheres

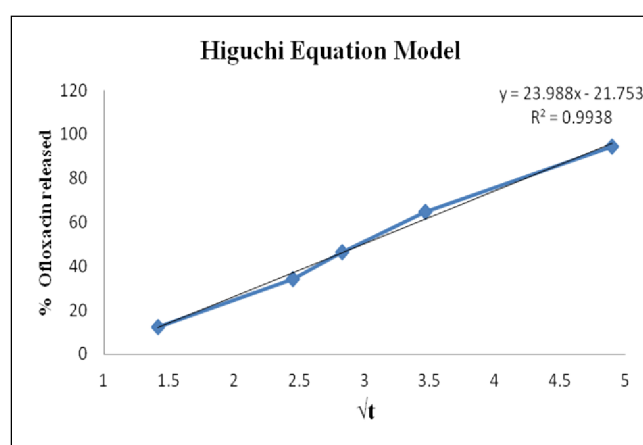


Figure 8: Higuchi Release Model of Ofloxacin Microspheres

release within 6 hrs. So the study must be discontinued from proceeding further. The similar kind of drug release was formed in F2 and it was not so acceptable release as it was within 8 hrs. This study has also been suspended for further. F3, F4 and F5, the drug release was continued up to 24 hrs. In all the formulations, with the increase in the polymer concentration, the rate and amount of drug release was found to be decreased, which can be attributed to the higher binding of the drug with the polymer. The release data of the formulation, F5 showed better drug loading and release characteristics, and was fitted into the equations of various kinetic models. The linear regression value was calculated and it was found to be 0.952, which means ofloxacin from the microspheres does not obey controlled fashion of zero-order release. So, the drug release was dependent on the concentration gradient. In the first-order kinetics, the linear regression value was found to be 0.987, which was evident that the release of Ofloxacin from microsphere obeys first order kinetics (rate of release dependent on concentration gradient). Higuchi equation explains the diffusion controlled release mechanism. The plot was linear and the linear regression coefficient value was 0.993, which was obvious that the drug release obeys diffusion mechanism from the microsphere. The release exponent 'n' was found to be 0.832 and the linear regression values from Korsmeyer-Peppas were 0.985. Therefore, this indicates a coupling of the diffusion and considerable swelling mechanism—so-called anomalous diffusion—and may indicate that the drug release was controlled by more than one process. According to Hixson-Crowell equation, the plot was not linear; the linear regression coefficient value was 0.998 that indicates a considerable erosion of the microsphere have taken place during the dissolution process.

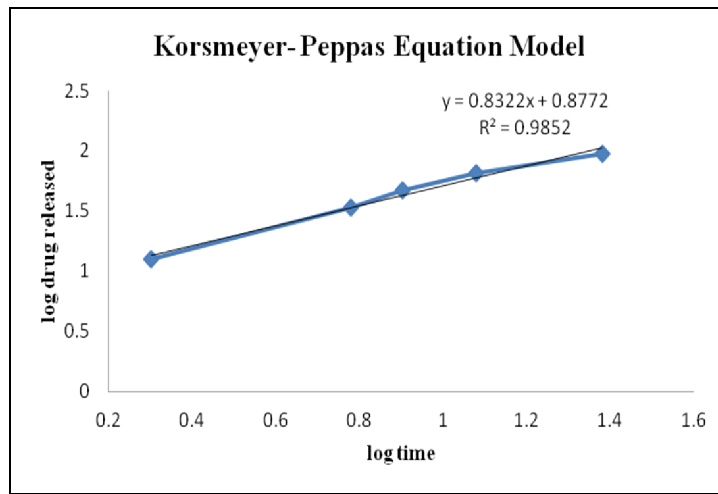


Figure 9: Korsmeyer-Peppas Release Model of Ofloxacin Microspheres

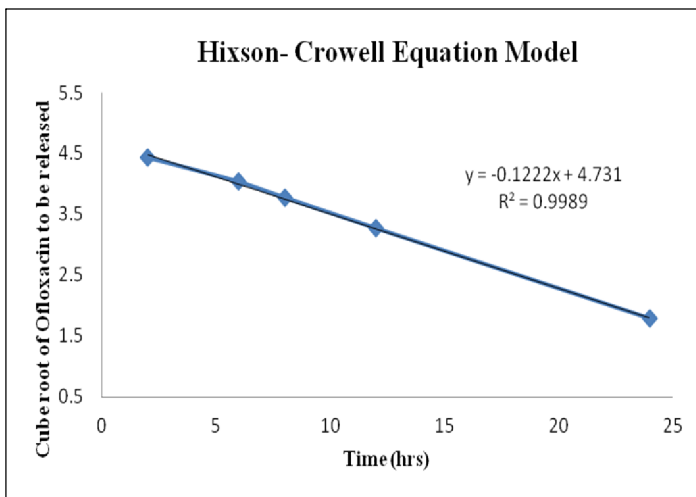


Figure 10 : Hixson-Crowell Release Model of Ofloxacin Microspheres

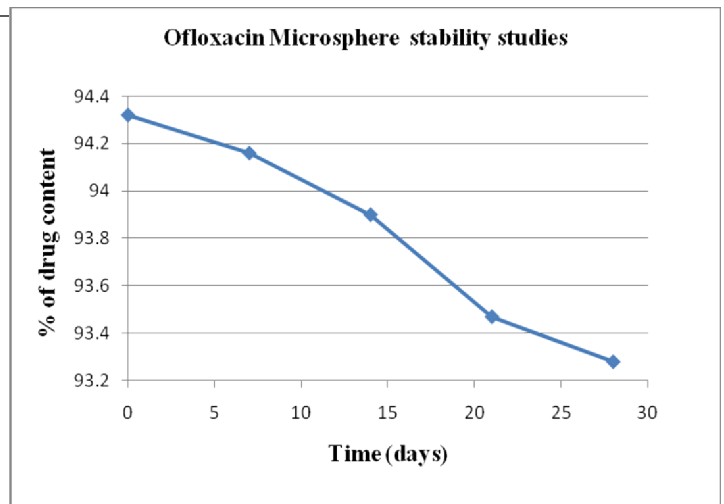


Figure 11: Stability Studies of final formulation

The Ofloxacin microsphere's stability was assessed at periodical the intervals through out 28 days and 93% of drug content were observed at the end of one month with no-observable physical changes. The microspheres were analyzed for their drug content. This indicates a good stability of the Ofloxacin microspheres.

CONCLUSION

In this study, the technique that was chosen, non-ionic crosslinking method with the use of chitosan as a polymer and gelatin as a co-polymer, the F5 was able to sustain the release effectively. It was evident in kinetics equations that the drugs were released in anomalous diffusion with a considerable swelling mechanism. Further studies are needed involving selectively on the *in-vivo* studies or develop a correlation between the *in-vivo* and *in-vitro* study of the release rate of Ofloxacin microsphere.

ACKNOWLEDGEMENT

The authors express their gratitude to the GCEO of Masterskill University College of Health Sciences, Dato' Sri Dr. Edmund Santhara for the research funding, Dr. Sudhahar, Mr. Muthappan, Mr. Muthu Mohamed and Dr. Ashok Kumar for their endless support and guidance. The authors are also thankful to the authorities of Masterskill University College of Health Sciences for providing laboratory facilities and their constant assistance. The authors are also grateful to Reachem Laboratory chemicals for providing gift sample of Ofloxacin.

REFERENCES

- [1] J.M.Covino, M. Cummings, B .Smith, S.Benes , K.Draft , M.William. Comparison of Ofloxacin and Ceftriaxone in the Treatment of Uncomplicated Gonorrhoea Caused by Penicillinase-Producing and Non-Penicillinase-Producing Strains. *Antimicrobial Agent Chemotherapy*, 1990, 34: 148–149
- [2] 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
- [3] 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
- [4] 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
- [5] 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 Development. Industrial Pharmaceutical*. 2003, 29, 713–724.
- [6] 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
- [7] K. Upadhye, S Bakhle, G. Dixit.Preparation and Evaluation of Gelatin Microspheres Containing Ciprofloxacin Hydrochloride. *Indian Drugs*., 2004,41(11): 665-668
- [8] S. Shiraiishi, T. Imai, M. Otagiri. Controlled-release preparation of indomethacin using calcium alginate gel. *Biology and Pharmaceutical Bulletin*., 1993, 16 (11): 1164–1168
- [9] A. Semalty & M. Semalty. Preparation and Characterization of Mucoadhesive Microspheres of Ciprofloxacin Hydrochloride.*Indian Drugs*.,2007,44(5): 368-373
- [10] S.P.Vyas & R.K.Khar .Targeted and Controlled drug delivery. 1st edition.,2006, 325-326: 417-457
- [11] 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
- [12] 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.
- [13] 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.
- [14] M.Shahar yar, A. Ahamed Siddiqui .Design of targeted dosage form of Ofloxacin. *Journal of Serbian Chemical Sciences*., 2006, 71 (12): 1269-1273

Indo Global Journal of Pharmaceutical Sciences, 2012; 2(2): 130-141

- [15] A.Henry Okeri & M. Ikhuoria Arhewoh. Analytical Profile of the fluoroquinolone antibacterials. African Journal of Biotechnology.,2008,7(6):670-680
- [16] T.P.Hadjioannou, G.D. Christian, M. Koupparis, P.E. Macheras. Quantitative Calculations in Pharmaceutical Practice and Research, New York: VCH Publishers Inc.,1993,345-348
- [17] A.W. Hixson and J.H. Crowell. Dependence of reaction velocity upon surface and agitation .I. theoretical consideration. Industrial and Engineering Chemical Research.,1931, 23: 923-931
- [18] R.W. Korsmeyer, R. Gurny, E.Doelker, P. Buri ,N.A.Peppas. Mechanisms of solute release from porous hydrophilic polymers. International Journal of Pharmaceutics., 1983, 15: 25.

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