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Original Article

EYE DROPS WITH NANOPARTICLES AS DRUG DELIVERY SYSTEMS

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ABSTRACT

Objective: The objective of this study was to examine and characterize topical eye drops with indomethacin-loaded poly(vinyl acetate) nanoparticles (IMC-p(VAc)-NPs).

Methods: IMC-p(VAc)-NPs were obtained by emulsifier-free radical homopolymerization of the monomers in the presence of indomethacin in water and in an aqueous solution of Carbopol®. Thus obtained indomethacin nanocarriers were included in topical ophthalmic formulations. (Hydroxypropyl)methyl cellulose was used in different concentrations to increase the viscosity of the eye drops. Rheological characteristics, the surface tension, the ocular tolerance according to *In vitro* hen's egg test–chorioallantoic membrane, and the indomethacin release from the eye drops models were studied.

Results: The investigation of the rheological characteristics and the surface tension of the (hydroxypropyl)methyl cellulosesolutions showed the suitable concentrations as an excipient increasing the viscosity of the eye drops with IMC-p(VAc)-NPs. *In vitro* study of the indomethacin release from the eye drops showed that the investigated nanocarriers had a different behavior according to the releaseddrugfrom the NPs in phosphate-phosphate buffer at *pH* 7.4. No signs of ocular irritation were detected within 5 min according to *In Vitro* hen's egg test–chorioallantoicmembrane - for the investigated IMC-p(VAc)-NPs, contrary to the indomethacin substance.

Conclusion: The obtained results prove the possibility to prepare topical eye drops with IMC-p(VAc)-NPs as a drug delivery systems and give reasons to continue the research in this direction.

Keywords: Indomethacin-loaded nanoparticles, HPMC, Carbopol coated nanoparticles, Eye drops, *In vitro* HET-CAM.

INTRODUCTION

In the recent years, there has been an increased interest in the wide range of nanocarriers as drug delivery systems [1]. Nanoparticles (NPs) [2], liposomes [3], nanosuspensions [4], nanoemulsions [5] etc, have been studied as drug delivery and drug releasing systems for different formulations. Nanocarriers have been used to increase the drug solubility [1, 4] and the drug stability in storage and in biological environment. These effects lead to increased drug bioavailability [1, 4] and reduction of the dose, the drug toxicity and the side effects [6]. Different nanocarriers have been also studied as systems for targeted delivery and controlled drug release [2-7].

Indomethacin (IMC), ([1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]-acetic acid) is a nonsteroidal anti-inflammatory drug, used in ophthalmology as topical eye drops for prevention of miosis during cataract surgery, cystoid macular edema and conjunctivitis [8, 9]. Its use in liquid formulations is limited due to its insolubility in water, low bioavailability and ocular mucosa irritation. In previous studies the possibility of *in-situ* including of IMC in poly(vinyl acetate) (pVAc) NPs via emulsifier free radical polymerisation was demonstrated [10] and a sustained drug release was proved [10, 11]. The main purpose of these studies was to include the obtained indomethacin loaded poly(vinyl acetate) NPs (IMC-p(VAc)-NPs) in an ophthalmic formulation.

There was no information in the available literature about the interaction between the IMC and the used monomers and initiator of the polymerization, as well as about the IMC influence on the stability of the monomer and polymer dispersions in water. The preliminary experiments allowed choosing the emulsion polymerization conditions, excluding chemical modification and degradation of the IMC molecule [10-12]. On the other hand, the IMC concentration (1% (w/v)) led to minimum coagulated formations during the polymerization with high yield of NPs [13]. Even more, stable polymer latexes with included IMC in nanosized latex particles, were produced without the usage of surfactants, an important advantage of this method for a drug formulation. The challenge was to find easily available and feasible technological parameters for the effective control of the IMC release from the polymer NPs. It was achieved by changing the mixture of the compatible polymers (pVAc, poly(3-dimethyl(methacryloyloxyethyl) ammonium propane sulfonate) (pDMAPS), Carbopol®, p(VA-co-DMAPS) and chitosan) from which the NPs with included IMC were prepared.

The obtained results confirm the efficiency of these approaches for the control of the IMC degree of loading, encapsulation efficiency, its release degree and also rate of release [13]. As drug releasing systems for IMC in topical ophthalmic formulations were selected models with IMC-p(VAc)-NPs which are characterized by high values of yield (%Y), encapsulation efficiency (%EE), drug loading (%DL), and zeta potential (ζP) of NPs in a Sorensen's PPB at pH 7.4 as an indicator of the physical stability of the system; they have an optimal average particle size (Z-average), and provide prolonged release of the drug. The objective of this research was to study and characterize topical eye drops with IMC- loaded p(VAc)-NPs.

MATERIALS AND METHODS

In this research IMC (European Pharmacopoeia reference material) as a drug and vinyl acetate (VAc) as a monomer were purchased from Fluka. Ammonium persulfate (Fluka) was used as an initiator. Potassium dihydrogen phosphate and *di*-sodium hydrogen phosphate from Merck (Darmstadt, Germany) were used for the preparation of a phosphate-phosphate buffer (Sorensen's phosphate buffer) (PPB). It was used as a medium for dissolution of IMC-p(VAc)-NPs. Carbopol 971 (BF Goodrich, Cleveland, OH) (Cbp) was used as a polymer to obtain some of the IMC-p(VAc)-NPs. (Hydroxypropyl) methyl cellulose F4M (Dou USA Chemical Corporation) (HPMC) was used for the preparation of the technological models of eye drops, and Indocollyre® 1mg/ml, 5 ml, (Bausch & Lomb Incorporated) was compared to the proposed ophthalmic formulations. Benzalkonium chloride (BC) (Fluka) was added as a preservative.

Preparation and characterization of IMC-loaded nanocarriers

IMC-loaded NPs were obtained by an emulsifier-free radical polymerization of VAc 10%(v/v) in the presence of IMC 1% (w/v) in water (IMC-p(VAc)), and in aqueous solution of Cbp 0.5% (w/v) (IMC-p(VAc)+Cbp). The preparation method has been detailed in previous studies [10, 11]. Briefly, the polymerization was conducted in a nitrogen atmosphere at a temperature of 55°C, for 90 min under ultrasonic impact (Ultrasonicator Siel UST7.8-200, Gabrovo, Bulgaria). Ammonium persulphate (AP) in concentration 1% (w/v) was used as an initiator. The model latexes were exposed to dialysis through membrane with MWCO 8000 Da for 7 h to eliminate the low molecular weight compounds (e. g. the initiator of the process, residual monomers or free IMC) from the primary latex, and then the samples were freeze-dried.

TEM and DLS were used to observe the microstructure and determine the particle size [11, 13]. XRD-, FTIR-, UV-spectroscopy and simultaneous DTA-TG analysis were applied for the determination of the IMC inclusion and *In vitro* release characteristics [11, 13]. To determine the kinetic model that best describes the release mechanism, the *In vitro* release data were analyzed according to zero-, first- and Higuchi models [13].

Preparing of the model eye drops with IMC-p(VAc)-NPs

In laminar flow accurately weighed quantity of IMC-p(VAc)-NPs was dispersed in a thermostatically controlled vessel with a volume of the dissolution medium 100.0 ml Sorensen's PPB with pH 7.4 at 20°C under continuous stirring with 100 min^{-1} for 1 h. After that the aqueous dispersions of IMC-p(VAc)-NPs were filtered through Chromafil® Xtra 0.22 μm and under aseptic conditions and continuous stirring an accurately weighed quantity of HPMC and BC 0.1% (w/v) as preservative were added at each formulations. Homogenization of the models continued until complete dissolution of the HPMC and the preservative.

Methods used for characterization of the eye drops

Viscosity of the HPMC solutions

The viscosity of the HPMC solutions was determined by Rheotest 2 (RHEOTEST Messgeräte Medingen GMBH) with a N cylinder at a temperature of 20°C in I station, "a" position with Z1 = 3.30 and Z2 = 31.70.

Tangential stress (T) [14] was calculated according to the equation:

$$T = \frac{\alpha \times Z}{10}, [Pa] (1)$$

The viscosity of the solutions (η) [14] was calculated according to the following equation:

$$\eta = \frac{T}{D}, [Pa.S] (2)$$

Surface tension of the HPMC solutions at 35°C

For the purpose of the experiment an interface tensiometer was designed, based on the principle of the "ring-method" [14-17]. A platinum ring was connected to a tension transducer TRI 201 (20 mN - Isometrical force transducer LSi LETICA; Panlabsl, Barcelona, Spain). The value of the surface tension was measured by a fully developed tension transducer interface system for registration and analysis of the change of the applied mechanical pressure due to the generated surface tension after a vertical ring translation in the direction of the liquid free surface. The transformation of the signal in a digital form was done by a 13bit analog-to-digital converter based on a programmable microcontroller. The measurements were performed after calibrating the apparatus with control solutions at a constant temperature.

Equal amounts of the tested samples (5 ml) were placed into a Petri dish and using the system of vertical microtranslation, the ring was immersed into the liquid. Then in the opposite direction, a force was gradually applied with the screw until the ring was removed from the liquid. The values recorded by the tension sensor were transformed into a digital form, stored in two-dimensional data arrays and the recorded maximum force was determined and presented in a graphical form. Nine measurements were carried out for each of the experimental groups. The obtained data was statistically analyzed by Kruskall-Wallis non-parametric test at a significance level of $p < 0.05$.

***pH* of the solutions**

pH of the solutions was determined by a Corning *pH*-meter 440 (Corning Incorporated, Corning, NY). Each measurement was repeated three times and the result was presented as a mean value ± SD.

***In vitro* study of the IMC release from the eye drops**

The study was performed in a thermostatically controlled vessel with equal amounts of the tested models under perfect "sink" conditions. The acceptor medium for dissolving was 100.0 ml Sorensen's PPB at pH 7.4, the donor medium was 10 ml and the dialysis membrane MWCO 8000 Da was 4 cm^2[18]. The temperature was 37°C and the stirring speed was 100 min^{-1}.

For the purpose of this study samples for analysis were taken at regular intervals, depending on the type of the polymeric carrier. The quantitative determination of the released IMC was carried out spectrophotometrically at λ = 320 nm [18] with Ultrospec 3300 pro (Biochrom Ltd., Cambridge, UK) after filtration of the samples through Chromafil® Xtra 0.45 μm filter. The measurements were performed compared to the tested medium - Sorensen's PPB at pH 7.4. Each experiment was repeated six times and the results were presented as mean values. The concentration of IMC was calculated on a standard curve with linear coefficient (r) = 0.999.

Ocular tolerance assays with *In vitro* HET-CAM test

The risk of ocular irritation by the NPs was assessed using the hen's egg test–chorioallantoic membrane (HET-CAM) [17]. The HET-CAM is based on direct application onto the CAM and the subsequent reactions, such as hemorrhage, intravascular coagulation or lysis of blood vessels, which are microscopically assessed along a time-course [17, 19]. These irritation effects may occur within 300 seconds after mucosal administration of the sample onto the HET-CAM, according to the Invittox protocol [18, 20]. Fresh (not older than seven days), clean, fertile, White Leghorn chicken eggs weighing between 50 and 60 grams were incubated for 9 days and after this time defective eggs were discarded. The shell around the air cell was removed and the inner membranes extracted to reveal the CAM. A 0.9% NaCl negative control and a 0.1 N NaOH positive control were used in each experiment in order to provide a baseline for the assay endpoints and to ensure that the assay conditions do not inappropriately result in an irritant response. 6 eggs per group were used (negative and positive controls, IMC-p (VAc), IMC-p(VAc)+Cbp, and IMC substance). After moistening the inner membrane with 0.9% NaCl, 0.3 mg IMC-p(VAc)-NPs under test and IMC substance was applied to the CAM so that at least 50% of the CAM surface area was covered and left in contact for 300 seconds. The intensity of the reactions was semi-quantitatively assessed on a scale from 0 (no reaction) to 3 (strong reaction). The time of onset and the intensity of reactions occurring within 5 min were recorded. The ocular irritation index (OII) [19, 20] was then calculated using the following equation

$$OII = (301 - h) \times 5/300 + (301 - l) \times 7/300 + (301 - c) \times 9/300 (3)$$

Where

"h" is hemorrhage time = observed start (in seconds) of hemorrhage reactions on CAM;

"l" is lysis time = observed start (in seconds) of vessel lysis on CAM;

and "c" is coagulation time = observed start (in seconds) of coagulation formation on CAM.

The following classification was used: OII ≤ 0.9, slightly irritating; 0.9 < OII ≤ 4.9, moderately irritating; 4.9 < OII ≤ 8.9, irritating; and 8.9 < OII ≤ 21, severely irritating.

Statistical assessment of the data

The statistical analysis was performed using SPSS 11.5 (SPSS Inc, Chicago, IL, USA). The data were processed through a detailed and a

comparative analysis. The appropriate statistical analysis was determined after checking the distribution for normality of the variables with Kolmogorov-Smirnov test. Non-parametric tests for independent samples - Kruskal Wallis test, were used when the parametric tests did not meet the conditions for application. After that Post Hoc analysis was performed using Dunn`s Multiple Comparison Test and adjusted according to the number of comparisons level of statistically significant difference [21, 22].

RESULTS AND DISCUSSION

The IMC-p(VAc) and IMC-p(VAc)+Cbp were chosen for the preparation of the eye drops models. The factors, which determined that choice were: the zeta potential (ζP) of NPs in a Sorensen's PPB at pH 7.4 as an indicator of the physical stability of the system, the NP size distribution (PSD), average particle size (Z-average), yield (%Y) of the NPs, encapsulation efficiency (%EE) and drug loading (%DL), the time for the release of 85% of the included IMC ($T_{85\%}$) and the kinetics of this release (Table 1) [13].

As an excipient increasing the viscosity of the eye drops was used HPMC in concentration of 0.25, 0.50, 1.0, and 2.0% (Solution 1, 2, 3 and 4). Sorensen's PPB at pH 7.4 was used as a medium for the dissolution of HPMC. The rheological parameters of the proposed HPMC solutions were investigated in order to choose an appropriate concentration for application in the ophthalmic liquid formulation models.

Table 1: Zeta potential (ζP) of IMC-NPs in a Sorensen's PPB at pH 7.4, NP size distribution (PSD), average particle size (Z-average), yield (%Y) of the NPs, encapsulation efficiency (%EE) and drug loading (%DL), the time for the release of 85% of the included IMC ($T_{85\%}$), and the kinetics of this release

Polymer carrier of IMC	ζP±SD, [mV]	PSD	Z-average±SD, [nm]	%Y ±SD	%EE ±SD	%DL±SD	$T_{85\%}$, [h]	Kinetic model of IMC released from IMC-p(VAc)-NPs
IMC-p(VAc)	-31.50 ±1.2	Mono-modal	128.1 ±3.4	98.32 ±1.33	82.92±1.01	7.67 ±0.32	7	First order
IMC-p(VAc)+Cbp	-29.50 ±1.32	Bi-modal	178.2 ± 2.86	89.02 ±1.01	48.82±0.77	4.77 ±0.65	16	First order

SD - standard deviation, n=3

Rheological characteristics of the HPMC solutions

Fig. 1 clearly shows that with the increase of the gradient the viscosity of the solutions decreases. The solutions with 0.25 and 0.5% concentration show a non-Newtonian flow at low values of the gradient up to 100s^{-1} and then with the increase of the gradient they turn into Newtonian fluids. 1% HPMC solution has a similar behavior - the non-Newtonian flow is observed at gradient values up to 250s^{-1}. 2% HPMC solution shows a Pseudo plastic flow.

The correlation between the solution viscosity and the HPMC concentration at a gradient of 437 s^{-1} shows that the increase of the HPMC concentration from 0.25% to 0.50% results in a threefold increase of the viscosity: from 0.005 Pa. s to 0.016 Pa. s (fig. 2). The increase of the HPMC concentration from 0.50% to 1.00% results in viscosity increase up to 0.087 Pa. s - about fivefold greater compared to that of 0.50% and 17-fold greater compared to the 0.25% solution. Fig. 2 shows that the change in the viscosity of the solutions in correlation with the HPMC concentration at 437s^{-1} gradient corresponds to an exponential dependence.

A 2% HPMC solution has a high viscosity at low gradient values up to 100 s^{-1} (fig. 1). With such a viscosity the formulation will be retained longer on the cornea and thus a better contact will be provided, but on the other hand the high viscosity will blur the vision, creating inconvenience and discomfort. It is important to know that the viscosity of the lacrimal fluid of a healthy eye is in the range between 1 and 10 cP [23].

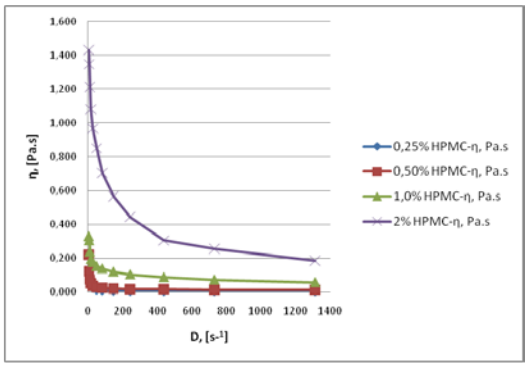

Fig. 1: The correlation between the change of the viscosity of HPMC solutions and the change of the gradient

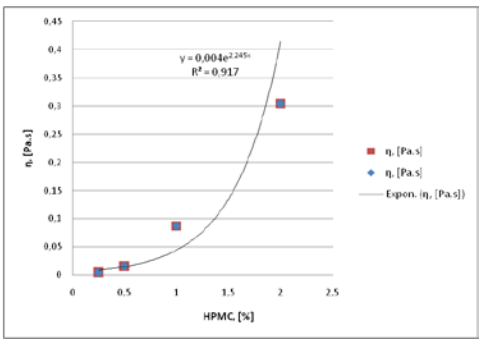

Fig. 2: The correlation between the solution viscosity and the HPMC concentration at 437s^{-1} gradient

Surface tension of the HPMC solutions

For the purpose of the experiment an interface tensiometer was designed, based on the principle of the "ring-method". The HPMC solutions with concentration 0.25%, 0.50% and 1.0% were used as carriers for the eye drops models and their surface tension was measured. The obtained values were compared to the surface tension of the commercial eye drops Indocollyre®. The measurement was carried out at 35°C. The results of the measurement are shown in fig. 3.

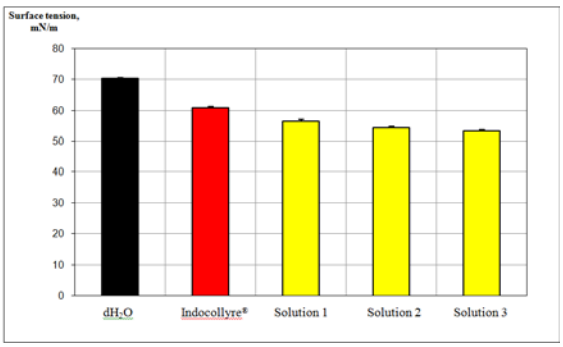

Fig. 3: Surface tension of Indocollyre® and HPMC solutions with concentration 0.25% (Solution 1), 0.50% (Solution 2) and 1.0% (Solution 3) at 35°C

The HPMC solution with 0.25% concentration has 56.53 ± 0.47 mN/m surface tension, for the 0.50% HPMC solution this value is 54.34 ± 0.51 mN/m, and for the 1% solution - 53.21 ± 0.54 mN/m at 35°C. The surface tension of the lacrimal fluid is 42÷46 mN/m [23]. It is clear that the solutions have a lower surface tension compared to the conventional eye drops Indocollyre® (60.82 ± 0.60 mN/m). There is a statistically significant difference (P <0.050) between: 1) the surface tension of the eye drops Indocollyre® and the tested HPMC solutions; 2) the surface tension of Solution 1 and the other solutions - Solution 2 and Solution 3. There is no statistically significant difference between the surface tension of Solution 2 with a 0.50% concentration of HPMC and Solution 3 with a 1.00% concentration of HPMC (P> 0.050).

Preparation of the eye drops models

Table 2 shows the content of the tested eye drops models, in which IMC - p (VAc)-NPs were included. In the literature there is plenty of data "for" and "against" the use of different preservatives in the ophthalmic formulations. Since there is no clear evidence for incompatibility between BC and IMC and having in mind that it has been widely used in the commercial formulations on the market, BC was chosen as an antimicrobial agent in the eye drops models [24, 25].

***In vitro* study of the IMC release from the eye drops models**

In vitro study of the release of IMC from the eye drops models was performed within 24 hours. Fig. 4 shows the release profiles of IMC from Models 1, 3 and 5 with IMC-p (VAc) as a drug carrier.

Table 2: Models of liquid ophthalmic formulations and their content

Model	IMC-p(VAc), [mg]	IMC-p(VAc)+Cbp, [mg]	%EE, [%]	Solution of HPMC up to 100 ml	Benzalkonium chloride, [%]
Model 1	130.38	-	82.92	Solution 1	0.01
Model 2	-	209.64	48.82	Solution 1	0.01
Model 3	130.38	-	82.92	Solution 2	0.01
Model 4	-	209.64	48.82	Solution 2	0.01
Model 5	130.38	-	82.92	Solution 3	0.01
Model 6	-	209.64	48.82	Solution 3	0.01

The physiological pH of the tear fluid of a healthy eye is in the range of 7.3 to 7.7. This value depends on the dissolved substances in the tears, especially the buffer system bicarbonate-carbon dioxide [23]. The proposed technological models have pH 7.4±0.05.

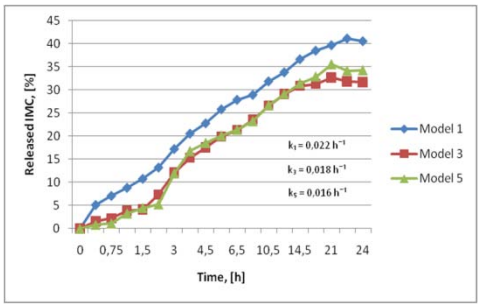

Fig. 4: The release profile of IMC from Models 1, 3 and 5 with IMC-p(VAc) as a drug carrier, where k_1, k_3 and k_5 are the rate constants according to first order kinetics

Within the time of the study none of the carriers released 100% of the included active substance. The largest amount of IMC was released from Model 1 (fig. 4). Model 3 and Model 5 released the included active substance at the same rate and extent. The release of IMC from the three models is according to the first-order kinetics with correlation coefficients (R) 0.99035, 0.98087, and 0.99043, respectively for Model 1, Model 3, and Model 5.

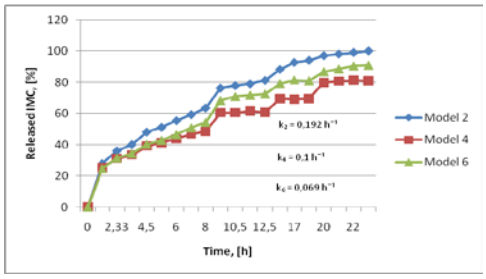

Fig. 5: The release profile of IMC from Models 2, 4 and 6 with IMC-p(VAc)+Cbp as a drug carrier, where k_2, k_4 and k_6 are the rate constants according to first order kinetics

The added HPMC changes the rate and extent of IMC release within the time of the study, but not the kinetic model. Possible reasons for that could be: the viscosity of the solution (the higher the viscosity, the harder it is for the water molecules to diffuse into the hydrophobic pVAc-matrix) and the adsorption of HPMC on the pVAc-particles, eventually leading to steric hindrance or the formation of a gel layer around the particles, which result in a prolonged release of the active substance [24 26]. These processes limit the rate and extent of the IMC dissolution. Fig. 5 shows the release profile of IMC from Models 2, 4 and 6 with IMC-p (VAc)+Cbp as a carrier for the active substance.

Within the time of the test only Model 2 released 100% of the included IMC. The release process again follows the first-order kinetic model with correlation coefficients (R) 0.98333, 0.97892, and 0.96107 for Models 2, 4, and 6, respectively. The comparison between the release process of IMC from IMC-p(VAc)+Cbp (Table 1) in buffer with pH 7.4 and IMC from Model 2 (fig. 5) shows that both models release equal amount of IMC for 16 hours - approximately 85%. The addition of 0.25% HPMC leads to achieving a certain viscosity of the solution, but does not prolong the release time of the included active substance. 0.25% HPMC does not affect the rate, the extent and the kinetics of the drug release. Model 4 and Model 6 possess higher viscosity thus they release the IMC prolonged in comparison with Model 2. Model 4 reaches 90% released IMC and Model 6 – 80% for the time of the experiment. Fig. 5 shows that Models 2, 4 and 6 released about 30% of the included IMC in the first hour. After 4.5 hours they released 40 ÷ 50%, and after 8 hours – 50 ÷ 60% of the drug.

According to Tiwari et al. [26] the combination of the different types of carbomers with HPMC leads to increase of the viscosity in the matrix tablets, because of the formation of hydrogen bonds between the polymers. Similarly, between the Cbp-shells of IMC-p(VAc)+Cbp and HPMC in the solution should occur such interactions. The difference in the rate and the extent of the IMC release from the models with IMC-p(VAc) (Model 1, 3 and 5) (fig. 4) and those with IMC-p(VAc)+Cbp (Model 2, 4 and 6) (fig. 5) is probably due to: 1) the larger surface area of the NPs with IMC-p(VAc) (average particle size 128.10 nm) compared to that of the NPs with IMC-p(VAc)+Cbp (178.20 nm); 2) concentration of Cbp, which is insufficient to establish strong hydrogen bonds (bimodal particle size distribution); 3) the hydrophilic properties of the Cbp-shell which forms at this pH values a gel layer around the p(VAc)-core; 4) a difference in the surface structure of the NPs; 5) a difference in the zeta potential of the model (table 1).

The results from an *In vitro* study of the IMC release from the proposed eye drops models were compared to the dissolution profile of IMC from the commercial product Indocollyre®. The latter contains as excipients hydroxypropyl-*ß*-cyclodextrin, arginine, diluted hydrochloric acid, thiomersal as a preservative and water for injection. Fig. 6 shows that 80% of the active substance included in the commercial eye drops was released after 45 min. It is a certain evidence for the prolonged release of IMC from the tested technological models.

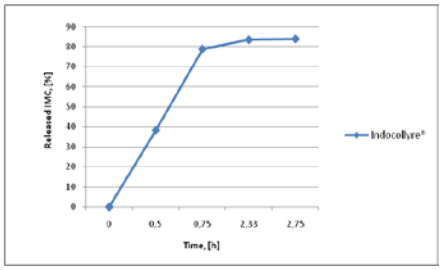

Fig. 6: The release profile of IMC from Indocollyre® eye drops

***In vitro* HET-CAM**

The CAM is a non-innervated complete tissue containing arteries, veins and capillaries, and it is technically easy to study. It responds to injury via an inflammatory process similar to that observed in the conjunctival tissue of a rabbit eye. The well-developed CAM vascularization provides an ideal model for ocular irritation studies. Ocular tolerance assays for IMC substance showed slight irritation. In contrast, when the different NP formulations, developed in this work, were tested, no signs of ocular irritation were detected within 300 seconds: the irritation index was zero. With respect to the effect of particle size on ocular irritation, as described by Schoenwald and Stewart [25 27], only particles with 20 µm mean diameter induced irritation. So, the IMC-p(VAc)-NPs seem to be suitable systems for ocular administration.

CONCLUSION

The established biocompatibility of pVAc [28], its stabilizing role on the *in-situ* included IMC and the research on IMC release from nano-sized carrier at pH 7.4, are the reasons which make NPs from p(VAc) homopolymers and its mixtures with hydrophilic and biocompatible Carbopol® a suitable drug delivery system in eye formulations. Model 2 is of special interest, because it releases 100% of the included drug for 24 hours and thus it can provide: 1) therapeutic concentrations of the active substance within a period of 24 hours and 2) less frequent applications of the formulation, which is convenient for the patient.

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CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Wilczewska AZ, Niemirowicz K, Markiewicz KH, Car H. Nanoparticles as drug delivery systems. Pharmacol Rep 2012;64(5):1020-37.
2. Sadiq AA, Abdul Rassol AA. Formulation and evaluation of silibinin loaded solid lipid nanoparticles for peroral use targeting lower part of gastrointestinal tract. Int J Pharm Pharm Sci 2014;6(1):55-67.
3. Shaji J, Lal M. Preparation, Optimization and evaluation of transferosomal formulation for enhanced transdermal delivery of a COX-2 Inhibitor. Int J Pharm Pharm Sci 2014;6(1):467-77.
4. Sutradhar KB, Khatun S, Luna IP. Increasing possibilities of nanosuspension. J Nanotechnol 2013;2013:12.
5. Hyma P, Chandra A, Abbulu K. Formulation and characterization of telmisartan self microemulsifying drug delivery system. Int J Pharm Pharm Sci 2014;6(1):120-5.
6. De Jong WH, Borm PJ. Drug delivery and nanoparticles: applications and hazards. Int J Nanomed 2008;3(2):133-49.
7. Nagarwal RC, Kant S, Singh PN, Maiti P, Pandit JK. Polymeric nanoparticle system: A potential approach for ocular drug delivery. J Control Release 2009;136:2-13.
8. Sweetman S. Martindale: The complete drug reference. 33d Ed. London: Pharmaceutical Press; 2007.
9. Wickstrom K. Acute bacterial conjunctivitis-benefits versus risks with antibiotic treatment. Acta Ophthalmol (Oxf) 2008;86:2-4.
10. Andonova V, Georgiev G, Toncheva V, Kassarova M. Preparation and study of poly(vinyl acetate) and poly(styrene) nanosized latex with indometacin. Pharmazie 2012;67(7):601-4.
11. Andonova V, Georgiev G, Toncheva V, Petrova N, Kassarova M. Indomethacin nanoparticles for applications in liquid ocular formulations. Folia Med 2013;55(1):76-82.
12. Andonova V, Georgiev G, Toncheva V, Kassarova M. Influence of some technological factors on the preparation of polymeric nanoparticles with indomethacin. OASR 2013;2(4):703.
13. Andonova V, Georgiev G, Toncheva V, Petrova N, Karashanova D, Penkov D, *et al*. Indomethacin loading and *in vitro* release properties from vinyl acetate homo- and co-polymer nanoparticles, coated with polyzwitterion and carbopol® shells. Int J Pharm Pharm Sci 2014;6(1):691-9.
14. Hibbeler RC. Mechanics of Materials. New Jersey USA: Pearson Education; 2004.
15. McClenaghan BA, Literowich W. Fundamentals of computerised data acquisition in the human performance laboratory. Sports Med 1987;4(6):425-45.
16. Mcateer E, Neil D, Barr N, Brown M, Draper S, Henderson F. Simulation software in a life sciences practical laboratory. J Comput Educ 1996;26:101-12.
17. Kittnar O, Mlcek M. Analysis of the electrical heart field. Physiol Res 2010;59(1):19-24.
18. Czajkowska-Kośnik A, Sznitowska M, Mirkowska K. Self-emulsifying oils for ocular drug delivery. II. *In vitro* release of indomethacin and hydrocortisone. Acta Poloniae Pharm-Drug Res 2012;69(2):309-17.
19. Tavaszi J, Budai P. The use of HET-CAM test in detecting the ocular irritation. Commun Agric Appl Biol Sci 2007;72:137.
20. Appendix B3 of "ICCVAM test method evaluation report: current validation status of *in vitro* test methods proposed for identifying eye injury hazard potential of chemicals and products" NIH Publication; 2010; Available at: http://iccvam.niehs.nih.gov/methods/ocutox/MildMod-TMER.htm.
21. Levesque R. SPSS Programming and Data Management: A Guide for SPSS and SAS Users. 4th ed. SPSS Inc, Chicago Ill; 2007;184-9.
22. Howard CJ. Multiple comparisons procedures. Circ 2008;17:698-701.
23. Epstein AA. Solution optimization and physical properties of healthy human tears. Contact Lens Spectrum 2010;4:880-2.
24. Elder D, Crowley P. Antimicrobial Preservatives Part Two: Choosing a Preservative. American Pharmaceutical Review; 2012.
25. Kaur IP, Lal S, Rana C, Kakkar S, Singh H. Ocular preservatives: associated risks and newer options. Cutaneous Ocul Toxicol 2009;28(3):93–103.
26. Tiwari SB, Rajabi-Sianboomi AR. Colorcon Inc. Modulation of drug release from hydrophilic matrices. Pharm Tech Europe 2008;437:217-43.
27. Schoenwald R, Stewart P. Effect of particle size on ophthalmic bioavailability of dexamethasone suspensions in rabbits. J Pharm Sci 1980;69:391-4.
28. Kostova B, Kamenska E, Momekov G, Rachev D, Georgiev G, Balashev K. Synthesis and characterization of novel drug delivery nanoparticles based on polyzwitterionic copolymers. Eur Polym J 2013;49:637-45.