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Development of a Thermogelling Ophthalmic Formulation of Cysteine

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ABSTRACT Preliminary studies carried out with cysteine 2% solution showed that pH adjusted to isoelectrical pH (i.e., 4.9) led to enhance stability during autoclaving and ensured no significant degradation during at least 14 days if stored at 2–8°C protected from light. Optimized formulations combined either cysteine(2%)/Poloxamer407(16.5%) or cysteine(2%)/Poloxamer407(20%)/Poloxamer188(5%) and were characterized by an adequate temperature of gelification (TG) (25.9°C and 26.9°C, respectively), an important gel strength (5.1daN and 5.3daN, respectively) and a drastic increase in the apparent viscosity between 24°C and 32°C (multiplication factor of 78 and 77-fold, respectively). Cysteine addition produced only slight but significant decrease in temperature of gelification and increase in gel strength.

KEYWORDS Cysteine, Stability, Ophthalmic, Poloxamer

INTRODUCTION

L-cysteine (CySH) is an amino acid with a thiol function which is responsible for redox activity and complexing properties. L-cysteine (CySH) plays a major role in various enzymatic reactions. Complexing properties of CySH favor heavy metal detoxification and, in addition, xenobiotic metabolism is promoted by sulfate generated by CySH (Niroshini & Walts, 2003). Recent studies present CySH as playing a key role in preventing oxidative damages. This action is mainly based on the synthesis of reducted glutathione (GSH). As about 50% of CySH is used for GSH synthesis, CySH is generally considered as the major limiting factor of GSH synthesis (Li et al., 2002; Meister, 1991). In ophthalmology, CySH (Menna et al., 1982; Saracco et al., 1982; Bovis et al., 1978; Berard et al., 1976) or prodrugs of CySH like N-acetylcysteine (NAC) (Busch et al., 1999; Aldavood et al., 2003) provide interesting properties for photochemical damages or corneal wounds in humans or animals. These compounds yielded important levels of GSH in cultured rat lens and may have very promising anti-cataract potential (Holleschau et al., 1996). Anti-oxidative properties come from free radical scavenging (Halliwell & Gutteridge, 1999). Reactive species of oxygen are neutralized by formation of disulfide compounds. In addition, both substances (CySH and NAC) probably act by mechanisms of binding to the Zn^{2+} site of specific enzymes like collagenase. Nevertheless, during pharmaceutical development, a major hurdle is raised by the drastic degradation of CySH into cystine (CyS–SCy) by

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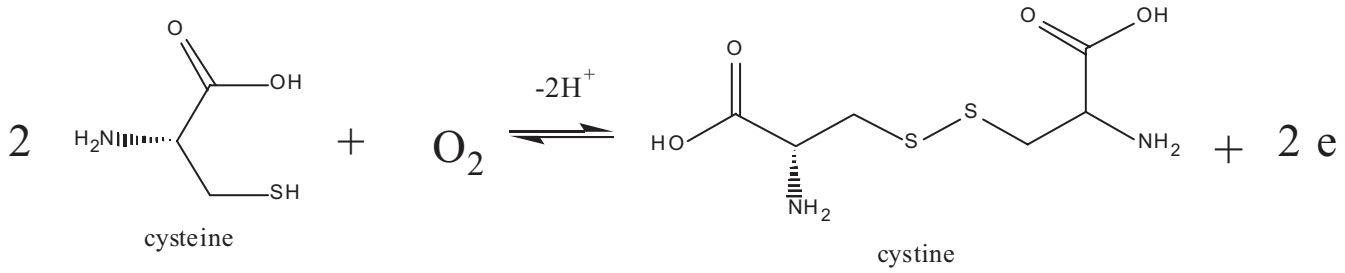

FIGURE 1 **Degradation of Cysteine into Cystine.**

oxidation in the presence of oxygen (Fig. 1) (Merck Index, 2002). Hydrosulfite derivatives have been proposed to favor stability of CySH in solution.

The first part of this article studies CySH stability and addresses the factors which may prevent CySH from oxidation into CyS–SCy in order to develop a sterile solution free of anti-oxidative agent, in particular during autoclaving process. The second part presents possible ophthalmic formulations which may prolong drug residence in the precornea area. Indeed, conventional formulations are characterized by a drastic drainage after eye-drop instillation leading to reduce the ocular drug bioavailability. Gel-forming formulations, including thermosensible gels, have been presented as offering several advantages by many authors (Bourlais et al., 1998; Sklubalova, 2005). Consequently, the objectives of this second part were to obtain a thermosensible gel with an appropriate temperature of transition, which remains stable after autoclaving. Poloxamer block copolymers have been introduced in the late 1950s and, since then, have been proposed for various pharmaceutical applications (Smolka, 1972, 1974). They are listed in the United States and European Pharmacopoeia (Kabanov et al., 2002). This group of copolymers consists of ethylene oxide (EO) and propylene oxide (PO) blocks arranged in a triblock structure EOx–POy–EOz. Their chemical formula is $HO[CH_2\text{-}CH_2O]_x$ $[CH(CH_3)\text{-}CH_2O]_y[CH_2\text{-}CH_2O]_zH$ in which y is higher than 14. Poloxamers 188 (P188, MW: 7680–9510, HLB>24) and 407 (P407, MW: 9840–14600, HLB: 18–23) present thermogelling properties. The phenomenon of thermogelling is perfectly reversible and is characterized by a sol-gel transition temperature (Tsol→gel). Below this temperature, the sample remains fluid though above the solution becomes semi-solid. The thermogelation results from interactions between different moieties of the copolymer (Dumortier et al., 1991). As temperature increases, the copolymer molecules aggregate into micelles. This micellization is due to the dehydration of hydrophobic PO blocks. These micelles are spherical with a dehydrated polyPO core with an outer shell of hydrated swollen polyEO chains (Juhasz et al., 1986). Micellar entanglements produce high viscosity, partial rigidity, and slow dissolution.

MATERIALS AND METHODS

Preformulation (Stability Studies with CySH Solution)

Defining the protective factors against oxidative degradation of CySH represents a critical step during preformulation. Different factors have been studied like pH value, use of nitrogen gas to reduce oxidation, temperature, and light.

CySH Solutions

Different batches plus a control solution have been formulated with CySH (Sigma-Aldrich, Saint Quentin, France). L-Systone (CySH) (2%, w/v) was solubilized with purified water. For the different batches, pH was adjusted using either NaOH 0.1N or hydrochloric acid 0.1N (Sigma-Aldrich) to 4.9 (isoelectric pH), 4, 7, and 8, respectively, using protection against oxidation (i.e., light and oxygen dissolved in water). Protection from light was carried out with opaque aluminium tinfoil. The reduction in oxygen dissolved in water was conducted by bubbling the solution with nitrogen gas (2 L/min) during 15 min. As control, a batch of 2% CySH solution adjusted to pH 4.9 was prepared without any protection.

Stability Studies

Stability including CySH concentration, osmolarity, and clarity was studied before and after autoclaving (121°C, 20 min) (Preciclave ®, Commodore, Blanquefort, France) (n = 4). Numerous assays have been conducted with the CySH control solution (no protection, pH 4.9) before and after autoclaving to estimate the reproducibility (n = 32). Then, stability was studied during 14 days with 2% CySH solution (pH 4.9, protected or not) (n = 4).

A solution has been considered clear if its clarity was the same as that of purified water in respect to the European Pharmacopoeia (5th Edition) method.

L-Cysteine (CySH) concentration was determined according to the European Pharmacopoeia (5th Edition) method by titration. A blank titration has been carried out in the same conditions.

Osmolarity and pH were measured with a Fiske Mark 3 osmometer (Norwood, MA, USA) and a Meterlab pHmeter (Copenhagen, Denmark), respectively.

Formulation of Poloxamer Thermogelling CySH Gels

Preparation of the Thermogelling Formulations

P188 and P407 (Lutrol® F68 and F127, respectively) (BASF Levallois Perret, France) were solubilized using the cold method. Solutions containing either P407 alone (15–27% w/w) or mixture of both poloxamers (P407 20% plus various concentrations of P188 1–6%) were prepared to optimize Tsol→gel. Poloxamer was mixed at 4–5°C with purified water previously cooled until an homogeneous solution was obtained. Before adding CySH, the poloxamer solutions were gently saturated with nitrogen gas bubbling (2 L/min) during 15 min. L-cysteine (CySH) 2% (w/v) was then solubilized in two thermogelling gels containing either P407 16.5% or P407/P188 (20%–5%), respectively. pH was adjusted to 5.0+/–0.1 with hydrochloric acid 1 N (Sigma-Aldrich) to favor stability. The preparations were protected from light and stored at 2–8°C.

CySH Dermination

CySH/P407 (2%/16.5%) and CySH/P407/P188 (2%/20%/5%) were also sterilized by autoclaving (121°C, 20 min). As previously, stability including determination of CySH concentration and clarity was studied with CySH-Poloxamer formulations. The only difference, concerning the CySH assay, corresponded to the use of a colorimetric method based on Ellman's reaction (Ellman, 1959). This procedure represents a possible substitute to the European Pharmacopoeia method and has been proposed in case of complex pharmaceutical formulations (Motoyama et al., 1989). This assay is based on the use of disulfide 5,5′-dithiobis2-nitrobenzoic acid (DTNB). Various concentrations of CySH (0–0.7 mM) were prepared with purified water, then 500 µl of each sample was mixed with 2240 µl HEPES buffer and 100 µL DTNB solution. Absorbance was measured at 412 nm (Spectronic, Ivyland, PA, USA) [DNTB solution: 200 mg DTNB added to 33.6 mg EDTA Na_2 (Cooper, Melun, France) in 50 mL phosphate buffer] [HEPES buffer: 2.60g HEPES (Sigma-Aldrich) plus 2.92g NaCl and 33.6 mg EDTA Na_2 dissolved in 1000 mL purified water, then adjusted to pH 7.5 with hydrochloric acid 1 N].

Tsol→Gel Determination

The method used has been fully described elsewhere (Kim et al., 2002; Ryu et al., 1999; Fawaz et al., 2004). Poloxamer solutions either with or without CySH were heated progressively (0.5°C/min) using constant stirring (100 rpm). The temperature was set up using a thermostated bath and was controlled inside the sample with a precise thermometer (+/–0.1°C). When the magnetic bar (length 25 mm, diameter 6 mm) stopped moving due to gelation, the temperature displayed was considered to be Tsol→gel.

Viscosity Studies

The viscosity studies were carried out with a Brookfield Rheoset Viscometer (Stoughton, MA, USA) using a spindle/guardleg geometry. three different spindles (spindle references: #21, #27, and #29 for fluid, intermediate, and viscous samples, respectively) were chosen according to the range of viscosity of the samples which drastically increased with temperature. The apparent viscosity was calculated by submitting progressively the sample (5–10 mL) to a rate of 50 rpm. Temperature was controlled using a thermostated chamber surrounding the spindle/guardleg system (+/–0.1°C) and set up at 24, 26, 28, 30, and 32°C, respectively.

Adhesion Study

Adhesion was assessed according to Gurny's method (Charrueau et al., 2001). Adhesion studies were carried out with a dynamometer (Lhomargy DY 20B, Ivry, France) which measured a tensile force (daN) necessary to detach two aluminium/inox pieces (surface of contact: 7.07 cm^2) of a chamber containing the gels to be tested. The tensile force (daN) necessary for breaking the gel/support system was recorded at an extension rate of 10 mm per min. Each preparation was tested four times at 33°C.

Statistical Analysis

Anova and Fisher exact tests were used for quantitative (CySH concentration, osmolarity, Tsol→gel) and qualitative (clear or not items), respectively (Statview 5, SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Preformulation (Stability Studies with CySH Solution)

Autoclaving does not alter CySH solution adjusted to pH 4 and 4.9 (Table 1). L-cysteine (CySH) concentration, osmolarity, and aspect remained stable while significant modifications were detected with other batches. For the CySH solutions adjusted to pH 7 and 8, significant losses of CySH were equal to 8.2% and 17.3%, respectively. For both batches, autoclaving led to significant precipitation (p = 0.03) (Table 1). The precipitation was probably due to CyS–SCy that is poorly water-soluble. Significant decrease in osmolarity (CySH solution adjusted at pH 7 and 8, respectively) was in concordance with CyS–SCy formation (Table 1). In solution, different forms of CySH coexist and the dissociation of CySH is pH-dependent according to the Henderson–Hasselbach equation (Eqs. 1, 2, and 3). The dissociation of the three pH-dependant groups are represented as following H_2A^{+-} corresponding to the zwitterion form:

$$ka_3 = \frac{[H_2A^{+/-}][H_3O^+]}{[H_3A^+]} \quad (1)$$

$$ka_2 = \frac{[HA^{+/2-}][H_3O^+]}{[H_2A^{+/-}]} \quad (2)$$

$$ka_1 = \frac{[A^{2-}][H_3O^+]}{[HA^{+/2-}]} \quad (3)$$

$$pHi = \frac{pka_3 + pka_2}{2} = 4.94 \quad (4)$$

Direct dissolution of CySH in water led to a pH close to pHi [i.e., isoelectrical pH, Eq. (4)]. Below pH 6.18, the thiol function is not dissociated. Isoelectrical pH (pHi) generally promotes stability and our data are in concordance with these observations. The zwitterion is globally neutral and corresponds to the major form of CySH in the 3.71–6.18 range of pH. Elevation or reduction in pH beyond this interval produces drastic oxidation of CySH (Fig. 1). In addition, according to the Nernst equation (Eq. 5), redox potential is directly a function of the pH value and can be presented as following in the pH interval where the zwitterion form is predominant:

$$E = E_0 + 0.06\,\mathrm{Log}\,(|\,COO^-NH_3^+\text{S-S}NH_3^+COO^-\,| \,|\,H^+\,|^2)/|\,NH_3^+COO^-SH|^2 \quad (5)$$

$COO^-NH_3^+$ S-$SNH_3^+COO^-$ represents CyS-SCy zwitterion form.

Stability studies were then conducted according to these preliminary observations. Solution protected or not, pH 4.9 CySH were selected because they presented better stability during autoclaving. Both batches only differed on the condition of preparation and storage (Table 2). Our observations clearly showed that without any protection, CySH solution presented significant instability. Two days after the preparation, significant degradation in CySH concentration was detected and a precipitation was observed at day 10 (Table 2). On the contrary,

$$NH_3^+/COOH/SH \Leftrightarrow NH_3^+/COO^-/SH \Leftrightarrow NH_3^+/COO^-/S^- \Leftrightarrow NH_2/COO^-/S^-$$

$$H_3A^+ \Leftrightarrow H_2A^{+/-} \Leftrightarrow HA^{+/2-} \Leftrightarrow A^{2-}$$

$$pka_3 = 1{,}71 \qquad pka_2 = 8{,}18 \qquad pka_1 = 10{,}28$$

TABLE 1 **Stability Studies Carried Out with Cysteine Solution (2%) Either Non-protected (NP) or Protected (P) Adjusted at Different pH**

NP, pHi 4.9			Concentration (%)	Osmolarity (mosm/l)	Clarity
Before autoclaving	*n* = 4	Mean (+/–SD)	93.2 (+/–9.0)	161.5 (+/–2.0)	yes (*n* = 4)
After autoclaving	*n* = 4	Mean (+/–SD)	101.1 (+/–1.7)	158.8 (+/–1.5)	yes (*n* = 4)
			NS	NS	NS
P, pHi 4.9					
Before autoclaving	*n* = 32	Mean (+/–SD)	99.7 (+/–1.9)	159.4 *(+/–5.8)*	yes (*n* = 32)
After autoclaving	*n* = 32	Mean (+/–SD)	99.2 (+/–3.0)	160.3 (+/–5.0)	yes (*n* = 32)
			NS	NS	NS
P, pH 4					
Before autoclaving	*n* = 4	Mean (+/–SD)	99.6 (+/–1.9)	161.9 (+/–2.3)	yes (*n* = 4)
After autoclaving	*n* = 4	Mean (+/–SD)	96.6 (+/–1.7)	159.9 (+/–1.5)	yes (*n* = 4)
			NS	NS	NS
P, pH 7					
Before autoclaving	*n* = 4	Mean (+/–SD)	96.7 (+/–0.5)	173.3 (+/–1.2)	yes (n = 4)
After autoclaving	*n* = 4	Mean (+/–SD)	88.5 (+/–0.6)	159.8 (+/–2.5)	no (*n* = 4)
			$p < 0.001$	$p < 0.001$	$p = 0.03$
P, pH 8					
Before autoclaving	*n* = 4	Mean (+/–SD)	100.5 (+/–0.4)	214.0 (+/–5.5)	yes (*n* = 4)
After autoclaving	*n* = 4	Mean (+/–SD)	83.2 (+/–2.1)	189.6 (+/–19.2)	no (*n* = 4)
			$p < 0.001$	$p = 0.03$	$p = 0.03$

Protection corresponded to protect the batches from light and to use nitrogen gas bubbling during the preparation. (NS-non significant $p > 0.05$).

TABLE 2 **Stability Studies Carried Out with Cysteine Solution (2%, pH :4.9) Either Non-protected (NP) or Protected (P)**

			Concentration (%)				
			D1	D2	D3	D10	D14
NP	Mean (+/–SD)	*n* = 4	111.0 (+/–0.9)	94.4 (+/–2.9)*	99.6 (+/–1.2)*	93.3 (+/–1.8)*	86.6 (+/–3.2)*
P	Mean (+/–SD)	*n* = 4	94.4 (+/–2.9)	98.0 (+/–1.3)	105,6 (+/–4.2)	97.5 (+/–1.4)	96.0 (+/–0.7)
			Osmolarity mosmol/l				
			D1	D2	D3	D10	D14
NP	Mean (+/–SD)	*n* = 4	155.9 (+/–3,7)	160.8 (+/–2,6)	167.9 (+/–5,6)	153.5 (+/–12,8)	158.1 (+/–2,1)
P	Mean (+/–SD)	*n* = 4	160.8 (+/–2.6)	159.4 (+/–2.7)	159.1 (+/–5.0)	159.3 (+/–7.1)	159.3 (+/–1.5)
			Clarity (yes/no)				
			D1	D2	D3	D10	D14
NP	Mean	*n* = 4	yes	yes	yes	no**	no**
P	Mean	*n* = 4	yes	yes	yes	yes	yes

*Significant degradation versus D1 ($p < 0.05$).
**Fischer exact test $p = 0.03$.
Protection corresponds to store at 2–8°C, protect from light, and use nitrogen gas bubbling during preparation (Day: D).

protected batch presented a better stability. The solution remained limpid during 14 days (Table 2). Use of nitrogen gas, protection from light and storage at 2–8°C clearly favored stability of the preparation. pH dependency of CySH stability has been previously showed and have been conducted to lower the pH of the solution (Mezyk, 1995; Merck Index, 2002). Nevertheless, most of the studies reported in the literature deal with amino acid products in parenteral nutrition solutions and described incompatibilities

like precipitation of cysteine (Bohrer et al., 2003; Allwood & Kearney 1998; Cochran & Boehm, 1992; Nekliudov & Verem'ev, 1984). The authors have generally resolved the cysteine instability by adding anti-oxidative agents like metabisulfite. Nevertheless, hydrosulfite derivatives may induce serious side effects like systemic allergic shock. Consequently, formulations free of anti-oxidative agent have to be developed and our preliminary study showed that this objective may be reached by optimizing the formulation and the storage conditions.

Formulation of CySH-P407 Thermogelling Gel

Tsol→gel varied from 14.8°C to 36.3°C (Fig. 2). The procedure was reproductive for the 15–27% range of concentrations. Tsol→gel increased while poloxamer concentration was decreasing. A linear correlation between P407 concentration and Tsol→gel was retrieved in the 16.5–27% range of concentration (r = 0.988). This observation was in concordance with data available in the literature (Edsman et al., 1998). Thermosensible gels generally sustain drug residence in the precorneal area and reduce drainage into the nasolachrymal duct (Bourlais et al., 1998; Sklubalova, 2005). Nevertheless, to achieve these objectives, the P407 concentration must be carefully chosen based on preformulation evaluations. For ophthalmic applications, Tsol→gel should ideally range between 26–31°C (i.e., higher than 25°C, which represents the higher limit of the room temperature defined by the European Pharmacopoeia, and lower than 32–33°C, which corresponds to the precorneal temperature). Twenty percent P407 concentration has been generally used for many pharmaceutical applications but this one presents a Tsol→gel far too low (i.e., 21.8°C) to remain fluid at room temperature (15–25°C). On the contrary, the 16.5% P407 concentration was selected as the most appropriate formulation because its Tsol→gel (27.1°C, Table 3) was more compatible with an ophthalmic administration at room temperature. Nevertheless, another phenomenon of great importance, corresponding to the immediate dilution, has to be taken into consideration. The dilution of poloxamer occurring in the lachrymal fluids is related to the possible loss of the thermosensible properties for lower P407 concentrations (e.g., 16%) (Edsman et al., 1998). Consequently, suitable combinations of copolymers must be determined with the objective of optimizing Tsol→gel and limiting the potential loss of thermosensible properties due to the rapid in vivo dilution. To specify possible interest of adding P188 to P407, different combinations of P188 (1–6%) and P407 (20%) have been tested. In our experiment, Tsol→gel increased with P188 concentration from 21.8 to 30.2°C (Fig. 3). The formulation combining P407(20%) and P188(5%) was selected in regards to a

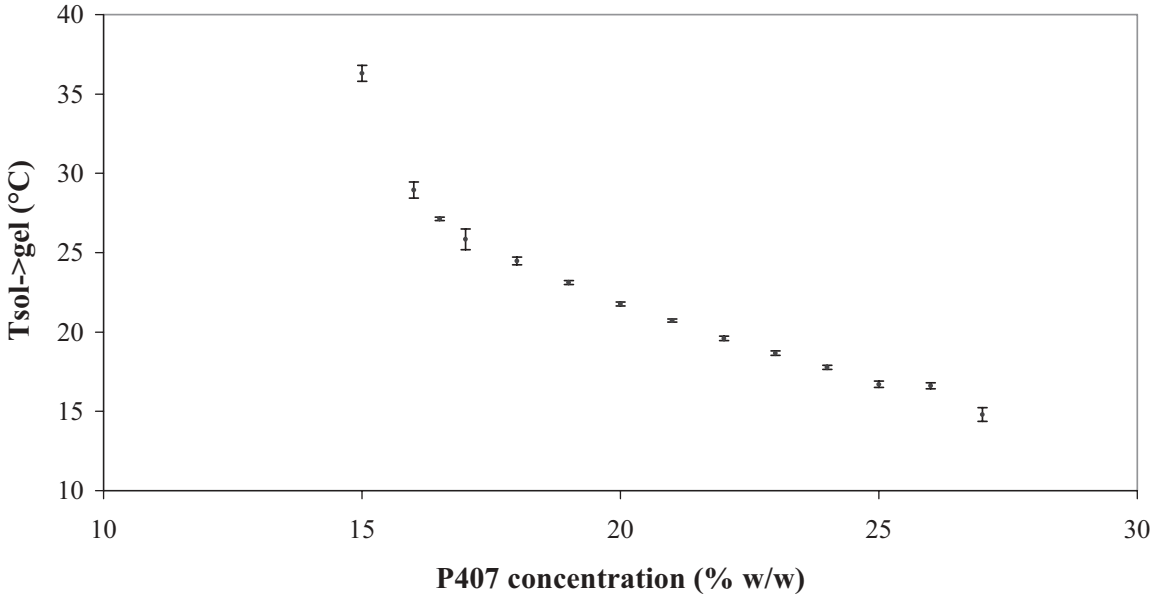

FIGURE 2 **Temperature of Gelification (Tsol → Gel) Versus P407 Concentration (with Standard Error Bar).**

TABLE 3 **Influence of Cysteine (CySH) on the Temperature of Gelification (Tsol→gel)**

Formulations	Tsol→gel (+/– standard deviation) (n = 5)
P407 16.5%	27.1°C(+/–0.1)
P407 16.5% /CySH 2%	25.9°C(+/–0.1)*
P407 20% / P188 5%	28.4°C(+/–0.2)
P407 20% / P188 5% / CySH 2%	26.9°C(+/–0.1)*

*Significant ($p < 0.001$) versus the formulation without CySH.

28.4°C Tsol→gel (Table 3). Similar optimizations were observed with different formulations. A formulation containing P407/P188 (16%/14% ratio) and rhEGF (human epithelial growth factor) characterized by a Tsol→gel of 35.5°C produced a drastic increase in lachrymal AUC (Kim et al., 2002). Addition of P188 (10%) to P407 (21%) solution promoted local availability of technetium-99m-diethylentriamine pentacetic acid (radiotracer) in relation to the optimization of Tsol→gel from 17.5°C (P407 21%) to 26.5°C [P407/P188 (21%/10%)](Wei et al., 2002).

Adding CySH (2%) yielded a significant decrease of Tsol→gel for the P407 (16.5%, $p < 0.001$) and the P407(20%)/P188(5%) combination ($p < 0.001$) (Table 3). L-cysteine (CySH) probably acts by interfering with P407 micellization and by altering the dehydration of hydrophobic PO blocks. Dehydration of hydrophobic PO blocks represents the very first step in the gelling process. The influence of CySH was confirmed by the apparent viscosity measurement as a function of the temperature (Fig. 4). The gelling process appeared less drastic when CySH was added to the P407(20%)/P188(5%) combination. Conversely, CySH increased the gel strength as it has been observed with the adhesion studies (Table 4). Including drugs or various additives may greatly modify Tsol→gel and adhesive forces, generally in the opposite way (Gilbert et al., 1987). Molecules like diclofenac, ethanol, propylene glycol, and HCl reduce the gel strength and bioadhesive force and increase Tsol→gel while others do the opposite (e.g., NaCl, Na_2HPO_4, NaH_2PO_4, sodium alginate, polycarbophil, and carbopol) (Choi et al., 1999; Yong et al., 2001; Chi & Jun, 1990, 1991; Ryu et al., 1999). Nevertheless, despite the significant though limited modifications observed with CySH addition, both CySH-poloxamer formulations [either P407 16.5% or P407/P188 (20%/5%)] still met the criteria necessary to obtain thermosensible properties compatible with an ophthalmic use:

- In the presence of CySH, Tsol→gel were still included in the 26–31°C temperature range, which ensures fluid administration at room temperature and satisfactory thermogelling transition in the lachrymal medium (Table 3).

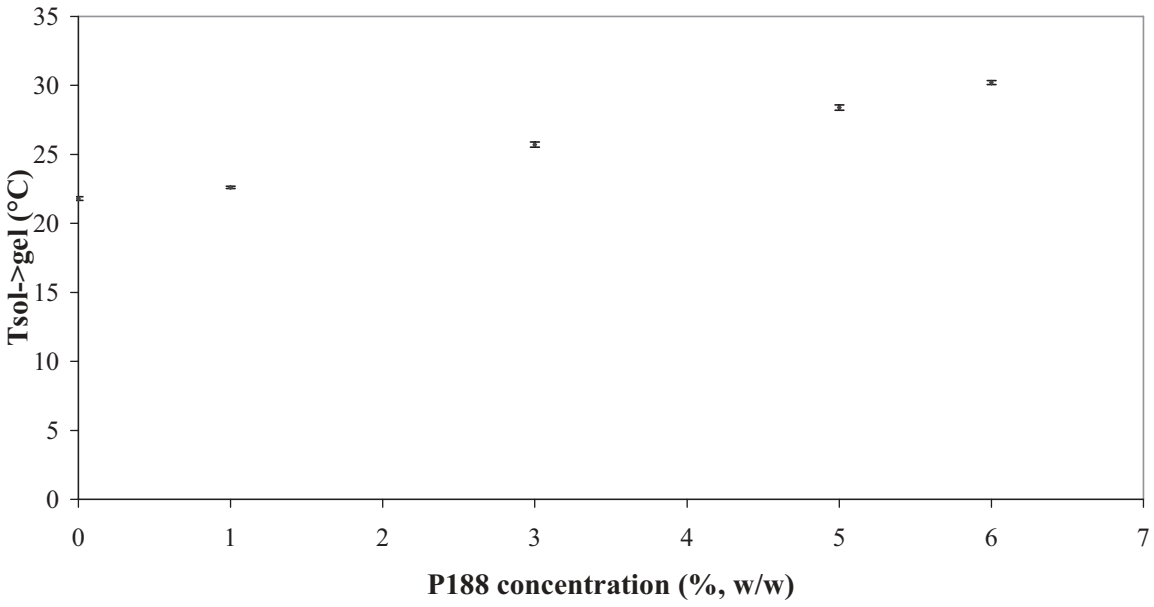

FIGURE 3 **Temperature of Gelification (Tsol→Gel) Versus P188 Concentration (w/w) in a Combination with 20% (w/w) P407 Formulations (with Standard Error Bar).**

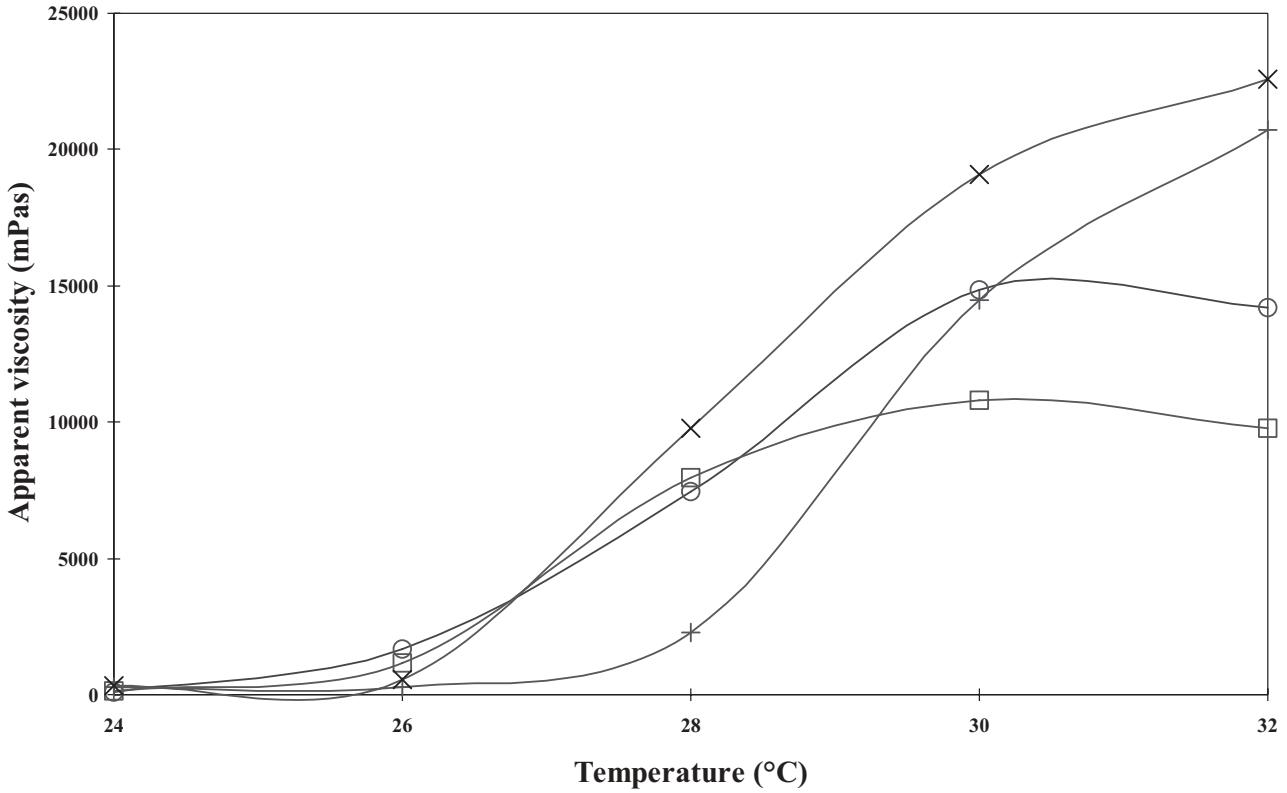

FIGURE 4 **Apparent Viscosity Versus Temperature. + = P407(20%)/P188(5%); X = CySH(2%)/P407(20%)/P188(5%); ❍ = P407(16.5%); ❑ = CySH(2%)/P407(16.5%).**

- CySH addition resulted in higher adhesive characteristics, which may prolong drug residence in the precorneal area (Table 4).
- With or without CySH, the apparent viscosity increased at least 70-fold between 24°C and 32°C, which underlined the great temperature dependency of our thermosensible preparations (Fig. 4).

Stability of CySH-Poloxamers Formulation During Autoclaving

Ellman's assay was linear ($r > 0.999$) and reproducible (coefficient of variation <5%) in the 0.1–0.7 mM CySH concentration interval. The presence of poloxamer did not significantly interfere in the CySH determination. This method presents advantages like rapidity and lack of use of iodine (possibility of allergy) compared to the European Pharmacopoeia method.

TABLE 4 **Influence of Cysteine (CySH) on the Tensile Force**

Formulations	Tensile force (mean+/– SD) (n = 4)
P407 16.5%	4.1 daN (+/–1.9)
P407 16.5%/CySH 2%	5.1 daN (+/–1.1)
P407 20%/P188 5%	4.1 daN (+/–1.9)
P407 20%/P188 5%/ CySH 2%	5.3 daN (+/–0.9)

A non-significant CySH loss of 1% was observed after autoclaving for P407 (16.5%), whereas P407(20%)/P188(5%) formulations presented significant and reproducible loss higher than 10% ($p < 0.001$) (Table 5). All formulations were clear after autoclaving but some samples of P407(20%)/P188(5%) formulation became unclear a few hours after autoclaving. For P407, this is in relevance with previous studies showing that sterilization by autoclaving (120°C, 15 min, 1 bar) appears compatible and does not significantly alter characteristics of P407 solution (Dimitrova et al., 2000; Veyries et al., 1999). Unfortunately, no data is available on P188 stability during autoclaving and this copolymer might promote oxidation. This promotion may be due to differences in the chain length of the different blocks or in the HLB value compared to P407. These findings indicate possible incompatibilities which

TABLE 5 **Stability of 2% Cysteine (CySH) in P407 (16.5%) and P407(20%)/P188(5%) Formulations Before and After Autoclaving (121°C, 20 min)**

CySH Concentration	CySH 2% P407 (16,5%) (n = 4)	CySH 2% P407(20%)/P188(5%) (n = 10)
Before autoclaving	92.4% (+/–4.4%, SD)	106,9 (+/–4.3%, SD)
After autoclaving	92.5% (+/–4.4%, SD)	95.6% (+/–3.3%, SD)*
Clarity		
Before autoclaving	Yes (n = 4)	Yes (n = 10)
After autoclaving	Yes (n = 4)	Yes (n = 10)

*Significant (p < 0.001) after versus before autoclaving.

require further studies and suggest that other sterilizing methods may be more adequate with the P407(20%)/P188(5%) formulation.

CONCLUSION

Formulation of CySH solution must take into account the drastic oxidation into CyS–SCy. This study emphasized that adjusting pH value in the formulation and storing the preparation at 2–8°C exerted a preventive action against oxidation, and CySH remained stable in aqueous solution after autoclaving. Ophthalmic thermogelling solution may offer interesting prospects to increase CySH residence after ocular administration according to satisfactory in vitro formulation. Nevertheless, if combining P407 and P188 led to satisfactory in vitro characteristics, further investigations are required to explain why these combinations promote instability of CySH during autoclaving.

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