Recent Advances on Bioadhesive Ocular Dosage Forms

Jens CEULEMANS*, Annick LUDWIG*°

**Summary:** Continuous secretion of tears, frequent blinking and a surface epithelium with low permeability are the main factors protecting the eye against external factors. Obviously, this protective barrier also reduces the efficacy of ocular drugs applied topically. The use of mucoadhesive dosage forms can be employed to increase the bioavailability of ocular drugs.

The suitability of polymer preparations (both liquid and solid dosage forms) to lengthen the precorneal residence time of the drug and to achieve an increase of the ocular bioavailability, by means of a mucoadhesive interaction, is evaluated. The clarification of the mucoadhesive interaction mechanism(s) is accomplished by the development of an in vitro technique using oscillatory shear rheology. Implementation of several rheological procedures enables the characterisation of the degree and type of network formation between the polymer dispersion and mucin. The results obtained with the in vitro technique are verified in vivo by ocular fluorophotometry determining the ocular elimination kinetics.

To influence significantly the precorneal residence time and the ocular bioavailability, the use of a mucoadhesive minitablet seems to be inevitable. Liquid polymer dispersions can also interact with mucin, but the interaction mechanisms as demonstrated by the in vitro experiments are insufficient to achieve a significant in vivo effect.

**Key Words:** Mucoadhesion, Minitablet, Oscillatory shear rheology, Fluorophotometry, Optimisation, In vitro, In vivo, Polyacrylic acid, Acceptability

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**INTRODUCTION**

Upon instillation conventional aqueous eye drops are drained rapidly from the precorneal area and diluted by tear turnover, so that only a small amount of drug remains in the cul de sac to exert a local action or to be absorbed by the eye tissues. Moreover, the resorption of the drug is hampered by the anatomical structure and the barrier function of the cornea. Therefore the bioavailability of eye drops is very low (1 to 10%) and only a pulse-entry of the drug is provided into the eye.¹²

* Laboratory Pharmaceutical Technology & Biopharmacy Universiteitsplein 1, 2610 Antwerp, Belgium.

° Correspondence
Several strategies were developed to improve the bioavailability of eye drops. First, viscolysers were added to the formulation in order to prolong the precorneal residence time of the solution instilled and to improve the resorption of the drug. The viscolysers used in ophthalmology are water soluble, naturally occurring, semi-synthetic or synthetic polymers. Cellulose ethers (HPMC, HPC, HEC, MC and NaCMC), dextran, PVA, PVP were commonly and abundantly used. In general the viscous ophthalmic solutions exhibited a Newtonian or pseudoplastic rheological behaviour. Pseudoplastic solutions offer less resistance to the eyelids during blinking and, therefore are expected to be more comfortable in the eye than Newtonian solutions.

The increase in bioavailability was only small in the case of most viscolysers due to blurred vision and irritation at high polymer concentration. The ideal viscosity is estimated at 15 - 30 mPa.s, except in the case of viscoelastic polymers like sodium hyaluronate were a higher viscosity is well tolerated by the patient.

A first modified approach to deal with the viscosity related problem is the application of phase transition systems or in situ gelling systems, which are instilled in a liquid form and shift to a gel or solid phase in the cul-de-sac. The only polymer known to have a buffer capacity, which is low enough to gel effectively in the cul-de-sac of the eye, is cellulose acetate hydrogen phthalate (CAP). The pH change of 2.8 units after instillation of the native formulation (pH 4.4) into the tear fluid (pH 7.2) leads to an almost instantaneous transformation of the highly fluid latex into a viscous gel. Several types of polymers (e.g. poloxamer 407) have been employed to increase the ocular residence time by thermosensitive gels. The temperature increase to 32°C, which is the temperature at the surface of the cornea, causes the gelation of the polymer. Gelrite® is a low-acetyl gellan gum, which forms a clear gel in the presence of mono- or divalent cations. The concentration of mono- (K+) and divalent (Ca++, Mg++) cations in the tear fluid is particularly suited to cause gelation of the polymer when topically instilled into the cul-de-sac. Cohen et al. demonstrated that also alginates with a high guluronic/mannuronic ratio can be applied as a drug carrier for the prolonged ophthalmic delivery of pilocarpine through the in situ formation of calcium-crosslinked alginate gels. Verschueren et al. reported a marked increase in viscosity when potassium chloride is added to iota- and kappa-carrageenans.

Besides the viscosity increase of the vehicle, drug carrier systems such as nanoparticles, microspheres and liposomes were demonstrated to reduce the drainage loss of topically applied solutions and to increase the bioavailability of the drugs instilled.

The ocular mucoadhesive concept comprises the use of suitable natural and synthetic polymers, which attach to the mucus film coating the external surface of the eye. By increasing the precorneal residence time of the preparation, mucoadhesive polymers may have a good potential to increase the bioavailability of the drug instilled. Polymers incorporated most frequently in order to obtain an interaction with the mucus film are polyacrylic acid, hyaluronic acid and chitosan.

Peppas et al. defined bioadhesion as 'the attachment of natural or synthetic macromolecules to a biological substrate'. When the attachment occurs more specifically to a mucosal epithelium, this phenomenon is referred to as mucoadhesion. Both conjunctival and corneal epithelial cells can secrete mucus. The cornea does not possess goblet cells but mucus is secreted through a non-goblet cell pathway in addition to the membrane mucin on the apical cell surface (glicocalyx). The majority of mucins present in conjunctival and corneal mucus is likely to be of conjunctival goblet cell origin and hydrated by the lacrimal fluid.

At least three of the human mucins (MUC1, MUC4 and MUC5AC) are expressed by the epithelia of the ocular surface. The membrane-spanning mucin (MUC1) is produced by the entire corneconjunctival epithelium. MUC4 mucin is expressed by the human conjunctival epithelium, but is also found in a soluble form in the tear fluid. It has been spec-
ulated that the cleavage of transmembrane mucins can occur as a consequence of both proteolysis and/or alternative splicing events. The secretory gel-forming mucin (MUC5AC) is derived from the conjunctival goblet cells. Although the role of all mucins is assumed to protect the eye against desiccation and microbial invasion, the specific role of each mucin in the tear film remains to be determined.

A complete understanding of how and why certain macromolecules attach to a mucosal surface is not yet available but certain elements of the process are clear:

- the bioadhesive must spread over the substrate to initiate intimate contact and to increase the contact area,
- chains of the adhesive can interdiffuse into the mucus substrate to create a greater area of contact,
- forces of attraction and repulsion develop and, for successful mucoadhesion, the attraction force must dominate.

The general aim of the research work consists of improving bioavailability of ocular drugs by formulating mucoadhesive dosage forms. The first part focuses on the development of an oscillatory rheological method, contributing to the clarification of the exact nature of interactions between a polymer and mucin. Secondly, the in vitro technique developed is employed to assess the degree of interaction between various polymers and mucin, as well as the interaction mechanism. Finally, in vivo experiments on rabbits and human volunteers are performed to examine whether the results obtained with the in vitro rheological technique are in accordance with in vivo data. By comparing the in vitro results with the results obtained in vivo, the influence of the mucoadhesive interaction on the ocular kinetics of a tracer and/or a model drug is investigated.

MATERIALS

Carbopol® 974P NF (CP974), Carbopol® 980 NF (CP980), Carbopol® 1342 NF (CP1342) and Noveon AA1 (NOV) were obtained as a gift from BF Goodrich (Brussels, Belgium). Drum Dried Waxy Maize (DDWM) and xanthan gum (XG) were supplied by Cerestar (Vilvoorde, Belgium) and Kelco Co. (Surrey, UK), respectively. Na-Stearyl fumarate (NaSF) was a gift from Edward Mendell Co. Inc. (New York, USA). 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, fluorescein-sodium and Mucin - Type II: Crude from porcine stomach (Sigma Chemicals, Bornem, Belgium). Purified water was used throughout the experiments.

METHODS

a) Oscillatory shear rheology (mechanical vibrational spectroscopy)

A small strain experiment performed to investigate the linear viscoelastic behaviour of a viscoelastic liquid is the oscillatory rheological experiment, during which the viscoelastic sample is deformed sinusoidally.

\[ \gamma = \gamma_0 \cdot \sin(\omega t) \]  

(Eq. 1)

After a few cycles of start-up, the stress will oscillate sinusoidally at the same frequency \( \omega \) but in general shifted by a phase angle \( \delta \) with respect to the strain wave because of the viscoelastic time-dependent effect (Figure 1).

![Figure 1. Sinusoidally oscillating shear strain (dotted line) producing a sinusoidal stress phase (full line) shifted by an amount \( \delta \).](image)
\[
\sigma = \sigma_0 \cdot \sin(\omega t + \delta)
\]  
(Eq. 2)

The data are analysed by decomposing the stress wave into two waves of the same frequency, one in phase (\(\sin \omega t\)) and one \(\pi/2\) or 90° out of phase (\(\cos \omega t\)) with the strain wave, representing the elastic solid-like response and viscous liquid-like response, respectively. Thus,

\[
\sigma = \sigma' + \sigma'' = \sigma'_0 \sin \omega t + \sigma''_0 \cos \omega t
\]  
(Eq. 3)

And,

\[
\tan \delta = \frac{\sigma''_0}{\sigma'_0}
\]  
(Eq. 4)

This decomposition suggests two dynamic moduli \(G' = \sigma'_0/\gamma_0\) (the in-phase, storage, or elastic modulus) and \(G'' = \sigma''_0/\gamma_0\) (the out-of-phase, loss, or viscous modulus). After considering equation 4,

\[
\tan \delta = \frac{G''}{G'}
\]  
(Eq. 7)

b) Oscillatory shear rheological procedures to characterise polymer networks\(^{59-61}\)

The in vitro method adopted to determine mucocoehesion is based upon the difference between the viscoelastic behaviour of a polymer solution, a physically entangled polymer network and a polymer network with secondary bonds (graphical illustration in Figure 2). The left part of the figure is a schematic representation of the different kinds of polymer configurations studied, while the right part shows the resulting conformations after applying a stress. The polymer solution (Figure 2.A) consists of non-interacting polymer chains. The polymer dispersion is not stabilised through the formation of a network. Imposing a stress results in an alignment of the linear chains in the direction of the stress. Interactions between the polymer chains can result in network formation. The basic principle of the interaction mechanism can be a physical interpenetration or a secondary chemical bond. Physical interpenetration results in a network structure, which is generally characterised as 'physically entangled' (Figure 2.B). The polymer chains are solely entangled but not chemically connected to each other. Therefore, the network is called 'transient'. By applying a stress, the polymer network is severed due to disentanglement of the polymer chains. However, when the polymer network is strengthened by secondary bonds (Figure 2.C), a 'permanent' instead of a 'transient' network is created. If the stress applied is smaller than the strength of the secondary bond, the deformation is small and does not result in a lasting deformation.

The main rheological strategy adopted in general to distinguish between entanglement network solutions, and physical and chemical gels is the following:

1. Measuring the elastic and viscous modulus versus an increasing oscillatory stress (strain) at a constant radial frequency \(\omega\). A double logarithmic plot of
G' and G" versus the stress can be generated and the linear viscoelastic region can be determined.

This procedure is called 'Dynamic Stress Sweep'.

2. Measuring the elastic and viscous modulus versus an increasing oscillatory angular frequency \( \omega \) at a constant stress (strain) situated in the linear viscoelastic region. This 'Dynamic Frequency Sweep' allows to derive the mechanical spectrum, where log G' and log G" are plotted versus log \( \omega \).

Rheological analyses were performed with a controlled stress rheometer (Carri-Med CSL² 100, TA Instruments, Brussels, Belgium) equipped with a 6 cm plate (low viscous samples) or a double concentric cylinder (highly viscous samples). Each procedure (dynamic stress sweep/dynamic frequency sweep) is performed three times on each dispersion. Mean values and standard deviations are calculated.

Although the main purpose of the stress dependence experiment is to determine the linear viscoelastic region, the results can also be applied effectively in the structural characterisation of the polymer network. For a dispersion of a high molecular weight polymer, three different situations can be encountered in the Dynamic Stress Sweep: G' >> G" for a chemically cross-linked system, G' >> G" for a network consisting of secondary bonds and G' >> G" for a physically entangled polymer dispersion.

The frequency dependence or mechanical spectrum of a dilute polymer solution is such that G" is proportionally related to \( \omega \) and G' to \( \omega^2 \), and G" >> G'. For higher polymer concentrations (Figure 3), G' and G" approach \( \omega^2 \) and \( \omega \) dependence only at very low frequencies. As the radial frequency is increased, G' equals G", resulting in a cross-over frequency \( \omega_c \). At still higher frequencies, G' and G" become much less \( \omega \) dependent, and an entanglement network with a high frequency plateau (G' > G") exists. Due to the high oscillation frequency, the time available is too short to enable disentanglement of the polymer chains. The time needed to disentangle is the relaxation time \( \tau \), which is generally defined by the reverse of the cross-over frequency \( \omega_c \).

For both strong and weak gels (Figure 4), the results are very different with G' > G" and both moduli being largely independent of frequency. This behaviour is sustained even if the experiment is extended down to very low frequencies. Due to the secondary interactions, the bonds are fixed irrespective of the angular frequency (time scale) applied.

In the case of an entangled dispersion, this structural behaviour results in a limiting slope = 2 for G' and slope = 1 for G" at low frequency in a log-log plot of moduli versus frequency, while at intermediate fre-
quency a plateau develops (Figure 3). For a network of secondary bonds, an almost constant value of $G'$ and $G''$ is observed over the whole frequency range, with the value of $G'$ exceeding that of $G''$ (slope = 0) (Figure 4). However in practice, the situation is generally not as straightforward as explained here; both $G'$ and $G''$ are slightly frequency-dependent, both increasing with $\omega$.

c) In vitro rheological determination of mucoadhesion

To prepare a polymer dispersion ($P_d$) the required amount of polymer powder (\%, w/w) is dispersed in an iso-osmotic phosphate buffer solution (PBS) pH 7.4. The dispersion is stirred at the appropriate temperature using a magnetic stirrer until the polymer powder is hydrated. To ensure complete hydration the dispersions are stored in the refrigerator ($6^\circ \pm 2^\circ$C) for a minimum of 12h.

Simulated Lachrymal Fluid (SLF) is an electrolyte solution containing 1.7893 g/1 KCl, 6.3118 g/1 NaCl, 2.1842 g/1 NaHCO$_3$, 44.4 mg/1 CaCl$_2$ and 47.6 mg/1 MgCl$_2$. The pH was adapted to a physiological value (7.4 ± 0.1) by adding the required amount of 0.1 N HCl$^2$.

To prepare the mucin dispersion (M), the required amount of mucin powder (\%, w/w) is dispersed in SLF using a magnetic stirrer. The mucin dispersions are stirred for 24 h at room temperature to allow complete hydration.

To study the mucoadhesive interaction, equal amounts (w/w) of polymer dispersion $P_d$ and mucin dispersion M are mixed, which results in reducing the actual concentration of both polymer and mucin in half (Fig. 5). This dilution procedure simulates as much as possible the in vivo situation after the instillation of an eye drop. The maximum volume of the precorneal tear film is about 10 µl, while the maximum volume which can be held into the cul-de-sac without overflow to the cheek is about 20 µl. This means that instillation of an ideal 10 µl eye drop results in a 1:1 dilution with lachrymal fluid$^{63,64}$.

An interaction between the polymer and mucin - either physical entanglements or secondary bonds - should be regarded as a synergistic effect in the rheological properties, which means that the rheological response of the $P_d$/M mixture should be larger than the "sum" of the rheological responses of the "single components"$^{65}$. Therefore, it is essential to characterise the "single components" as well as the $P_d$/M mixture. The first single-component reference dispersion ($P_d$/SLF) is a mixture containing equal amounts of $P_d$ and SLF. The rheological behaviour of this dispersion depends on polymer/polymer, polymer/PBS and polymer/SLF interactions. The second single-component reference dispersion (M/SLF) consists of M mixed with an equal amount of SLF, and characterises the mucin/mucin and mucin/SLF interactions$^{64}$.

Whereas the interpretation of the dynamic stress sweep results is mainly directed at the assessment of the degree of interaction (MucoAdhesive Index MAI) between a polymer and mucin [calculation of MAI (G) and MAI(G')] (eq. 8,9), the determination of the type of interaction can be derived from the mechanical spectra derived from the dynamic frequency sweep results$^{66-69}$.
MAI(G') = mucoadhesive index calculated with G'
= G'(P/M) \cdot G'(P/SLF) \cdot G'(M/SLF) \quad \text{(Eq. 8)}

MAI(G") = mucoadhesive index calculated with G"
= G"(P/M) \cdot G"(P/SLF) \cdot G"(M/SLF) \quad \text{(Eq. 9)}

If the mucoadhesive indexes are positive, which means that there are interactions between mucin and the polymer, the dynamic frequency sweep results can indicate whether physical entanglements or secondary chemical bonds are responsible for these interactions. As was mentioned before, it is important that the measurements are performed in the low frequency range because then the difference between the slope values of physically entangled networks and secondary bond networks is most obvious (Figure 3 and 4). Several response parameters can be considered. The slope of logG'/logω varies between 0 and 2 for respectively a perfect network of secondary bonds and a solution without any secondary bonds; the slope of logG"/logω varies between 0 and 1 under the same conditions. Therefore, the kind of interaction can be derived from the difference between the slope values of the mechanical spectra of P/M, P/SLF and M/SLF mixtures. If the slopes of the mechanical spectra of the P/M mixture are smaller (closer to zero) than the corresponding slopes of the mechanical spectra of P/SLF and M/SLF, the formation of additional secondary bonds after mixing the polymer (dispersion or powder) and mucin can be confirmed.

Another parameter which can be used as a response parameter to verify the kind of interaction is the cross-over frequency ωc (angular frequency at which log G' equals log G") or the relaxation time τ (=1/ωc). If a mucoadhesive interaction by additional secondary bond formation is present, ωc of the P/M mixture is smaller than ωc(P/SLF) and ωc(M/SLF) [or τ(P/M) is larger than τ(P/SLF) and τ(M/SLF)]69.

d) Multifactorial analysis using an experimental design approach

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 setup of the design and the calculation of the effects of the different factors are performed with Statgraphics® software version 4.0 (Manguistics Inc., Rockville, MD, USA).

The different effects and their interactions are estimated using equation 10.

\[ \Sigma(F+) - \Sigma(F-) \]
\[ \text{Effect } (F) = \frac{\Sigma(F+) - \Sigma(F-)}{n} \quad \text{(Eq. 10)} \]

with F = factor (or combination of factors) under investigation
\[ \Sigma(F+) = \text{sum of responses at a positive level of } F_{(+)} \]
\[ \Sigma(F-) = \text{sum of responses at a negative level of } F_{(-)} \]
\[ n = \text{number of responses at } F_{+} \text{ or } F_{-} \]

Apart from the main effects, also the interactions between the factors are calculated. An interaction is significant if the effect of a factor depends on the level of another factor. If the effect of changing factor A from level -1 to level +1 is different depending on the level of factor B, the interaction AB between factor A and B is significant.

To test the statistical significance of the effects, a variance analysis (ANOVA) is performed, which partitions the variability in the response factor into separate parts 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, centerpoints (0,0) and replicates are incorporated in the design. Only when an effect has a p-value less than 0.05, it is considered significantly different from zero at the 95.0% confidence level70,71.

e) In vivo performance of opthalmic formulations showing an in vitro interaction with mucin

Performing in vivo measurements is an essential part
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in the investigation of the mucoadhesive behaviour of ophthalmic formulations in order to verify whether a polymer defined as mucoadhesive by an *in vitro* technique is capable to accomplish a significant *in vivo* effect. More specifically, the ability of the polymer preparations to prolong the precorneal residence time of a tracer (fluorescein sodium) is examined.

Fluorophotometry of the anterior segment of the human eye was performed to investigate the anterior eye kinetics after application of a liquid or a solid ocular dosage form containing a fluorescent tracer (FluoroTron™ Master, Ocumetrics, Mountain View, CA, USA). A special lens (anterior segment adapter) is employed in the commercial fluorophotometer instead of the standard lens for detailed scanning of the anterior segment of the eye. The magnification of this special lens is about two times larger compared to the standard lens. On a scan made with the anterior chamber adapter only the fluorescence from the first half of the eye (cornea + tear film, anterior chamber and lens) is registered (Figure 6).

The apparent fluorescein sodium concentration (ng/ml) in the cornea/tear film compartment and in the anterior chamber compartment are determined at regular time intervals after application. The measuring protocols applied in the present study were those approved within the framework of a concerted action of the European Community biomedical program on ocular fluorophotometry. The basic principles of clinical research formulated in the World Medical Association Declaration of Helsinki were also taken into account.

After a thorough explanation of the aim of the study, eight healthy volunteers (5 men/3 women) agreed to participate in the study and signed an informed consent. Each preparation was applied three times to each volunteer, after which mean values and standard deviations were calculated.

Before the application of each preparation, the autofluorescence level (the intrinsic fluorescence due to the presence of endogenous fluorophores) of the cornea/tear film and anterior chamber compartment is determined to correct the values obtained after application of the preparation for autofluorescence. To verify whether the autofluorescence is not increased by the presence of the preparation, a blank preparation without fluorescein sodium is applied, after which the autofluorescence is determined as a function of time.

After determination of the autofluorescence pattern, 10 µl of the liquid preparation (sterile solution or polymer dispersion) is instilled using a sterile Eppendorf pipette or one solid preparation (minitablet) is applied into the cul-de-sac. At well-defined time periods, the concentration of the tracer in the cornea/tear film and the anterior chamber is measured. In case of a liquid preparation, the tracer concentration in both compartments is determined every 2 minutes during 1/2 hour. In the case of the solid formulation, the time interval after application can be divided into two separate parts: during the first hour after application, the measuring frequency is very high (every 5 minutes during the first quarter, every 10 minutes during the second and the third quarter and every 15 minutes during the fourth quarter); during the following 8 hours, one measurement is performed each hour (2, 3, 4, 5, 6, 7, 8 and 9 hours after application).

The Tear TurnOver (TTO; %/min) is the percentage decrease of the fluorescein concentration in the tear film per minute due to the basal tear flow. The TTO...
after instillation of the preparation, expressed as percent per minute, is defined as:

\[ TTO = 100 \cdot (1 - e^{-kt}) \quad (\% / \text{min}) \quad (\text{Eq. 11}) \]

Further assuming that the fluorescence measured is proportional to the fluorescein concentration in the cornea/tear film compartment, \( k_t \) is equal to the decay constant as obtained by exponential regression to the data points obtained (Fig. 7).

In case of a solution, the fluorescence decay is due to normal lachrymal drainage. However, in case of a polymer dispersion or a solid dosage form, the decay of the tracer is influenced by the elimination of the preparation (hydration of the preparation, dissolution and diffusion of the tracer out of the polymer network). Therefore, an Apparent Fluorescein TurnOver (AFTO) is defined:

\[ \text{AFTO} = 100 \cdot (1 - e^{-ke}) \quad (\% / \text{min}) \quad (\text{Eq. 12}) \]

Where \( k_e \) = apparent elimination coefficient (min\(^{-1}\)) of the monophasic decay of the cornea/tear film fluorescein concentration after application of a preparation. The first three measuring points of the fluorescence decay curve, which are influenced by reflex blinking and tearing, are excluded to calculate \( k_e \) and AFTO (Figure 7).

Besides the measurement of the tracer concentration in the tear film, the bioavailability of both liquid and solid dosage forms is investigated by measuring the apparent fluorescein concentration in the anterior chamber as a function of time. The parameters used to characterise the kinetic profiles of the preparations are \( C_{\text{max}} \) (maximum apparent fluorescein concentration achieved; ng/ml), \( t_{\text{max}} \) (time at which \( C_{\text{max}} \) is reached; min.) and \( C_{9h} \) (apparent fluorescein concentration 9 hours after application of the preparation; ng/ml).

**RESULTS AND DISCUSSION**

a) *In vitro* investigation of the mucoadhesive capacity of polyacrylic acid derivatives

In the present study, an experimental design approach is implemented to investigate simultaneously the effect of various parameters (polyacrylic acid concentration, mucin concentration, kind of Carbopol and influence of sonication) and the interaction between these parameters on the polymer/mucin interaction. 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. Statistical analysis of the experimental design data allows assessing the parameters influencing most significantly 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 clarified fully by carrying out a thorough rheological analysis of the carbomer/mucin mixture.

**Rheological characterisation of carbomer dispersions**

Rheological analysis of the 0.05% (w/v) polyacrylic acid dispersions demonstrate that CP1342, having a long alkyl chain, behaves most elastically. CP974 is
the least elastic carbomer examined, and CP980 shows intermediate behaviour. This order of ranking is maintained at higher concentrations, although the difference between CP974 and CP980 is reduced to a minimum at a 0.20% (w/v) carbomer concentration. After sonication, CP980 behaves most elastically.

The formation of secondary bonds can be observed already at 0.05% (w/v) for CP1342 and CP980. In the case of CP974P, the critical polymer concentration for the formation of secondary bonds amounts 0.125% (w/v). The sonication procedure increases this critical concentration to 0.125% (w/v) for CP980, and 0.20% (w/v) in the case of CP1342 and CP974.

Final conclusion is that the sonication procedure results 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 behaves most elastically, while CP980 seems to be the polymer being most resistant to the decrease of elasticity due to sonication.

Determination of factors influencing the response factors

The factors and levels employed 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.

<table>
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<tr>
<th>Factor</th>
<th>Level</th>
<th>A: Polymer concentration (% w/v)</th>
<th>B: Mucin concentration (% w/v)</th>
<th>C: Kind of Carbopol</th>
<th>D: Sonication</th>
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Table 2. Experimental design. Responses

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<tr>
<td>0 0 0 -1/+1</td>
<td>0.00 / 0.01</td>
<td>0.03 / 0.02</td>
</tr>
<tr>
<td>+1 -1 0 -1/+1</td>
<td>0.03 / 0.00</td>
<td>0.05 / 0.00</td>
</tr>
<tr>
<td>+1 +1 0 -1/+1</td>
<td>0.24 / 0.02</td>
<td>0.13 / 0.05</td>
</tr>
<tr>
<td>-1 -1 +1 -1/+1</td>
<td>0.02 / 0.08</td>
<td>0.04 / 0.10</td>
</tr>
<tr>
<td>-1 +1 +1 -1/+1</td>
<td>0.25 / 0.32</td>
<td>0.23 / 0.24</td>
</tr>
<tr>
<td>0 0 +1 -1/+1</td>
<td>0.12 / 0.01</td>
<td>0.16 / 0.02</td>
</tr>
<tr>
<td>0 0 +1 -1/+1</td>
<td>0.18 / 0.07</td>
<td>0.21 / 0.09</td>
</tr>
<tr>
<td>0 0 +1 -1/+1</td>
<td>0.06 / 0.02</td>
<td>0.10 / 0.01</td>
</tr>
<tr>
<td>+1 -1 +1 -1/+1</td>
<td>0.05 / 0.06</td>
<td>0.06 / 0.06</td>
</tr>
<tr>
<td>+1 +1 +1 -1/+1</td>
<td>0.33 / 0.08</td>
<td>0.30 / 0.07</td>
</tr>
</tbody>
</table>

The effect of polymer concentration (factor A), mucin concentration (factor B) and sonication (factor D) is calculated for each Carbopol® examined (Table 3). For each Carbopol®, the interaction between the polymer and mucin is improved significantly when the mucin concentration is increased from 8 to 16% (w/v), as can be derived from the p-values of factor B being smaller than 0.05 for both MAI(G') and MAI(G") (p>0.05 for both MAI(G') and MAI(G") for CP1342, CP974 and CP980 NF. Sonication (Factor D), which can on the contrary result in the conversion of an elastic to an entangled network does not significantly influence the interaction as well (p>0.05 for both MAI(G') and MAI(G") for CP1342, CP974 and CP980 NF. Interactions AB, AC and BC (p>0.05) are not shown.)
In the 0.05-0.20% (w/v) concentration 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 is not influenced by the kind of polymer network (physically entanglements or secondary bonds). Several researchers 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 polymer employed. Although increasing the polymer concentration in the present study results in an increase of the secondary bond formation, the polymer chains probably remain sufficiently flexible to be able to interpenetrate into the mucin dispersion, even at the 0.20% (w/v) concentration level. Madsen et al. reported that rheological synergism due to an interaction between a polymer and mucin is found to arise only within a certain concentration range of the polymer. Although the polycrylic acid derivatives investigated by Madsen et al. (Carbopol 2984, Noveon AA-1 and Pemulen TR-1&2) are different from the derivatives examined in the present study, it can be assumed that the concentrations applied are smaller than the maximum polymer concentration corresponding with optimal mucoadhesion. However, polymer concentrations higher than 0.2% (w/v) were not considered to be applicable in ocular formulations investigated in this study. Increasing the polymer concentration results in an increase in viscosity, which causes discomfort to the patient. An uncomfortable feeling elicits an increase in blinking frequency and drainage rate, which is responsible for faster drug elimination. Sonication of the dispersions on the other hand, seems 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® dispersions were also compared two by two. The two-by-two analysis reveals that the positive effect of increasing the mucin concentration (factor B) on the degree of polymer/mucin interaction can again be observed for both response factors, as well before as after sonication (results not shown).

**Rheological characterisation of the optima**

The results of the experimental design analysis can be applied to define the optimal combination of the parameters investigated. Independently on the kind of Carbopol® used, the optimum is situated at the highest mucin concentration. The kind of Carbopol® or the application of a sonication procedure do not influence significantly the mucoadhesive interaction. Neither does the polymer concentration influence the interaction. 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 kinds of Carbopol® investigated is not considered significant in the experimental design study, an optimum is defined for each kind of Carbopol®. Although the mucoadhesive indexes are very useful and actually essential to be able 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 MAI(G') optimum (=0.54) is different from the MAI(G') optimum (=0.47). However, since the mucoadhesive interaction is mainly correlated with the formation of an elastic network, only the MAI(G') optimum is further analysed rheologically (Fig. 8). Both stress and frequency sweep curves show that the CP1342/M mixture behaves more elastically compared to CP1342/SLF and M/SLF. In the dynamic stress sweep curves the synergistic rheological effect after mixing CP1342 and mucin is illustrated by: (1) CP1342/M having the longest linear region, (2) G'(CP1342/M) being larger than G'(CP1342/M) and (3) the dimensional difference between the dynamic moduli of the various mixtures. Furthermore, the frequency sweep data show that the cross-over frequency \( \omega_c \) of the CP1342/M mixture (=0.1 rad/s or 0.016 Hz) is lower compared to \( \omega_c \) M/SLF (=0.34 rad/s or 0.05 Hz). The relaxation
time \(\tau = 1/\omega_c\) of the bonds present in CP1342/M \((\tau = 63 \text{ s})\) is longer compared to M/SLF \((\tau = 18 \text{ s})\), confirming the formation of additional secondary bonds in the CP1342/M mixture. The \(\omega_c\) value of CP1342/SLF cannot be determined because of the absence of a linear region and is therefore neglected.

The interaction between CP974 and mucin is optimal when mixing a sonicated 0.05\% (w/v) dispersion with mucin 16\% (w/v) [Table 2; MAI\(_G\) = 0.39 and MAI\(_{G'}\) = 0.34]. The rheological analysis of the CP974 optimum is shown in Figure 9. The linear region being the longest in the case of the CP974/M mixture and the dimensional difference between the CP974/M, CP974/SLF and M/SLF mixtures are factors which are comparable to CP1342, but \(G'(\text{CP974/M}) > G''(\text{CP974/M})\) is not as pronounced. Furthermore, the CP974/SLF curves (values of \(G' > G''\)) indicate that the electrolytes present in simulated lachrymal fluid can also increase the elasticity due to interaction with CP974 molecules. The cross-over frequencies of CP974/M and M/SLF are comparable \((\omega_c = 0.26 \text{ rad/s} \text{ or } 0.04 \text{ Hz})\), which indicates that the bonds in the CP974/M mixture are comparable to the bonds in M/SLF.

The CP980 optimum (rheological analysis presented in Figure 10) is achieved after mixing the unsonicated 0.20\% (w/v) dispersion with mucin 16\% (w/v). The stress sweep data are very comparable to the CP1342...
actions of the different kinds of Carbopol® derivatives are not significantly different. When MAI\(_{\text{G}'}\) and MAI\(_{\text{G}''}\) mentioned in Table 2 are taken into consideration, the optimal mucoadhesive interaction can be assigned to the sonicated CP1342 0.20% (w/w)/mucin 16% (w/v) mixture (MAI\(_{\text{G}'}\) = 0.54 and MAI\(_{\text{G}''}\) = 0.38). However, the full rheological characterisation of the three mixtures prepared to study the polymer/mucin interaction (CP/M, CP/SLF and M/SLF) reveals that the cross-over frequency shift to the low frequency region after mixing the polymer and mucin, is most pronounced in the case of CP980 (0.34 rad/s \(\rightarrow\) < 0.1 rad/s). This cross-over frequency shift is a clear indication for the additional formation of strong elastic bonds after mixing both components.

When CP1342, CP974 and CP980 are the Carbopol® derivatives available to prepare a viscous eye drop, CP980 is the best choice if no additional sonication procedure is required to prevent clump formation. 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.

**CONCLUSIONS**

The 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, independently on the kind of Carbopol® used. This finding implicates that if any interaction between a poly(acrylic acid) derivative and ocular mucin occurs, this interaction is possible only close to the 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 tear film can be excluded.

Although the experimental design study indicated that the difference between the mucoadhesive interactions of the Carbopol® derivatives is not significant,
the full rheological characterisation enabled a further
discrimination between the derivatives. The 0.20% (w/v) Carbopol® 980 dispersion shows a clear sec-
ondary bond interaction with mucin 16% (w/v). The
formation of secondary elastic bonds also occurs
when mixing the sonicated 0.20% (w/v) Carbopol®
1342 dispersion and mucin 16% (w/v), but not as
pronounced as with Carbopol® 980. The interaction
between Carbopol® 974 and mucin is very limited,
indpendently on the conditions applied during
preparation.

When incorporating polyacrylic acid into an oph-
thalmic formulation to prepare viscous eye drops,
both Carbopol® 980 and Carbopol® 1342 can be em-
ployed. The preparation procedure determines
whether Carbopol® 980 or Carbopol® 1342 should be
used. If an additional sonication procedure is imple-
mented to prevent clump formation, maximum mu-
coadhesive interaction is obtained with the 0.2% (w/
w) Carbopol® 1342 dispersion. If, however, no ad-
ditional sonication procedure is necessary, the use of
the 0.2% (w/w) Carbopol® 980 dispersion is re-
commended.

c) Influence of xanthan gum and Carbopol® 980 on
the elimination kinetics of a fluorescent tracer

A polymer incorporated in a liquid ocular formula-
tion can be considered mucoadhesive if it is able to
interact with the mucus layer in the tear film through
the formation of secondary bonds, after the physical
entanglement of both components.

The preceding study reveals that a significant mu-
coadhesive in vitro effect can be obtained by dis-
persing 0.2% (w/v) Carbopol® 980 in a proper aque-
ous medium (5.07% mannitol solution pH 7.4). How-
ever, since the experimental conditions of an in vitro
study can never simulate completely the in vivo sit-
uation, the implementation of an additional in vivo
study to verify the results obtained with the in vitro
rheological approach is imperative.

The 0.2 % (w/w) Carbopol® 980 dispersion and a
1.0% (w/w) xanthan gum dispersion (another poly-
mer considered to be mucoadhesive) are prepared
aseptically by dispersing the required amount of pol-
ymer powder in an iso-osmotic 5.07% (w/v) man-
nitol solution and a phosphate buffer solution (PBS)
pH 7.4, respectively. To adjust the pH of the carbom-
er dispersion to physiological conditions, the re-
quired amount of NaOH 1M is added. A sterile refer-
cence solution containing only 0.1% (w/v) Fluorescein
sodium in an iso-osmotic phosphate buffer solution
pH 7.4 is also prepared.

The results of the calculation of the Apparent Fluo-
rescein TearTurnover (AFTO) for both reference solu-
tion and polymer dispersions are presented in Table
4. The mean value for reference solution, xanthan
gum dispersion and carbomer dispersion is 8.33 %/min,
10.11 %/min and 8.68 %/min, respectively. The
AFTO data were analysed statistically using a two-
factorial ANOVA-analysis to investigate the in-
fuence of the experimental factors (volunteer and
preparation). The analysis indicates that the factor
'preparation' has a significant influence on the AFTO
(p=0.025), while the factor 'volunteer' has no in-
fuence (p<0.05). There is no significant interaction
between the two factors (p<0.05). Comparison of the
three preparations investigated using a Student New-
man-Keuls (SNK) test reveals that the AFTO after ap-
lication of the xanthan gum dispersion is signifi-
cantly different from both the reference solution (Fluo-
rescein sodium) (p = 0.010) and the CP 980 dispersion
(p = 0.030). Furthermore, the SNK test indicates that
the AFTO data after the application of the reference
solution and the CP980 dispersion are not signifi-
cantly different (p < 0.05). Most important conclusion is
that none of the polymer dispersions investigated is
able to decrease the Apparent Fluorescein TearTurn-
over (AFTO) of fluorescein sodium in a bio-
pharmaceutically significant way. All liquid formula-
tions studied are unable to increase the fluorescence
of the anterior chamber compartment.
Table 4. Mean Apparent Fluorescein Turn Over (%/min)

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Reference solution</th>
<th>Xanthan gum</th>
<th>Carbopol 980</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11.09 (3.99)</td>
<td>10.67 (0.92)</td>
<td>7.58 (2.24)</td>
</tr>
<tr>
<td>B</td>
<td>7.08 (1.27)</td>
<td>7.43 (2.60)</td>
<td>10.50 (0.46)</td>
</tr>
<tr>
<td>C</td>
<td>5.65 (0.68)</td>
<td>10.55 (0.55)</td>
<td>8.27 (0.67)</td>
</tr>
<tr>
<td>D</td>
<td>9.74 (0.80)</td>
<td>8.46 (0.81)</td>
<td>8.39 (1.85)</td>
</tr>
<tr>
<td>E</td>
<td>8.10 (1.59)</td>
<td>9.93 (1.31)</td>
<td>9.14 (0.76)</td>
</tr>
<tr>
<td>F</td>
<td>8.32 (0.33)</td>
<td>9.89 (2.66)</td>
<td>9.78 (1.41)</td>
</tr>
<tr>
<td>G</td>
<td>8.91 (6.35)</td>
<td>12.12 (6.07)</td>
<td>6.56 (1.83)</td>
</tr>
<tr>
<td>H</td>
<td>7.96 (2.17)</td>
<td>11.86 (0.95)</td>
<td>9.25 (0.03)</td>
</tr>
</tbody>
</table>

(n=3) (SD between brackets)

The explanation of the difference between the in vitro and in vivo results obtained with Carbopol® 980 is related with the following explanations: (1) the in vitro and in vivo mixing methods are different (stirring vs. blinking); (2) the in vitro mucin concentrations are purely hypothetical; (3) the test molecules diffuse out of the polymer network due to their small molecular size. However, the results from the in vitro experiments allow formulating these explanations more clearly. Obtaining a significant interaction between the polymer dispersion and mucin seems to be feasible only when the mucin concentration is sufficiently high (>16%). Considering the theory of the mucin gradient in the tear film, a highly concentrated mucus layer can be situated only close to the epithelium. If the presence of a mucus layer having a concentration close to 16% could be confirmed (which is not the case until now), the mixing efficiency of the blinking movement has to be very high to accomplish an interaction between the incorporated polymer and the concentrated mucus layer.

Only when these conditions (presence of a highly concentrated mucus layer and high mixing efficiency) are fulfilled, the polymer can possibly interact with the mucus layer (physical entanglements/secondary bonds) and behave as a mucoadhesive. However, to obtain a significantly increased bioavailability, the precorneal residence time of the drug (or tracer) should be lengthened, which can be achieved only if the molecular size of the molecule is higher than the meshes present in the polymer/mucin network. Otherwise, the drug (or tracer) diffuses out of the network and the bioavailability remains at a low level.

d) In vivo examination of the effect of an ocular mini-tablet on the elimination kinetics of a fluorescent tracer in human volunteers

Although an in vitro mucoadhesive interaction can be obtained if the experimental variables are optimised, the in vivo capability of liquid polymer formulations to lengthen the precorneal residence time or to increase the bioavailability is comparable to an aqueous solution and therefore very limited. In the case of fully hydrated polymers the mucoadhesion mechanism is almost solely related to surface energy thermodynamics and/or interpenetration phenomena. The mucoadhesion mechanism of a dry polymer formulation is, however, probably partly different from the interaction mechanism of a fully hydrated polymer dispersion. This can be explained by the water transfer from mucus to the dosage form, since the adhesive and cohesive nature of the mucus gel increases when the water content of the formulation is decreased. Therefore, the use of dry solid preparations to obtain an ocular mucoadhesive effect is investigated.

The in vivo elimination kinetics of fluorescein sodium is assessed after applying a solid and a liquid dosage form, prepared with a polymer mixture consisting of 5% Carbopol® 974P, 1% stearyl fumarate sodium and 94% Drum Dried Waxy Maize Starch (CPDD). The preparation of the polymer dispersion is similar to that of the CP980 dispersion. A sterile reference solution containing only 0.1% (w/v) fluorescein sodium in an iso-osmotic phosphate buffer solution (PBS) pH 7.4 is also prepared. Minitablets (Ø: 2 mm, weight: 5 mg) containing 2% fluorescein sodium, 1% stearyl fumarate sodium, 5% Carbopol® 974P and 92% Drum Dried Waxy Maize are prepared by direct compression at a compression force of 0.16-0.18 kN. As a reference preparation, blank tablets without fluorescein sodium are made. The acceptability of the polymer preparations is assessed by a questionnaire (Table 5).
Table 5. Questionnaire to evaluate preparation acceptability.

<table>
<thead>
<tr>
<th>Parameter evaluated</th>
<th>Question</th>
<th>Irritation scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular irritation (general)</td>
<td>Does the preparation cause a painful sensation at the eye?</td>
<td>No irritation (0), Mild (1), Hurting (2-3), Stinging (4-5)</td>
</tr>
<tr>
<td>Ocular irritation (puncti)</td>
<td>Does the preparation cause a painful sensation at the eye?</td>
<td>No irritation (0), Mild (1), Hurting (2-3), Stinging (4-5)</td>
</tr>
<tr>
<td>Lachrymation</td>
<td>Does the preparation increase lachrymation?</td>
<td>Eye watery (0-2), Lachrymation (3-4), Overflow onto the cheek (5)</td>
</tr>
<tr>
<td>Sensation</td>
<td>How does the preparation sense?</td>
<td>Smooth (0), Thick (1), Sticky (2), Gritty (3), Sandy (4-5)</td>
</tr>
<tr>
<td>Vision</td>
<td>Does the preparation cause blurring of the vision?</td>
<td>Clear (0), Blurred Vision (1-5)</td>
</tr>
</tbody>
</table>

Elimination from the cornea/tear film compartment

According to the measuring protocols applied, the presence of a monophasic elimination pattern is an important requisite to carry out an exact calculation of the Apparent Fluorescein Turnover. As this monophasic elimination pattern was not shown by volunteer F after application of the minitablet, probably due to a high and irregular blinking frequency, the data were excluded when calculating mean values and performing the statistical analysis.

The results of the calculation of the Apparent Fluorescein Turnover for both liquid and solid preparations are presented in Table 6. The AFTO data were analysed statistically using a two-factorial ANOVA-analysis to investigate the influence of both factors: volunteer and preparation. The analysis revealed that the factor 'preparation' has a significant influence on the AFTO (p<0.001), while the factor 'volunteer' has no influence (p>0.05). The interaction between both factors is not statistically significant (p>0.05). Comparison of the three preparations investigated using a Student Newman-Keuls (SNK) test reveals that the AFTO after application of the minitablet is significantly different from both the reference solution (p = 0.001) and the dispersion (p = 0.001). Furthermore, the SNK test indicates that the AFTO data after the application of the reference solution and the polymer dispersion are not significantly different (p > 0.05).

Table 6. Mean Apparent Fluorescein TurnOver (%/min) (n=3) (SD between brackets) after application of reference solution (0.1% fluorescein sodium in PBS), polymer dispersion (3.00 % CPDD and 0.1% fluorescein sodium in 5.07% mannitol solution) and minitablet (2 % fluorescein sodium in CPDD)

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Reference solution</th>
<th>Polymer dispersion</th>
<th>Minitablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11.09 (3.99)</td>
<td>9.99 (0.73)</td>
<td>0.63 (0.29)</td>
</tr>
<tr>
<td>B</td>
<td>7.08 (1.27)</td>
<td>12.31 (1.23)</td>
<td>0.93 (0.24)</td>
</tr>
<tr>
<td>C</td>
<td>5.65 (0.68)</td>
<td>8.98 (0.63)</td>
<td>0.37 (0.19)</td>
</tr>
<tr>
<td>D</td>
<td>9.74 (0.80)</td>
<td>10.08 (1.85)</td>
<td>0.55 (0.19)</td>
</tr>
<tr>
<td>E</td>
<td>8.10 (1.59)</td>
<td>7.61 (1.53)</td>
<td>1.02 (0.09)</td>
</tr>
<tr>
<td>F</td>
<td>8.32 (0.33)</td>
<td>8.47 (2.43)</td>
<td>NM (NM)</td>
</tr>
<tr>
<td>G</td>
<td>8.91 (0.35)</td>
<td>7.12 (3.34)</td>
<td>0.55 (0.37)</td>
</tr>
<tr>
<td>H</td>
<td>7.96 (2.17)</td>
<td>9.09 (0.71)</td>
<td>0.95 (0.23)</td>
</tr>
</tbody>
</table>

(NM = not measurable due to a high and irregular blinking frequency)

Elimination from the anterior chamber compartment

An increase of the apparent fluorescein concentration in the anterior chamber is achieved only after application of the minitablet into the cul-de-sac. In case of a liquid dosage form, the apparent fluorescein concentration remains at autofluorescence levels.

The mean values and standard deviations of the parameters characterising the kinetic profile \(C_{\text{max}}, t_{\text{max}}\) and \(C_{9\%}\) of each volunteer after application of a minitablet are summarised in Table 7. A typical corresponding kinetic profile is shown in figure 11. The average value of \(C_{\text{max}}, t_{\text{max}}\) and \(C_{9\%}\) for the 8 volunteers is 22.2 ng/ml, 317 min and 9.7 ng/ml, respectively. These data demonstrate that CPDD is able
to act as a prolonged release preparation when formulated as a minitablet.

**Table 7.** Mean value of the kinetic parameters characterising the decrease of the apparent fluorescein sodium concentration in the anterior chamber as a function of time after application of a minitablet (n=3) (SD between brackets)

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>( C_{\text{max}} ) (ng/ml)</th>
<th>( t_{\text{max}} ) (min)</th>
<th>( C_{\text{sh}} ) (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9.65 (1.20)</td>
<td>292 (3)</td>
<td>3.65 (0.81)</td>
</tr>
<tr>
<td>B</td>
<td>12.45 (5.58)</td>
<td>340 (26)</td>
<td>5.55 (2.36)</td>
</tr>
<tr>
<td>C</td>
<td>27.45 (4.31)</td>
<td>334 (36)</td>
<td>17.32 (3.19)</td>
</tr>
<tr>
<td>D</td>
<td>55.02 (4.12)</td>
<td>295 (6)</td>
<td>17.81 (2.35)</td>
</tr>
<tr>
<td>E</td>
<td>13.02 (3.05)</td>
<td>336 (33)</td>
<td>6.72 (1.00)</td>
</tr>
<tr>
<td>F</td>
<td>19.61 (11.52)</td>
<td>254 (34)</td>
<td>7.95 (4.31)</td>
</tr>
<tr>
<td>G</td>
<td>15.64 (11.67)</td>
<td>390 (25)</td>
<td>6.70 (0.77)</td>
</tr>
<tr>
<td>H</td>
<td>22.26 (18.25)</td>
<td>324 (42)</td>
<td>11.26 (5.64)</td>
</tr>
</tbody>
</table>

**Acceptability**

The acceptability scores of the polymer dispersion and the minitablet are shown in Figure 12. The general irritation score of the polymer dispersions is zero, indicating that the dispersions are well accepted by the volunteers. The dispersion causes a slight sensation in the eye, minimal lachrymation and blurred vision.

After a short instruction and training session, all volunteers were perfectly able to apply the minitablet.

**CONCLUSIONS**

An optimisation of the mucoadhesive interaction of a polyacrylic acid dispersion is performed *in vitro* using the oscillatory rheological technique. The formation of elastic secondary bonds is considered as the parameter to be optimised. The results indicate that the polymer concentration has to be as high as possible (0.2%), that is close to the maximum level ap-
Ceulemans, Ludwig

applicable without damaging the epithelium and still being comfortable after application. These highly concentrated polymer dispersions only interact significantly with highly concentrated mucin dispersions. The presence of such a highly concentrated mucus layer in the eye is questionable and still not demonstrated until now.

Main conclusion derived from the in vivo experiments is that liquid dispersions are unable to significantly increase the precorneal residence time or the bioavailability of fluorescein sodium. This negative effect is presumably due to the lack of secondary bond formation between the polymer and mucin. Furthermore, it is important to realise that the formation of a mucoadhesive junction could still not guarantee the increase of the precorneal residence time and bioavailability of the drug molecule (or tracer). If the molecular size of the drug is smaller than the meshes present in the polymer/mucin network, diffusion out of the network and drainage can occur, resulting in a decrease of the therapeutic effect. The negative results obtained with the liquid dispersions give rise to another approach consisting of the application of dry dosage forms. In vivo comparison of the biopharmaceutical value, evaluated by the precorneal residence time and the bioavailability, of a minitablet and a polymer dispersion, both containing Carbopol® 974P and drum dried waxy maize starch as auxiliary agents, demonstrates the pronounced effect of hydration. The minitablet influences significantly both evaluation parameters (precorneal residence time and the bioavailability), contrary to the liquid dispersion.

Interpenetration and secondary bond formation can possibly contribute to the interaction between a polymer and mucin but are on their own insufficient to obtain a significant in vivo effect on the precorneal residence time and the ocular bioavailability. This final statement explains the negative results obtained with the liquid polymer dispersions (viscous eye drops) and illustrates the necessity to use a dry dosage form when developing an efficient ocular mucoadhesive preparation with the polymers investigated in the present study.

ACKNOWLEDGEMENTS

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