Research paper

Formulation and in vitro evaluation of cysteamine hydrochloride viscous solutions for the treatment of corneal cystinosis

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Abstract

In the present study, viscous solutions of cysteamine hydrochloride (CH) were prepared by using 0.5%, 1.0%, 1.5% or 3.0% of hydroxypropylmethylcellulose (HPMC) and were evaluated for their in-vitro characteristics and stability. Osmolalities, pH and viscosity of the formulations were determined. The influence of benzalkonium chloride and autoclave sterilization on solution characteristics was also investigated. For stability assessment, the viscous solutions were stored at +4 and +25 °C over 12 months. In-vitro characteristics and CH contents of the stored solutions were monitored. Irritation tests for the formulations were evaluated on rabbit eyes. Dialysis sac technique was used to perform in vitro release study of the solutions containing 1.0% and 1.5% HPMC. All of the viscous solutions tested showed non-newtonian (dilatant) flow behavior. Osmolality values were ranked between 351.2 ± 6.2 and 355.1 ± 7.9 mOsm kg^{-1}, and pH values were between 3.97 ± 0.1 and 3.98 ± 0.2 for all the solutions. Furthermore, no significant changes in dilatant behavior, osmolality or pH values of the pure HPMC solutions were observed. After addition of the excipients or CH-excipients, increased viscosity values were noted in these formulations. Neither benzalkonium chloride nor autoclave sterilization had any influence on viscosity, pH or osmolality values of the solution containing 1.5% HPMC. Stability studies showed that a faster decrease in the concentration of CH was observed in the formulations stored at 25 °C compared to those kept at 4 °C; no changes were determined in osmolality values of the solutions at all storage conditions. Increased pH and decreased viscosity values were noted in HPMC solutions containing CH and excipients, while no changes in these values were observed for pure HPMC solutions kept at 4 and 25 °C. In vitro release tests revealed that 81.2% and 85.3% of CH were released from the viscous solutions containing 1.5% and 1% HPMC, respectively, in 8 h. No irritation was observed when the viscous solutions were tested on rabbit and human eyes.
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1. Introduction

Nephropathic cystinosis is a rare autosomal recessive metabolic disorder characterized by a defect in lysosomal cystine transport leading to the intralysosomal accumulation of cystine crystals in many tissues (kidneys, bone marrow, intestine, etc.) including the eye (retina, conjunctiva, iris, and cornea) [1–4]. In the eye, corneal cystine crystals cause severe photophobia, blepharospasm, and recurrent corneal erosions [5–7]. Because of their relatively low prevalence, cystinosis and cysteamine can be considered as an orphan disease and orphan drug, respectively [8]. Despite the lack of pharmaceutical industry interest in developing new medications to treat rare diseases such as cystinosis, many researches have been carried out in an effort to contribute to the treatment of both nephropathic and ocular cystinosis [9–13].

The mainstay of treatment for cystinosis, oral cysteamine, stabilizes glomerular function, improves growth velocity, and obviates the requirement for thyroid hormone replacement [14,15]. Thoene et al. [16] demonstrated the

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cystine-depleting efficacy of cysteamine (β-mercapto-ethyl-amine) in both in vitro and in vivo studies, and various types of its salts have been used as cystine-depleting agents in pediatric patients for more than 20 years [6,8]. The use of oral cysteamine products, which was approved by the U.S. Food and Drug Administration as "Cystagon" in 1994, has been shown to reduce cystine deposition systemically, leading to improvement in renal functions, but unfortunately, an effect on the cornea has not been clarified [6,7]. Cysteamine hydrochloride (CH) does not dissolve corneal crystals, most probably because of inadequate local cysteamine concentrations [5].

Cysteamine has also been valuable in eye formulations for relieving some of the ocular symptoms of cystinosis. Trials with topical cysteamine formulations (0.1–0.2%) have shown promising results, demonstrating substantial clearance of crystals following long treatment periods with topical cysteamine formulations [9–11].

The National Institutes of Health (NIH) developed an eye-drop formulation composed of a sterile solution of CH 0.55% w/v with benzalkonium chloride and sodium chloride. According to the NIH, their formulation is stable for one week at room temperature, two weeks under refrigeration, and for 18 months frozen [17]. Since this storage condition is not practical commercially [1], Hi-Tech Pharmaceuticals developed a new formulation which stabilizes cysteamine as the free thiol for 7 months at room temperature, and for 24 months under refrigeration. The formulation consists of CH (0.55% w/v), monosodium phosphate, disodium EDTA and benzalkonium chloride. Tsilou et al. [5] evaluated the safety and efficacy of the new formulation in a multicenter randomized double-masked clinical trial. The authors reported that no serious adverse reactions were observed with this formulation, but the new formulation was not as effective as the standard NIH formulation [5,17].

Topical cysteamine aqueous solutions should be administered either every hour while awake [10,11] or six times per day [18] for significantly better dissolution of corneal cystine crystals. Because of this frequent usage, it may lead to noncompliance to treatment, especially in childhood. In light of these data, it was aimed to prepare ophthalmic viscous solutions of CH to reduce the frequency of administration by increasing the precorneal residence time of the ophthalmic formulations, which may lead to a more effective treatment, and to perform in vitro assessment of the formulations developed prior to in vivo studies.

2. Materials and methods

2.1. Materials

Cysteamine hydrochloride (CH) was obtained from Sigma-Tau Pharmaceuticals (Sigma Chemicals, St. Louis, MO, USA). All other chemicals were purchased and used as received: benzalkonium chloride (Merck, Darmstadt, Germany); disodium EDTA (Merck, Darmstadt, Ger-many); monosodium phosphate (Merck, Darmstadt, Germany); acetic acid and hydrochloric acid (Merck, Darmstadt, Germany); phenolphthalein and hydroxypropylmethylcellulose) (HPMC, average Mn 86 000) (Sigma Chemicals, St Louis, MO, USA); iodide (Aklar Chemistry, Turkey); Carbopol 940 (Sigma Chemicals, St. Louis, MO, USA); Carbopol 980 (Sigma Chemicals, St. Louis, MO, USA); methanol (Merck, Darmstadt, Germany); Na_2S (Merck, Darmstadt, Germany); starch (Aklar Chemistry, Turkey); and sodium thiosulfate (Horasan Chemistry, Turkey). Salts for the preparation of Simulated Lachrymal Fluid (SLF) were obtained from Merck (Merck, Darmstadt, Germany) (KCl, $NaHCO_3$ and NaCl) and Sigma Chemicals Co. (St. Louis, USA) ($CaCl_2$ and $MgCl_2$).

2.2. Study conditions

Because CH is unstable in light, at room temperature, and in the presence of oxygen, special study conditions were established throughout the study. All experiments with CH were carried out in a darkened room. During the procedures of the formulation study, a continuous flow of nitrogen was applied to the formulations and equipment. Furthermore, all formulations were kept refrigerated.

2.3. Polymer selection

Carbopol 940, Carbopol 980 and HPMC polymers were used to prepare ophthalmic viscous solutions. Based on the results of pre-formulation studies, in which Carbopol 940 and Carbopol 980 were determined to be incompatible with drug substance and other formulation ingredients, HPMC was selected as the viscolysing agent.

2.4. Formulation and sterilization of viscous solutions

Four different concentrations of HPMC (0.5%, 1.0%, 1.5% and 3.0%, w/v) were used to prepare the formulations. In order to obtain the viscous solutions, properly weighted HPMC, monosodium phosphate (1.85%), disodium EDTA (0.1%) and benzalkonium chloride (0.01%) were dispersed and thoroughly hydrated in about 20–30% of the required amount of water. The dispersion was vigorously stirred and heated to 80–90 °C, and the remaining water was then added to the swollen solution. Cold water was then added to obtain the required volume [19]. Since CH (active substance, 0.55%, w/v) is unstable at high temperatures, the viscous solutions prepared with the excipients were sterilized at 121 °C in autoclave for 20 min prior to the addition of CH, as mentioned in American Pharmacopeia [20]. The preparations were kept in a darkened room at an ambient temperature for one day in order to obtain cool and clear viscous solutions. Afterward, aqueous CH solutions were sterilized by filtration through 0.22 μm sterile filter and added to the sterile HPMC solutions under the laminar flow hood in aseptic conditions.

Finally, solutions were placed in colored bottles previously sterilized at 170 °C for 2 h. Formulation codes are described in Table 1.

In order to evaluate the effect of benzalkonium chloride and autoclave sterilization on the formulations, 1.5% HPMC solution without drug and excipients (formulation H15, see Table 1) was selected to measure viscosity, pH and osmolality before and after sterilization.

2.5. Assay of cysteamine hydrochloride

The determination of CH was carried out using the iodometric titration [21–24]. Initially, CH and 4 g of potassium iodide were dissolved in 20 mL of distilled water and the solution was cooled in an ice bath. After addition of 5 mL of 3 N HCl, the solution was titrated with 25 mL of 0.1 N aqueous iodide solution and left for 20 min in a darkened room. Finally, back-titration was performed with 0.1 N $Na_2S_2O_3$ and 3 mL of aqueous starch solution as an indicator. In order to obtain calibration curves, six different concentrations of CH (10, 20, 40, 60, 125, and 250 mg) were used. The assay method was validated for linearity, sensitivity, precision, accuracy, and specificity.

Cysteamine oxidizes to its disulfide, cystamine, at room temperature making it unstable in the formal, chemical sense [1]. In order to confirm the iodometric titration assay specific to only cysteamine, the same assay method was carried out for cystamine, decomposition product of cysteamine. Before these experiments, cystamine was obtained by non-enzymatic oxidation of cysteamine to cystamine by sulfide according to the modified method of Cavallani et al. [25]. Cysteamine hydrochloride was dissolved in 0.3 mL of potassium phosphate buffer (pH 7.4) and water plus additions to a total volume of 3 mL. Then, 2 μmol Na_2S was added to this solution and mixed for 12 h with magnetic stirrer under the room conditions. Afterward, this solution (cystamine solution) was exposed to the same iodometric titration assay procedure. No color change was observed after back-titration step, indicating that this assay method was sensitive to the drug, and cystamine did not cause any interaction for cysteamine assay.

2.6. In-vitro characterization of viscous solutions

In vitro investigations were done using three different groups of the solutions: "HECH" solutions containing all components of the final formulation, "HE" solutions containing HPMC and other excipients without CH, and "H" solutions containing only HPMC (see Table 1). While viscosity, pH and osmolality experiments were performed with the solutions containing 0.5%, 1.0%, and 1.5% of HPMC, in vitro release experiments were done with the viscous solutions containing 1.0% and 1.5% of HPMC. Throughout these experiments, viscous solutions were homogenized before refrigeration for one night, and the measurements were carried out at room temperature.

Viscosity measurements were performed using a cone-plate viscosimeter (Brookfield Co., USA) at ten different rpm values (0.5, 1.0, 2.0, 2.5, 4.0, 5.0, 10.0, 20.0, 50.0, and 100.0). Viscosity (cps), shear stress (ss), and shear rate (sr) parameters were all obtained.

Osmolality measurements of the viscous solutions were carried out using Model 3300 Micro-Sample Osmometer (USA).

pH measurements were performed by ThermoOrion (USA)-pH meter to determine whether formulation ingredients or their concentrations have any effect on pH value of the whole formulation.

2.7. In vitro release studies

In vitro release of CH from HECH10 and HECH15 formulations was determined by dialysis bag method [26,27]. SLF was used as a release medium. SLF is an electrolyte solution composed of 1.7893 g/L KCl; 6.3118 g/L NaCl; 2.1842 g/L $NaHCO_3$; 0.0670 g/L $CaCl_2$ $2H_2O$; and 0.1572 g/L $MgCl_2$ $6H_2O$, adjusted with 0.1 N HCl to a pH of 7.4 ± 0.1 [28]. Fifteen milliliters of HECH10 formulation or HECH15 formulation was placed inside the cellophane membrane (cut-off 12,000–14,000 Da) and the bags were fitted into the glass tube containing 20 mL of SLF (Arthur H. Thomas Co., USA). The apparatus was fully immersed in a water bath which was stirred magnetically

Table 1
Contents of the viscous solutions

Code of formulation	Cysteamine hydrochloride (%)	HPMC (%)	Monosodium phosphate (%)	Disodium EDTA (%)	Benzalkonium chloride (%)
H05		0.5			
H10		1.0			
H15		1.5			
H30		3.0			
HE05		0.5	1.85	0.1	0.01
HE10		1.0	1.85	0.1	0.01
HE15		1.5	1.85	0.1	0.01
HE30		3.0	1.85	0.1	0.01
HECH05	0.55	0.5	1.85	0.1	0.01
HECH10	0.55	1.0		0.1	0.01
HECH15	0.55	1.5		0.1	0.01
HECH30	0.55	3.0		0.1	0.01

at 600 rpm (Heidolph, Germany) and kept at 32 °C. The tubes were prepared individually for each sample collection. At 0.25, 0.5, 0.75, 1, 3, 5, and 8 h, the whole release medium was withdrawn and the drug content of the samples was determined by the previously described iodometric titration. To calculate the release rate constant of CH, the percentage released-versus-time profile was obtained by curve-fitting analysis.

2.8. Stability studies

Three groups of the viscous solutions containing low (0.5%), medium (1%), and the highest (3%) amount of HPMC were prepared for the stability studies. Each viscous solution was separated into the flacons and kept at either 4 or 25 °C. The samples were evaluated for osmolality, pH, viscosity and CH content on days 0, 15, 30, and every month over a year. Measurements were carried out with three replicates for each formulation.

2.9. Ocular tolerability in animals and in volunteers

The potential ocular irritancy and/or damaging effects of the ophthalmic viscous solutions containing CH were evaluated both in animals and in volunteers according to a modified Draize test [29]. After receiving approval from the Hacettepe University Ethics Committee for Animals (decision number 2005/2), an irritation test was performed. For this purpose, six male Nippon albino rabbits (2.5–3 kg) were included in the study. In the first week, 50 µL of placebo viscous solutions (H05, H10, H15, and H30) was administered to the right eyes three times a day. Left eyes were taken as controls, and 0.9% NaCl solutions were used for control eyes. Following the tests with placebo, the test was repeated one week later with viscous solutions containing CH (HECH05, HECH10, HECH15, and HECH30). The eyes were evaluated according to irritation signs of conjunctiva, iris, and cornea before, during and every day after the test (see Table 2).

After written approval from the Erciyes University Ethics Committee (decision number 2008/161), six volunteers (5 females and 1 male, 30 ± 6) with no history and/or signs of any ocular diseases such as ocular allergy, dry eye, ocular inflammation, and ocular infection were informed about the study protocol. Both the volunteers and the ophthalmologist were blind for the drug and the placebo bottles. Since it was observed that HECH15- and HECH30-coded formulations were not convenient to be instilled into the rabbit eye, only HECH10-coded formulation was selected for human study. In the first week, 50 µL of placebo viscous solutions (H10) was administered to the right eyes four times a day. Left eyes were taken as controls, and 0.9% NaCl solution were used for control eyes. Following the tests with placebo, the test was repeated one week later with viscous solution containing CH (HECH10). The eyes were evaluated according to irritation signs of conjunctiva, iris, and cornea before, during and every day after the test.

Table 2
Scores for the evaluation of rabbit or human eyes in modified Draize test

Ocular presentations	Scores
A – Conjunctival edema (Chemosis)	
Absent	0
Any swelling	1
Prominent swelling along with partial lid eversion	2
Swelling with half-closed lids	3
Swelling with totally closed lids	4
B – Redness in conjunctiva	
Absent	0
Abnormal conjunctival injections	1
More diffuse and deeper hyperaemia, separate vessels can not be seen easily	2
Diffuse and dense hyperaemia	3
C – Secretion	
Absent	0
Any abnormal secretion	1
Secretion leading to wet eye lashes closer to lids	2
Secretion leading to wet lids and whole periorbital areas	3
D – Corneal opacity	
Absent	0
Scattered or diffused areas – details of the iris discernible	1
Easy discernable, transparent areas, detail of the iris slightly darkened	2
Opalescent areas, no details of the iris discernible, size of the pupil barely discernible	3
Opaque cornea, iris not discernible	4
E – Iris involvement	
Absent	0
Pronounced deep folds, congestion, deep swelling, circumcorneal injection, the iris still reacts to light	1
No response, haemorrhage, marked destruction	2

Moreover, the symptoms including itching, burning, dryness, pain, redness, and blurred vision were evaluated (0: absent to 5: very severe) everyday in each of the volunteers.

2.10. Statistics

Statistical analysis of data was performed with ANOVA by using the Statistica® (Statsoft, Tulsa, USA) software.

3. Results and discussion

Although it was shown that the standard NIH cysteamine eye-drop formulation is effective against corneal cystinosis, due to its restrictive storage conditions, a new formulation was developed by Hi-Tech Pharmaceuticals to increase the stability of the standard formulation [17]. However, Tsilou et al. [5] reported that the new stable formulation was not as effective as the standard NIH formulation and that further work was needed to develop an alternative treatment for corneal cystine crystals that does not have limiting storage requirements. For this reason, in the present study, we prepared the same formulation as that of Hi-Tech Pharmaceuticals and, in order to obtain a stable formulation, added HPMC polymer to determine whether we could increase the corneal residence time of

the Hi-Tech formulation when compared with the standard NIH formulation. We also aimed to increase viscosity of the formulations with HPMC and increase patient compliance, which might be obtained by reducing the frequency of drug application.

3.1. Selection of polymer and analytical method

Polymers are used in ophthalmic formulations in order to increase the residence time on the ocular surface and thereby increase bioavailability. The most common polymer agents employed in ophthalmic preparations are polyvinyl alcohol, Carbopol 940, Carbopol 980, and cellulose derivatives (HPMC, carboxymethylcellulose) [30]. In the pre-formulation part of the present study, 0.1%, 0.5%, and 2.0% of Carbopol 940 and Carbopol 980 viscous solutions were prepared and evaluated for in-vitro characteristics. These studies showed that the chemical structure of viscous solutions was spoiled and pH values declined following the addition of monosodium phosphate and CH to Carbopol viscous solutions. As a result, it was decided that there was an incompatibility between CH, monosodium phosphate and Carbopol, and thus HPMC, which showed no sign of incompatibility, was chosen for the formulation. Over the past years, many researches have addressed whether HPMC could be used safely for ophthalmic formulations [31–35]. HPMC was selected as the best choice of polymer in the current study due to its common use in ophthalmic formulations, reliability, and the appropriateness of its physicochemical structure.

In this study, iodometric titration method was chosen to assay CH since it has been reported as reliable for the determination of thiol groups [21–24]. Iodometric method

is recommended by American Pharmacopeia [20] and European Pharmacopeia [36] for cysteine, which is another thiol-containing compound and is similar to cysteamine in molecular structure. Kast et al. [21] used iodometric titration for the determination of thiol groups of the polymer–cysteamine complex. Another study using iodometric titration to determine thiol groups was carried out by Bernkop-Schnürch et al. [22] Iodometric titration was considered an appropriate method for in vitro studies because it is reliable, easy to perform, produces rapid results, and is inexpensive.

3.2. In-vitro characterization studies

The flow curves obtained from the viscosity measurements of H05, H10, H15; HE05, HE10, HE15; and HECH05, HECH10, HECH15 formulations are shown in Fig. 1. Each formulation showed non-newtonian (dilatant) flow without any hysteresis. Chen et al. [37] and Kulicke et al. [38] reported the dilatant flow, and Ludwig et al. [39] and Tomoaki and Ford [40] reported the newtonian flow for 0.5–3% concentrations of HPMC solutions. Furthermore, Kulicke et al. [38] reported that the addition of sodium lauryl sulfate to HPMC solutions caused an increase in the dilatant flow properties of the solutions. In our study, however, neither addition of excipients nor drug plus excipients had any influence on the dilatant flow behavior of the solutions.

As shown in Fig. 1(a–c), increasing the concentration of HPMC from 0.5% to 1.5% caused an increase in viscosity of all the solutions. When viscosity results of the formulations H05, H10, and H15 were compared to HE05, HE10, HE15 or HECH05, HECH10, and HECH15 formulations,
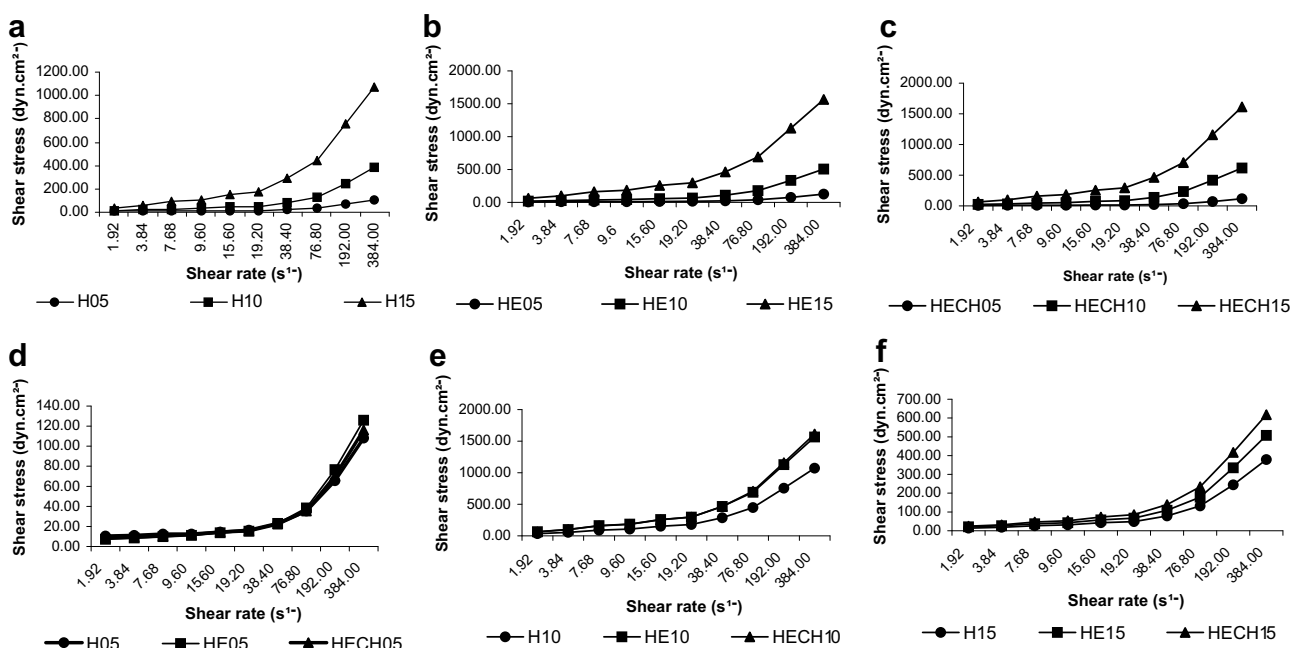

Fig. 1. Flow curves of the viscous solutions. (a–c) Effect of HPMC concentration; (d–f) effect of cysteamine hydrochloride and excipients ($n = 3$, $X \pm SD$).

it could be deduced that the addition of excipients or drug plus excipients to the solutions caused an increase in viscosity of all solutions ($p < 0.001$), except H05-, HE05-, and HECH05-coded formulations ($p = 0.217$) (Fig. 1d–f). Viscosities of H10, HE10, HECH10 formulations at 2.5 s^{-1} sr were 320.8, 443.7, and 552.9 cps, respectively, and these values for H15, HE15, and HECH15 were 1119.3, 1945.6, and 1911.0 cps, respectively. Similarly, Hino and Ford [40] reported that the addition of nicotinamide to the HPMC solutions caused an increase in viscosity of the solutions due to the hydrogen-bonding of the drug to HPMC molecules. A similar situation is very likely in our case due to the presence of sulfhydryl and amine groups of cysteamine.

Osmolality measurements showed that all of the formulations tested had osmolalities between 351.2 ± 6.2 and 355.1 ± 7.9 mOsm kg^{-1}, which are within the acceptable tonicity range of the eye. The presence of the excipients or drug plus excipients had no significant effect on osmolality values ($p = 0.578$). Similarly, pH values ranged between 3.97 ± 0.1 and 3.98 ± 0.2, and no significant changes were observed after addition of the excipients or CH plus excipients ($p = 0.189$).

HECH10 and HECH15 formulations were chosen for the in vitro release study due to their potential use in the in vivo studies. Furthermore, HECH30 was too viscous to instill properly into the eye and the lowest concentration (0.5% HPMC) was not preferred since the aim of this study was to increase the viscosity of the formulations developed to the extent possible. The release profiles of CH from the HECH10 and HECH15 viscous solutions are shown in Fig. 2. Release profiles were followed throughout 8 h, and faster drug release was observed for the HECH10 formulation compared to HECH15, which can be explained by the lower viscosity of the formulation. Fitting of the release profiles (percentage release-versus-time) proved the adequacy of Higuchi's square-root equation compared to zero order, first order and cube-root equations, as determined by F test for both the formulations. As shown in Fig. 2, 43.3% and 52.1% of the drug were rapidly released from the formulations HECH15 and HECH10, respectively, in the first hour,
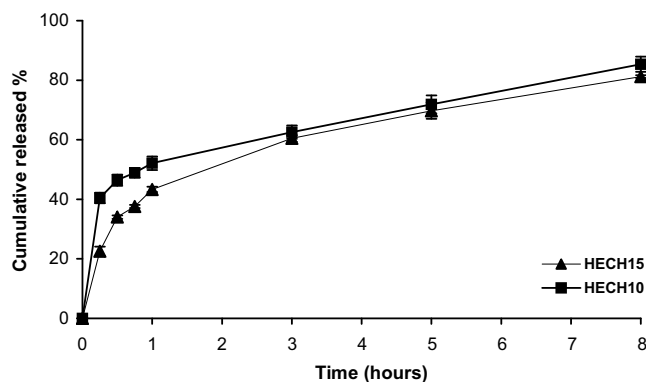
Table 3
pH and osmolality values of formulation H15 and benzalkonium chloride containing H15 before and after autoclave sterilization ($n = 3$, $X \pm$ SD)

Content	pH	Osmolality (mOsm.kg^{-1})
H15	4.32 ± 0.2	272 ± 6.1
H15 + BC (before autoclaving)	4.31 ± 0.1	280 ± 7.3
H15 + BC (after autoclaving)	4.33 ± 0.1	279 ± 5.9

H15: HPMC solution in water (1.5%).
BC, benzalkonium chloride.

and then the release continued slowly. In the first hour, release rates were 40.09 and 42.73 h$^{-1/2}$, which later decreased to 20.61 and 22.08 h$^{-1/2}$, for HECH15 and HECH10, respectively. Reddy et al. [41] reported Higuchi's release kinetics for minoxidil and Nounou et al. [27] for dibucaine from the viscous solutions with 2–6% HPMC.

The viscosity, pH and osmolality values of formulation H15 were evaluated in comparison with HPMC solutions with benzalkonium chloride, before and after autoclave sterilization. The results obtained from the measurements indicated that neither the presence of benzalkonium chloride nor the autoclave sterilization had any influence on viscosity
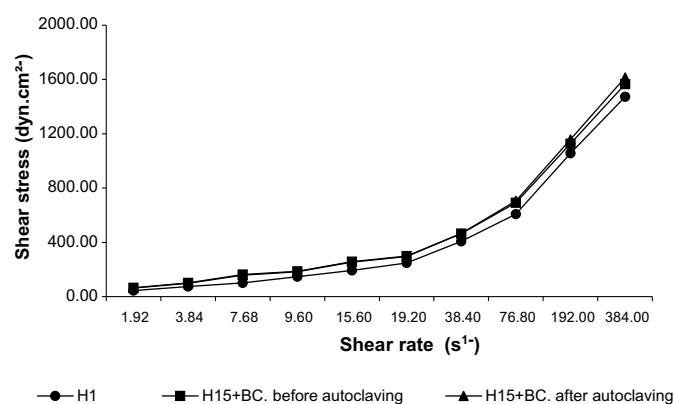
Fig. 3. Flow curves of formulation H15, benzalkonium chloride (BC) containing H15; before and after autoclave sterilization ($n = 3$, $X \pm$ SD).

Fig. 2. Release profile of cysteamine hydrochloride from HECH10- and HECH15-coded formulations ($n = 3$, $X \pm$ SD).
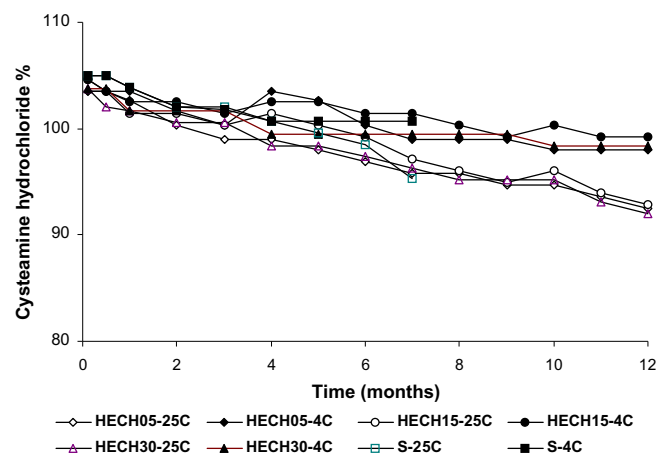
Fig. 4. Change in cysteamine hydrochloride content over time ($n = 3$, $X \pm$ SD).

($p = 0.127$), pH ($p = 0.108$) or osmolality ($p = 0.173$) values of the H15 formulation (Table 3 and Fig. 3). Similar results were observed in the literature [42–44].

3.3. Stability studies

Solutions without HPMC (S formulation) and with 3% HPMC (H30, HE30, and HECH30 formulations) were included in the stability studies in order to evaluate whether HPMC concentration has any effect on the stability of CH in the solutions. Fig. 4 illustrates the variation of CH concentrations in the solutions stored at 4 and 25 °C for over 12 months. As can be seen, a faster decomposition of CH was observed for the formulations stored at 25 °C compared to those kept at 4 °C ($p < 0.001$). The concentrations of CH in HECH05, HECH15, and HECH30 formulations stored at 4 °C remained at 97.9%, 99.2%, and 98.4% of the initial concentrations, respectively, while those of the solutions kept at 25 °C fell to 92.5%, 92.8%, and 92.0%, respectively, at the end of 12 months. These results are in accordance with the results taken from the formulation developed by Hi-Tech Pharmaceuticals [17]. Furthermore, similar decrease profiles were found for the formulations S, HECH05, HECH15 and HECH30 ($p = 0.897$). This indicated that the presence of HPMC in these concentrations had no influence on the stability of CH in the solutions at either storage temperature.

The results obtained from the pH measurements indicated that the pH alteration over time was not found to be significant ($p = 0.349$ and $p = 0.219$ for 4 and 25 °C, respectively) for the formulations H05, H15, and H30 at either storage temperature, especially when the values of the first day and the 12 month were considered (Fig. 5). However, for HECH05-, HECH15-, and HECH30-coded formulations, the increase in pH value after 12 months
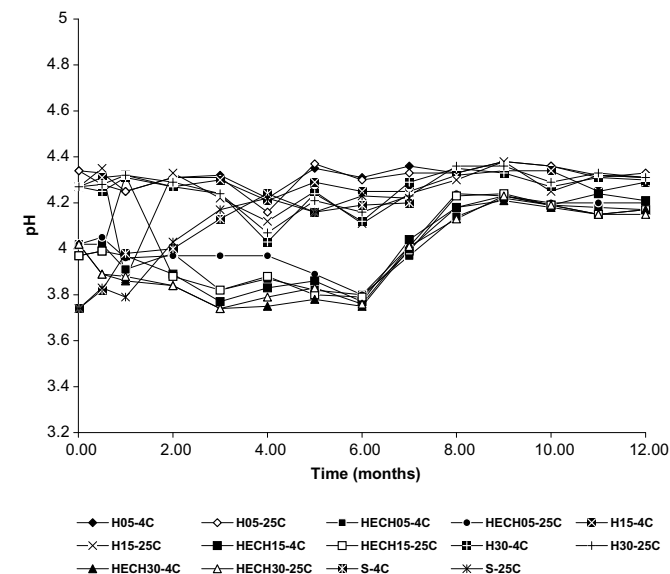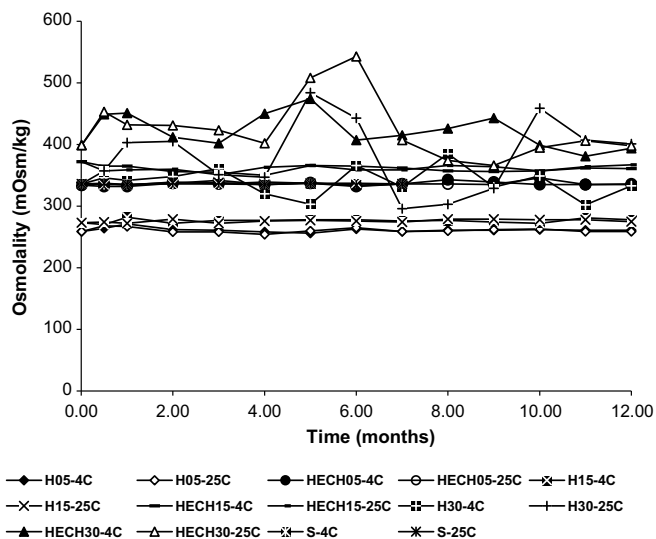
Fig. 6. Change in osmolality values of the viscous solutions over time ($n = 3$, $X \pm SD$).

was found statistically significant ($p < 0.001$). This might be attributed to the presence of drug and excipients in the formulation with ionization ability and to alteration over time in pH of the solutions. Furthermore, similar results were obtained for all the solutions at both storage temperatures. The data showed that increase in the storage temperature from 4 to 25 °C had no significant effect on the change in pH of the formulations ($p = 0.165$).

The differentiation in osmolality values of the formulations stored at 4 or 25 °C is shown in Fig. 6. As can be seen, no changes in the osmolality values were noted for any of the solutions at either storage condition over the 12 months ($p = 0.402$).

Fig. 7 shows viscosity changes at 2.5 s^{-1} sr for formulations stored at 4 and 25 °C over the 12 months. The viscosity decline over one year for formulations HECH15 and HECH30 was found statistically significant ($p < 0.001$), while it was not significant for HECH05-coded formulation ($p = 0.093$). This alteration could be attributed to the influence of pH changes resulting from some ionized groups in the formulations on viscosity of the solutions. Also, it seemed to be noticeable in formulations HECH15 and HECH30, which have much tighter and denser chain bonds, which may lead to change over time. Similarly, Bohic et al. [45] reported that the presence of divalent and trivalent ions has an effect on the polymer chain conformations of the HPMC solutions. Furthermore, there was no statistically significant difference in viscosity between the solutions kept at 4 and 25 °C over the 12 months (Fig. 7a–c). No changes were observed in dilatant flow behavior of the solutions after 12 months under the storage conditions used.

3.4. Ocular tolerability

To evaluate applicability of the viscous solutions prepared as ophthalmic drug delivery systems, the ocular
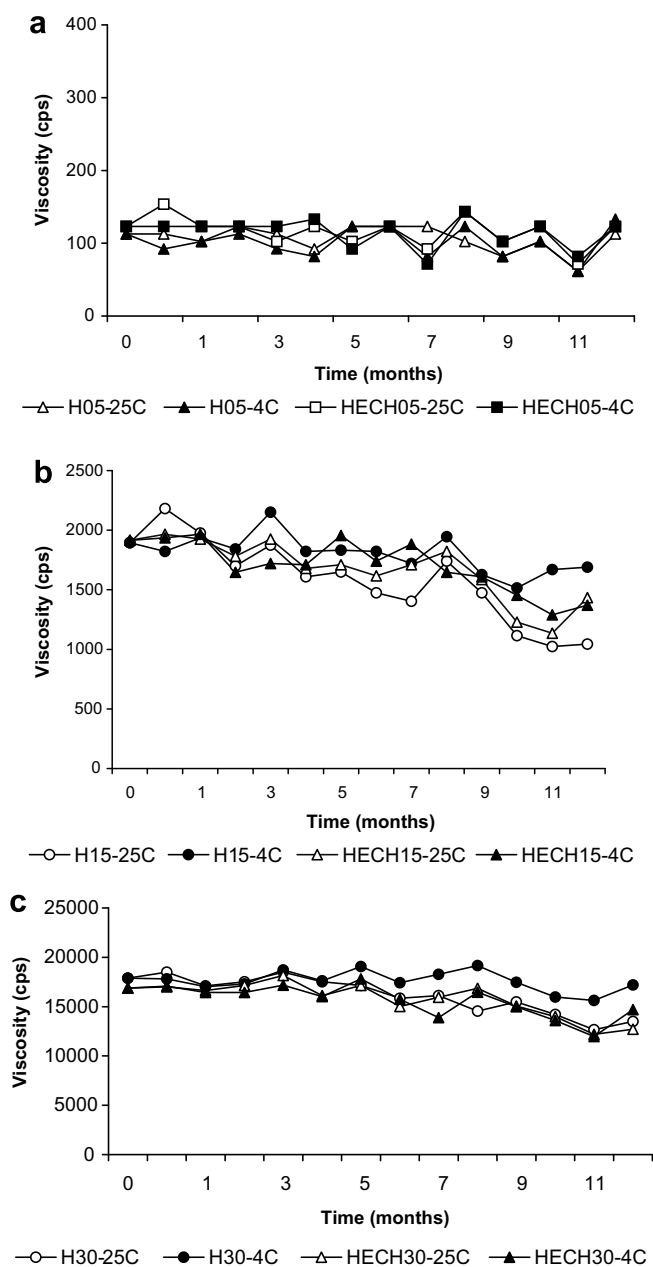
Fig. 5. Change in pH values of the viscous solutions over time ($n = 3$, $X \pm SD$).

a

—△— H05-25C —▲— H05-4C —□— HECH05-25C —■— HECH05-4C

b

—○— H15-25C —●— H15-4C —△— HECH15-25C —▲— HECH15-4C

c

—○— H30-25C —●— H30-4C —△— HECH30-25C —▲— HECH30-4C

Fig. 7. Change in viscosity of the viscous solutions at 2.5 s^{-1} shear rate over time($n = 3$, $X \pm$ SD).

tolerability was evaluated by the modified Draize test. For this purpose, HECH05, HECH10, HECH15, and HECH30 formulations were tested by comparison with blank HPMC solutions (formulations H05, H15, and H30) in rabbits. Ocular tolerability results showed no evidence of inflammation and/or discomfort in rabbit eyes with any of the formulations tested. The scores of ocular presentations were determined as zero at all observations. Although no irritation was observed with HECH15 and HECH30 formulations, these solutions were not convenient to instill into the rabbit eye due to their high viscosity.

Ocular tolerability results in volunteers revealed no irritation signs and symptoms with HECH10 formulation.

Solely, a slight burning sensation that was felt immediately after drug administration and continued for approximately five seconds was noted in all volunteers. The scores of modified Draize test were all determined as zero at all observations.

4. Conclusions

In the present study, cysteamine hydrochloride (CH) viscous eye solutions containing 0.5%, 1.0%, 1.5% or 3.0% hydroxypropylmethylcellulose (HPMC) were prepared and evaluated for their in-vitro characteristics and stability. According to the in-vitro characterization study, it can be concluded that all the solutions have non-newtonian (dilatant) flow behavior and that they have tonicity range acceptable to the eye. The initial viscosity of the HPMC solutions was not affected by CH and formulation excipients. Furthermore, addition of benzalkonium chloride or autoclave sterilization had no influence on viscosity, pH or osmolality values of blank solution with 1.5% HPMC. Faster drug release was observed with the formulation containing 1.0% HPMC compared to that with 1.5% HPMC.

Stability studies revealed that a faster decomposition of CH was observed in the formulations stored at 25 °C compared to those kept at 4 °C. In addition, the presence of HPMC (concentration from 0.5% to 3.0%) had no influence on the stability of CH at either storage temperature. Increases in pH values were noted for the complete solutions kept at 4 and 25 °C over the 12 months, while no changes were observed in the osmolality values. The decrease in viscosity over one year was obtained in all CH- and excipient-containing solutions, except solutions containing 0.5% of HPMC at both storage temperatures. There was no statistically significant difference in viscosity in the solutions kept at 4 and 25 °C over one year. The dilatant flow behavior of the solutions did not change after 12 months under the storage conditions used. The absence of irritant activity is promising for ophthalmic use of the formulations.

Considering the in-vitro characterization, stability and irritation test results, the HECH10 formulation (CH- and excipient-containing viscous solutions with 1.0% HPMC) seems suitable for further in vivo studies to evaluate their effectiveness.

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