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

Ashwani Jain, Pradeep Chauhan, Sandeep Jain

Abstract: ***Background: Ocular drug delivery is hindered by physiological barriers such as tear turnover and nasolacrimal drainage, leading to poor bioavailability of conventional formulations. Biodegradable hydrogel microspheres offer a promising approach for sustained ocular release of therapeutic agents.Objective: To formulate and evaluate sodium alginate–Pluronic F-68 hydrogel microspheres for ocular delivery of metronidazole using a factorial design approach.Methods: Hydrogel microspheres were prepared by ionic crosslinking of sodium alginate and Pluronic F-68, with metronidazole incorporated into the polymeric matrix. Nine formulations (MH1–MH9) were developed and evaluated for entrapment efficiency, particle size, zeta potential, swelling behaviour, and in vitro drug release in simulated tear fluid.Results: Entrapment efficiency ranged from 33.62% to 67.37%, with MH7 showing the highest drug loading (33.6%) and encapsulation efficiency (67.37%). Particle size varied between 40.44 µm and 148.28 µm, with MH7 exhibiting a zeta potential of –22.1 mV, indicating good stability. Swelling studies revealed that higher sodium alginate concentrations increased water uptake, whereas higher Pluronic F-68 concentrations reduced it. In vitro release demonstrated that MH7 achieved sustained release, with 49.4% drug released over 24 h. Kinetic modelling indicated that drug release followed the Higuchi model (R^2 = 0.9925), suggesting a diffusion-controlled mechanism.Conclusion: The optimised formulation MH7 demonstrated high encapsulation efficiency, stable particle characteristics, and prolonged drug release, making it a promising candidate for sustained ocular delivery of metronidazole.***

Keywords: Ocular Drug Delivery; Biodegradable Hydrogel Microspheres; Metronidazole; Sustained Release; Sodium Alginate

I. INTRODUCTION

Ocular drug delivery remains a significant challenge in pharmaceutical research because of the eye's unique anatomical and physiological barriers, including tear turnover, nasolacrimal drainage, and corneal impermeability, which limit drug bioavailability and therapeutic efficacy [1,2].

Conventional dosage forms such as eye drops often suffer from poor patient compliance and inadequate drug retention, necessitating advanced delivery systems [3].

Biodegradable hydrogels and microspheres have emerged as promising carriers for sustained ocular drug release. Hydrogels, owing to their high-water content and biocompatibility, can mimic natural tissue environments, while microspheres provide controlled release through polymeric encapsulation [4,5]. Among natural polymers, sodium alginate has been widely used because it gels in the presence of divalent cations, forming stable hydrogel matrices [6]. Similarly, Pluronic F-68, a non-ionic surfactant with amphiphilic properties, enhances drug entrapment and modulates release kinetics [7].

Metronidazole, a nitroimidazole derivative, is widely used to treat ocular infections caused by anaerobic microorganisms [8]. However, its conventional administration is limited by rapid clearance from the precorneal area, necessitating frequent dosing [9]. Incorporating metronidazole into biodegradable hydrogel microspheres offers the potential for sustained release, improved patient compliance, and enhanced therapeutic outcomes [10].

In this study, a factorial design approach was employed to formulate and evaluate sodium alginate–Pluronic F-68 hydrogel microspheres for ocular delivery of metronidazole. The prepared formulations were characterised for entrapment efficiency, particle size, zeta potential, swelling behaviour, and in vitro release kinetics. The aim was to identify an optimised formulation that provides prolonged drug release with high encapsulation efficiency, addressing the limitations of conventional ocular dosage forms.

II. MATERIAL AND METHODS

Metronidazole was obtained as a generous gift from Medreich Pharmaceuticals, Bengaluru; Pluronic was purchased from Sigma-Aldrich; sodium alginate and calcium chloride were purchased from Oxford Fine Chemicals, Mumbai.

A. Preformulation Studies

The obtained metronidazole sample was assessed for organoleptic features, melting point, and solubility. The FTIR spectrum was obtained to identify any physical incompatibility amongst the drug and excipients used.

B. Preparation of Calibration Curve in PBS pH 7.4 [11]

Accurately weighed 10 mg of metronidazole was taken in a 100 mL volumetric flask, dissolved in water,

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*Correspondence Author(s)
Ashwani Jain*,IPS College of Pharmacy, Gwalior (Madhya Pradesh), India. Email ID:mrashwani360@gmail.com
Pradeep Chauhan,IPS College of Pharmacy, Gwalior (Madhya Pradesh), India. Email ID: rbsofficework@gmail.com
Sandeep Jain, IPS College of Pharmacy, Gwalior (Madhya Pradesh), India. Email ID: sandeepjainips@gmail.com

and the volume was made up to the mark with PBS pH 7.4. The solution was suitably diluted with PBS pH 7.4 to obtain solutions of 10-50 ppm. The calibration curve was prepared by measuring the absorbance of the solutions at 340 nm using a UV spectrophotometer.

C. Formulation of Hydrogel Microspheres

The hydrogel microspheres loaded with metronidazole were prepared using a 32-factorial approach, with sodium alginate as variable X_1 and Pluronic F-68 as variable X_2. Both variables were used at three different levels (+1, 0, -1) to obtain 9 different formulations. Table 1 presents the design table for the formulations. Percentage drug release and per cent drug encapsulation were used as the dependent variables. Table 2 presents the quantities of drug and polymers used.

Table I:Design Table for Formulation of Hydrogel Microspheres

Formulation Code	MH1	MH2	MH3	MH4	MH5	MH6	MH7	MH8	MH9
X_1	-1	0	+1	-1	0	+1	-1	0	+1
X_2	-1	-1	-1	0	0	0	+1	+1	+1

The ionic crosslinking method was used to prepare sodium alginate and Pluronic F-68 hydrogel microspheres [12]. Sodium alginate was dissolved in water by continuously stirring to obtain a homogeneous solution. Pluronic F-68 was dispersed in this solution and stirred overnight to obtain a homogeneous solution. Then, metronidazole was dissolved in the above polymer solution. The drug-polymer solution was placed in a 100 mL plastic syringe and dropped dropwise at a constant flow rate through a 26 G blunt-tipped metal needle into the gelling solution (1.0% w/v $CaCl_2$ solution). The $CaCl_2$ solution was homogenised using the magnetic stirrer. The prepared hydrogel microspheres were filtered and dried at 45°C.

Table II:Composition of Hydrogel Microspheres

Formulation Code	Sodium alginate (g)	Pluronic F-68 (%)	Metronidazole (mg)
MH 1	0.15	1	150
MH 2	0.2	1	150
MH 3	0.25	1	150
MH 4	0.15	2	150
MH 5	0.2	2	150
MH 6	0.25	2	150
MH 7	0.15	3	150
MH 8	0.2	3	150
MH 9	0.25	3	150

III. EVALUATION OF HYDROGEL MICROSPHERES

A. Entrapment Efficiency

Metronidazole content in the microspheres was estimated in phosphate buffer (pH 7.4). Microspheres (10 mg) were powdered using a 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 was then analysed by UV spectrophotometry at λmax = 340 nm after suitable dilution with phosphate buffer (pH 7.4). The % encapsulation efficiency was calculated as

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

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

B. Particle Size and Zeta Potential Measurement

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

C. FTIR Spectral Measurements

FTIR spectra of the blank microspheres, drug-loaded microspheres and the neat drug were obtained using a Bruker Alpha FTIR spectrometer. Scanning was performed from 400 to 4000 cm^{-1}.

D. Swelling Study

Equilibrium water uptake by the hydrogel microspheres was determined by measuring swelling in distilled water for 24 h. Excess liquid drops adhered to the surface were removed by blotting with 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 the weight of the dried samples no longer changed. The % equilibrium water uptake was calculated as

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

E. In Vitro Release Study

Simulated tear fluid was prepared by dissolving NaCl (6.8 g), NaHCO3 (2.2 g), CaCl2·2H2O (0.08 g), and KCl (1.4 g) in distilled deionised water to 100 mL.The *in vitro* release of metronidazole from the hydrogel microspheres was determined using a tablet dissolution tester utilising simulated tear fluid as the dissolution medium. A weighed quantity of the hydrogel microspheres was placed in dissolution baskets maintained at 37°C and rotated at 100 rpm. Samples were withdrawn at predetermined intervals and analysedusing a UV spectrophotometer at λmax = 340 nm after suitable dilution with phosphate buffer (pH 7.4).

IV. RESULTS AND DISCUSSION

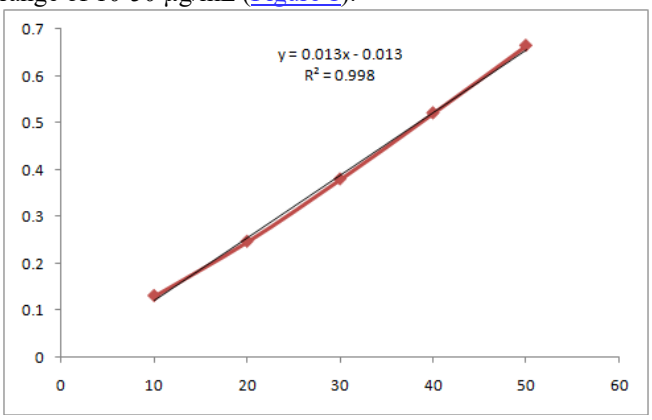
Table 3 presents the organoleptic observations of the acquired metronidazole sample.

Table III:PreformulationParameters of Metronidazole

S.No	Test	Observation
1	Physical Appearance	White
2	Odour	Odourless
3	Melting Point	157-159°C
4	Solubility Characters	Soluble in water, ethanol, and 0.1N HCl

A. Calibration Curve of Metronidazole

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



[Fig.1: Standard Curve of Metronidazole]

B. Drug-Excipient Compatibility Study

To confirm drug-excipient compatibility, a physical mixture of sodium alginate, pluronic F-68, and metronidazole was subjected to FT-IR analysis. The spectrum was examined for stretching and bending vibrations. The mixture spectra 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.

Table IV: FTIR Peaks of Physical Mixture of Metronidazole and Metronidazole, Sodium Alginate and Pluronic F-68

S. No.	Wave Number, cm^{-1} in Metronidazole	Wave Number, cm^{-1} in Physical Mixture	Functional Group
1	-	3705	OH stretching
2	3228	3227	Primary amine stretching
3	3104, 2970	3100, 2970	CH Stretching
4	1696	1696	O=C-NH_2
5	-	1456	C-N stretching
6	695, 658	695, 658	Out-of-plane N-H Wagging

C. Formulation of Hydrogel Microspheres

We prepared 9 formulations using sodium alginate and Pluronic F-68 as the two independent variables at three levels (-1, 0, +1). The formulation design was as described in Table 5.4, and ionic crosslinking was used to prepare the hydrogel microspheres. The amount of drug encapsulated and the percentage drug released from the hydrogel microspheres were evaluated to describe the best formulation.

D. Evaluation of the Hydrogel Microspheres

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

E. Entrapment Efficiency

The drug loading and encapsulation efficiency of the hydrogel microspheres were determined using the reported method, and encapsulation was found to depend on the polymeric blend ratio (Table 5). **MH7** exhibited the highest encapsulation efficiency (67.37%), whereas MH showed the lowest encapsulation (33.62%). As discussed previously, the presence of Pluronic F-68 enhances the viscosity of the emulsion, thereby increasing drug entrapment in the matrix globules.

Table V:Drug Loading and Entrapment Efficiency of Hydrogel Microspheres

Formulation Code	% Drug Loading	% Entrapment Efficiency	Mean Particle Size (µm)
MH1	21.5	43.04	67.4
MH2	14.4	33.62	53.92
MH3	13.9	37.9	40.44
MH4	26.7	53.49	121.32
MH5	22.3	52.11	94.36
MH6	18.4	49.13	53.92
MH7	**33.6**	**67.37**	148.28
MH8	25.4	59.39	134.8
MH9	22.3	59.57	107.84

F. Particle Size and Zeta Potential

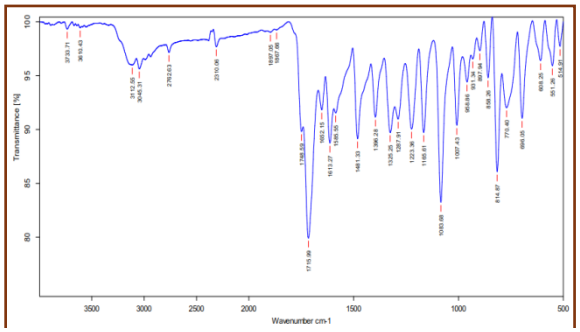
Particle size measurements of all microspheres were done using a calibrated ocular micrometre. Particle size was also found to depend on the amount of Pluronic F-68. As the concentration of Pluronic increased, particle size increased. The particle size ranged from 40.44 µm for **MH3** to 148.28 µm for **MH7** (Table 5).

Although MH7 had the largest particle size, it exhibited the highest drug loading and entrapment; therefore, its particle size was verified using a Malvern Zetasizer. The zeta potential of MH7 was also observed. The size of hydrogel microspheres MH7 was 116.7 µm, and the zeta potential was -22.1 mV.

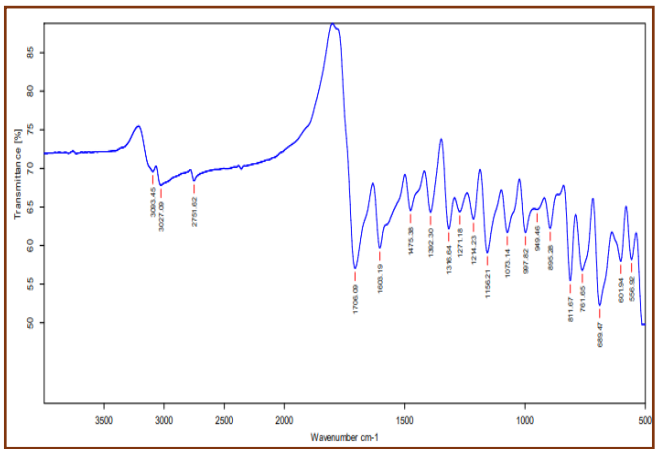
The high zeta potential confers stability to the particles, preventing their accumulation due to electrostatic repulsion between the adjacent similarly charged particles.

G. FTIR Spectral Measurements of Hydrogel Microspheres

The FTIR spectra of blank and drug-loaded microspheres were compared with the pure drug, and the drug-loaded microspheres showed all the peaks of the pure drug. In contrast, most characteristic metronidazole peaks were absent in the blank hydrogel microspheres (Figure 6.7, 6.8).



[Fig.2: FTIR of Drug-Loaded Hydrogel Microsphere (MH7)]



[Fig.3: FTIR of Blank Microsphere]

H. Swelling Study

The water uptake results indicate that the per cent water uptake of the hydrogel microspheres was affected by the sodium alginate concentration. Higher sodium alginate levels increased water uptake. In contrast, a higher Pluronic F68 ratio decreased water uptake in the microspheres. **MH3** exhibited the highest water uptake (300.5%), whereas MH7 exhibited the lowest water uptake (67.5%). Table 6 shows the swelling results.

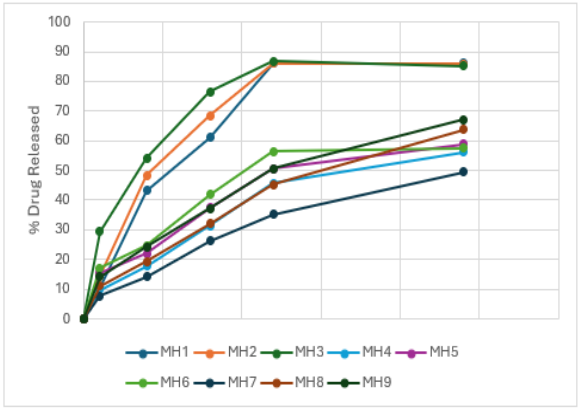
Table VI: Per Cent Water Uptake by Hydrogel Microspheres

Formulation Code	% Water Uptake
MH1	156.5
MH2	191
MH3	300.5
MH4	133.5
MH5	167.5
MH6	210.5
MH7	67.5
MH8	97
MH9	106

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.

i. In Vitro Release of Metronidazole from Hydrogel Microspheres

In vitro drug release from hydrogel microspheres was evaluated using a dissolution test apparatus in simulated tear fluid. The results of *the in vitro release study over* 24 h are presented in Figure 4.



[Fig.4: Release of Metronidazole from Hydrogel Microspheres]

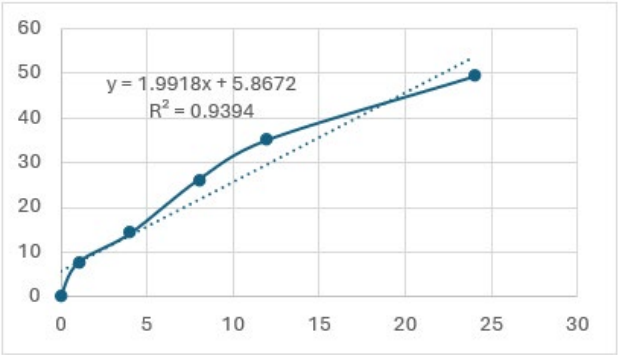
As inferred from the results, the quantity of Pluronic F68 was instrumental in controlling metronidazole release from the microspheres. Higher Pluronic F68 concentration prolonged the release. In contrast, higher chitosan levels reduced the prolonging effect of Pluronic F68. **MH7** released around 49.54% drug after 24 h, thereby indicating that the release might be prolonged for around 48 h with 3% Pluronic F68 and 0.25 g sodium alginate.

I. Selection of the Best Formulation

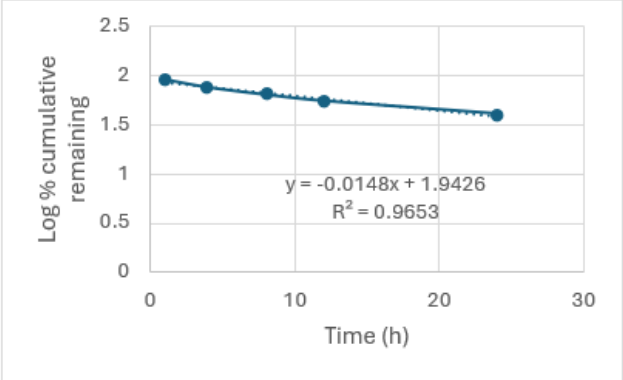
The dependent variables selected to optimise the formulation were per cent encapsulation efficiency and per cent drug release. The results indicated that **MH7** had the highest drug encapsulation (67.37%) and controlled drug release for the longest duration (49.4% in 24 h). Formulation **MH4** also controlled release to a great extent, with 56.2% of the drug released at the end of 24 h, but it exhibited an encapsulation efficiency of 53%. Hence, MH7 was considered to be the most optimised formulation. The optimised formulation, MH7, was subjected to stability and release kinetics studies.

J. Release Kinetics of MH7

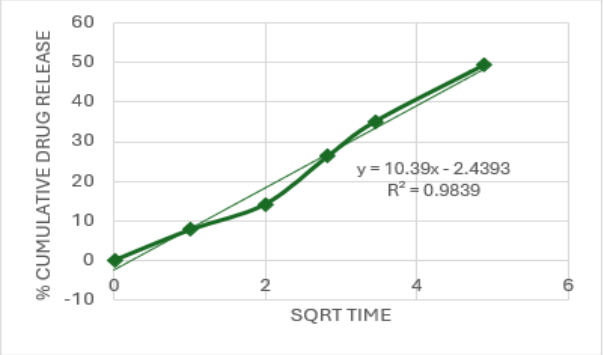
Kinetic modelling of drug release from MH7 was performed using mathematical models to determine the possible mechanism of drug release from the hydrogel microspheres. The release data were subjected to zero-order, first-order, Higuchi model and Korsmeyer-Peppas models to analyse the regression coefficients (Figure5-8). The Higuchi model yielded the best regression coefficient (0.9925), suggesting that drug release occurs by both diffusion and polymer dissolution (non-Fickian diffusion).



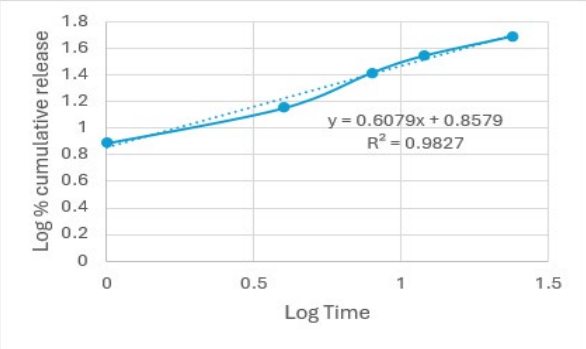
[Fig.5. Zero-Order Release Profile of MH7]



[Fig.6: First-Order Release Profile of MH7]



[Fig.7: Higuchi Model for Release Profile of MH7]



[Fig.8: Korsmeyer-Peppas Model for Release Profile of MH7]

V. CONCLUSION

The present study successfully formulated and evaluated biodegradable hydrogel microspheres of metronidazole using sodium alginate and Pluronic F-68. The factorial design approach enabled systematic optimisation of polymer ratios, revealing that Pluronic F-68 concentration significantly influenced drug entrapment and release kinetics. Among the nine formulations, MH7 emerged as the most optimised, exhibiting high encapsulation efficiency (67.37%), stable particle size (116.7 µm), and prolonged drug release (49.4% over 24 h). The release kinetics followed the Higuchi model, indicating a diffusion-controlled mechanism. These findings suggest that sodium alginate–Pluronic F-68 hydrogel microspheres can serve as an effective sustained-release ocular delivery system for metronidazole, potentially improving therapeutic efficacy and patient compliance compared to conventional eye drops.

DECLARATION STATEMENT

Some of the cited references are older and are noted explicitly as [1], [2], [3], [4], [5], [6], [7], [8], [9], [10] and [11]. However, these works remain significant for the current study, as they are pioneering in their fields.

After aggregating input from all authors, I must verify the accuracy of the following information as the article's author.

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- **Funding Support:** This article has not been funded by any organisations or agencies. This independence ensures the research is conducted objectively and without external influence.
- **Ethical Approval and Consent to Participate:** The content of this article does not necessitate ethical approval or consent to participate, with supporting documentation.
- **Data Access Statement and Material Availability:** The adequate resources of this article are publicly accessible.
- **Authors' Contributions:** The authorship of this article is contributed equally to all participating individuals.

REFERENCES

1. Gaudana R, Jwala J, Boddu SHS, Mitra AK. Recent perspectives in ocular drug delivery. Pharm Res. 2009;26(5):1197-216. DOI: 10.1007/s11095-008-9694-0, Works remain significant; see the declaration
2. Bourlais CL, Acar L, Zia H, Sado PA, Needham T, Leverge R. Ophthalmic drug delivery systems—recent advances. Prog Retin Eye Res. 1998;17(1):33-58. DOI: 10.1016/S1350-9462(97)00002-5, Work remains significant; see the declaration
3. Urtti A. Challenges and obstacles of ocular pharmacokinetics and drug delivery. Adv Drug Deliv Rev. 2006;58(11):1131-5. DOI: 10.1016/j.addr.2006.07.027, Works remain significant; see the declaration
4. Peppas NA, Hilt JZ, Khademhosseini A, Langer R. Hydrogels in biology and medicine: from molecular principles to bionanotechnology. Adv Mater. 2006;18(11):1345-60. DOI: 10.1002/adma.200501612, Works remain significant; see the declaration
5. Sahoo SK, Panyam J, Prabha S, Labhasetwar V. Residual polyvinyl alcohol associated with poly (d,l-lactide-co-glycolide) nanoparticles affects their physical properties and cellular uptake. J Control Release. 2002;82(1):105-14. DOI: 10.1016/S0168-3659(02)00127-X, Works remain significant; see the declaration
6. Lee KY, Mooney DJ. Alginate: properties and biomedical applications. Prog Polym Sci. 2012;37(1):106-26. DOI: 10.1016/j.progpolymsci.2011.06.003, Works remain significant; see the declaration
7. Alexandridis P, Hatton TA. Poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: thermodynamics, structure, dynamics, and modelling. Colloids Surf APhysicochem Eng Asp. 1995;96(1-2):1-46. DOI: 10.1016/0927-7757(94)03028-X, Works remain significant; see the declaration
8. Brook I. Metronidazole therapy for anaerobic infections. Clin Infect Dis. 1994;18(Suppl 4): S285-9. DOI: 10.1086/647939, Works remain significant; see the declaration
9. Schoenwald RD. Ocular drug delivery. Pharm Biotechnol. 1998; 6:13-58. DOI: 10.2165/00003088-199018040-00001, Works remain significant; see the declaration
10. Jain AK, Jain S, Jain NK. Novel ocular delivery systems: concepts and applications. Indian J Pharm Sci. 2003;65(4):337-43. DOI: 10.1089/jop.2012.0200, Works remain significant; see the declaration
11. Naveed S, Qamar F. Simple UV Spectrophotometric Assay of Metronidazole. Open Access Library Journal. 2014; 1: e615, DOI: 10.4236/oalib.1100615, Works remain significant; see the declaration
12. Savi´c Gaji´c, I.M.; Savi´c, I.M.; Svir˘cev, Z. Preparation and Characterisation of Alginate Hydrogels with High Water-Retaining Capacity. Polymers 2023, 15, 2592. DOI: 10.3390/polym15122592