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Reconstituted ocular gel for cystinosis treatment

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Cystinosis is a rare genetic disorder that causes an accumulation of cystine into cells and its corneal crystal deposition. The main treatment for this ocular disorder remains the continuous administration of cysteamine, which may lead to non-compliance, especially in childhood. Reconstituted gels (RG) are described here as a possible alternative to the current formulations consisting of aqueous solutions of cysteamine salts. RG are dried powders containing cysteamine, tartaric acid, carbopol and a polyhydroxylated compound that are reconstituted at the time of use with water, thus obtaining immediately the final gel. The rheological behavior of the gels prepared with lactose and mannitol was pseudoplastic, while the gel prepared with sorbitol was not markedly pseudoplastic. All the formulations tested were physically and chemically stable for one year at 25 °C and cysteamine, present in the gel obtained after reconstitution, was stable for at least one week at 4 °C.

Keywords: Cystinosis – Cysteamine – Cysteamine delivery – Ocular gels.

Cystinosis is a rare genetic disorder that causes an accumulation of the amino acid cystine into cells, forming crystals that can build up and damage cells. Cystinosis arises due to a defect in the lysosomal transport mechanism for cystine. This results from mutations in the CTNS gene found on chromosome 17p13, which codes for cystinosin, a lysosomal membrane transport protein [1].

Cystine accumulates in all organs and tissues in the body. Corneal crystal deposition is one of the most troublesome complications affecting patients, especially as their prognosis improves and life expectancy increases. Crystals deposit in the cornea slowly through infancy until they become apparent at an age of approximately 16 months. The deposition becomes a problem when the entire peripheral stroma and endothelium have become packed, usually around the age 6-8 years, although this varies [2]. Photophobia and, ultimately, blepharospasm affect the quality of life such that the slightest glimmer of sunlight can be debilitating. In addition, the crystals accumulation over a period of years can cause corneal scars, keratitis and cataracts, as well as band keratopathies to form [3].

Although novel pro-drug strategies are being researched, the main treatment for the disorder remains the administration of the aminothiol cysteamine [4]. The molecule lowers intracellular levels of cystine by forming a cysteamine-cysteine mixed disulfide (*Figure 1*). The structure of the disulfide is spatially similar to the amino acid lysine and can cross the lysosome membrane using the undamaged excretion pathway for lysine [5].

The oral form of the drug has no effect on depleting corneal crystals due to a lack of vasculature in the cornea, thus cysteamine must be administered topically.

Topical cysteamine aqueous solutions should be administered either every hour while awake [6] or six times a day [7] in order to significantly improve the dissolution of corneal cystine crystals. Because of this frequent usage, it may lead to non-compliance to treatment, especially in childhood. In light of these data, it was decided to study a new ophthalmic formulation to reduce the frequency of administration by increasing the precorneal residence time and overcoming the problem of low stability in cysteamine solution [8].

Cysteamine is a small molecule which is very soluble in aqueous medium, consequently the efficiency of the ocular delivery route could be increased with a hydrogel formulation, avoiding the nasolacrimal duct drainage that represents the protective mechanism of the eye, which takes place when the volume of the fluid in the eye exceeds the normal lacrimal volume of 7-10 μL [9]. This drainage mechanism decreases the effective dosage of the drug for ocular tissue.

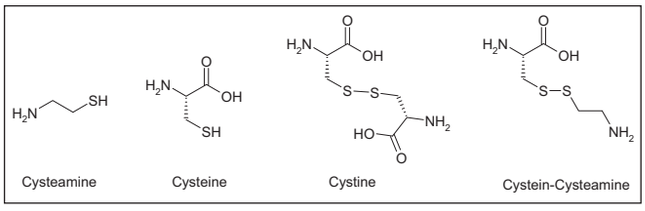

Figure 1.

Hydrogel formulations could be interesting formulations for cysteamine delivery but the stability problem still remains. In aqueous solution and at room temperature, cysteamine easily degrades forming oxidated products.

To overcome this problem, the possibility of a solid formulation was evaluated. The use of oral cysteamine products, approved by the FDA (USA) such as Cystagon in 1994, has been shown to reduce the cystine deposition systematically, leading to improved renal function, but unfortunately, an effect on the cornea has not been clarified [10]. Minitablets are among the novel solid formulations for ocular delivery, which have been proposed in literature in the recent years [11, 12]. Many studies show that the minitablets could be an interesting new drug delivery system, but have different characteristics compared with hydrogel.

In this paper, the reconstituted gel has been studied as a new solid formulation for ocular delivery of cysteamine, which could combine the advantages of the solid formulations and the gel formulations.

Reconstituted gels (RG) are gels that are obtained after lyophilization or spray-drying of a primary gel (or viscous solution), that are then reconstituted at the time of use with water, thus obtaining immediately the final gels [13].

I. MATERIALS

Cysteamine, mannitol (mannitolum Ph. Eur.), D-sorbitol, benzalkonium chloride and L-(+)-tartaric acid were purchased from Sigma-Aldrich. Lactose monohydrate (Lactosum monohydricum Ph. Eur.) was purchased from Fluka. Carbopol 974P NF was a gift from Noveon (New York, United States).

II. METHODS

1. Gel preparation

1.1. Preparation of RG-1, RG-2, and RG-3 formulations

Carbopol 974P NF was solubilized in aqueous solution containing benzalkonium chloride. The gels were then allowed to equilibrate for 24 h at 25 °C. Subsequently tartaric acid, mannitol or sorbitol and cysteamine were added in order. Osmotic pressure of the gels was controlled and adjusted to 320-340 mOsm/Kg by adding NaCl (ex. for 100 g of gel RG-3 500 mg of NaCl were added). The gel compositions are reported in *Table I*. Each gel thus obtained was divided into portions of 1 and 5 g and dried by lyophilization.

The lyophilization was performed in a Virtis SP Scientific Freeze Drier apparatus, model Advantage EL (United States). Lyophilization conditions were: for the freezing phase: 3 h at - 45 °C, for drying phase, 3 h at - 45 °C and 100 mTorr, 3 h at - 20 °C and 100 mTorr, 3 h at - 10 °C and 100 mTorr, 3 h at 10 °C and 100 mTorr.

By adding 950 µL or 4.75 mL of water (for 1 and 5 g portions, respectively) to the lyophilized powder, immediately the gels at 0.5% of cysteamine were reconstituted.

1.2. Preparation of RG-4 formulation

Carbopol 974P NF was solubilized in water. Subsequently tartaric acid, lactose and cysteamine were added. The slightly viscous solution, continuously kept under stirring, was dried through a spray-drying Buchi (Switzerland) model B-191 apparatus. Spray-drying conditions: air inlet temperature 60 °C, spray flow 500 L/h, aspirator flow rate 82% and pump flow 2 mL/min.

The finely divided powders, reconstituted with appropriate amount of water, formed a gel immediately.

2. Stability study

Preliminary stability studies were performed on the solid formulations and on fluid reconstituted gels. The stability of the solid formulations was tested for one year at 25 °C by analytical control after one, three, and six months and one year. For fluid reconstituted gels, the stability was tested after one weak at 5 and 25 °C.

An HPLC method was used to control the cysteamine title.

3. HPLC method

HPLC Shimadzu (Japan) instrument equipped with controller SCL-10A, Pump LC-10AT, Autosampler SIL-10 AD and Detector UV-PDA SPD10.

Cysteamine was analyzed on a Spherisorb SCX 5 µm (250 mm × 4.6 mm I.D.) column (Waters, Milford, MA, United States) using a mobile phase containing acetonitrile/buffer phosphate 50 mM at pH 3 (65/35) at a flow rate of 1.5 mL/min. A volume of 20 µL was injected into the HPLC system and LC run time was set to 20 min. The elution time of cysteamine was 12 min.

The samples were prepared by dilution of 1 g of reconstituted gel with 50 mL of water. Cysteamine used as a reference compound was accurately weighted, solubilized in water and immediately injected. The concentration, expressed as mg/mL of cysteamine in the gel, was calculated by the following formula:

mg/mL = [chromatographic area of cysteamine in the gel × mg/mL reference compound]/chromatographic area reference compound

4. Rheological studies

The rheological studies were carried out with a rotational viscometer of the concentric cylinder type (Viscometer TV-10 Tokyo Sangyo equipped with a small sample adapter and rotor type M3). The viscosity and shear stress of the samples were measured at different rpm (0.5, 1.0, 2.0, 2.5, 4.0, 5.0, 10.0, 20.0, 50.0 and 100) at 25 °C. The temperature was maintained within ± 0.1 °C by a re-circulating bath connected to the viscometer. The samples were equilibrated for five minutes prior to each measurement.

5. *In vitro* release studies

In vitro release experiments were carried out with a dissolution instrument Sotax AT 7 smart (Switzerland) equipped with a paddle over disk system (*Figure 2*). About 2.00 g exactly weighted of gel containing cysteamine were weighed in the disk and covered with dialysis membrane Diachema (Diachema AG, Switzerland) mw-cut off 10000 Da, and placed in a 1 L vessel containing 500 mL of Simulated Lacrimal Fluid (aqueous solution composed of 1.79 g/L KCl; 6.31 g/L NaCl; 2.18 g/L $NaHCO_3$; 0.067 g/L $CaCl_2$ $2H_2O$; 0.16 g/L $MgCl_2$ $6H_20$ adjusted at pH 7.4 with 0.1 N HCl). The experiments were carried out at 33 °C and with a 50 rpm stirring paddle. At regular intervals, 1.0 mL of solution was withdrawn from the vessel and filtered using a 0.2 µm filter and analyzed by HPLC to determine cysteamine concentration.

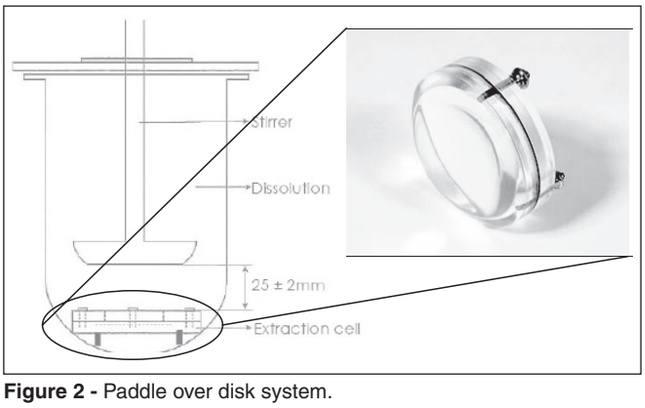

Figure 2 - Paddle over disk system.

III. RESULTS AND DISCUSSION

In literature [14, 15], the use of eye-drop formulations is reported as consisting of a sterile solution of cysteamine at 0.55 % and other excipients for six-eight daily applications.

In this work, gels with cysteamine concentration 0.5 and 1 % have been prepared with the aim of maximizing the interval of administration and to promote a controlled release of cysteamine. The gels were also designed to take into account the chemical and physical characteristics to provide good bioadhesion. Bioadhesion or mucoadhesion is established via electrostatic interaction, hydrophobic interaction, van der Waals intermolecular interactions and hydrogen bonding with the ocular mucus substrate. In particular, hydrogen bonding interaction appears to play a significant role in the mucoadhesion process [16]. Carbopol 974P was selected as gelling material. In fact poly acrylic acid has shown good bioadhesive properties in comparison to other gelling materials [17]. The ability of these polymers to increase ocular

Table I - Gel composition after reconstitution.

	Cysteamine	Carbopol	Benzalkonium chloride	Tartaric acid	Mannitol	Sorbitol	Lactose
RG-1	0.5 %	1.5 %	0.01 %	0.25 %	3 %		
RG-2	0.5 %	1.5 %	0.01 %	0.25 %		3 %	
RG-3	0.5 %	1.5 %	0.01 %	0.25 %			3 %
RG-4	1.0 %	1.5 %		0.5 %			3 %