CONTRIBUTIONS ON FORMULATION AND PRELIMINARY EVALUATION OF OCULAR COLLOIDAL SYSTEMS OF CHITOSAN AND POLOXAMER 407 WITH BUPIVACAINE HYDROCHLORIDE

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Abstract

The aim of this paper was the formulation and preliminary evaluation of colloidal systems with improved mechanical and mucoadhesive properties in order to increase ocular retention time and drug bioavailability. Polymeric mixtures of chitosan (0.3%), a pH-sensitive polymer, and poloxamer 407 (12%, 15%, 18%), thermoresponsive polymer, with bupivacaine hydrochloride (0.25%) were prepared. Gelling temperature, wettability, rheological behaviour and *in vitro* drug release were evaluated. The formulation containing 15% poloxamer 407 is converted to a colloidal dispersion at 35°C, the temperature of the ocular surface. Contact angle values of colloidal systems at physiological temperature (35°C) were lower than those corresponding to formulations at non-physiological temperature (25°C), indicating a higher spreadability over the ocular surface. Rheological studies have shown pseudoplastic behaviour and the poloxamer concentration increased the viscosity of the formulations. *In vitro* bupivacaine hydrochloride delivery from colloidal systems revealed that drug release mechanism followed Higuchi model. The colloidal dispersions with chitosan and poloxamer 407 could be viable alternatives to increase the bioavailability of bupivacaine hydrochloride in topical ocular anaesthesia.

Rezumat

Obiectivele acestei lucrări sunt formularea și evaluarea preliminară a unor sisteme coloidale cu proprietăți mecanice și mucoadezive sporite care să asigure un timp de rețență crescut la nivel ocular și o biodisponibilitate a substanței medicamentoase îmbunătățite. S-au preparat amestecuri polimerice de chitosan (0,3%), un polimer sensibil la variația de pH, și poloxamer 407 (12%, 15%, 18%), polimer cu proprietăți termoreversibile, în care a fost încorporată bupivacaină clorhidrat (0,25%). Pentru acestea s-au evaluat: temperatura de gelificare, udabilitatea, comportamentul reologic și cedarea *in vitro* a substanței active. Formularea conținând 15% poloxamer 407 s-a transformat într-o dispersie coloidală la 35°C, temperatura de la suprafața oculară. Valorile unghiurilor de contact pentru sistemele coloidale la temperatura fiziologică (35°C) au fost mai mici decât cele corespunzătoare formulărilor la temperatura nefiziologică (25°C), fapt ce indică o etalare corespunzătoare pe suprafața oculară. Studiile reologice au arătat un comportament pseudoplastic, iar la creșterea concentrației de poloxamer s-a înregistrat creșterea vâscozității preparatelor. Cedarea *in vitro* a bupivacainei clorhidrat din sistemele coloidale s-a realizat în conformitate cu modelul Higuchi. Dispersiile coloidale de chitosan și poloxamer 407 pot reprezenta alternative viabile de creștere a biodisponibilității bupivacainei clorhidrat în anestezia topică oculară.

Keywords: chitosan, poloxamer 407, ocular delivery, colloidal systems

Introduction

One important challenge for the ophthalmic drug administration is to obtain and maintain an optimal concentration of the drug substance in the eye [40]. Conventional eye drops are widely used due to the easy way of administration and compliance of the patient, but they have a drug bioavailability less than 5% [26]. Physiological barriers such as tear fluid, cornea or nasolacrimal drainage limit the bioavailability of therapeutics in order to protect the eye from the penetration of microorganisms or toxic agents [34]. An attempt to increase ocular retention time through numerous topical pharmaceutical

formulations has not been entirely accepted due to disadvantages such as blurred vision (ointments) or low patient compliance (inserts) [21].

An alternative to improve ocular retention time is represented by colloidal systems. There are polymeric colloidal dispersions that undergo phase transition under the influence of temperature in a physiological pH. The main interactions responsible for the sol-gel transition are physical intermolecular forces such as hydrophobic interactions between polymeric chains [25].

Chitosan is a polysaccharide composed of D-glucosamine and N-acetyl-D-glucosamine units linked β -(1-4). It is obtained by deacetylation of chitin, a

structural element from exoskeleton of crustaceans such as crab or shrimp and insects cuticles [6, 27]. Chitosan is soluble in acid up to 6.2 due to the protonation of the free amino groups. Acidic solutions exposed to alkaline pH cause a decrease in apparent charge density with physical gel formation due to the occurrence of hydrogen bonds and hydrophobic interactions [23, 29, 39]. The benefits of including chitosan as an excipient in ophthalmic preparations are good mechanical strength and mucoadhesive character, biocompatibility, biodegradability, non-toxicity, improvement of ocular permeability through paracellular permeation, antimicrobial activity and corneal wound healing effect [8, 20]. Chitosan exhibits pseudoplastic and viscoelastic behaviour. Mucoadhesiveness is the result of electrostatic interactions between positively charged amine groups of chitosan and negatively charged residues of sialic acid in the mucin structure, enhancing contact time with the ocular surface [5].

Poloxamers (Pluronic[®]) are triblock copolymers composed of a hydrophobic polyoxypropylene (PPO) unit flanked by two hydrophilic polyoxyethylene (PEO) chains [2]. Poloxamer 407 known as Pluronic F127 is a non-ionic surfactant with good solubility, low toxicity, compatibility with the cells and fluids of the body. Aqueous solutions of Poloxamer 407 exhibit thermoreversible properties [14]. At concentrations of 18% (w/w) or more in aqueous solution, poloxamer 407 become a non-crosslinked gel by exposure to room temperature. Keeping the preparation in the refrigerator enables the ease of administration, but can cause ocular irritation due to low temperature [28]. The main drawback of this polymer is poor mechanical strength that leads to rapid erosion of its matrix [30]. The combination of chitosan with poloxamer 407 results in sterile, non-irritating formulations, and the gels have improved mechanical strength and mucoadhesive character, providing increased contact time with the ocular surface [15].

Bupivacaine hydrochloride is a local anaesthetic with amide structure, slightly soluble in water, with long-lasting action (2 - 9 h). Analgesia persists after the regaining of sensation reducing the need for strong analgesics [3]. The mechanism of action is determined by the stabilization of neuronal membranes that prevents the transmission of nerve impulses [9]. Ocular procedures requiring the use of local anaesthetics include cataract surgery, trabeculectomy, pars plana vitrectomy or refractive surgery [32]. Topically applied local anaesthetics block the trigeminal nerve terminations in the cornea and conjunctiva, leaving the intraocular structures of the anterior segment non-anesthetized. Interventions in the iris can irritate the ciliary nerves with discomfort, so association with intracameral anaesthesia is required [22]. In addition to analgesic action, bupivacaine possesses antimicrobial activity on Gram positive and Gram negative bacteria [1, 24].

The United States Pharmacopeia mentions that the systemic absorption rate of bupivacaine hydrochloride is determined by the drug concentration, the route of administration, the vascularization of the administration site and the presence or absence of epinephrine. A diluted concentration of epinephrine (1:200000) with vasoconstrictor action reduces the absorption rate of bupivacaine hydrochloride and prolongs its duration of action [36].

The purpose of this paper was to obtain ophthalmic colloidal systems with improved mechanical and mucoadhesive properties in order to provide enhanced drug bioavailability. In this purpose, polymeric mixtures of chitosan, a pH-sensitive and mucoadhesive polymer, and poloxamer 407, a thermosensitive polymer and gelling agent, were prepared. The same concentration of chitosan (0.3%) was used and poloxamer 407 concentration (12%, 15%, 18%) was varied. Bupivacaine hydrochloride was added in a concentration of 0.25% according to the literature references for topical application [10, 31]. The formulations were evaluated for gelling temperature, wettability, rheological profiles and *in vitro* release of the drug substance.

Materials and Methods

Preparation of polymeric dispersions

Chitosan (Medium MW 190000 - 310000, DD 75 - 85%) and Poloxamer 407 were obtained from Sigma-Aldrich (USA). Bupivacaine hydrochloride was also supplied by Sigma-Aldrich. All other chemicals and solvents used were of analytical grade.

Simulated tear fluid (STF, pH 7.4) was prepared by dissolving the following substances: sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride dehydrate 0.008 g in distilled water (q.s. ad 100 g) [16].

Polymeric dispersions based on chitosan and poloxamer were prepared using the "cold method" [15, 30]. 0.3% (w/w) chitosan solutions were prepared by dispersing the corresponding amount in 2% (w/v) acetic acid solution under continuous stirring until complete dissolution. Formulations with Poloxamer 407 (12%, 15%, 18% w/w) were obtained by dispersing the corresponding amount of poloxamer in distilled water under continuous stirring for 1 h at room temperature. The partially dissolved poloxamer dispersions were stored in the refrigerator (4°C) for 24 h when the entire amount of polymer was completely dissolved. Chitosan/pluronic mixtures were obtained by dispersing the solutions of poloxamer into chitosan solutions under continuous stirring for 1 h. The partially dissolved systems were refrigerated for 24 h until they were thoroughly mixed. Depending on the concentration of Poloxamer 407 (12%, 15%, 18%), the formulations were coded as A, B, C. Preparation of polymeric mixtures with bupivacaine hydrochloride was achieved by adding 0.25% drug substance to the

chitosan/poloxamer dispersions under continuous stirring. The pH of the formulations was adjusted to 4.5 with NaOH 10% and they were stored at 4°C

until further use. Each mixture was analysed in triplicate. The composition of the polymeric systems with bupivacaine hydrochloride is shown in Table I.

Table I

Composition of polymeric systems

Formulation ingredients	A	B	C
P407 (% w/v)	12.00	15.00	18.00
Chitosan (% w/v)	0.30	0.30	0.30
Bupivacaine (w/20 mL)	0.05	0.05	0.05
NaOH 10% (mL)	2.00 ± 0.14	2.06 ± 0.07	1.96 ± 0.21
Water q.s. ad mL	20.00	20.00	20.00

Visual aspect and gelling temperature

The gelling capacity for each formulation was determined by adding a volume of the preparation into a transparent vial containing freshly prepared STF and placed in a thermostated water bath. The gel formation was visually evaluated and the phase transition temperature was noted [17, 38]. Determination of gelling temperature (GT) was performed before and after dilution of each polymeric mixture with STF in 40:7 ratio. It was considered that the average volume of a drop released from an eye drop bottle was approximately 40 µL and the available lacrimal fluid volume was 7 µL [28]. The visual aspect of the formulations was determined by visual examination alternately on a white or black background under intense light [19].

Contact angle and wettability studies

The wetting capacity was evaluated by measuring the contact angle of the formulations at room temperature and at 35°C. Contact angles were measured by the sessile drop technique using a CAM 101 Goniometer. The materials to be analysed were coated with glass slides which had water contact angles similar to the ocular surface. 10 µL of liquid were placed on the surface of the coated glass slide using a syringe and the drop image was analysed by an automated curve-fitting program. Data for contact angles are presented as mean values ± standard deviations measured in triplicate on three locations on each surface [12, 33].

Rheological studies

Viscosity measurements were performed using a rotational viscometer Fungilab equipped with a low viscosity adaptor (LCP). To simulate physiological conditions, polymeric mixtures were diluted with STF in 40:7 ratio [28]. The formulations were placed in a sample tube and analyzed at physiological temperature (35°C) by connecting a ThermoHaake

P5 Ultrathermostat. The experimental conditions for rheological analysis were previously described. The rotational speed of the spindle was increased from 1 to 100 and the viscosity of each formulation was measured [13, 27].

In vitro release of bupivacaine hydrochloride

To determine the *in vitro* release profile of bupivacaine hydrochloride from polymeric mixtures, immersion cells for semisolids (small volume) were adapted to a dissolution equipment such as USP2 paddle method (Hanson Vision® G2 Classic 6™, USA) with flat bottom 150 mL dissolution vessels. 1 mL of sample was weighed and placed in the compartment of the enhancer cells. Previously, filter membranes with 0.45 µm pore size and 25 mm of cellulose acetate provided by Sartorius Biolab Products were soaked for 8 h in phosphate buffer (pH 7.4), then placed on the samples surface. The enhancer cells were assembled as *per* the manufacturer's instructions. The cells were suspended in the dissolution vessels containing 100 mL of phosphate buffer medium and the stirring rate was maintained at 150 rpm. The apparatus was pre-set at physiological temperature (35°C). At predetermined time intervals (5, 15, 30, 45, 60, 75, 90, 120, 150 min), 1 mL sample of the release medium was withdrawn and replenished with fresh medium. The concentration of bupivacaine hydrochloride was spectrophotometrically assessed at 262 nm [4, 9, 18, 35].

Results and Discussion

Table II shows the visual appearance of each sample at room temperature (25°C), pH, as well as phase transition temperature (PCT) for polymeric mixtures with bupivacaine hydrochloride before and after dilution with STF.

Table II

Physical properties of the polymeric systems

Formulation	Clarity	pH	PCT before dilution (°C)	PCT after dilution (°C)
A	Clear	4.51 ± 0.01	37.06 ± 0.07	38.40 ± 0.35
B	Clear	4.53 ± 0.02	33.33 ± 0.21	35.40 ± 0.14
C	Not clear	4.54 ± 0.05	25.36 ± 0.28	28.80 ± 0.07

It was visually observed that formulations with poloxamer 407 12% (series A) and 15% (series B)

were clear, transparent at room temperature. The series with 18% poloxamer (series C) was slightly

opalescent at room temperature. This was in accordance with the literature which mentioned that formulations with poloxamer 18% were converted from low viscosity solutions to non-crosslinked gels [7, 38].

Regarding the gelling temperature (GT), this was influenced by the concentration of poloxamer 407. The ideal phase transition temperature should be between 25°C and 35°C, the temperature at the ocular surface. Due to this fact, poloxamer was chosen as a gelling agent [15]. The reversible thermogelling phenomenon is the consequence of hydrophobic interactions between polymer chains. At low temperatures, the molecules are surrounded by a hydration layer, while the increase in temperature results in breakage of the hydrogen bonds between the aqueous solvent and the hydrophilic chains of the polymer. Increasing the temperature determines the aggregation of the chains with formation of a micelle structure due to the dehydration of the hydrophobic units of polyoxypropylene [11, 14]. The increase in poloxamer concentration resulted in GT reduction before and after dilution with STF. After dilution with STF, all formulations showed a translucent clear matrix and a superior PCT value. In the case of series A, the concentration of poloxamer 407 was relatively low, so it was not sufficient for a rapid gelling and PCT values were the highest. Formulations with poloxamer 407 15% were converted to a colloidal solution after dilution at 35°C, the temperature of the ocular surface. The visual appearance of polymeric mixtures before and after gelation is shown in Figure 1.

Figure 1.

Polymeric mixtures before (a) and after gelling (b)

Gelling temperature of polymeric mixtures before and after dilution with STF is illustrated in Figure 2. The amino groups of chitosan provide an increased charge density that can affect the formation of water channels around the polyoxypropylene units [37]. Gratieri et al. showed that a chitosan concentration between 0.5% and 1.5% did not significantly affect sol-gel transition of poloxamer [15]. Thus, the poloxamer gelling capacity was not influenced by the 0.3% chitosan concentration. However, chitosan, whose solubility is affected by an increase in pH and the presence of water, can also contribute to the occurrence of turbidity after dilution.

Figure 2.

Phase Change Temperature (PCT) of chitosan/poloxamer mixtures before and after dilution

The contact angle (CA) values for polymeric mixtures at room temperature are presented in Table III.

Table III

Contact angle values of formulations at non-physiological conditions (25°C)

Formulation	CA (°)
A	16.97 ± 0.75
B	29.36 ± 0.95
C	32.44 ± 1.83

Table IV highlights the contact angle values for colloidal dispersions with chitosan and poloxamer 407 at 35°C.

Table IV

Contact angle for colloidal systems at physiological conditions (35°C)

Formulation	CA (°)
A	18.32 ± 0.38
B	28.01 ± 0.90
C	27.99 ± 1.33

Increasing polymer concentration and enhancing viscosity, the measured contact angle values slightly increased; consequently, spreading of the formulations on the surface decreased. Figure 3 shows polymeric mixtures drops images from the optical contact angle goniometer for the series A, B, C.

Figure 3.

Images of polymeric mixtures drops for series A, B, C

Contact angle values of colloidal dispersions at 35°C are lower than those corresponding to formulations at 25°C, indicating a higher spreadability over the ocular surface. The colloidal systems drops images from the optical contact angle goniometer for the series A, B, C at 35°C are shown in the Figure 4.

Figure 4.

Images of colloidal systems drops for the series A, B, C

The formulations with low contact angle values (those from series A-poloxamer 10% and from series B- poloxamer 15%) spread rapidly and form a flat, transparent monolayer that enables clear vision without blurring and disturbing observation of anterior segment or retinal structures, so important for performing intraocular procedures. The ocular surface was found to be hydrophilic due to tear film and hence the improved hydrophilic properties of thermogelling systems allow them to make proper contact with cornea and conjunctiva. Evaluation of rheological profiles for the analysed systems was performed at 35°C.

The rheological curves recorded as viscosity *versus* shear rate are given in Figure 5.

The viscosity of the thermogelling dispersions was proportionally with the poloxamer concentration. As can be noticed from Figure 5, the viscosity decreased with the shear rate increase, which showed pseudoplastic flow behaviour. The pseudoplastic flow was due to breakage of physical bonds between polymer

molecules and alignment of disordered molecules in the direction of flow [13]. The addition of chitosan to the aqueous solutions of poloxamer 407 has led to improve the viscosity and elasticity of the preparations.

Figure 5.

Plots of viscosity as a function of shear stress for colloidal systems

The rheological data were fitted according to Power law model (eq. 1):

$$\frac{Wt-Wd}{Wd} \text{ g/g}, \quad (1)$$

where, m is the viscosity for shear rate 1 s^{-1} and n – flow index.

The values of the rheological parameters characteristic for the analysed systems are shown in Table V.

Table V

Values of the rheological parameters characteristic for the analysed systems

Tested samples	m	n	R^2
Serie A	195.701 ± 3.38	0.789 ± 0.02	0.9930 ± 0.0005
Serie B	267.497 ± 4.50	0.795 ± 0.04	0.9945 ± 0.0006
Serie C	405.201 ± 6.52	0.815 ± 0.05	0.9969 ± 0.0008

The influence of poloxamer concentration on the cumulative drug released bupivacaine hydrochloride percentage as a function of time is represented in Figure 6.

The kinetic data respected the Higuchi model, the values of the determination coefficients being higher than 0.99 (Table VI). The quantification of the release kinetics of bupivacaine hydrochloride from the polymeric mixtures was evaluated by the diffusion coefficient parameter:

$$D = \frac{q^2 \cdot \pi}{4 \cdot C_0^2 \cdot t}, \quad (2)$$

where, D is the diffusion coefficient of the active substance (cm^2/min), q – the amount of the drug released on the surface unit of the membrane (g/cm^2), C_0 – the initial concentration of the active substance (g/mL) and t – the time of release (min).

Figure 6.

Cumulative release profiles of bupivacaine hydrochloride from colloidal systems as a function of time

Table VI illustrates the values obtained for the kinetic parameter mentioned above. An increase in poloxamer concentration by 6 units (from 12% to 18%) resulted in a decrease of the diffusion coefficient, after gelling, with 74.4% (from $4.22 \cdot 10^7$ (cm²/s) to $3.14 \cdot 10^7$ (cm²/s) and consequently a reduction in the release rate of bupivacaine hydro-

chloride. A higher poloxamer concentration represented a real barrier to bupivacaine hydrochloride release due to lowering the number and size of water channels and the appearance of the micelles. Chitosan has contributed to the prolonged release of the active substance by formation of micelle junction points with poloxamer.

Table VI

Diffusion coefficient values, using a USP2 device, the correlation coefficient specific to Higuchi model

Delivery system	System responses	
	Diffusion coefficient $D \cdot 10^7$ (cm ² /s)	Determination coefficient (Higuchi model)
A	4.22 ± 0.11	0.9935 ± 0.0003
B	3.80 ± 0.09	0.9940 ± 0.0006
C	3.14 ± 0.21	0.9913 ± 0.0008

Conclusions

This paper aims to formulate and evaluate colloidal dispersions with chitosan and poloxamer 407 as promising delivery systems in ocular surgery. The topical anaesthetic bupivacaine hydrochloride (0.25%) was successfully incorporated in thermoreversible polymeric systems with the same concentration of chitosan (0.3%) and three different concentrations of poloxamer 407 (12%, 15%, 18%). The formulation with 15% poloxamer exhibited a gelling temperature at 35°C that is the temperature at the ocular surface. Contact angle values of colloidal mixtures at physiological conditions are lower than those corresponding to polymeric dispersions at non-physiological conditions. They spread rapidly and form a flat, transparent monolayer that enables clear vision without blurring and disturbing observation of anterior segment or retinal structures. The rheological profiles showed a pseudoplastic flow for all the three types of formulations. The increase in poloxamer concentration resulted in a lowering of the diffusion coefficient after gelling and a decrease in the bupivacaine hydrochloride release rate which was performed according to the Higuchi model. In conclusion, these thermogelling systems could be promising alternatives to ocular drug delivery, improving the bioavailability of bupivacaine hydrochloride in the eye.

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