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Preformulation of cysteamine gels for treatment of the ophthalmic complications in cystinosis.

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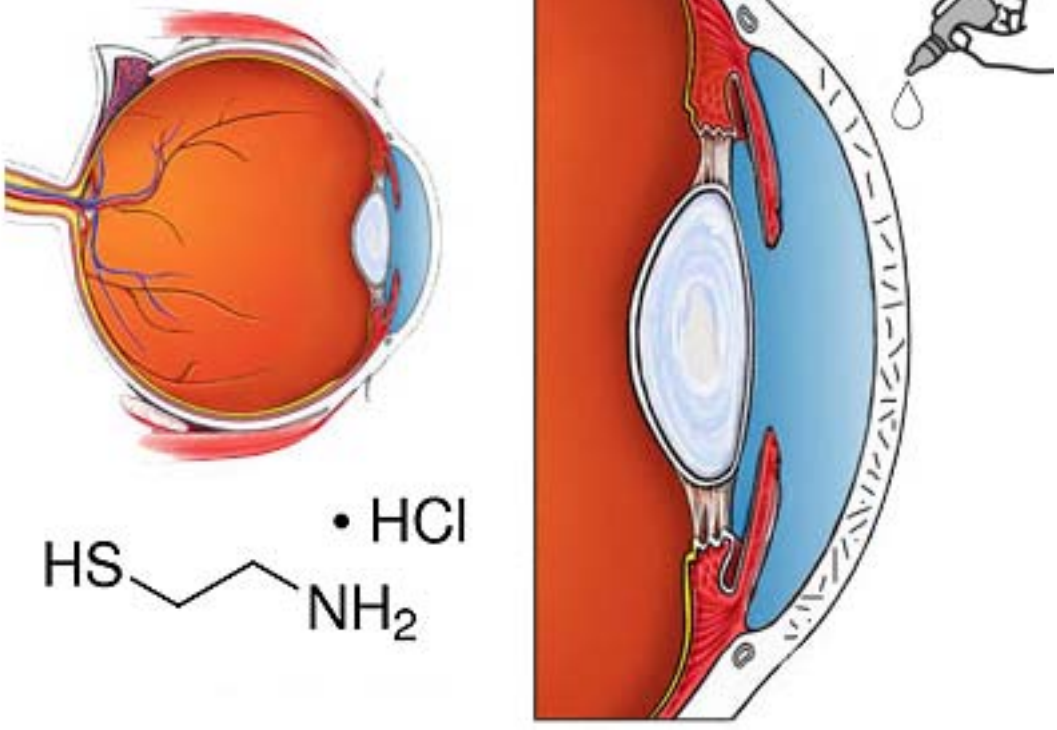

Abstract

Nephropathic cystinosis is a rare autosomal recessive disease characterised by raised lysosomal levels of cystine in the cells of all organs. It is treated by regular administration of the aminothiol, cysteamine. Corneal crystal deposition is one of the most troublesome complications affecting patients and requires the hourly administration of cysteamine eye drops. In an attempt to reduce this frequency and improve the treatment, the preformulation and evaluation of cysteamine containing gels is reported. Suitability for ophthalmic delivery was determined by analysis of rheology, bioadhesion, dissolution and stability. The results demonstrated that three polymers were suitable for ophthalmic delivery of cysteamine; namely sodium hyaluronate, hydroxyethyl cellulose and carbomer 934. Sodium hyaluronate displayed optimum performance in the preformulation tests, being pseudoplastic (reduction in apparent viscosity under increasing shear rate), bioadhesive, releasing cysteamine over 40 minutes and displaying stability over time. In conclusion these results offer the possibility to formulate cysteamine in an ocular applicable gel formulation.

Keywords: Cystinosis, Ophthalmic delivery, Gel, Cysteamine, Preformulation

1. Introduction

Nephropathic Cystinosis is a rare genetic disease, characterised by extremely high lysosomal levels of cystine, the oxidized dimer form of the amino acid cysteine and manifested by a general failure to thrive (Buchan, et al. 2012). The accumulation of cystine as crystals in most tissues leads to the progressive

impairment and dysfunction of multiple organs, such as the pancreas, eye, brain and thyroid (Syres, et al. 2009). Without treatment, this autosomal recessive disease can result in multi-organ failure and death before the onset of puberty (Gahl, 2009). The main treatment for the disorder remains the administration of the aminothiol, cysteamine (figure 1) (as the bitartrate salt, Cystagon®)(Thoene, et al. 1995). Cysteamine therapy produces rapid depletion of cystine from leukocytes, with minor side effects (Schneider, et al. 1976; Schneider 2004).

Corneal crystal deposition is one of the most troublesome complications affecting patients with cystinosis and persists even as their prognosis improves and life expectancy increases. Photophobia and, ultimately, blepharospasm affect the quality of life to such an extent that the slightest glimmer of sunlight can be debilitating. In addition, crystal accumulation over a period of years can cause the formation of corneal scars, keratitis and cataracts, as well as band keratopathies (Gahl et al., 2000). The oral form of Cystagon has no effect on depleting corneal crystals due to poor drug availability stemming from the absence of vasculature in the cornea (Gahl, et al. 1987; Gahl and Kuehl, 2000), thus cysteamine must also be administered topically in the form of eye drops.

Compliance with the use of eye drops is a major issue however, as in order to achieve the maximum benefit in their current formulation these drops must be routinely administered every hour while awake (Gahl and Kuehl, 2000; Gahl, et al. 2007). This is due to a low bioavailability commonly reported for topical eye treatments, with tissue contact time varying from 1-2 minutes (Gangrade, et al. 1996; Jansook, et al. 2010; Le Bourlais, et al. 1998) to 5 minutes (McKenzie and Kay 2015; Robinson, 1989; Shell, 1984; Urtti and Salminen 1993). This short

ophthalmic residence time is due to a multitude of protective mechanisms such as blinking and high tear fluid production and turnover (Ahmed and Patton, 1987; Kaur and Kanwar, 2002; Saettone and Salminen, 1995; Morrison et al, 2014; Morrison et al 2013). The current drops, which contain 0.55% cysteamine hydrochloride in saline also cause frequent stinging and redness upon application (Gahl 2009).

By formulating cysteamine as a bioadhesive ophthalmic gel with controlled drug release, it is hypothesised that administration frequency may be reduced, perhaps allowing once or twice-daily dosing instead of the current hourly requirement. These formulation changes may reduce the burden of treatment and improve compliance, producing long-term prevention of ophthalmic morbidity, such as blindness. Hydrogels which are pseudoplastic, transparent and bioadhesive are highly desirable for topical ophthalmic application, and have the potential for less frequent application and improved patient compliance.

2. Materials and methods

Cysteamine hydrochloride (CH), hydroxyethyl cellulose (HEC), hydroxypropylmethyl cellulose (HPMC), xanthan gum (XG), potassium chloride, sodium chloride, sodium carbonate, calcium carbonate and magnesium chloride were purchased from Sigma, UK. Polyvinylpyrrolidone (PVP) was purchased from Fluka. Ellman's Reagent, 5,5'-dithiobis(2-nitrobenzoate) (DTNB) was purchased from Molekula (Gillingham, UK). Tris buffer (1M, pH7.4) was bought from Fisher. Carbomer 934 (C934) was purchased from Universal Biologicals, UK. Carbomer 974 (C974) was purchased from Surfachem, UK. Sodium hyaluronate (HA) was

purchased from Aromantic (Moray, UK). Benzalkonium chloride (BZK) was purchased from Aldrich. Tubing membrane (12–14,000 kDa) was purchased from Visking, UK. All other chemicals were of pharmaceutical grade.

As part of initial pre-formulation work, potential gel carriers were screened for suitability as ophthalmic vehicles. A literature review was undertaken, investigating the suitability of polymers available for ophthalmic use. Inclusive parameters were non-toxicity in the eye, pseudoplastic rheology, bioadhesive nature, good optical clarity, stability and compatibility with cysteamine. Eight gel carriers met these criteria. The eight gels were subjected to unmedicated pre-formulation testing of rheology and optical clarity. The polymers were dissolved in water for injection (WFI) and neutralised to pH 7.4 with sodium hydroxide if required, and allowed to fully hydrate at 4°C for 24 hours before testing. The concentrations used corresponded to those used commercially (table 2). This maintained the apparent viscosity of the gels within the limits tolerated by the eye. Gels which performed satisfactorily were loaded with 0.55%w/w CH and subjected to further testing: dissolution, bioadhesion, toxicity, stability and surface tension. Unmedicated gels were used as controls. Simulated Lachrymal Fluid (SLF) at pH 7.4 was used to mimic tear fluid during tests. It was also used in the production of the ophthalmic gels to provide additional buffering capacity. The following salts were weighed out and stirred in a 1 L volumetric flask: potassium chloride 0.179% w/v, sodium chloride 0.631% w/v, sodium carbonate 0.218% w/v, calcium carbonate 0.004% w/v and magnesium chloride 0.005% w/v. Hydrochloric acid (0.1M) was used to adjust the pH to 7.4. Sorensen's Modified Phosphate Buffer (SMPB) was also added to the gels to stabilise the gels

at pH7.4. It is one of the most common ophthalmic buffers used. Briefly, a solution of 0.2M monosodium phosphate and disodium phosphate was made in WFI.

2.1 Rheology

The rheological properties of the gel were studied using an Advanced Rheometer AR1000 from TA Instruments (Delaware, USA). A 60 mm, 2° angle cone geometry was used, with a truncation value of 65 μm. All measurements were made at 34°C, the temperature at the corneal surface. Continuous shear measurements were made initially, using a linear mode and a continuous ramp of 0-600 s^{-1}, and 600-0 s^{-1} over 20 minutes, to establish flow types such as Newtonian or pseudoplastic.

Oscillatory measurements were performed on the gels to characterise the linear visco-elastic behaviour and relate the rheological parameters to molecular structure. A linear mode was used with a frequency of 1-10 Hz, and 20 sample points. The controlled variable was percentage strain. The sample volume was approximately 1.5 ml. All tests were performed in triplicate.

2.2 Optical transmission

The optical transmission of each gel was measured using a Cecil CE 3021 Spectrometer (Cambridge, England). The transmission was measured as a ratio of the amount of light unabsorbed by the gel to the total amount of light the gel was exposed to, expressed as a percentage. A wavelength of 480 nm was used, the middle of human light wavelength perception. The gels were measured with

1cm, 5mm and 2mm path lengths; although *in situ* they would be less than a millimetre thick. The gels were referenced to deionised water at room temperature, which was taken as 100% transmission. A figure greater than 90% was classed as transparent, between 10 and 90% as translucent, and less than 10% as opaque (Buchan, et al. 2010). Each test was performed in triplicate.

2.3 Evaluation of cysteamine release *via* dissolution

A 100 ml round-bottomed flask was held in a water bath, heated to 34°C. To the flask 50 ml aqueous solution of SLF and Tris buffer (90:10) was added, and an equimolar quantity (to the concentration of cysteamine in the gel) of Ellman's reagent was added, and this solution was stirred magnetically using an IKA RET basic hotplate stirrer (Staufen, Germany). A dialysis membrane (12-14,000 kDa) containing 7 ml of the gel and, tied in a rod shape (length 2.23 cm; radius 1 cm, average of 3 measurements) to exclude air bubbles, was added to the flask at time zero. The quantity of gel used was chosen to avoid reaching the limit of detection of the UV spectrometer. The medium was sampled every 2 minutes for the first ten minutes, every 5 minutes for an hour, and every 15 minutes after the first hour. Samples were analysed at 440nm, the λmax for DTNB. Dissolution tests were performed in triplicate.

2.4 Bioadhesion

Bovine corneal tissue, which is reported as being similar in structure to the human cornea, was used as a control (Loch C, et al. 2012). A Texture Analyser (Stable Micro Systems, Surrey, UK) was used to measure the force required to remove

the gel from an area of bovine cornea (Thirawong, et al. 2007). Fresh bovine eyes were collected immediately after slaughter, and washed with deionised water. The whole cornea was then excised and washed in SLF at room temperature. Prior to testing, the corneas were placed on a tissue to remove excess fluid. Cyanoacrylate glue was then used to attach a cornea to a 2 cm^2 stainless steel plate. Care was taken not to allow the glue to come into contact with the upper surface of the tissue. Immediately after this, the steel plates were attached (in pairs) to the Texture Analyser, one positioned directly above the other. Each gel sample was placed between the cornea samples and held together for 60 seconds; the force required to separate the plates was then measured (contact force of 0.05 N, contact time 60 s, probe speed 0.5 mm/s). The force was plotted against distance; the area under the curve (AUC) being equal to the work of adhesion (Wad) (Thirawong, et al. 2007; Varum, et al. 2010). Each individual test was undertaken nine times. Statistical significance was determined using a Mann-Whitney test.

2.5 Toxicity

The effect of the gel carriers on healthy human fibroblasts was determined using an alamar Blue test (Fisher Scientific, UK).

2.6 Stability testing

A 12 week stability study was performed to elucidate the effect of storage containers and conditions on cysteamine oxidation. Storage was at 4°C or 21°C, under air or nitrogen, in beakers, crimped bottles or sealed glass ampoules. Stability was quantified by measuring the cysteamine content in the gels using

Ellman's reagent; cysteamine content over time was compared to 100% at the beginning of the test (T_0).

2.7 Surface tension

Surface tension studies were performed using a torsion balance (Malvern Wells, UK). Tests were performed on the C934 and HA gels in triplicate at 20°C, with and without benzalkonium chloride to ascertain the effect of the preservative on the gel.

3. Results

As a result of the initial literature search, eight polymers were identified as suitable candidates for ophthalmic vehicles (Table 1). The polymers were tested for light transmission and rheology. The polymer concentrations used initially in this project were based on published values or commercial concentrations.

As a result of the initial light transmission and rheology analysis, five gels were found to be most suitable for optical delivery: two grades of carbomer (934 and 974), xanthan gum, sodium hyaluronate and HEC. PVP and HPMC were eliminated due to unsuitable optical clarity and rheology. The carrageenan became a solid mass upon cooling, and was therefore unsuitable for use in this project. Also, PVP possessed a yellow colour which would be noticeable to the patient.

The concentrations of the five gels were adjusted to obtain a gel within the desired viscosity range, i.e. 10 mPa.s – 30 mPa.s (Oechsner and Keipert, 1999; Zhu and

Chauhan, 2008) (table 2). All five gels contained cysteamine hydrochloride at a concentration of 0.55% w/v as per the current eye drop. Further analysis was performed to determine which of the five medicated gels was most suitable for ophthalmic delivery of cysteamine. Benzalkonium chloride is the most widely used preservative in ophthalmic preparations (Lewis, et al. 2007; Marple, et al. 2004). More recently, the safety of this compound in the eye has been questioned, particularly in multiple-dose preparations (Baudouin, et al. 2010). Alternatives include sodium perborate and polyquaternium 1. Polyquaternium 1 is licensed for exclusive use by Alcon pharmaceticals for use in the eye, therefore sodium perborate was tested as an alternative to benzalkonium chloride. Sodium perborate was also used as an alternative to benzalkonium choride, to determine any precipitation effect particularly for carbomer. All of the gels' parameters were unchanged except for hydroxyethyl cellulose and sodium hyaluronate which were four to five times more viscous, and carbomer 974 and xanthan gum, which were both 5-10% more optically clear. It was therefore decided to continue testing using benzalkonium chloride as a preservative.

3.1 Rheology

The rheology measurements of the gels provide information about the nature of the gel structure. All of the gels tested in Table 2 displayed a network like that of an 'entangled solution' apart from xanthan gum (Garrec and Norton 2012).

3.2 Optical transmission

Concerns were expressed about gel opacity, particularly for carbomer 934, and as a result, optical path lengths of 2mm were also used (figure 2). It is likely that, *in vivo*, the gels will be much thinner, in the region of 1.5 μm (Robinson and Mlynek, 1995). As such, the optical clarity at this thickness should be greatly improved, reducing the initial blurred vision after installation and allowing the possibility of daytime administration.

3.3 Toxicity

The effect of the gel carriers on healthy human fibroblasts was determined using the alamar Blue assay (Figure 3). All four of the gels tested displayed a non-toxic nature, similar to that of the control.

3.4 Stability

Long term stability tests were performed to elucidate the effect of storage containers and conditions on cysteamine oxidation. Gels were stored at four different conditions, and the percentage CH remaining over time compared. The order of oxidation stability was found to be: ampoules > crimped bottles > beakers (Table 3, Figure 5).

3.5 Bioadhesion

As a result of poor long-term stability, carbomer 974 and xanthan were eliminated from the study. Tests continued on the remaining three gels. Table 4 summarises the bioadhesion test results.

3.6 Dissolution

Dissolution tests using sodium hyaluronate, carbomer 934 and HEC were performed in triplicate (Figure 4). The three gel carriers each release cysteamine over a 45-50 minute period.

3.7 Surface tension

HEC was eliminated from the study at this point. Surface tension studies were performed using a torsion balance (Malvern Wells, UK) on the final two medicated gels, sodium hyaluronate and carbomer 934. The surface tension of a liquid will give an indication of the wetting capabilities on the corneal surface. A gel with a lower surface tension value will be able to spread more easily than a gel with a higher surface tension, and subsequent adhesion and absorption of the drug should therefore be improved (Abdelkader, et al. 2012). As the surfactant benzalkonium chloride is used as a preservative, this should lower the surface tension further still. Tests were performed on the two gels in triplicate at room temperature (20°C), with and without benzalkonium chloride (Table 5).

HEC and carbomer 934 were discounted, as neither gel demonstrated comparable long-term stability to sodium hyaluronate. Therefore, the experiment and results which follow report only the sodium hyaluronate gel, at a concentration of 0.3% w/w, medicated with 0.55% w/w cysteamine hydrochloride.

4. Discussion

Current understanding of ocular pharmacokinetics involves mixing of the eye drops with lachrymal fluid, produced at a rate of 0.5-2.2 $\mu Lmin^{-1}$, resulting in a short contact time with ocular tissue (Ahmed and Patton 1985; Ahmed and Patton 1987). Subsequent drainage towards the naso-lachrymal duct during blinking results in extensive elimination of the applied solution. An ophthalmic gel may overcome these physical barriers and improve bioavailability through improved contact time with the corneal surface.

Sodium hyaluronate is a biopolymer which is found throughout the human body in tissues such as skin, cartilage and vitreous humour (Sadhasivam, et al. 2013). It therefore provides an inherent biocompatibility with the eye (Chung, et al. 2013). It also promotes wound healing, and has been used commercially in artificial tears preparations for over 30 years (Kuo, 2005) with an established safety record. It is a high-molecular weight polysaccharide (107 kDa) consisting of a backbone of alternating groups of (1-4) glucuronic acid and (1-3)-N-acetyl glucosamine (Sadhasivam, et al. 2013).

The physical hydrogel formed during this project was both transparent and bioadhesive and these attributes are highly desirable for topical ophthalmic application. In addition to these physico-chemical properties, the gel is isotonic due to the inclusion of SPMB buffer as an excipient, and exhibits a physiological pH of 7.4, minimising the potential for irritation.

The gels demonstrated a bioadhesive nature ($p<0.01$), particularly for area under the curve (AUC) (Buchan, et al. 2010). Various theories of bioadhesion

mechanisms exist, however it is widely proposed that there are two main mechanisms once the surfaces initially come into contact. Physical bioadhesion likely occurs when the polymer chains become physically entangled with natural glycoproteins on the surface of the tissue. This physical interaction can be further strengthened with chemical bonding, the type of which is dependent on the polymer's sub-groups (Thirawong, et al. 2007). The force required to remove the physical entanglements is measured as the AUC, while the force required to separate the probe and the plate measures the secondary chemical interactions. Bioadhesion is a useful property in an ophthalmic treatment, particularly for a chronic condition. By holding the active compound at the site of action for a prolonged period, the frequency which the eye drop needs to be administered may be reduced.

It has been hypothesised that a viscosity of 12-15 mPa.s is optimal for ophthalmic delivery, demonstrated through *in vivo* work with rabbits (Rupenthal, et al. 2011). A viscosity of around 20 mPa.s is known to be acceptable to patients, and preparations for ophthalmic instillation such as eye gels should ideally be less than 30 mPa.s to maximise patient comfort. The sodium hyaluronate gel possessed a viscosity of 14 mPa.s, which is within the acceptable range. The gels also demonstrated pseudoplastic flow, which is ideal for ophthalmic vehicles as it permits blinking without running out of the eye (Buchan, et al. 2010).

Dissolution tests using sodium hyaluronate, carbomer 934 and HEC were performed in triplicate (Figure 3). The three gel carriers each release cysteamine over a 45-50 minute period. This extended release, in combination with the bioadhesive nature of the gel, may increase bioavailability compared to the current

eye drop. This may permit a reduction from the multiple dosing regimen currently experienced.

Long term stability tests were performed to elucidate the effect of storage containers and conditions on cysteamine oxidation. The order of oxidation stability was found to be: ampoules > crimped bottles > beakers (Table 3, Figure 5). Some samples displayed a 65% improvement in stability over six weeks. Historically, cysteamine preparations have been unstable at room temperature, and have been frozen to prolong shelf life (Bozdag, et al. 2008). Many attempts have been made to overcome these stability issues, such as using prodrugs (Cairns, et al. 2008; Cairns, et al. 2008) and reformulation (Buchan, et al. 2012), however in this project cysteamine stability was improved through chemical synthesis and storage under nitrogen, and restriction to air.

It has been reported that both carbomer 934 and hyaluronic acid possess antioxidative properties, and reduce the cell damage that would otherwise have been caused by benzalkonium chloride (Baudouin, et al. 2010). In addition, sodium hyaluronate is used commercially in dry eye preparations (Table 1), and therefore will have a dual-action effect.

The surface tensions of the two gels sodium hyaluronate and carbomer 934 are similar, and both gels demonstrate a large reduction in surface tension with the addition of benzalkonium chloride (table 5). This will be a useful property in an ophthalmic formulation, and may facilitate drug absorption into the cornea. In addition, benzalkonium chloride has a penetration enhancing effect due to corneal epithelial disruption, which can further promotes drug absorption (Abdelkader, et

al. 2012). The analysis was performed at room temperature due to the logistics of encapsulating the entire texture analyser in a heated environment. Due to the limitations of the use of 'room temperature' it would be worth investigating the effect of temperature on this parameter.

5. Conclusions

Polymer vehicles suitable for the ophthalmic delivery of CH have been investigated. The candidate gel carriers have been through initial laboratory-based characterisation, and the gel which possesses the most suitable characteristics for ophthalmic delivery of cysteamine determined. Sodium hyaluronate demonstrated good optical transparency, a pseudoplastic rheology, significant bioadhesion, sustained release of CH, low surface tension, good long-term stability and non-toxic characteristics. Sodium hyaluronate may be a suitable alternative to the current aqueous-based eye drop formulation of cysteamine.

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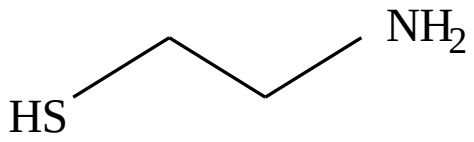

Figure 1. Cysteamine.

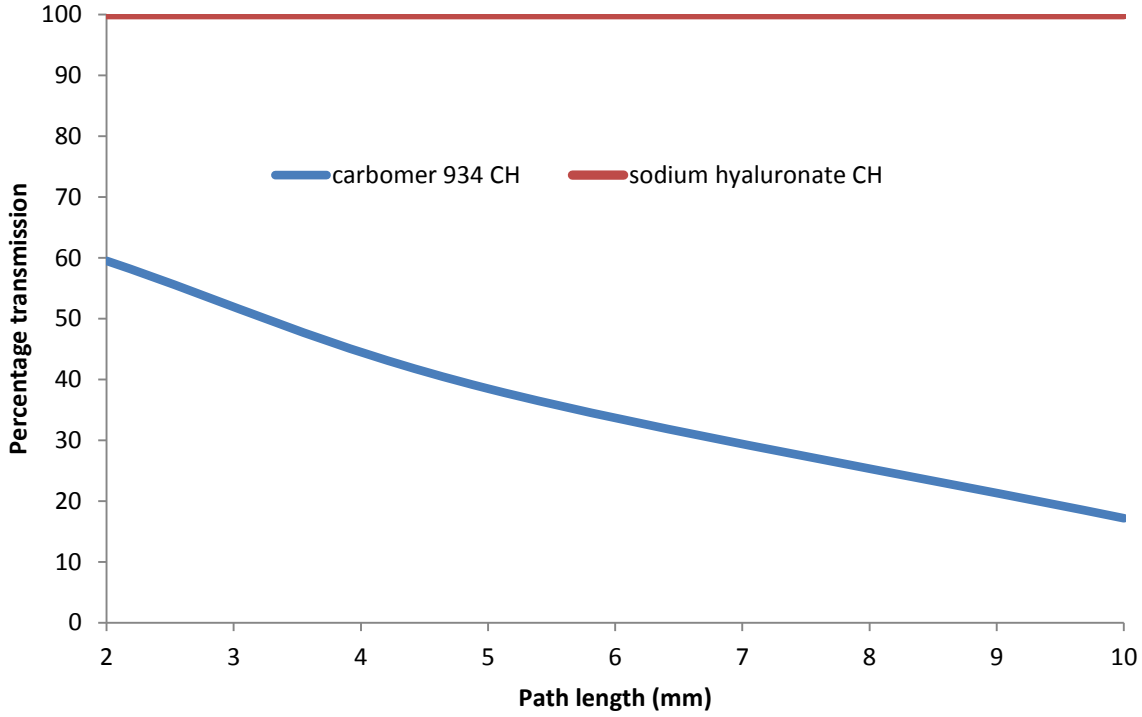

Figure 2. Effect of active addition upon the optical clarity of medicated sodium hyaluronate and carbomer 934 gels at different gel thicknesses.

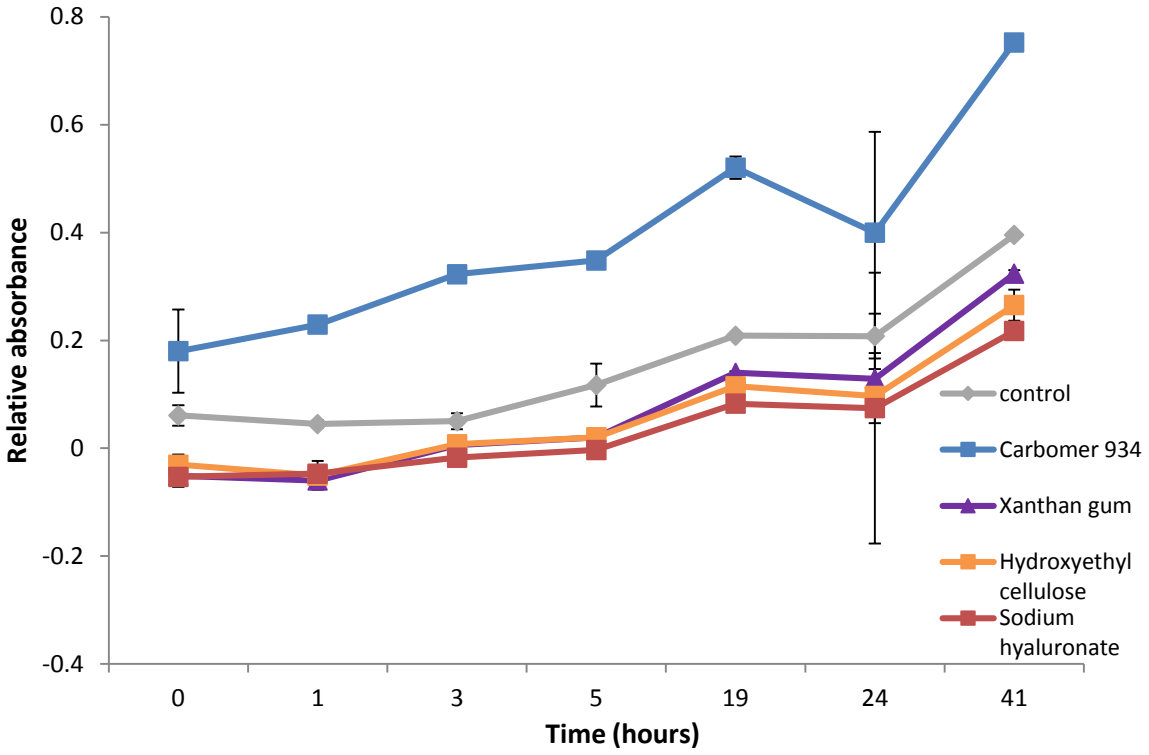

Figure 3. Toxicity tests of the final four gels.

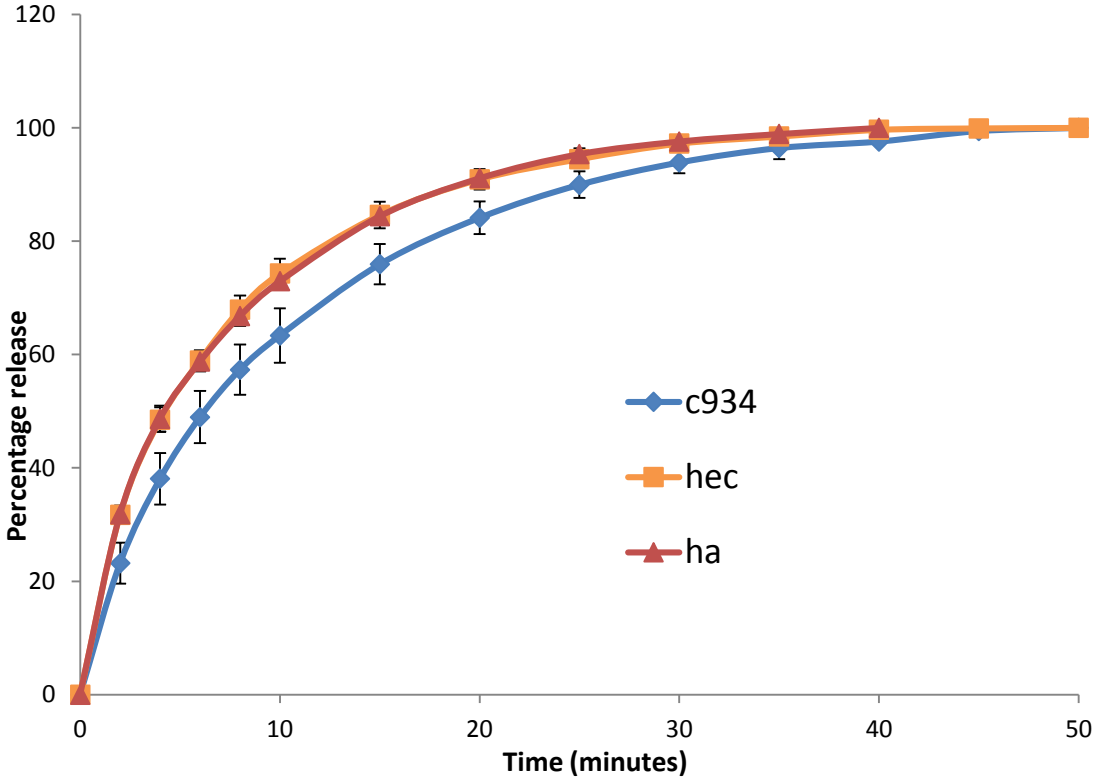

Figure 4. The release of cysteamine hydrochloride from the three gel carriers: sodium hyaluronate, hydroxyethyl cellulose and carbomer 934.

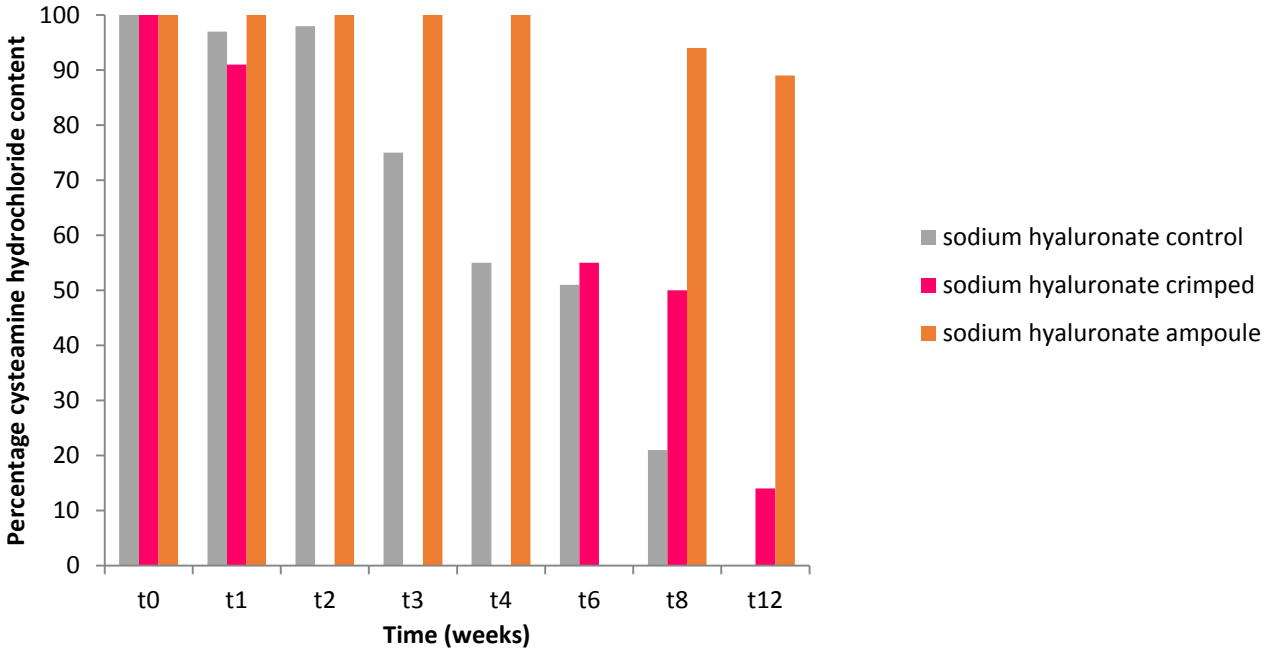

Figure 5. Percentage cysteamine HCL content over 12 week period. The sodium hyaluronate control was a beaker sealed with parafilm, maintained at 4°C.

Table 1. Summary of the eight gels which were selected from the literature as suitable carriers, with the preformulation data.

Unmedicated gel in aqueous solution, pH 7.4	**Concentration: commercial preparation (%w/w)**	**Optical transmission 1cm % (n = 3, ± SD)**	**Optical transmission 0.5cm % (n = 3, ± SD)**	**Rheology (n = 3, ± SD)**	**Gel structure/ viscosity mPa.s (n = 3, ± SD)**
Carbomer 934 (Johnson ME, et al. 2008) Published work	0.3	71.9 (±0.89)	85.7 (±0.5)	pseudoplastic	G'* > G''$^{\Delta}$, tan delta† <1 Viscosity 150 (±0.05)
Xanthan gum (Stewart, et al. 2002) Timolol GFS®	0.3	36 (±1.06)	58.9 (±0.64)	Pseudoplastic thixotropic	G' > G'', tan delta <1 Viscosity 12 (± 0.0)
Hydroxypropylmethyl cellulose (HPMC) (British Medical Association, Royal Pharmaceutical Society 2011) Genteal®, BionTears®, Tears Naturale Forte®	0.3	98.7 (±0.17)	99.2 (±0.06)	Newtonian/ dilitant	G' < G'', tan delta >1 Viscosity 1.9 (± 0.06)
Hydroxyethyl cellulose (HEC) (British Medical Association, Royal Pharmaceutical Society 2011) Minims®	0.44	99.9 (±0.06)	99.7 (±0.11)	Pseudoplastic thixotropic	G' < G'', tan delta >1 Viscosity 63 (±0.02)
Polyvinylpyrrolidone (PVP) (British Medical Association, Royal Pharmaceutical Society 2011) FreshKote®	2	98.8 (±0.06)	99.7 (±0.06)	Dilitant	G' < G'', tan delta >1 Viscosity 1.6 (± 0.01)
I-carrageenan (Bonferoni, et al. 2004) Published work	1.5	-	-	-	-
Sodium hyaluronate (British Medical Association, Royal Pharmaceutical Society 2011) Oxyal®, Ocusan®, Hycosan®	0.1-0.2	100 (±0)	100 (±0)	pseudoplastic	G' < G'', tan delta >1 Viscosity 10.7 (±0.03)
Carbomer 974 (British Medical Association, Royal Pharmaceutical Society 2011) Liquivisc®	0.25	94.0 (±0.52)	97.3 (±0.75)	pseudoplastic	G' > G'', tan delta <1 Viscosity 260 (±0.06)

*G' = elastic modulus
ΔG" = viscous modulus
†Tan delta = G"/G'

Table 2. Overview of all 5 gels, medicated with 0.55% w/w cysteamine hydrochloride with benzalkonium chloride as preservative.

	Concentration (% w/w)	OT 1cm % (±SD)	OT 5mm % (±SD)	OT 2mm % (±SD)	Gel structure/viscosity (mPa.s)	100% Dissolution (mins)	Bioadhesion significant
Carbomer 934	0.3	17 (±0.2)	38 (±0.51)	59 (±0.36)	G'' > G', tan delta > 1 Viscosity 7	45	yes
Carbomer 974	0.25	20 (±0.23)	40 (±0.06)	66 (±2.17)	G'' > G', tan delta > 1 Viscosity 4	35	yes
Xanthan gum	0.3	25 (±3.47)	54 (±2.42)	66 (±1.03)	G'' < G', tan delta < 1 Viscosity 10	37	yes
Hydroxyethyl cellulose	0.8	100 (±0)	100 (±0)	100 (±0)	G'' > G', tan delta > 1 Viscosity 27	40	yes
Sodium Hyaluronate	0.3	100 (±0)	100 (±0)	100 (±0)	G'' > G', tan delta > 1 Viscosity 14	45	yes

OT = optical transmission
G' = elastic modulus
G" = viscous modulus
Tan delta = G"/G'

Table 3. Summary of the stability tests for the final five medicated gels.

	Stability at 4°C, in a beaker sealed with film, darkness (control)	**Stability at 21°C, sealed container with sunlight**	**Stability at 4°C in a crimped container, darkness (with nitrogen)**	**Stability at 4°C in a crimped container darkness (with minimal air exposure)**
Carbomer 934	100% @t0 100% @t1week 91% @t3weeks 89% @t4weeks 81% @t6weeks 74% @t8weeks 72% @T9weeks 58% @t12weeks 60% @t13weeks	65% @t9weeks	100% @t0 100% @t2weeks 93% @t6weeks 67% @t8weeks 68% @t10weeks 66% @t12weeks 60% @t15weeks	-
Carbomer 974	100% @t0 67% @t6days 85% @t3weeks 73% @t4weeks 62% @t9weeks	-	-	-
Xanthan gum	100% @t0 86% @t6days 65% @t4weeks 61% @t9weeks	-	-	-
Hydroxyethyl cellulose	100% @t0 88% @t1week 81% @t2weeks 69% @t3weeks 67% @ t4weeks 35% @t6weeks 0% @t10weeks	66% @t9weeks	100% @t0 85% @t2weeks 42% @t6weeks 69% @t8weeks 67% @t10weeks	-
Sodium hyaluronate	100% @t0 97% @t1week 98% @t2weeks 75% @t3weeks 55% @t4weeks 51%@t6weeks 21% @t8weeks 0% @t12weeks	0% @t5weeks	100% @t0 91% @t2weeks 55% @t6weeks 50% @t8weeks 33% @t10weeks 14% @t12weeks 0% @t15weeks	100% @t0 100% @t2weeks 100% @t3weeks 100% @t4weeks 100% @t5weeks 94% @t8weeks 90% @t9weeks 90% @t10weeks 88% @t14weeks 77% @t16weeks

Table 4. Summary of bioadhesion tests, n = 9.

	Force (N)	AUC
Hyaluronic acid		
Tissue vs plain gel	0.034	0.026[b]
Tissue vs gel with cysteamine HCl	0.048	0.053[b]
Hydroxyethyl cellulose		
Tissue vs plain gel	0.03	0.026[b]
Tissue vs gel with cysteamine HCl	0.082[a]	0.081[b]
Carbomer 934		
Tissue vs plain gel	0.067[b]	0.205[b]
Tissue vs gel with cysteamine HCl	0.107[b]	0.177[a]

p<0.05 a, p<0.01 b

Table 5. Summary of the surface tensions of the gels, with and without benzalkonium chloride (n = 3).

	Surface tension N/m (±SD)
Sodium hyaluronate, cysteamine HCl, BZK	0.044 (±0.0003)
Sodium hyaluronate, cysteamine HCl, without BZK	0.071 (±0.0001)
Carbomer 934, cysteamine HCl, BZK	0.039 (±0.0001)
Carbomer 934, cysteamine HCl, without BZK	0.073 (±0.0004)