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Formulation and Evaluation of Biodegradable Microsphere Hydrogel for Ocular Delivery of Ofloxacin

Tanu Dubey[1], Kaushelendra Mishra[2], Kajal Sharma[3], Parulben D Mehta[4]

[1,2,3,4]Lakshmi Narain College of Pharmacy, Bhopal

Abstract

The objective of the present investigation was to explore the hydrogel microspheres loaded with Ofloxacin to obtain controlled release for ocular delivery. The FTIR spectra of pure drug and the drug excipient mixture revealed no chemical interaction. Ofloxacin loaded hydrogel microspheres were prepared using Chitosan/Pluronic F127 blend and emulsion crosslinking method. **F7** with blend ratio 3.33:1 exhibited the highest encapsulation efficiency (77.85%) whereas the lowest encapsulation was witnesses in the formulation **F3** (32.10%) which has a blend ratio of 15:1. The particle size ranged from 29.3 µm for **F3** to 146.5 µm for **F7.** The microspheres exhibited negative zeta potential and the value was found to be -17.5mV for F7. **F3** exhibited the highest water uptake (316.0 %) whereas **F7** exhibited the lowest water uptake (81.0 %). **F7** released around 58.4% drug after 24 h. Hence **F7** was considered to be the most optimized formulation. The release of Ofloxacin from F7 followed Higuchi mathematical model suggesting Non-Fickian diffusion.

Keywords: Ofloxacin, hydrogen, ocular, delivery, microsphere

Introduction

With conventional dosing formulations, the concentration of the released drug is initially high, peaks and then declines sharply below the minimum therapeutic level [1, 2]. Hydrogels are three-dimensional networks composed of hydrophilic polymers cross linked through covalent bonds or held together via physical intermolecular attractions. Hydrogels can absorb large amounts of water or bio logical fluids, from 20% up to several thousand %, and swell readily without dissolving. The high hydrophilicity of the hydrogels is mainly due to the presence of a number of hydrophilic moieties such as amino, carboxyl, amide and hydroxyl groups distributed along the backbone of the polymeric strands. In the swollen state the hydrogels are so ft and have a rubbery structure, resembling to a great extent the living tissues. Some hydrogels, such as alginate-based gels, also offer excellent biocompatibility [3, 4].

Recently, natural polysaccharides have shown to be very useful for drug entrapment and controlled release of drugs [5-12]. The natural polymers used in controlled release technology have the great advantage of being nontoxic, biocompatible and biodegradable. Chitosan is a hydrophilic, biocompatible and biodegradable polymer of low toxicity and has been investigated extensively in pharmaceutical and medical applications. Chitosan has been used for the preparation of microcapsules and microspheres with encapsulated proteins, enzymes, DNA and cells. Studies have also highlighted the potential use of chitosan as an absorption-enhancing agent. Owing to its bioadhesive properties, chitosan has also received a

substantial attention as novel bioadhesive drug delivery system, which is aimed at improving the bioavailability of drugs by prolonging the residence time at the site of absorption.

Pluronics, are recognized as pharmaceutical multi-purpose excipients capable of increasing aqueous solubility and stability of drugs. Pluronic F127 in aqueous solution at the concentrations of 15–20% and higher exhibits the unique property of reversible thermal gelation. This unique property of being liquid at 4–8°C and in a semi solid gel at ambient or body temperature provides an attractive means for controlled release of drugs.

Ofloxacin (Ofx) (Figure 1) is one of the most commonly available ocular antibiotic solutions. Unfortunately, it suffers from rapid tear turnover and therefore requires administration every 4 h or even every hour for serious eye infections; moreover, its solubility is pH-dependent and very low at the pH of corneal fluid (7.2–7.4), and it can form a white crystalline deposit on the cornea. Hydrogels are hydrophilic polymer networks, which absorb from 10–20% (an arbitrary lower limit) up to thousands of times their dry weight in water. This enables a controlled release of drug from the hydrogels.

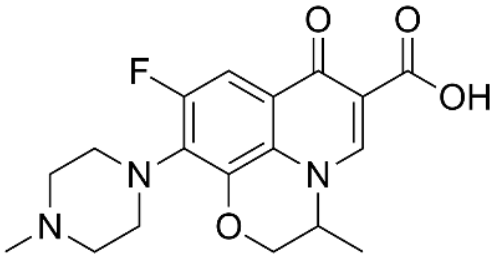

Figure 1. Structure of ofloxacin

The objective of the present investigation was to explore the hydrogel microspheres loaded with ofloxacin in order to improve the biological half-life of the drug by controlling the drug release. These hydrogel microspheres would be developed as ocular delivery system for the release of ofloxacin, to reduce its frequency of administration.

Material and Methods

Drug-excipient compatibility study

FT-IR spectra matching approach was used for detection of any possible chemical interaction between drug and excipients. A physical mixture (1:1:1) of drug, chitosan and Pluronic F-127 was prepared. It was scanned from 4000 to 400 cm^{-1} in FT-IR spectrometer. The IR spectrum of the physical mixture was compared with that of pure drug to detect any appearance or disappearance of peaks.

Calibration Curve of olfoxacin in phosphate buffer pH 7.2 [13]

Ofloxacin 100 mg was weighed and dissolved in methanol in a 100 ml volumetric flask. The flask was shaken and volume was made up to the mark with phosphate buffer pH 7.2 to give a solution containing 1 mg/ml. From the primary stock solution, pipette out 2 ml and placed into 10 ml volumetric flask. The volume was made up to mark with phosphate buffer pH 7.2 to give a stock solution containing 200 µg/mL. Appropriate volume of aliquots (0.25 to 1.25 mL) from olfoxacin secondary stock solution was transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with phosphate buffer pH 7.2 to obtain concentrations of 5, 10, 15, 20 and 25 µg/mL. Absorbance of each dilution against phosphate buffer pH 7.2 as blank was measured at 302 nm.

Formulation of hydrogel microspheres

The hydrogel microspheres loaded with ofloxacin were prepared using 3^2 factorial approach using Chitosan as the variable X_1 and Pluronic F-127 as variable X_2. Both the variables were used at three different levels (+1, 0, -1) to obtain 9 different formulations. The design table for formulations has been presented as table 1. The percentage drug release and percent drug encapsulation were taken as the dependent variables. The quantities of drug and polymers used are presented in table 2.

Table 1 Design table for formulation of hydrogel microspheres

Formulation Code	HM1	HM2	AF3	HM4	HM5	HM6	HM7	HM8	HM9
X_1	-1	0	+1	-1	0	+1	-1	0	+1
X_2	-1	-1	-1	0	0	0	+1	+1	+1

Emulsion-crosslinking method was utilized for preparing the hydrogel microspheres of chitosan and Pluronic F-127 [14]. Chitosan was dissolved in 2% aqueous acetic acid solution by continuously stirring to obtain a homogeneous solution. To this solution, Pluronic-127 was dispersed and stirred overnight to form a homogeneous solution. Then, ofloxacin was dissolved in the above polymer solution. This solution was added slowly to light liquid paraffin (100g, w/w) containing 1% (w/w) Span-80 under constant stirring at 600 rpm for 15 min. To this w/o emulsion, glutaraldehyde was added slowly and stirring was continued for 2 h post addition. The hardened microspheres were separated by filtration and washed with n-hexane. Microspheres were washed with 0.1 M glycine solution to mask the unreacted glutaraldehyde followed by washing with distilled water to remove the unreacted glutaraldehyde. The microspheres were vacuum dried at 40°C for 24 h and stored in a desiccator.

Table 2 Composition of hydrogel microspheres

Formulation Code	Chitosan (mg)	Pluronic F-127 (mg)	Glutaradehyde (mL)	Ofloxacin (mg)
F1	100	10	20	150
F2	150	10	20	150
F3	200	10	20	150
F4	100	20	20	150
F5	150	20	20	150
F6	200	20	20	150
F7	100	30	20	150
F8	150	30	20	150
F9	200	30	20	150

Evaluation of hydrogel microspheres

Entrapment Efficiency

Ofloxain content in the microspheres was estimated in phosphate buffer (pH 7.4). Microspheres (10 mg) were powdered using mortar & pestle and then extracted with 50 mL phosphate buffer (pH 7.2) for 1 h followed by sonication for 30 min. The solution was centrifuged and washed twice with phosphate buffer (pH 7.4) to complete the drug extraction. The clear supernatant solution was then analyzed by UV

spectrophotometer at the λ_{max} value of 286 nm by suitable dilution with phosphate buffer pH 7.2. The % encapsulation efficiency was calculated as

$$\%\ Drug\ Loading = \frac{Amount\ of\ drug\ in\ HMs}{Weight\ of\ HM} \times 100$$

$$\%\ Encapsulation\ Efficiencey = \frac{Actual\ Drug\ Loading}{Theoretical\ loading} \times 100$$

Particle Size and Zeta Potential Measurement

Particle size and size distributions were measured using ocular micrometer and was confirmed by laser light scattering technique. The zeta potential of the best formulation was measured using a zetasizer.

FTIR spectral measurements

FTIR spectra of the blank microspheres, drug-loaded microspheres and the neat drug were obtained using Bruker alpha FTIR spectrometer. The scanning was done from 400-4000 cm^{-1}.

Swelling Study

Equilibrium water uptake by the hydrogel microspheres was determined by measuring the amount of swelling of the hydrogel matrix in distilled water for a period of 24 h. Excess liquid drops adhered on the surface were removed by blotting with a filter paper and the swollen microspheres were weighed on an electronic balance. The hydrogel microspheres were then dried in an oven at 60°C for 5 h until there was no change in the weight of the dried mass of the samples. The % equilibrium water uptake was calculated as

$$\%\ Water\ Uptake = \frac{Weight\ of\ swollen\ microspheres - Weight\ of\ dry\ microspheres}{Weight\ of\ dry\ microspheres} \times 100$$

***In vitro* release study**

Simulated tear fluid was prepared by dissolving NaCl 06.8 g, $NaHCO_3$ 2.2 g, $CaCl_2{\cdot}2H_2O$ 0.08 g and KCl 1.4 g, in distilled deionized water to 100 mL. The *in vitro* release of ofloxacin from the hydrogel microspheres was determined using a tablet dissolution tester utilizing simulated tear fluid as the dissolution medium. A weighed quantity of the hydrogel microspheres were placed in the dissolution baskets maintained at 37°C and rotated at a speed of 100 rpm. Samples were withdrawn at predetermined intervals and analyzed by UV spectrophotometer at the λ_{max} value of 286 nm after suitable dilution with phosphate buffer pH 7.2.

Results and Discussion

Drug-Excipient Compatibility Study

In order to confirm the compatibility of the drug and the excipients, a physical mixture of chitosan, pluronic F-127 and Ofloxacin was subjected to FT-IR analysis. The spectrum was observed for the occurrence of stretching and bending vibrations. The spectra of the mixture exhibited all the peaks of the pure drug as well as some peaks due to the functional groups of the excipients. No peak of the pure drug was removed though the position of the peak changed marginally due to the vibrations of the functional groups of the excipients (Figure 2).

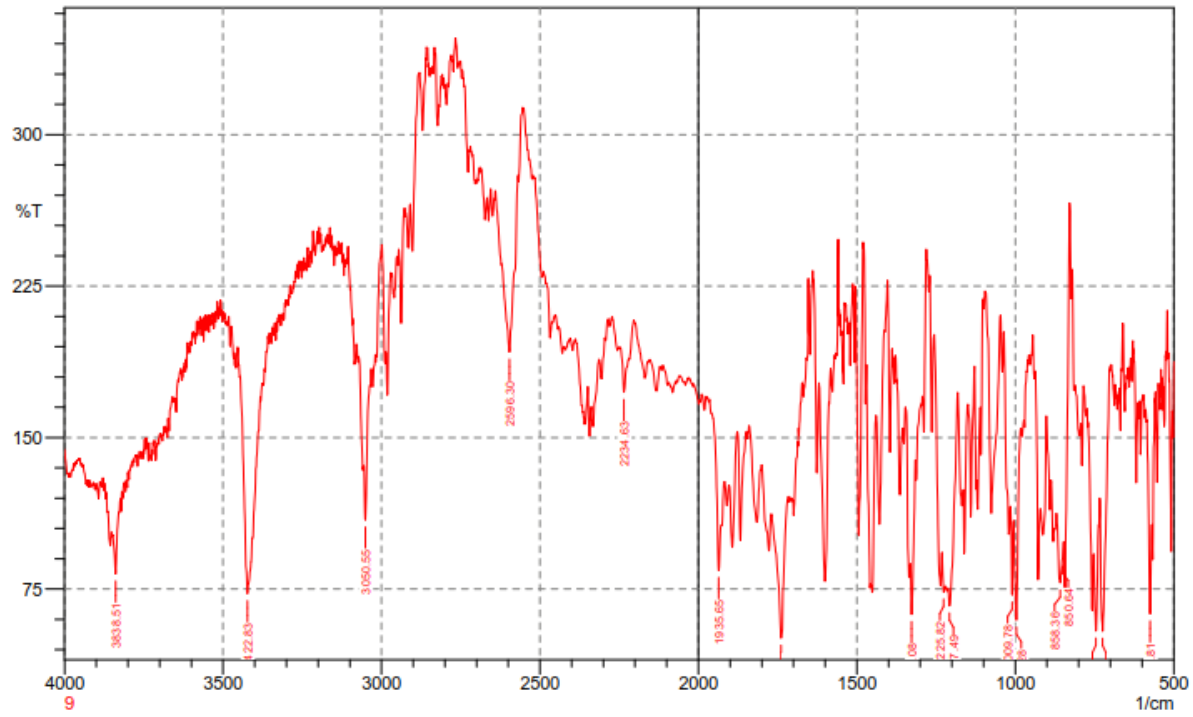

Figure 2 FT-IR spectrum of physical mixture of Ofloxacin, chitosan and pluronic F-127

Calibration curve of Ofloxacin

The 20µg/ml solution in phosphate buffer pH 7.2 of Ofloxacin was subjected to scanning from 400-190 nm in UV spectrophotometer and the absorption maximum was found to be at 286 nm. The absorbance of working standards was plotted against the concentration of solution to obtain the calibration curve of the drug in phosphate buffer pH 7.2. The regression coefficient of the calibration curve was found to be 0.9945 and the equation for prediction of concentration of unknown sample was Abs = 0.0041 (conc) + 0.0036 (Figure 3).

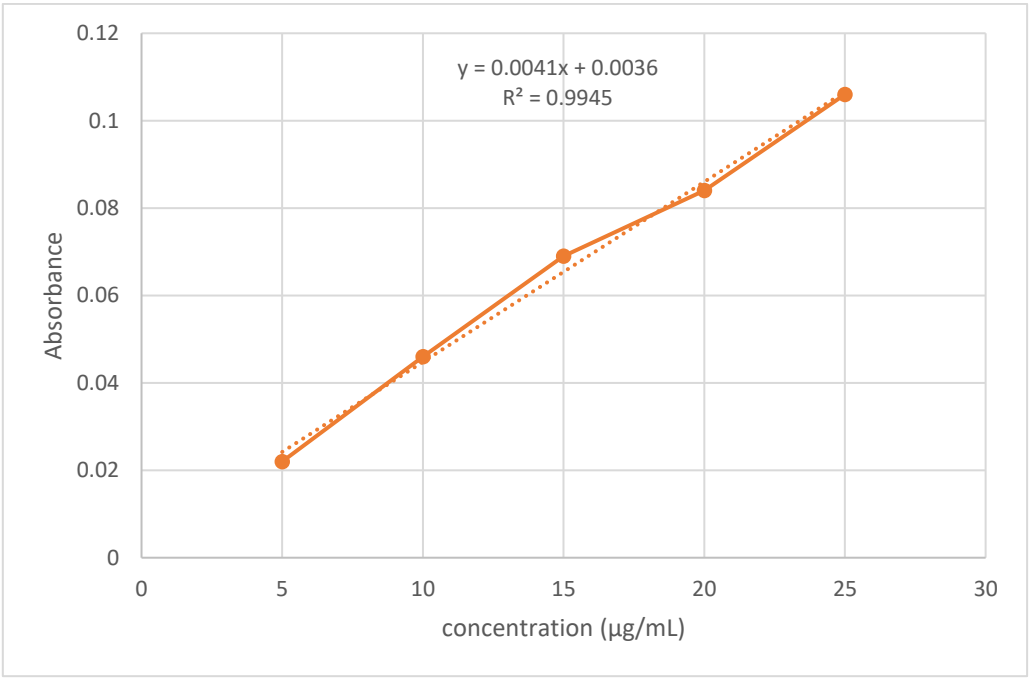

Figure 3 Calibration curve of Ofloxacin

Formulation of hydrogel microspheres

A total of 9 formulations were prepared using chitosan and pluronic F-127 as the two independent variables at three different levels (-1, 0, +1). The design of formulations was done as mentioned in Table 5.4 and emulsion crosslinking method was used to prepare the hydrogel microspheres. The amount of drug encapsulated and the percent drug released from the hydrogel microspheres was evaluated for describing the best formulation. Glutaraldehyde (2-3% w/w) was used as the crosslinking agent for the formulation of microspheres.

The glutaraldehyde mediated crosslinking of the polymeric blend of chitosan and Pluronic F-127 may involve interaction of the aldehydic group of glutaraldehyde with the amine group of chitosan leading to a stable imide linkage [15]. This crosslinking with glutaraldehyde provides the necessary stability and hardness to the particles. The presence of Pluronic F-127 affects the particle size of the formulation due to enhanced viscosity of the emlusion [16]. The effect of crosslinker on the drug release characteristics has also been reported previously which suggested that a higher concentration of glutaraldehyde causes a decreased release of drug from the polymeric matrix of the particles due to the contraction of the microvoids which is a result of the enhanced rigidness of the polymeric chains.

Evaluation of the hydrogel microspheres

Entrapment Efficiency

The drug loading and encapsulation efficiency of the hydrogel microspheres was determined using the reported method and it was found that the encapsulation was dependent on the ratio of the polymeric blend (Table 3). The drug loading and encapsulation efficiency were highest when the ratio of chitosan/Pluronic F-127 was low. **F7** with blend ratio 3.33:1 exhibited the highest encapsulation efficiency (75.27%) whereas the lowest encapsulation was witnesses in the formulation **F3** (29.23%) which has a blend ratio of 15:1. As discussed previously, the presence of Pluronic F-127 enhances the viscosity of the emulsion thereby increasing the entrapment of the drug in the matrix globules.

Table 3 Drug loading and Entrapment efficiency of hydrogel microspheres

Formulation Code	% Drug Loading	% Entrapment Efficiency
F1	22.8	48.45
F2	13.1	30.85
F3	11.2	29.23
F4	27.6	61.54
F5	22.5	56.02
F6	17.1	46.76
F7	**32.1**	**75.27**
F8	25.2	64.76
F9	20.3	57.65

Particle size and zeta potential

The particles size measurements of all the microspheres was done using calibration ocular micrometer. The particle size of the formulations was also found to be dependent on the amount of Pluronic F-127. As the concentration of Pluronic increased, the particle size was found to be higher. The particle size ranged from 43.95 µm for **F3** to 161.15 µm for **F7** (Table 4).

Since F7 exhibited the highest drug loading and entrapment, its particle size was verified using Malvern zetasizer. The size of hydrogel microspheres F7 was found to be 159.65 µm.

Swelling Study

The results of the water uptake study indicate that the percent water uptake by the hydrogel microspheres were affected by the concentration of chitosan. The higher levels of chitosan increased the water uptake. On the contrary a higher ratio of Pluronic F127 decreased the water uptake by the microspheres. **F3** exhibited the highest water uptake (316.0 %) whereas **F7** exhibited the lowest water uptake (81.0 %) (Table 4). The amphiphilic nature of Pluronic F127 could be attributed to lower swelling owing to the formation of a rigid gel on increasing the concentration of Pluronic F127.

Table 4 Particle Size of hydrogel microspheres

Formulation Code	Mean Particle Size (µm)	% Water Uptake
F1	73.25	175.5
F2	58.6	206.5
F3	43.95	316.0
F4	131.85	147.5
F5	102.55	182.0
F6	58.6	220.5
F7	161.15	81.0
F8	146.5	114.0
F9	73.25	119.5

FTIR spectral measurements of hydrogel microspheres

The FTIR spectra of blank and drug loaded microspheres were compared with the pure drug and it was seen that the drug loaded microspheres presented all the peaks of the pure drug while most of the characteristic peaks of Ofloxacin were absent in the blank hydrogel microspheres (Figure 4).

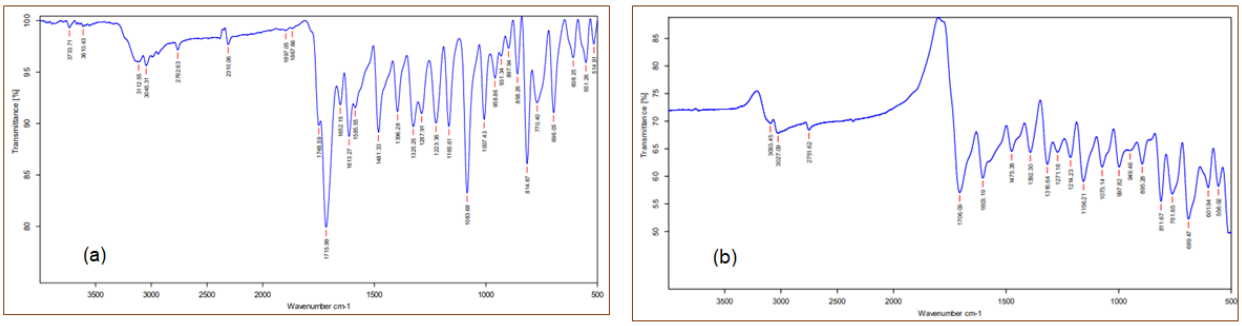

Figure 4 FTIR of (a) drug loaded hydrogel microsphere (F7) (b) blank microsphere

***In vitro* release of Ofloxacin from hydrogel microspheres**

The in vitro drug release from hydrogel microspheres was evaluated using dissolution test apparatus in simulated tear fluid. The results of *in vitro* release study over a period of 24 h (Figure 5).

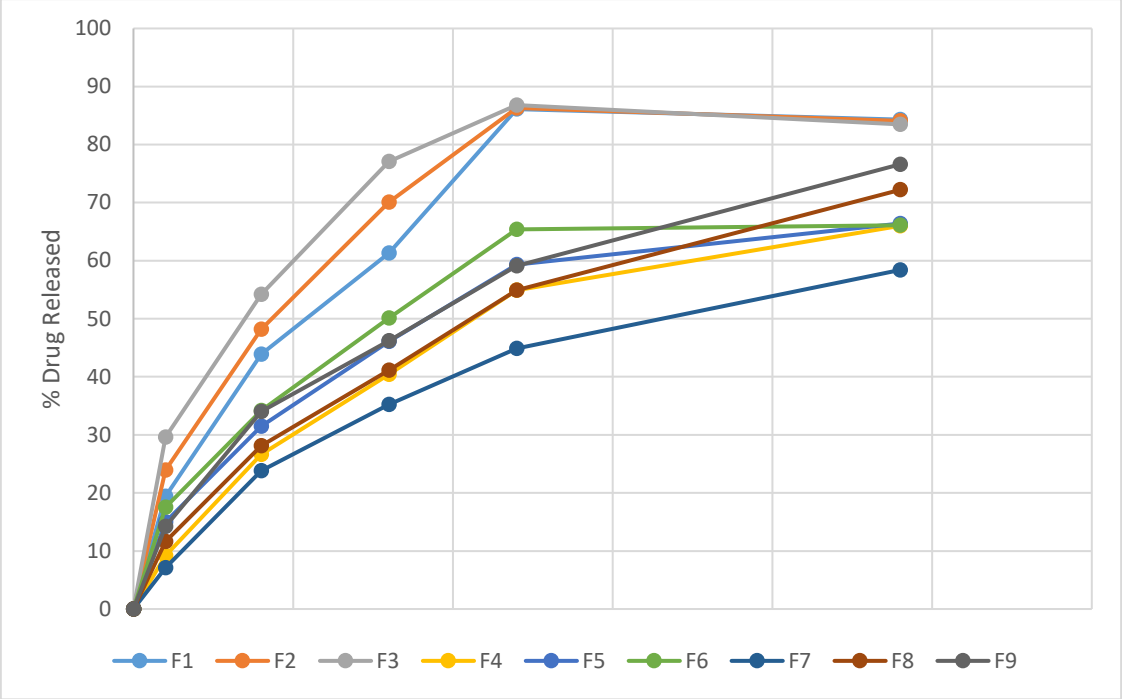

Figure 5 Release of Ofloxacin from hydrogel microspheres

As it can be inferred from the results, the quantity of Pluronic F127 was instrumental in controlling the release of Ofloxacin from the microspheres. Higher the concentration of Pluronic F127, more prolonged was the release. On the other hand, higher levels of chitosan reduced the prolonging effect of Pluronic F127. **F7** released around 58.4% drug after 24 h thereby indicating that the release may be prolonged for almost 48 h with 1:3 ratio of Pluronic F127 to chitosan.

Selection of the best formulation

The dependent variables selected for optimizing the formulation based on the 3^2 factorial approach were percent encapsulation efficiency and percent drug release. The results indicated that **F7** had the highest encapsulation of the drug (75.27%) and was able to control the release of the drug to the maximum duration (58.4% in 24 h). Hence **F7** was considered to be the most optimized formulation. The ratio of chitosan to Pluronic F127 in the formulation **F7** was 3.33:1.

The optimized formulation **F7** was subjected to stability release kinetic and stability study.

Release kinetics of F7

The kinetic modeling of drug release from F7 was done using mathematical models to determine the possible mechanism of drug release from the hydrogel microspheres. The release data was subjected to zero order, first order, Higuchi model and Korsemeyer-Peppas models to analyze the regression coefficients (Figure 6). The best regression coefficient was obtained with Higuchi mathematical model (0.9879) suggesting that the release of drug occurs by both diffusion and the dissolution of polymer (Non-Fickian diffusion).

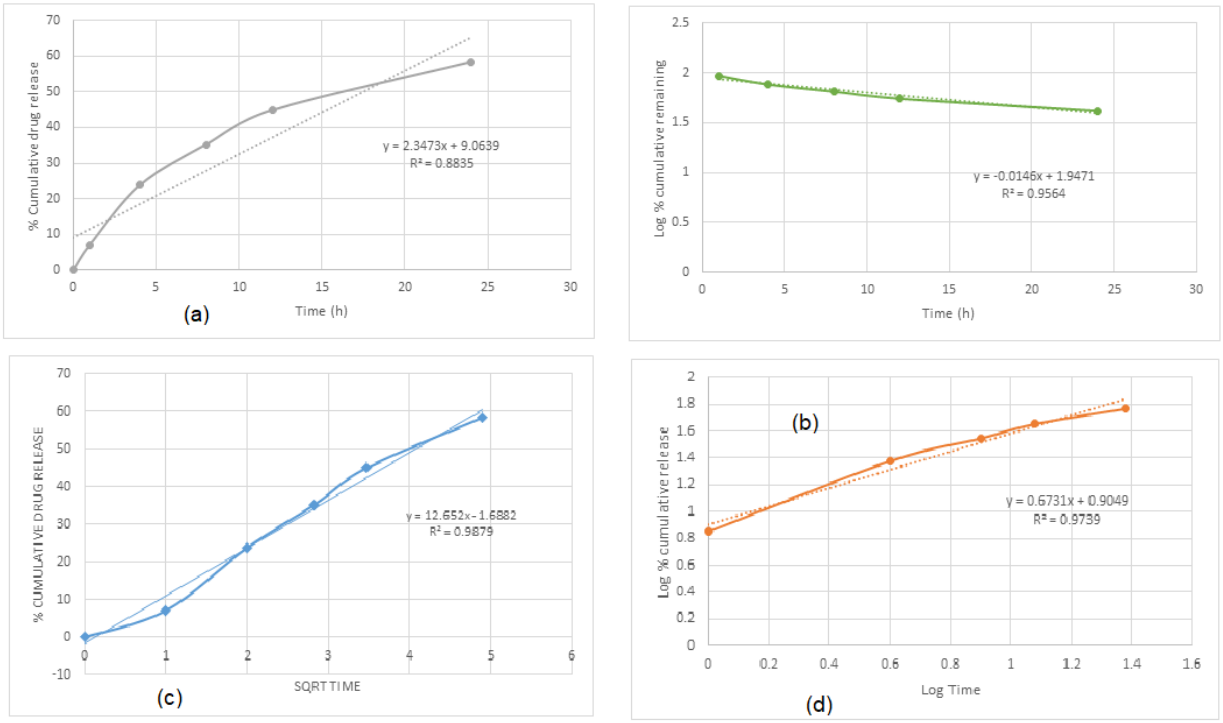

Figure 6 Release profile of F7 (a) Zero order (b) First order (c) Higuchi release (d) Korsemyer-Peppas release

Conclusion

Chitosan/Pluronic F127 was successfully used for preparation of hydrogel microspheres loaded with Ofloxacin using emulsion crosslinking method to control the release of the drug and to render ocular delivery. The formulation was able to control the release at a steady state for almost 48 h. The study led to the conclusion that hydrogel microspheres could be a viable alternative for a delivery of Ofloxacin via the ocular route in once a day dosing schedule.

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