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Research paper

Optimisation of carbomer viscous eye drops: an in vitro experimental design approach using rheological techniques

Jens Ceulemans, Annick Ludwig*

Laboratory of Pharmaceutical Technology and Biopharmacy, Department of Pharmaceutical Sciences, University of Antwerp (UIA), Antwerp, Belgium

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Abstract

The optimisation of the in vitro interaction between several poly(acrylic acid) derivatives (Carbopol® 1342P NF, Carbopol® 974P and Carbopol® 980 NF) and mucin was performed by an analysis technique combining oscillatory shear rheology and experimental design in order to improve the formulation of carbomer viscous eye drops.

First, standard oscillation procedures were used to characterise the polyacrylic acid and mucin dispersions, and to investigate the influence of several polymer-related factors (concentration, preparation, type of polymer used) on the rheological properties. Second, an experimental plan design was developed to investigate the effect of polymer-related factors on the mucoadhesive indexes ($MAI_{(G')}$ and $MAI_{(G'')}$) which were calculated using the viscoelastic data obtained from polymer/mucin, polymer/tearfluid and mucin/tearfluid mixtures. Optimal mucoadhesive interactions were determined based on the experimental design results. Finally, the optima were fully characterised rheologically to further verify the mucoadhesive capacity. The main conclusion is that the factor influencing most explicitly the mucoadhesive interaction of the viscous eye drop is the mucin concentration and neither the type of polyacrylic acid, nor its concentration.

Keywords: Mucoadhesion; Poly(acrylic acid); Optimisation; Shear rheology; Viscoelasticity

1. Introduction

The use of bioadhesive polymers for ocular drug delivery was the subject of several studies published during the last decade and several review articles are available [1,2]. A polymer investigated very frequently in this context is carbomer, a polyacrylic acid polymer available in a wide range of molecular weights and either being linear, branched or cross-linked [3]. The acidic carboxyl groups partially dissociate in water, producing a flexible coil. After the addition of alkaline compounds, solvatation, salt formation and electrostatic repulsion between the anionic groups cause gel formation [4]. The structural features responsible for the interaction between this anionic polymer and mucin were described by Leung and Robinson [5], while several other studies focussed on the effect of polyacrylic acid on the ocular contact time and bioavailability of several drugs [6–10]. Various techniques have been used to study the in vitro bioadhesive capacity of polyacrylic acid: tensile testing [11–13], turbidimetry [14], ^{13}C-NMR [13], steady shear flow rheology [15–17] and finally oscillatory rheology [17–21].

The aim of the present study is to gain more insight into factors influencing the interaction between a carbomer viscous eye drop and mucin, and to optimise the eye drop formulation by using the information obtained. As is mentioned in the review of Lee et al. [22] about bioadhesive dosage forms, concentration, molecular weight and chain flexibility are polymer-related factors, influencing the mucoadhesive process. Furthermore, some application site related factors (pH, physiological variables and contact time) influence mucoadhesion as well.

A first parameter investigated in the present study is the polyacrylic acid concentration. Madsen et al. [23] demonstrated that the degree of the polyacrylic acid/mucus interaction strongly depends on the concentration of the polymer. The concentration applied varies from 0.05 to 0.20% (w/v), which is the range between the concentration being sufficiently fluid to be comfortable after instillation into the human eye [24] and the maximum concentration applied in commercial preparations.

Second, the influence of the mucin concentration is exam-

* Corresponding author. Laboratory of Pharmaceutical Technology and Biopharmacy, Department of Pharmaceutical Sciences, University of Antwerp (UIA), Universiteitsplein 1, B-2610, Antwerp, Belgium. Tel.: +32-3-8202716; fax: +32-3-8202734.

E-mail address: ludwig@uia.ua.ac.be (A. Ludwig).

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ined. In contrast to the traditional three-layer model of the tearfilm, Robinson [25] presented the multilayer model showing a mucin gradient in the tearfilm instead of a separated mucin layer in contact with the eye surface. In the aqueous part beneath the superficial lipid layer, the mucin concentration is very low. However, the epithelial cells at the eye surface are covered by a highly concentrated mucus gel attached to the glycocalyx. The concentration used in the present study ranges from 8 to 16% (w/v), which is a hypothetical approach of the mucin gradient in the tearfilm. Since ocular mucin is not commercially available, crude gastric mucin is used. The molecular conformation of mucin in the eye is simulated as much as possible by dispersing the gastric mucin in a salt solution, simulating the lachrymal fluid [26]. Recently, typical properties of epithelial mucins on the ocular surface were discussed by Argüeso and Gipson [27]. The results reported in this review that support the use of gastric mucin when investigating the interaction between mucin and ocular drug delivery systems include: (1) ocular mucins are characterised by an extensive sialylation and sulfatation of the carbohydrate side chains; both sialic acid and sulphate groups are generally considered as the structural characteristic being responsible for the mucoadhesive interaction observed with gastric mucin; (2) the rheological behaviour of ocular mucin is predominantly determined by the gel-forming MUC5AC mucin, which is also responsible for the rheological behaviour of gastric mucin [27].

A third variable is the kind of Carbopol® employed. Carbopol® 974P NF is the benzene-free alternative of Carbopol® 934P NF. Edsman et al. [6] already used Carbopol® 974P NF to prepare carbomer gels for ophthalmic use. Carbopol® 980 NF is the most efficient thickener of all Carbopol® polymers on the market. Furthermore, it is already incorporated in commercial ophthalmic gels to treat dry eye syndrome. Carbopol® 1342 NF contains a long chain alkyl acrylate. The lipophilic modification of the backbone increases the resistance to dissolved ions. Davies et al. [28] reported the use of Carbopol® 1342 NF to coat liposomes intended to be administered ocularly.

The influence of sonication of carbomer dispersions on the polymer/mucus interaction is the last parameter investigated in the experimental design approach. Sonication can be applied as a dispersion technique to promote the formation of homogeneous dispersions without clumps. However, since several researchers demonstrated that the sonication procedure can decrease the molecular weight of natural and synthetic polymers [29,30], the effect of the sonication procedure on the mucoadhesive interaction has to be taken into account. After all, a critical polymer chain length is necessary to achieve interpenetration and molecular entanglements [31].

Other parameters kept constant to prevent the experimental design from being too complex are: pH (physiological conditions), temperature (32°C which is the temperature at the corneal surface) and isotonicity of the preparations.

In the present study, a time-saving experimental design approach is implemented to investigate simultaneously the effect of the various parameters mentioned and the interaction between them on the polymer/mucin interaction. The characterisation of the poly(acrylic acid) and mucin dispersions, and the calculation of the degree of interaction are performed by applying an oscillatory rheological method. Statistical analysis of the experimental design data enables the identification of the parameters which have the most significant influence upon the polymer/mucus interaction. After the identification of these parameters, the eye drop formulation and its preparation are optimised. Finally, the mechanism of the interaction between the optimised eye drop formulation(s) and mucin is fully clarified by performing a thorough rheological analysis of the carbomer/mucin mixture.

2. Materials and methods

2.1. Materials

Carbopol® 974P NF (CP974), Carbopol® 980 NF (CP980) and Carbopol® 1342 NF (CP1342) were obtained as a gift from BF Goodrich (Brussels, Belgium). All other chemicals were purchased and used as received: potassium chloride, sodium hydrogen carbonate, sodium hydroxide (Merck, Overijse, Belgium); mannitol (Bufa, Zwevegem, Belgium); sodium chloride (Federa, Brussels, Belgium); calcium chloride, magnesium chloride and Mucin-Type II: crude from porcine stomach (Sigma Chemicals, Bornem, Belgium); Rossi et al. [14] justified the use of commercial mucins by the lower batch-to-batch variability shown by commercial samples with respect to those freshly prepared.

Purified water was used throughout the experiments.

2.2. Methods

2.2.1. Sample preparation

To prepare a Carbopol® dispersion the required amount of the polymer powder was dispersed in an iso-osmotic solution containing 5.07% (w/v) mannitol. Physiological pH (7.4 ± 0.1) was adjusted by adding the required amount of NaOH. To ensure complete hydration the solutions were stored at 6°C for a minimum of 12 h. An additional sonication procedure of half minute four times imposing 80 W to the sample was performed using a Sonifier® B12 (Branson Sonic Power Company, CT, USA). After each sonification step of half minute, the dispersion was mixed manually.

Simulated lachrymal fluid (SLF) is an electrolyte solution containing 1.7893 g/l KCl, 6.3118 g/l NaCl, 2.1842 g/l $NaHCO_3$, 44.4 mg/l $CaCl_2$ and 47.6 mg/l $MgCl_2$ [26]. Physiological pH (7.4 ± 0.1) was adjusted by adding the required amount of 0.1 N HCl. The SLF solution was used to prepare the mucin dispersions (MUC). After dispersing the required amount of mucin powder in SLF using a

magnetic stirrer, the solution was stirred for 24 h at room temperature to allow complete hydration.

2.2.2. Rheological measurements

Rheological analyses were performed with a controlled stress rheometer (Carri-Med CSL^2 100, TA Instruments Ltd, Leatherhead, UK) at 32 ± 0.1°C (temperature at corneal surface) [32].

Two types of oscillatory measurements were performed. On the one hand, a dynamic stress sweep was applied in which the dynamic moduli were recorded at a constant frequency of 1 rad/s and at a range of stress amplitudes (0.001–10 Pa). The stress sweeps were used to investigate the influence of the stress on the dynamic moduli. The linear viscoelastic region is determined by the maximum stress which can be applied without affecting the elastic modulus (G') and the viscous modulus (G''). In the linear viscoelastic region, the response of the material is characteristic for its microstructure at rest. For stresses above the linear limit, the structure is affected or even destroyed by the measurement. The relative magnitude of the moduli is a qualitative indication for the structure in the sample. For a solution of a high molecular weight polymer, three different situations can be encountered: $G' \gg G''$ for a chemically cross-linked system, $G' > G''$ for a network consisting of secondary bonds and $G' \leq G''$ for a physically entangled polymer solution [33]. Experiments have also been performed in which the stress amplitude was kept constant and the frequency was varied. The structure of the system can be kept intact during the measurement by choosing the amplitude of the oscillations small enough (within the linear viscoelastic region). By performing such small stress oscillations at a whole range of frequencies (0.1–10 rad/s), the type of network structure present in the sample can be revealed. The main difference between a network of secondary bonds and one of physical entanglements is located in the low frequency range: in an entanglement network the polymers can disentangle if the available time is long enough (low frequency); in a network of secondary bonds the bonds are fixed irrespective of the time scale. This structural behaviour results for an entangled solution in a cross-over in G' and G'' in the low-frequency region. For a network of secondary bonds an almost constant value of G' and G'' is observed over the whole frequency range (slope ≈ 0), with the value of G' exceeding that of G'' [34] and no cross-over in G' and G''. The critical cross-over frequency (ω_c) can be used as a measure of the longest relaxation time τ ($= 1/\omega_c$) of the bonds present in a polymer mixture. A secondary bond network is characterised by an infinitely small ω_c and consequently a very long relaxation time τ, while in the case of an entangled solution, ω_c is situated at a relatively higher frequency, and the relaxation time τ is shorter [35].

Each procedure was performed three times on each sample. Mean values and standard deviations were calculated. However, standard deviations are not graphically shown to preserve the readability of the graphs.

2.2.3. Mucoadhesive evaluation

To study the mucoadhesive interaction, equal amounts of the Carbopol® dispersion and MUC were mixed. This dilution procedure simulated as closely as possible the in vivo situation after the instillation of an eye drop. The maximum volume of the precorneal tearfilm is about 10 μl [36], while the maximum volume which can be held in the cul-de-sac without overflow to the cheek is about 20 μl [37]. This means that an eye drop of 10 μl should be instilled to avoid loss by overflow. Higher volumes instilled (as is the case of eye drops of commercial preparations being 15–50 μl) induce rapid drainage and reflex blinking resulting in low bioavailability. Therefore, an instillation of a 10 μl eye drop should be considered as an ideal situation from a biopharmaceutical point of view.

After instillation of a 10 μl eye drop causing no overflow, a 1:1 dilution with lachrymal fluid is obtained.

An interaction between polymer and mucin – either physical entanglements or secondary bonds – should be seen as a synergistic effect in the rheological properties [15], which means that the rheological response of the Carbopol®/MUC mixture should be larger than the sum of the rheological responses of the single components Carbopol® and mucin. Therefore, it is essential to rheologically characterise the single components as well as the Carbopol®/MUC mixture. The first single-component solution (Carbopol®/SLF) is a mixture containing equal amounts of Carbopol® dispersion and SLF. The rheological behaviour of this dispersion depends on polymer/polymer and polymer/SLF interactions. The second single-component solution (MUC/SLF) consist of MUC, mixed with an equal amount of SLF, and characterises the mucin/mucin and mucin/SLF interactions.

2.2.4. Experimental design

Experimental design is an analysis technique developed to test simultaneously the effect of several parameters (called factors) on a certain response using only a limited number of measurements. The set-up of the design and the calculation of the effects of the different factors were performed with Statgraphics® software (Manguistics Inc., Rockville, MD, USA).

The factors and levels applied in the present study are summarised in Table 1, while the experimental design plan and the responses calculated are shown in Table 2.

Table 1
Factors and levels applied in the experimental design experiment

Factor	Level		
	−1	0	1
A: Polymer concentration (%, w/v)	0.05	0.125	0.2
B: Mucin concentration (%, w/v)	8	12	16
C: Type of Carbopol	CP1342	CP974	CP980
D: Sonication	No		Yes

Table 2
Experimental design and responses

A	B	C	D	MAI(G') (y_{D-1}/y_{D+1})	MAI(G'') (y_{D-1}/y_{D+1})
−1	−1	−1	−1/+1	0.02/0.05	0.06/0.05
−1	+1	−1	−1/+1	0.43/0.32	0.47/0.16
0	0	−1	−1/+1	0.16/0.13	0.12/0.07
0	0	−1	−1/+1	0.09/0.04	0.09/0.05
0	0	−1	−1/+1	0.11/0.04	0.11/0.04
+1	−1	−1	−1/+1	0.07/−0.00	0.01/−0.00
+1	+1	−1	−1/+1	0.17/0.54	0.25/0.38
−1	−1	0	−1/+1	0.02/0.02	0.04/0.04
−1	+1	0	−1/+1	0.16/0.39	0.15/0.34
0	0	0	−1/+1	0.08/0.04	0.10/0.06
0	0	0	−1/+1	0.06/0.01	0.06/0.04
0	0	0	−1/+1	0.00/−0.01	0.03/0.02
+1	−1	0	−1/+1	0.03/0.00	0.05/−0.00
+1	+1	0	−1/+1	0.24/0.02	0.13/0.05
−1	−1	+1	−1/+1	0.02/0.08	0.04/0.10
−1	+1	+1	−1/+1	0.25/0.32	0.23/0.24
0	0	+1	−1/+1	0.12/−0.01	0.16/0.02
0	0	+1	−1/+1	0.18/0.07	0.21/0.09
0	0	+1	−1/+1	0.06/−0.02	0.10/0.01
+1	−1	+1	−1/+1	0.05/0.06	0.06/0.06
+1	+1	+1	−1/+1	0.33/0.08	0.30/0.07

The calculation of the response factors was based on the method of Rossi et al. [17] taking the rheological synergism in the Carbopol®/MUC mixture into account. Elastic modulus G' and viscous modulus G'' were measured at an oscillation stress situated within the linear region and a frequency of 1 rad/s.

$$\mathrm{MAI}_{(G')} = \text{Mucoadhesive index calculated with } G'$$
$$\mathrm{MAI}_{(G')} = G'_{(\text{Carbopol}^{®}/\text{MUC})} - [G'_{(\text{Carbopol}^{®}/\text{SLF})} + G'_{(\text{MUC/SLF})}] \quad (1)$$

$$\mathrm{MAI}_{(G'')} = \text{Mucoadhesive index calculated with } G''$$
$$\mathrm{MAI}_{(G'')} = G''_{(\text{Carbopol}^{®}/\text{MUC})} - [G''_{(\text{Carbopol}^{®}/\text{SLF})} + G''_{(\text{MUC/SLF})}] \quad (2)$$

The effect of the factors and the interactions were estimated using Eq. (3) [38].

$$\mathrm{Effect}_{(F)} = [\sum_{(F+)} - \sum_{(F-)}]/n \quad (3)$$

with F = factor (or combination of factors) under investigation, $\Sigma_{(F+)}$ = sum of responses at a positive level of $F(+1)$, $\Sigma_{(F-)}$ = sum of responses at a negative level of $F(-1)$, n = number of responses at $F+$ or $F-$.

To test the statistical significance of the effects, an analysis of variance (ANOVA) was performed, which partitions the variability in the response factor into separate pieces for each of the effects. It then tests the statistical significance of each effect by comparing the mean square of the effect (sum of squares/degrees of freedom) against an estimate of the experimental error using an F-test. To determine the experimental error, centre points were implemented in the design [38]. Only if an effect has a P-value less than 0.05, it is considered significantly different from zero at the 95.0% confidence level.

3. Results and discussion

3.1. Rheological characterisation of carbomer dispersions

Stress sweep curves (Fig. 1) recorded at the minimum concentration (0.05%, w/v) demonstrate that CP1342, having a long alkyl chain, behaves most elastically. CP974 is the least elastic, and CP980 shows intermediate

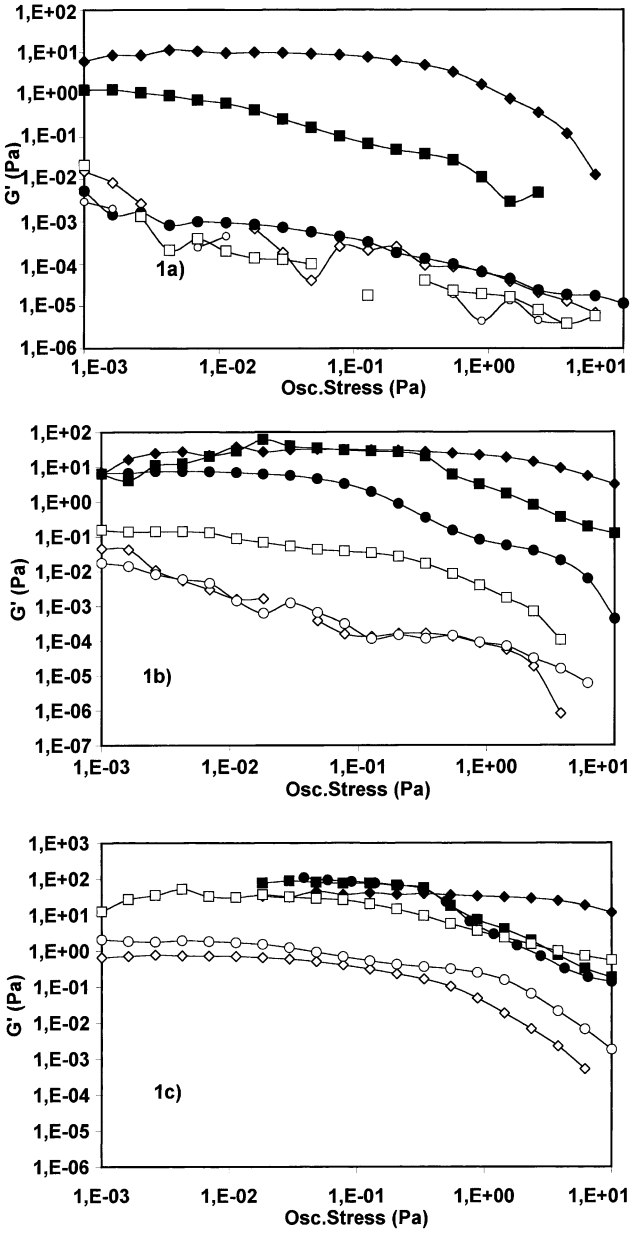

Fig. 1. Dynamic stress sweep measurements (log G'/log stress) of a 0.05% (w/v) (a), 0.25% (w/v) (b) and 0.20% (w/v) (c) Carbopol® dispersion. Diamonds: CP1342; squares: CP980; circles: CP974. Filled symbols: before sonication; open symbols: after sonication.

behaviour. This gradation was maintained at higher concentrations, although the difference between CP974 and CP980 was reduced to a minimum at 0.20% (w/v). After sonication, CP980 behaved most elastically and can be therefore considered as most resistant to the sonication procedure. The formation of secondary bonds as indicated by G' being larger than G'' (data not shown) was already observed at 0.05% (w/v) for CP1342 and CP980. In the case of CP974P, the critical concentration for the formation of secondary bonds was 0.125% (w/v). The sonication procedure increased this critical concentration to 0.125% (w/v) for CP980, and 0.20% (w/v) for CP1342 and CP974, respectively.

Frequency sweep data (Fig. 2) confirmed the secondary bond formation in both low and highly concentrated CP1342 and CP980 dispersions because $G' > G''$ and both moduli were independent of the frequency. CP974 showed frequency dependence at a concentration of 0.125% (w/v), and became fully frequency independent at 0.20% (w/v). The frequency sweeps which were performed after sonication (Fig. 2d) [only at 0.20% (w/v) since concentrations below 0.20% did not show a linear viscoelastic region] confirmed that CP980 was less frequency dependent than CP1342 and CP974, and that CP980 was most resistant to the sonication procedure.

Both stress and frequency sweep data indicated that the sonication procedure resulted in a decrease of the elasticity of the polymer network and an increase of the critical concentration to form secondary bonds. Both effects can be probably attributed to the decrease of the molecular weight due to the scission of the poly(acrylic acid) polymer chain. Before sonication, CP1342 behaved most elastically, while CP980 seemed to be the most resistant to the decrease of elasticity due to sonication.

3.2. Rheological characterisation of mucin dispersions

As can be derived from Table 2, the MUC/SLF dispersion was prepared six times in the case of mucin 8% (w/v) (level $B - 1$) and mucin 16% (w/v) (level $B + 1$), while mucin 12% (w/v) (centre point, level $B0$) was analysed nine times. The difference between the viscoelastic moduli of the dispersions having the same mucin concentration was large due to the heterogeneity of the dispersions prepared, even after stirring for 24 h. Therefore, to calculate the mucoadhesive indexes and to investigate the mucoadhesive interaction mechanism, the mean dynamic moduli of all the MUC/SLF dispersions prepared were calculated for each mucin concentration. The mean elastic and viscous modulus of MUC/SLF as a function of stress for mucin 8, 12 and 16% (w/v) are presented in Fig. 3a. Independently of the mucin concentration, $G' \geq G''$ in the low stress region which indicated that secondary bonds were formed between the mucin molecules. However, when the stress was slightly increased to 0.01 Pa, the secondary bonds were broken and the dispersion behaved as a purely viscous medium. Frequency

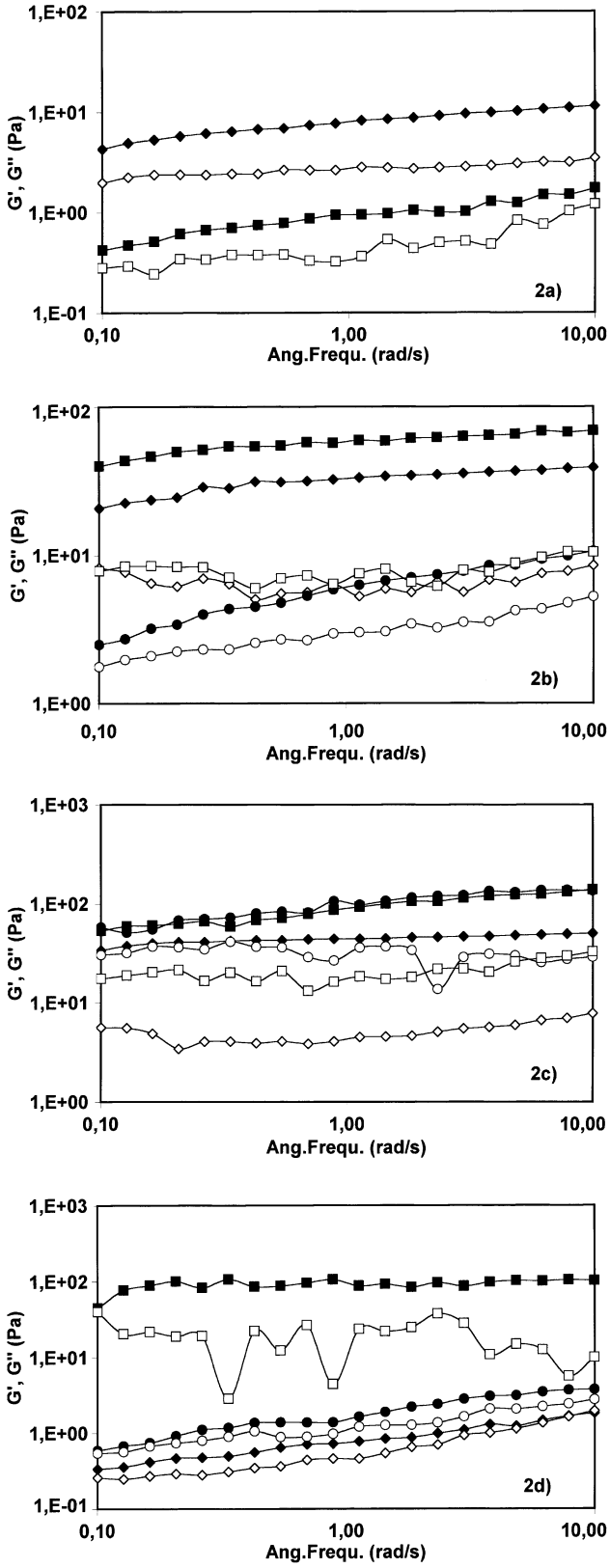

Fig. 2. Dynamic frequency sweep measurements (log $G'(G'')$/log ω) of a 0.05% (w/v) (no sonication) (a), 0.125% (w/v) (no sonication) (b), 0.20% (w/v) (no sonication) (c) and 0.20% (w/v) (sonication) (d) Carbopol® dispersion. Diamonds: CP1342; squares: CP980; circles: CP974. Filled symbols: storage modulus; open symbols: loss modulus.

sweeps performed with the 12% (w/v) (Fig. 3b) and 16% (w/v) mucin dispersion (Fig. 3c) [in the case of the mucin 8% (w/v) dispersion, the linear viscoelastic region was too small], showed a cross-over point ($G' = G''$) at approximately 0.26 rad/s, which corresponded with a relaxation time of 24 s. These results were in agreement with the work of Crowther et al. [39], who reported the presence of secondary bonds with the ability to break and reform with relative ease in addition to the network structure due to physical entanglements.

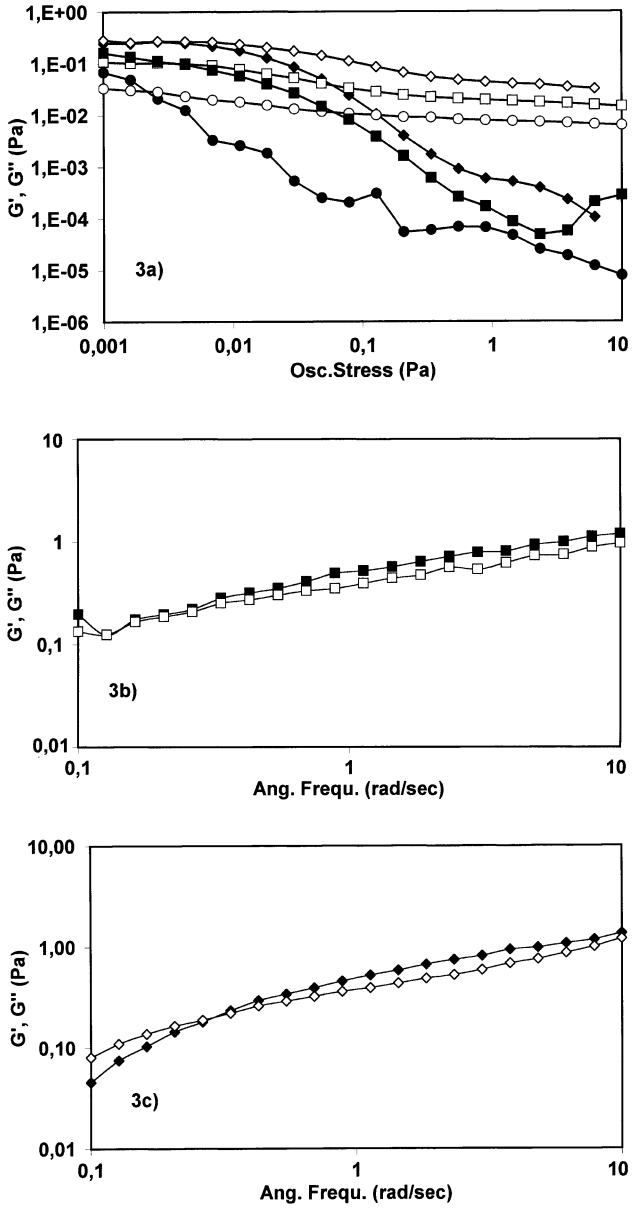

Fig. 3. Rheological spectra of MUC/SLF. Stress–sweeps 8, 12 and 16% (w/v) (a); frequency sweep 12% (w/v) (b); frequency sweep 16% (w/v) (c). Diamonds: mucin 16% (w/v); squares: mucin 12% (w/v); circles: mucin 8% (w/v). Filled symbols: storage modulus; open symbols: loss modulus.

3.3. Determination of factors influencing the response factors

The mucoadhesive indexes $MAI_{(G')}$ and $MAI_{(G'')}$ calculated at the different levels of the factors under investigation are presented in Table 2. The effect of polymer concentration (factor *A*), mucin concentration (factor *B*) and sonication (factor *D*) is calculated for each Carbopol®. The data (Table 3) indicate that for each Carbopol®, the interaction between the polymer and mucin can only be improved significantly by increasing the mucin concentration from 8 to 16% (w/v). Increasing the polymer concentration from 0.05 to 0.20% (w/v), which can result in the conversion of an entangled network to an elastic network with secondary bonds, did not influence the polymer/mucin interaction. Sonication, which can, on the contrary, result in the conversion of an elastic to an entangled network (Section 3.1) did not significantly influence the interaction either. In the 0.05–0.20% (w/v) Carbopol® range, the most important condition to achieve an optimised polymer/mucin interaction seems to be the presence of a highly concentrated mucin layer. The interaction was not influenced by the type of polymer network applied (physical entanglements or secondary bonds).

Several researchers have pointed out that chain interpenetration during the mucoadhesive process depends to a high extent on the concentration, the molecular weight and the degree of cross-linking of the applied polymer [23,31,40]. Although increasing the polymer concentration in the present study resulted in an increase of the secondary bond formation, the polymer chains probably remained sufficiently long and flexible to be able to interpenetrate into the mucin dispersion, even at the 0.20% (w/v) level. The applied concentrations are probably smaller than the maximum polymer concentration corresponding to optimal mucoadhesion, reported by Madsen et al. [23]. However, higher polymer concentrations were not considered to be applicable in ocular formulations investigated in this study. Sonication of the dispersions on the other hand, seemed not to decrease the chain length below the critical molecular weight value, necessary to achieve interpenetration and mucoadhesion.

To investigate whether the polymer/mucin interaction depends on the kind of Carbopol® used, the Carbopol®

Table 3
P-values of factors influencing significantly ($P < 0.05$) MAI(G') and MAI(G'')of Carbopol 1342 NF, Carbopol 974 and Carbopol 980 NF[a]

	CP1342		CP974		CP980	
	$MAI_{(G')}$	$MAI_{(G'')}$	$MAI_{(G')}$	$MAI_{(G'')}$	$MAI_{(G')}$	$MAI_{(G'')}$
A	*	*	*	*	*	*
B	0.0059	0.0025	0.0178	0.0083	0.0136	0.0101
D	*	*	*	*	*	*

[a] Interactions AB, AC and BC ($P > 0.05$) are not shown. *: *P*-value of the effect is larger than 0.05.

Table 4
P-values of factors influencing significantly ($P < 0.05$) MAI(G') and MAI(G'') using a two by two comparison of the Carbopols investigated[a]

		No sonication		Sonication	
		$MAI_{(G')}$	$MAI_{(G'')}$	$MAI_{(G')}$	$MAI_{(G'')}$
CP1342 vs. CP974	A	*	*	*	*
	B	0.0042	0.0010	0.0131	0.0131
	C	*	0.0281(−)	*	*
	AB	*	*	*	*
	AC	*	*	*	*
	BC	*	0.0205	*	*
CP1342 vs. CP980	A	*	*	*	*
	B	0.0012	0.0004	0.0194	0.0294
	C	*	*	*	*
	AB	*	*	*	*
	AC	*	*	*	*
	BC	*	*	*	*
CP974 vs. CP980	A	*	*	0.0275(−)	0.0164(−)
	B	0.0008	0.0009	0.0298	0.0195
	C	*	0.0084	*	*
	AB	*	*	0.0467	*
	AC	*	*	*	*
	BC	*	0.0708	*	*

[a] *: *P*-value of the effect is larger than 0.05. (−): the effect is negative.

dispersions are compared pairwise (Table 4). Setting up one single design to determine simultaneously the influence of each factor, including the kind of Carbopol® used (factor *C*) is unfeasible, since this factor *C* is not a continuous factor.

The pairwise analysis reveals that the positive effect of increasing the mucin concentration (factor *B*) on the degree of polymer/mucin interaction can again be observed before as well as after sonication for both response factors. The effect of the kind of Carbopol® (factor *C*) seemed to be different before and after sonication. Before sonication, the rheological synergism calculated using the viscous modulus G'' was significantly lower for CP974 compared to CP1342 and CP980, which did not differ significantly. Moreover, the effect depended on the mucin concentration (interaction BC < 0.05). However, this difference between the unsonicated dispersions was not observed when considering the rheological synergism calculated with the elastic modulus G'. Therefore, it can be assumed that the effect observed with $MAI_{(G'')}$ is only due to a 'concentration effect'. The total polymer concentration in the polymer/mucin mixture was higher compared to the polymer/SLF and mucin/SLF mixture, which resulted in a synergistic increase of the viscous modulus G''. Improvement of the physical entanglement between the polymer and mucin would also result in a synergistic increase of the viscous modulus, but imperatively associated with an increase of the elastic modulus G'. After sonication, the difference between CP1342 and CP980 on the one hand and CP974 on the other hand was no longer observed. The main conclusion concerning the use of different Carbopol® dispersions was that the difference between the Carbopol®/mucin interactions was probably not significant.

Finally, the pairwise CP974/CP980 analysis revealed a negative polymer concentration effect after sonication of the dispersions. This effect indicates that the polymer concentration effect of a sonicated CP1342 dispersion is significantly different from that in CP974 and CP980. This is exemplified by the data at the highest mucin concentration level ($B + 1$) in Table 2. Increasing the sonicated polymer concentration from 0.05 to 0.20% (w/v) ($A - 1$ to $A + 1$ level) resulted in an increase of both $MAI_{(G')}$ ($0.32 \rightarrow 0.54$) and $MAI_{(G'')}$ ($0.16 \rightarrow 0.38$) in the case of CP1342 ($C - 1$ level), while both indexes decreased in the case of CP974 ($C0$ level) [$MAI_{(G')}$: $0.39 \rightarrow 0.02$; $MAI_{(G'')}$: $0.34 \rightarrow 0.05$] and CP980 ($C + 1$ level) [$MAI_{(G')}$: $0.32 \rightarrow 0.08$; $MAI_{(G'')}$: $0.24 \rightarrow 0.07$]. Both CP974 and CP980 are homopolymers of cross-linked acrylic acid. On the contrary, CP1342 is a copolymer of cross-linked acrylic acid, modified by long chain (C10–C30) alkyl acrylates. Regardless of the type of Carbopol®, the sonication procedure probably results in the splitting of the poly(acrylic acid) chain and the cross-links, as was indicated by the decrease of the elastic modulus in Fig. 1. However, the way the polymer chains are split seems to be influenced by the presence of long alkyl chains. If no long alkyl groups are present on the poly(acrylic acid) chain (CP974 and CP980), the molecular conformation obtained after sonication seems to be able to interact with the mucin dispersion only when the polymer concentration is sufficiently low. The significant interaction AB ($P = 0.0467$) indicated that a high mucin concentration was still necessary to optimise the interaction. At the low mucin level, the polymer concentration effect was different from the effect at the high mucin level. The presence of the long alkyl chains (CP1342) probably meant that the method of splitting the poly(acrylic acid chain) was changed, resulting in a molecular conformation

which was able to interact with the concentrated mucin dispersion mainly at high concentrations.

3.4. Rheological characterisation of the optima

The results of the experimental design analysis can be applied to define the optimal combination of the parameters investigated. Regardless of the type of Carbopol® used, the optimum is situated at the highest mucin concentration. The type of Carbopol® or the application of a sonication procedure did not influence significantly the mucoadhesive interaction. Nor did the polymer concentration influence the interaction, except after sonication of CP974 and CP980. Since actually only mucin seems to have a significant influence, the mucoadhesive indexes presented in Table 2 are further used to select the optimum. Since the difference between the types of Carbopol® investigated is not considered significant in the experimental design study, an optimum is defined for each Carbopol®. Although the mucoadhesive indexes are very useful and actually indispensable to perform the experimental design study, an additional thorough rheological characterisation of the optima remains an important requisite, since the calculation of the indexes is based on a single oscillation stress/frequency combination and not on the complete rheological spectra of the mixtures.

In the case of CP1342, the optimum derived with MAI(G') ($=0.54$) was different from the optimum derived with MAI(G'') ($=0.47$). However, since the mucoadhesive interaction is mainly correlated with the formation of an elastic network, only the MAI (G') optimum was further analysed rheologically (Fig. 4). Both stress and frequency sweep curves showed that the CP1342/MUC mixture behaved more elastically compared to CP1342/SLF and MUC/SLF. CP1342/MUC had the longest linear region, $G'_{(CP1342/MUC)}$ was larger than $G''_{(CP1342/MUC)}$ and the dimensional difference between the dynamic moduli of the various mixtures illustrated the synergistic rheological effect after mixing CP1342 and mucin in the dynamic stress sweep curves. Furthermore, the frequency sweep data showed that the cross-over frequency ω_c of the CP1342/MUC mixture ($=0.1$ rad/s) was lower compared to $\omega_{c(MUC/SLF)}$ ($=0.34$ rad/s). The ω_c value of CP1342/SLF could not be determined because of the absence of a linear region and was therefore neglected . The relaxation time τ ($=1/\omega_c$) of the bonds present in CP1342/MUC ($\tau=63$ s) was longer compared to MUC/SLF ($\tau=18$ s), confirming the formation of additional secondary bonds in the CP1342/MUC mixture.

The optimum for CP974 was achieved after mixing a sonicated 0.05% (w/v) dispersion with mucin 16% (w/v) [MAI(G') $=0.39$ and MAI(G'') $=0.34$]. The fact that the linear region was the longest in the case of the CP974/MUC mixture and the dimensional difference between the mixtures are both factors which were comparable to CP1342, but $G'_{(CP974/MUC)} > G''_{(CP974/MUC)}$ was not as pronounced. Furthermore, the CP974/SLF curves ($G' \gg G''$) indicate that the electrolytes present in SLF can also increase the elasticity. The cross-over frequencies of CP974/MUC and MUC/SLF are comparable, which indicates that the bonds in the CP974/MUC mixture are comparable to the bonds in MUC/SLF. These data indicate that the dimensional effect and the lengthening of the linear region in the stress sweeps are only due to the 'concentration effect'. This means that the effects observed are only due to the fact that the total polymer concentration in the CP974/MUC mixture is higher compared to the total polymer concentration in CP974/SLF and MUC/SLF (Fig. 5).

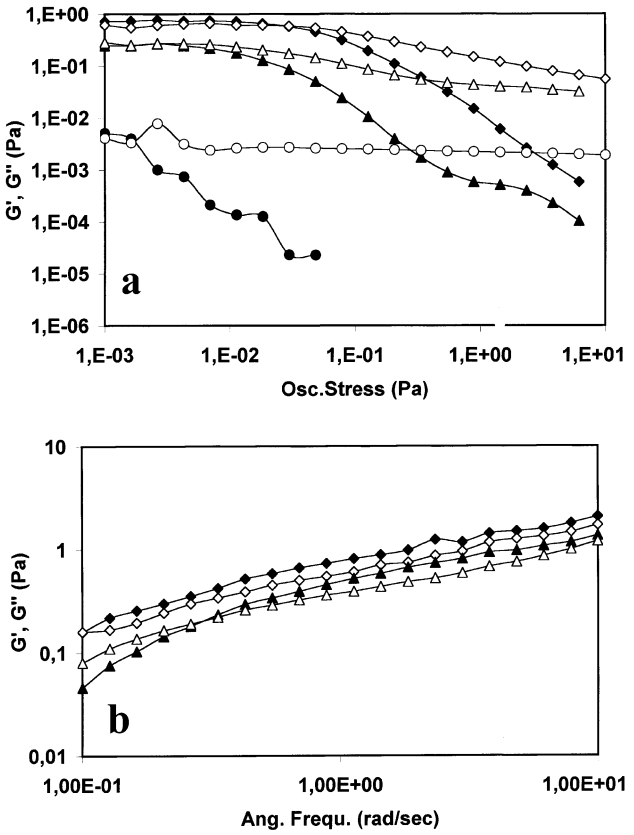

Fig. 4. Rheological analysis of the CP1342 optimum. Diamonds: CP1342/MUC; triangles: MUC/SLF; circles: CP1342/SLF. Filled symbols: storage modulus; open symbols: loss modulus.

The CP980 optimum was achieved after mixing the unsonicated 0.20% (w/v) dispersion with mucin 16% (w/v). The stress sweep data were very comparable to the CP1342 data. Only $G'_{(CP980/MUC)} > G''_{(CP980/MUC)}$ was somewhat less pronounced. However, the formation of additional elastic bonds after mixing CP980 and mucin 16% (w/v) was confirmed by the frequency sweep curves. The cross-over frequency ω_c of the CP980/MUC mixture is much smaller than 0.1 rad/s and tends to become infinitely small. Therefore, the relaxation time τ becomes infinitely large, pointing to a strong elastic bond. On the contrary, the relaxation time of the MUC/SLF mixture is 18 s. The relaxation time of the CP980/SLF mixture is neglected because of the absence of a linear region (Fig. 6).

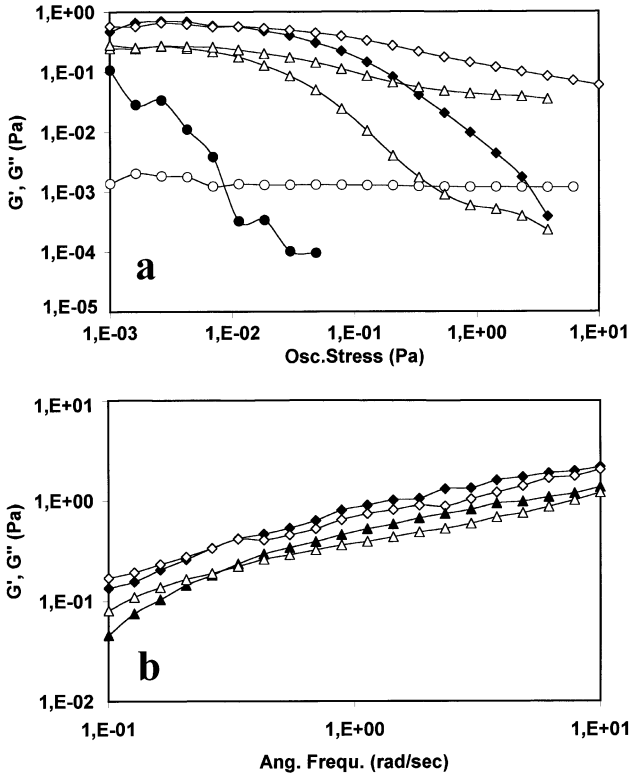

Fig. 5. Rheological analysis of the CP974 optimum. Diamonds: CP974/MUC; triangles: MUC/SLF; circles: CP974/SLF. Filled symbols: storage modulus; open symbols: loss modulus.

Statistical analysis of the complete set of data of each Carbopol® illustrated that the mucoadhesive interactions of the different kinds of Carbopol® derivatives were not significantly different. When MAI(G') and MAI(G'') mentioned in Table 1 were taken into consideration, the optimal mucoadhesive interaction was assigned to the sonicated CP1342 0.20% (w/v)/mucin 16% (w/v) mixture [$MAI_{(G')} = 0.54$ and $MAI_{(G'')} = 0.38$]. However, the full rheological characterisation of the three mixtures prepared to study the polymer/mucin interaction revealed that the cross-over frequency shift to the low-frequency region after mixing the polymer and mucin, was most pronounced in the case of CP980. This cross-over frequency shift is a clear indication for the additional formation of strong elastic bonds after mixing both components.

If no additional sonication procedure is required to prevent clump formation, CP980 is the best choice to prepare a viscous eye drop, presenting a significant interaction with mucin 16% (w/v). If, however, the additional sonication procedure is necessary, CP1342 is preferable to CP980. The mucoadhesive capacity of both the unsonicated and the sonicated CP974 dispersion is very limited. Further investigation will be aimed at the clarification of the molecular conformation of the sonicated poly(acrylic acid) derivatives to explain the concentration effects. Also in vivo experiments will be performed to verify whether the positive mucoadhesive effects obtained with the 0.20% (w/v) Carbopol® 980 and Carbopol® 1342 dispersion can be confirmed.

4. Conclusions

Most important conclusion which can be derived from the experimental design analysis is that increasing the mucin concentration from 8 to 16% (w/v) is actually the only factor increasing significantly the mucoadhesive interaction. At the lower mucin level, the interaction between the polymer and mucin is negligible and independent of the type of Carbopol® used. This finding implies that if any interaction between a poly(acrylic acid) derivative and ocular mucin occurs, this interaction is possible only close to the corneo-conjunctival epithelium which is covered by a highly concentrated mucin layer. Physical entanglement and secondary bond formation between the poly(acrylic acid) derivative and diluted mucin in the tearfilm can be excluded.

Although the experimental design study indicated that the difference between the mucoadhesive interactions of the Carbopol® derivatives is not significantly different, the full rheological characterisation enabled a further discrimination between the derivatives. The 0.20% (w/v) Carbopol® 980 dispersion shows a clear secondary bond interaction with mucin 16% (w/v). The formation of secondary elastic

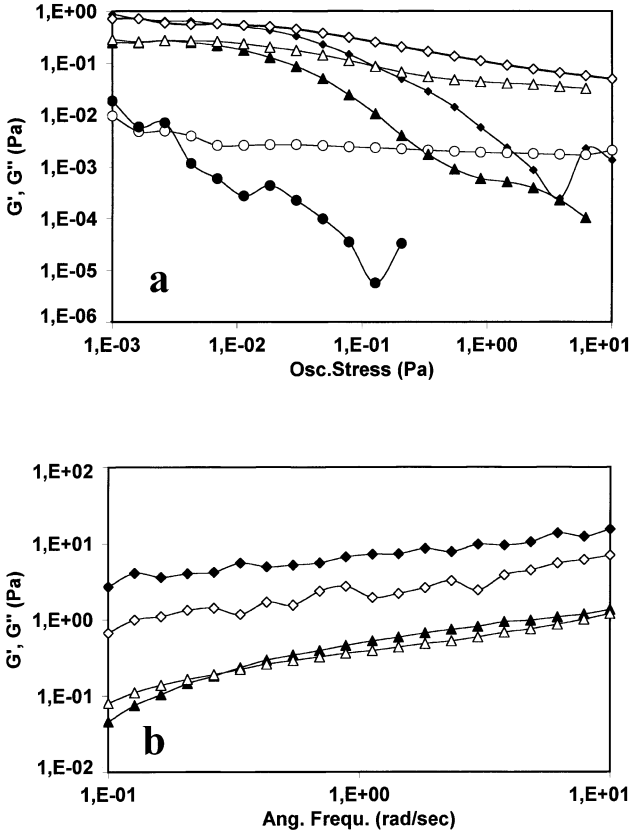

Fig. 6. Rheological analysis of the CP980 optimum. Diamonds: CP980/MUC; triangles: MUC/SLF; circles: CP980/SLF. Filled symbols: storage modulus; open symbols: loss modulus.

bonds also occurs when mixing the sonicated 0.20% (w/v) Carbopol® 1342 dispersion and mucin 16% (w/v), but is not as pronounced as with Carbopol® 980. The interaction between Carbopol® 974 and mucin is very limited, and independent of the conditions applied.

When incorporating polyacrylic acid into an ophthalmic formulation to prepare viscous eye drops, both CP980 and CP1342 can be used. The preparation procedure determines whether CP980 or CP1342 should be used. If an additional sonication procedure is implemented to prevent clump formation, maximum mucoadhesive interaction is obtained with the 0.2% CP1342 dispersion. If, however, no additional sonication procedure is necessary, the use of the 0.2% CP980 dispersion is recommended.

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