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FORMULATION AND EVALUATION OF BIODEGRADABLE MICROSPHERE HYDROGEL FOR OCULAR DELIVERY OF METRONIDAZOLE

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Abstract
The objective of the present investigation was to explore the hydrogel microspheres loaded with metronidazole to obtain controlled release for ocular delivery. The FTIR spectra of pure drug and the drug excipient mixture revealed no chemical interaction. Metronidazole loaded hydrogel microspheres were prepared using Chitosan/Pluronic F68 blend and emulsion crosslinking method. **MHM7** with blend ratio 3.33:1 exhibited the highest encapsulation efficiency (77.1505%) whereas the lowest encapsulation was witnessed in the formulation **MHM3** (34.71%) which has a blend ratio of 15:1. The particle size ranged from 26.96 µm for **MHM3** to 134.8 µm for **MHM7.** The microspheres exhibited negative zeta potential and the value was found to be -18.1 mV for MHM7. **MHM3** exhibited the highest water uptake (350.5 %) whereas **MHM7** exhibited the lowest water uptake (117.5 %). **MHM7** released around 59.54% drug after 24 h. Formulation **MHM4** was also able to control the release to a great extent with 67.37% drug released at the end of 24 h but it exhibited a lower encapsulation efficiency of 61.771%. Hence **MHM7** was considered to be the most optimized formulation. The release of metronidazole from MHM7 followed Higuchi mathematical model suggesting Non-Fickian diffusion.

Keywords: Metronidazole, chitosan, pluronic, hydrogel, controlled release

Introduction

Metronidazole is a commonly used antibiotic, belonging to the nitroimidazole class of antibiotics. It is frequently used to treat gastrointestinal infections as well as trichomoniasis and giardiasis, and amebiasis which are parasitic infections [1].

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 [2,3].

Recently, natural polysaccharides have shown to be very useful for drug entrapment and controlled release of drugs. 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 [4-8].

Pluronics, are recognized as pharmaceutical multi-purpose excipients capable of increasing aqueous solubility and stability of drugs. Pluronic F127/F68 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 [9].

The objective of the present investigation was to explore the hydrogel microspheres loaded with metronidazole 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 metronidazole, prolonging its release in the systemic circulation.

Material and Methods

Metronidazole was obtained as gift sample from Medreich Pharmaceuticals, Bengaluru. Chitosan was purchased from Himedia lab and PLuronic F68 was purchased from Sigma Aldrich.

Preparation of Calibration Curve in PBS pH 7.4 [10]

Accurately weighed 10 mg of metronidazole was taken in 100 mL volumetric flask and dissolved in water and the volume was made up to the mark with PBS pH7.4. The solution was suitably diluted with PBS pH7.4 to obtain solutions of 10-50 ppm. The calibration curve was prepared by measuring the absorbance of the solutions at 340 nm using UV spectrophotometer.

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-68 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.

Formulation of hydrogel microspheres

The hydrogel microspheres loaded with metronidazole were prepared using 3^2 factorial approach using Chitosan as the variable X_1 and Pluronic F-68 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	MHM1	MHM2	MHM3	MHM4	MHM5	MHM6	MHM7	MHM8	MHM9
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-68 [10]. Chitosan was dissolved in 2% aqueous acetic acid solution by continuously stirring to obtain a homogeneous solution. To this solution, Pluronic-68 was dispersed and stirred overnight to form a homogeneous solution. Then, metronidazole 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-68 (%)	Glutaradehyde (mL)	Metronidazole (mg)
MHM 1	150	1	20	150
MHM 2	200	1	20	150
MHM 3	250	1	20	150
MHM 4	150	2	20	150
MHM 5	200	2	20	150
MHM 6	250	2	20	150
MHM 7	150	3	20	150
MHM 8	200	3	20	150
MHM 9	250	3	20	150

Evaluation of hydrogel microspheres

Entrapment Efficiency

Metronidazole 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 7.4) 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 340 nm by suitable dilution with phosphate buffer (pH 7.4). 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 metronidazole 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 340 nm after suitable dilution with phosphate buffer (pH 7.4).

Results and Discussion

Calibration curve of metronidazole

The absorption maximum of metronidazole in PBS 7.4 was found to be 340 nm and the calibration curve was prepared for a range of 10-50 µg/mL (Figure 1).

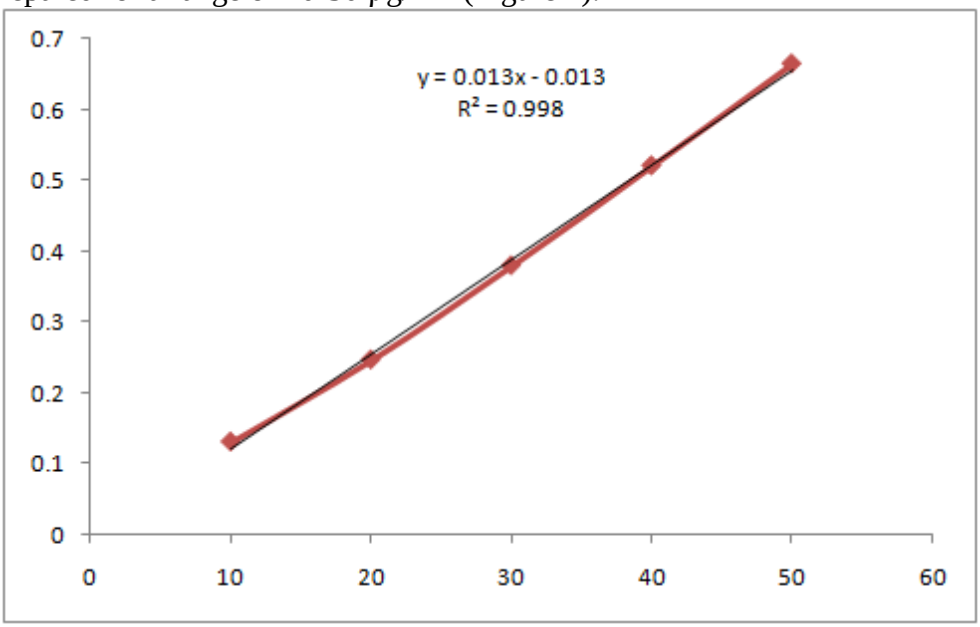

Figure 1 Standard curve of Metronidazole

Drug-excipient compatibility

The FT-IR spectrum of the procured sample of metronidazole revealed stretching vibrations of primary amine (3228 cm^{-1}), C-H (3104, 2970 cm^{-1}), amide carbonyl (1696 cm^{-1}) and out-of plane wagging of amine (695 and 658 cm^{-1}). 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).

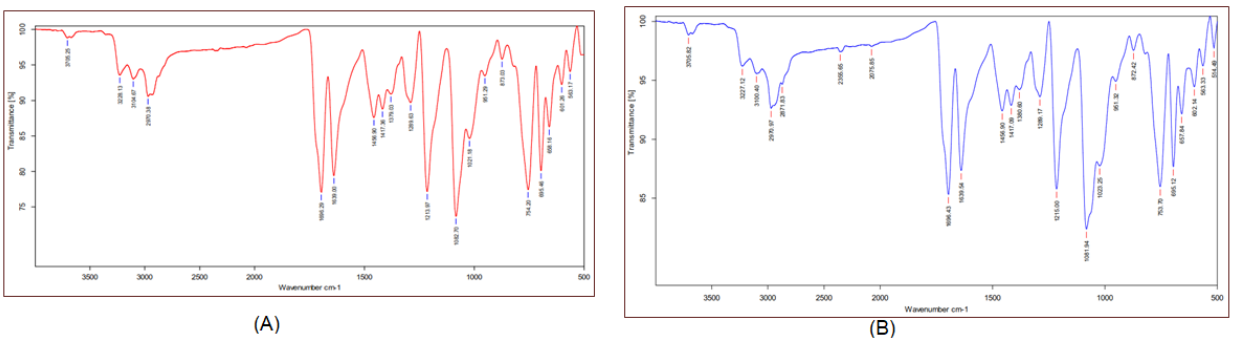

Figure 2 FTIR spectrum of (A) metronidazole (B) physical mixture of metronidazole, chitosan and pluronic F-68

Formulation of hydrogel microspheres

A total of 9 formulations were prepared using chitosan and pluronic F-68 as the two independent variables at three different levels (-1, 0, +1). The design of formulations was done as mentioned in Table 2 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 was used as the crosslinking agent for the formulation of microspheres.

The glutaraldehyde mediated crosslinking of the polymeric blend of chitosan and Pluronic F-68 may involve interaction of the aldehydic group of glutaraldehyde with the amine group of chitosan leading to a stable imide linkage [11]. This crosslinking with glutaraldehyde provides the necessary stability and hardness to the particles. The presence of Pluronic F-68 affects the particle size of the formulation due to enhanced viscosity of the emlusion [12]. 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

All the formulated hydrogel microspheres were evaluated for percent entrapment of drug, particle size and shape, zeta potential, swelling extent and drug release.

Entrapment Efficiency

The drug loading and encapsulation efficiency of the hydrogel micorspheres 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-68 was low. **MHM7** with blend ratio 3.33:1 exhibited the highest encapsulation efficiency (77.15%) whereas the lowest encapsulation was witnesses in the formulation **MHM3** (34.71%) which has a blend ratio of 15:1. As discussed previously, the presence of Pluronic F-68 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	Mean Particle Size (µm)	% Water Uptake
MHM1	21.7	46.1125	53.92	206.5
MHM2	13.9	32.7345	40.44	241
MHM3	13.3	34.713	26.96	350.5
MHM4	27.7	61.771	107.84	183.5
MHM5	22.1	55.029	80.88	217.5
MHM6	16.9	46.2215	40.44	260.5
MHM7	**32.9**	**77.1505**	134.8	117.5
MHM8	24.8	63.736	121.32	147
MHM9	21	59.64	94.36	156

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-68. As the concentration of Pluronic increased, the particle size was found to be higher. The particle size ranged from 26.96 µm for **MHM3** to 134.8 µm for **MHM7** (Table 3).

Though the particles size of MHM7 was highest but it exhibited the highest drug loading and entrapment, hence its particle size was verified using Malvern zetasizer. Also the zeta potential of MHM7 was observed. The size of hydrogel microspheres MHM7 was found to be 121.5866 µm and the zeta potential was observed to be -18.1mV.

The high zeta potential confers stability to the particles preventing their accumulation due to electrostatic repulsion between the adjacent similarly charged particles.

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 F68 decreased the water uptake by the microspheres. **MHM3** exhibited the highest water uptake (350.5 %) whereas **MHM7** exhibited the lowest water uptake (117.5 %) (Table 3).

The amphiphilic nature of Pluronic F68 could be attributed to lower swelling owing to the formation of a rigid gel on increasing the concentration of Pluronic F68.

***In vitro* release of metronidazole 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. As it can be inferred from the results, the quantity of Pluronic F68 was instrumental in controlling the release of metronidazole from the microspheres. Higher the concentration of Pluronic F68, more prolonged was the release. On the other hand, higher levels of chitosan reduced the prolonging effect of Pluronic F68. **MHM7** released around 59.54% drug after 24 h thereby indicating that the release might be prolonged for around 48 h with 1:3 ratio of Pluronic F68 to chitosan (Figure 3).

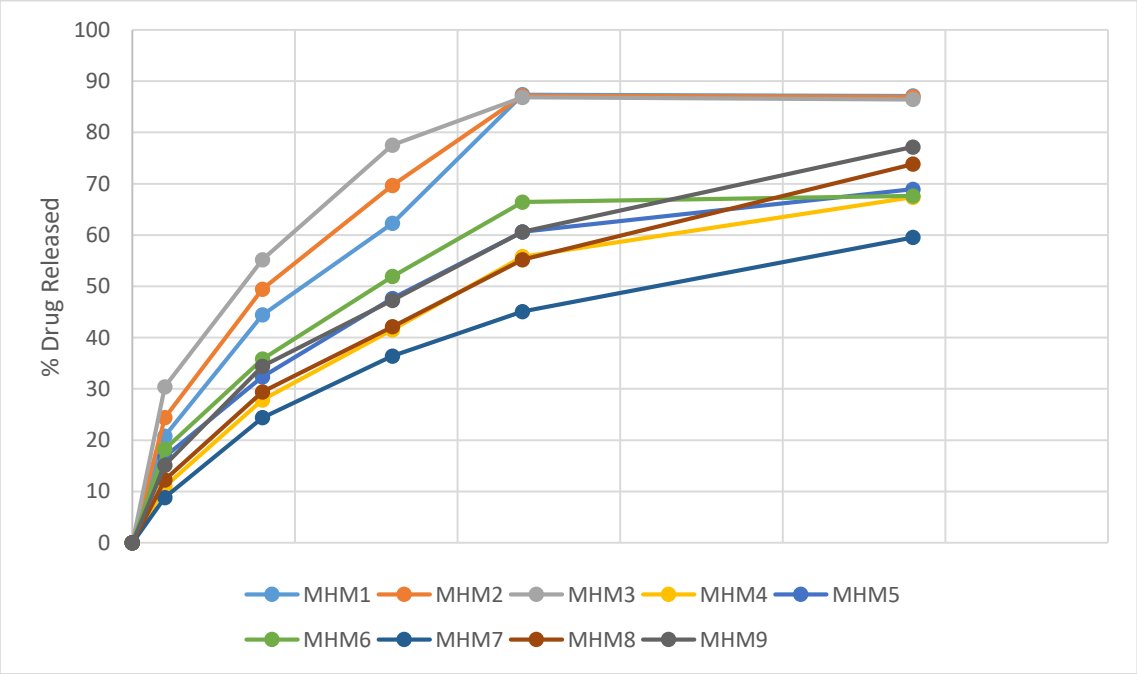

Figure 3 Release of metronidazole from hydrogel microspheres

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 **MHM7** had the highest encapsulation of the drug (77.1505%) and was able to control the release of the drug to the maximum duration (61.45% in 24 h). Formulation **MHM4** was also able to control the release to a great extent with 67.37% drug released at the end of 24 h but it exhibited an encapsulation efficiency of 59.64%. Hence **MHM7** was considered to be the most optimized formulation. The ratio of chitosan to Pluronic F68 in the formulation **MHM7** was 3.33:1.

The optimized formulation **MHM7** was subjected to stability release kinetic and stability study.

Release kinetics of MHM7

The kinetic modeling of drug release from MHM7 was done using mathematical models to determine the possible mechanism of drug release from the hydrogel microspheres (Figure 4). The best regression coefficient was obtained with Higuchi mathematical model (0.9925) suggesting that the release of drug occurs by both diffusion and the dissolution of polymer (Non-Fickian diffusion).

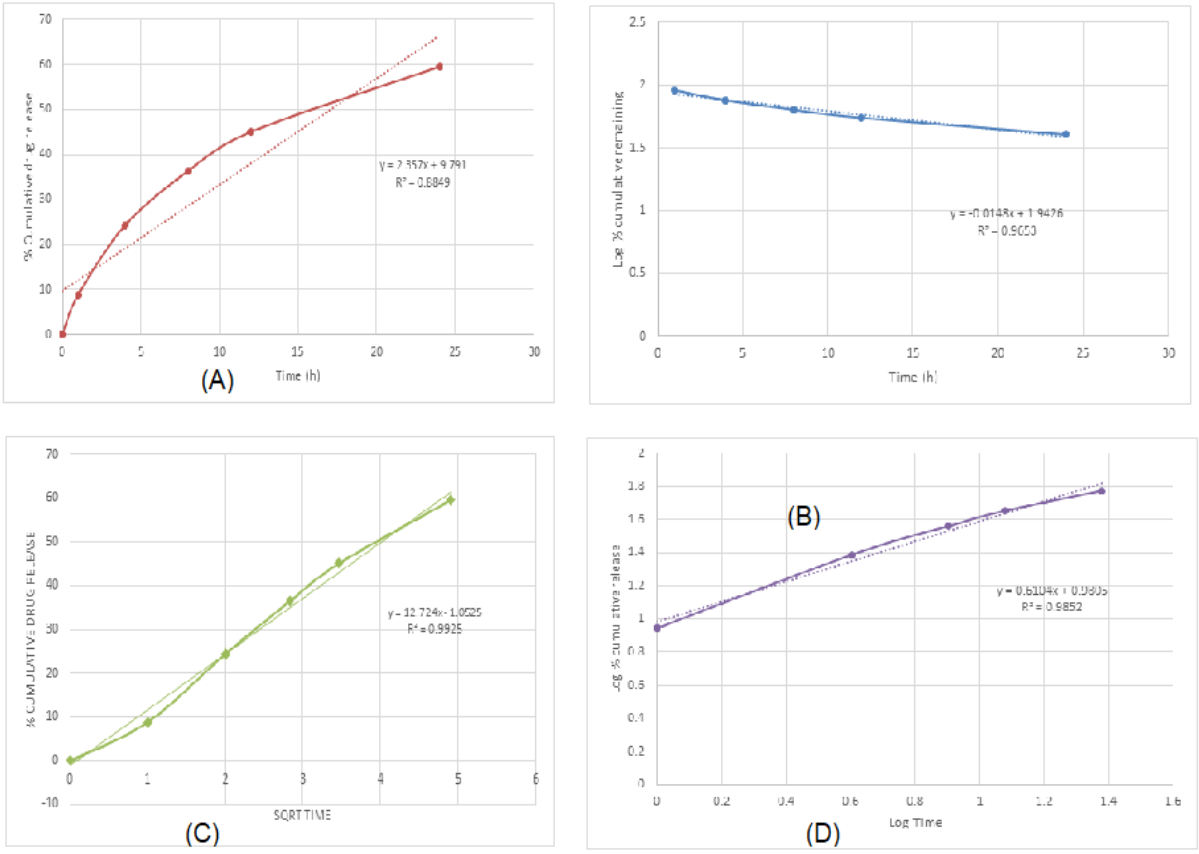

Figure 4 Plot of release (A) Zero order (B) First order (C) Higuchi (D) Korsemeyer-Peppas

Conclusion

Chitosan/Pluronic F68 was successfully used for preparation of hydrogel microspheres loaded with metronidazole 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 more than 24 h. The study led to the conclusion that hydrogel microspheres could be a viable alternative for a delivery of metronidazole via the ocular route in once a day dosing schedule.

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