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

In vitro assessment of the interaction mechanisms between mucin and a cationic cellulosic derivative by rheological methods using an experimental design

W. WEYENBERG and A. LUDWIG

An oscillatory rheological method is used for the determination of the mucoadhesive properties of a cationic cellulosic derivative (JR-30M) proposed for ocular dosage forms and dry eye viscous solutions. An experimental design was set up to study the influence of the concentrations of the polymer and mucin on mucoadhesion. From the rheological data it can be deduced that the interactions between mucin and JR-30M are mainly based on physical entanglements and less on ionic interactions.

1. Introduction

The bioavailability of drugs in conventional aqueous eye drops is low because of the rapid elimination after instillation due reflex blinking, drainage and the barrier function of the cornea. A prolonged precorneal residence of the active substance is a prerequisite to achieve a high therapeutic effect [1]. Increasing the viscosity of the vehicle is a possible strategy to reduce the drainage rate [2]. More efficient, however, should be the use of polymers interacting with the mucus present in the tearfilm covering the eye surface [3]. This phenomenon is called mucoadhesion. The interactions required between mucin and polymers in order to create mucoadhesion can be described as follows. The two polymer chains must first get into intimate contact, and then mix with each other. After these physical entanglements a third step can occur, being the formation of secondary bonds between the polymer and the mucin. Interactions can be due to hydrogen bonds between the sialic acid and/or sulphate groups of mucin molecules and specific motives on polymers tested [4].
In the case of dry eyes a reduced tearfilm stability could be due to a low mucin production by the goblet cells in the conjunctiva and results in discomfort for the patient. Instillation of hydrophilic polymers with mucin-like properties is prescribed to restore the hydration of the eye surface and lubrication [5]. The present study aimed to investigate in more detail the interaction mechanisms between a cationic cellulose derivative proposed for dry eye formulations and anionic mucins [6].

2. Investigations, results and discussion

2.1. Experimental design

The use of an experimental design has the advantage to obtain a maximum on information with a minimum of experiments. In present study the evaluation of the influence of the polymer and mucin concentrations on the mucoadhesion is performed. Five points in a 2^2 full factorial design with centre point are used to investigate the mucoadhesion characteristics (Fig. 1). Three different dispersions (i.e. mucin or M, native polymer tested or P and a mixture of mucin and polymer tested or M/P) are made to characterise a point of the design. The mucoadhesive response at each point will be derived from the rheological measurements of these dispersions. A mucin or a polymer effect on the response obtained can be derived by comparing the data at two levels of the design model. A mucin effect can be derived by comparing level 1–4 with 2–5. The polymer effect can be deduced by comparison of level 1–2 with 4–5.

2.2. Rheological characterization

The first method developed by Hassan and Gallo to quantify the mucoadhesion of aqueous polymer dispersions was based on flow measurements [7]. Mortazavi et al. proposed oscillation measurements to evaluate mucoadhesion, because during these measurements the network of polymer chains present is not destroyed contrary to flow measurements [8]. In present study a controlled stress rheometer is employed to carry out two series of oscillatory measurements. Dynamic Stress Sweep (DSS) and Dynamic Stress Frequency (DFS) measurements are employed for the evaluation of the elastic (G') and viscous (G'') parameters of the samples. First Dynamic Stress

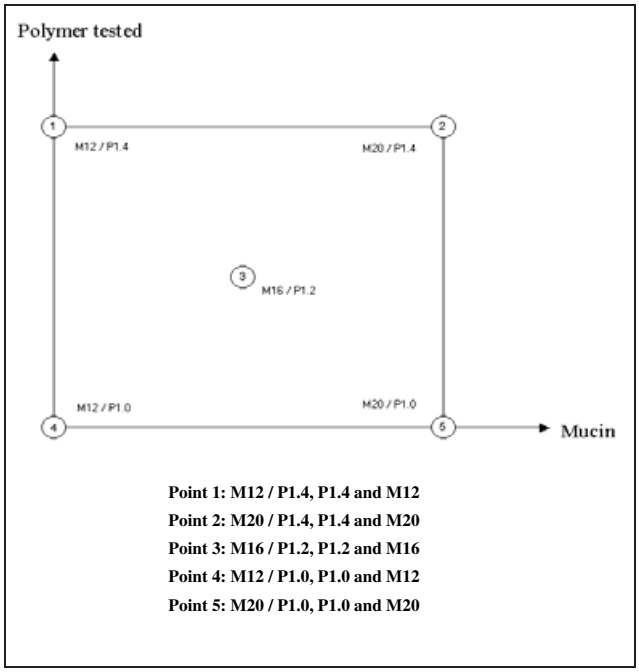

Fig. 1: Experimental design to evaluate mucoadhesion

Sweep measurements are performed to detect the linear viscoelastic region (LVER) of the dispersion, where the polymer network stays intact. The LVER is determined by the maximum stress which can be applied without decreasing G′ and G″ values. The relation between the strain and stress is only constant in this viscoelastic region. This relation is not more proportional after the LVER because of the destruction of the polymernetwork and a larger deformation of the sample due to the stress used resulting in a decrease of G′ values. Thus, analyses of DSS measurements allow the characterization of polymers, the force of their intermolecular bonds and their resistance towards the stress applied.

Three different situations can occur: G′ (elastic) $\gg$ G″ (viscous) for a chemically crosslinked system, G′ > G″ for a network consisting of secondary bonds and G′ < G″ values for a physically entangled polymer solution.

Another rheological response to be selected is the phase shift of the stress to the strain, defined by the loss angle δ, derived from tan (δ) = G″/G′. The larger (smaller) the phase difference between stress and strain, the larger (smaller) the loss angle δ and the more the sample has viscous (elastic) properties [9].

The Dynamic Stress Sweep measurement results indicate that a concentration increase of mucin from 12 to 20% (w/w) and cationic cellulosic derivative JR-30M from 1.0 to 1.4% w/w in the dispersion medium lead to an increase of the viscoelastic modulus G′ (Fig. 2). The G′ values obtained going from 0.15 Pa to 0.62 Pa at the oscillation stress of 0.01 Pa for M12/P1.0 and M20/P1.4 respectively. More interactions occur at higher concentrations and therefore the LVER is longer. The LVER for M20/P1.4 extends until 0.1 Pa, contrary to M12/P1.0 where it exists up to 0.04 Pa. Consequently more resistance is formed against the increasing oscillation stress at higher polymer concentrations.

The influence of the concentrations can also be derived from the delta versus oscillation stress curve (Fig. 3). The delta value (G″/G′) is lower in the case of M20/P1.4 due to a larger contribution by G′ (elastic properties) in comparison to M12/P1.0 as shown in Fig. 2. The delta values calculated are for each of the five points in the experimental design larger than 45°, which means that the dispersions prepared have mainly viscous properties [9].

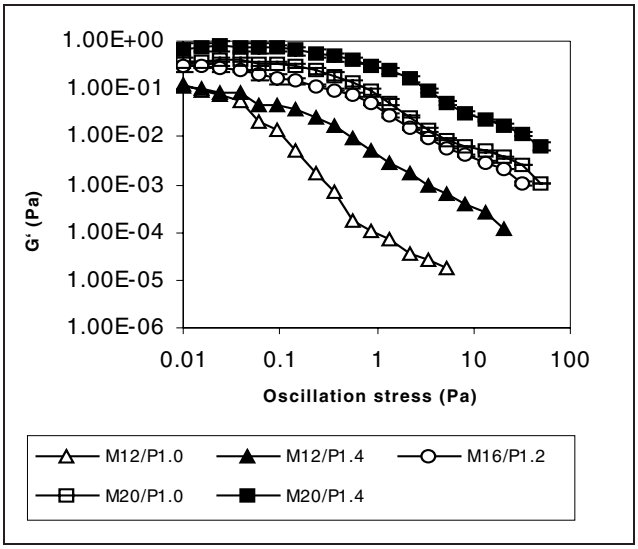

Fig. 2: The elastic properties of the different mucin/polymer dispersions derived from DSS measurements (n = 3)

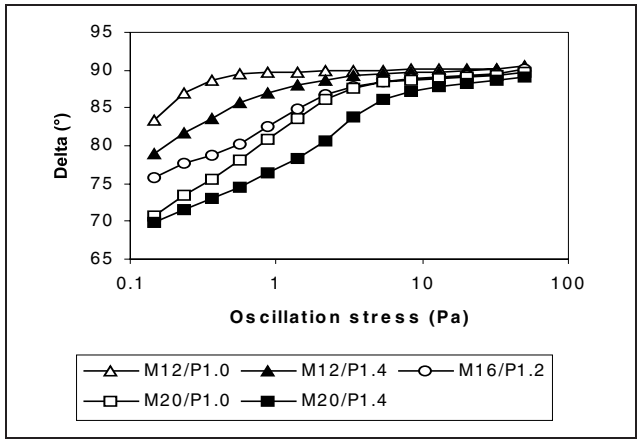

Fig. 3: The mean loss angle δ of the different mucin/polymer dispersions derived from DSS measurements (n = 3)

Interactions like physical entanglement or secondary bonds between the polymer and mucin should be seen as synergistic effects in the rheological properties. Consequently the rheological Dynamic Stress Sweep responses of the mucin polymer mixture (M/P) should be larger than the sum of the rheological responses of the single mucin (M) and polymer (P) dispersions. Therefore it is also essential to characterise the single components (M and P). Firstly the resulting Dynamic Stress Sweep graphs of M, P and M/P are compared. Mucin and the cationic polymer interact strongly if the elastic differences between P and M/P and between M and M/P are large. Little interactions between mucin and the polymer tested occur when the elastic differences between P and P/M and between P/M and M are small.

Fig. 4 shows that the G′ value of the mixture is larger than for the single mucin and polymer dispersions. This is due to an interaction between JR-30M and mucin, although the increase is rather low. The elastic compound of M/P indicates a larger resistance to the stress applied at higher oscillation stresses than the polymer dispersions M and P. Consequently interactions are present between mucin and the cationic cellulosic derivative JR-30M. The kind of such interaction can be derived from the Dynamic Frequency Sweep measurements. The DFS is carried out at an oscillation stress correspondingly to the LVER where the structure of the network is intact.

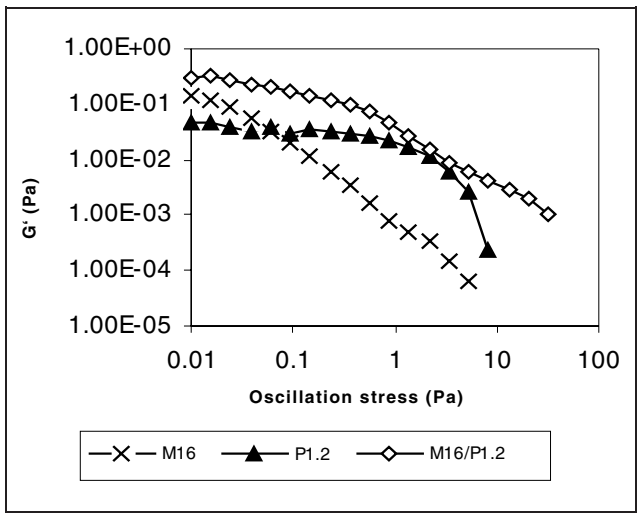

Fig. 4: The elastic properties of the dispersions used for the determination of mucadhesion (points of the experimental design (n = 3)

Two extreme situations in Dynamic Frequency Sweep can be considered: a sol and a gel state. In a sol state the polymer chains do not interact at low frequencies when the chains have enough time to disentangle. The viscoelastic properties of the system will decrease. However, when the oscillation frequency is larger the chains do not have enough time to unrafel, consequently more elastic properties appear at high frequencies. The structural behavior for an entangled solution results in a limiting slope value of 2 for G' and a value of 1 for G'' [10].

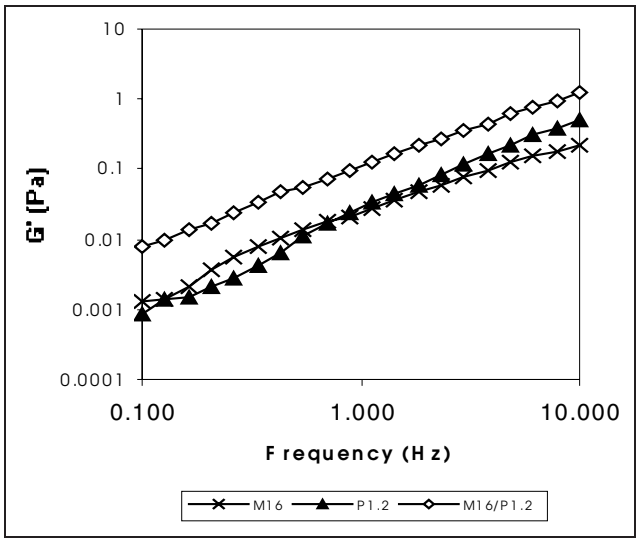

Fig. 5: The DFS measuements of point 3 of the experimental design (n = 2)

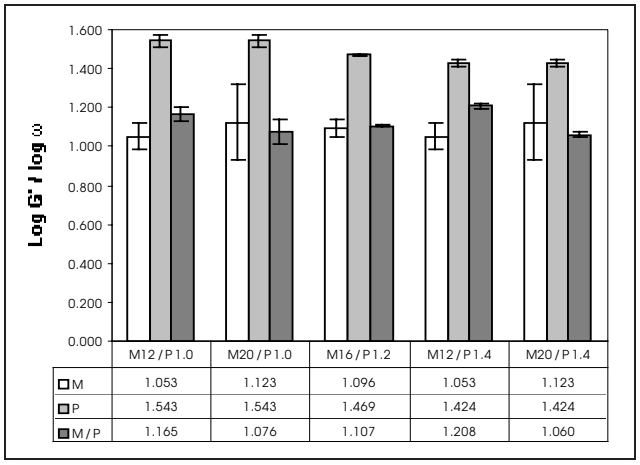

Fig. 6: The mean slope values and standard deviations of the log G'/log ω curves of M/P, M and P (n = 2)

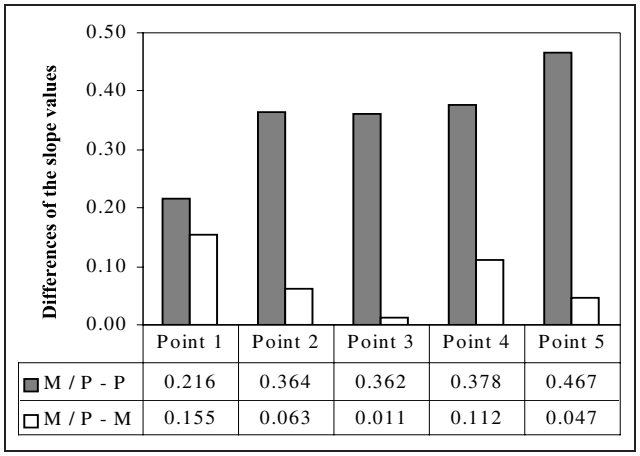

Fig. 7: Calculation of the smallest difference between the values obtained for the M/P mixture and M or P

The second possibility is the gel state in which the polymer chains interact, forming intermolecular bonds, even at low frequencies where they have enough time to disentangle. Consequently, G' and G'' values are not influenced by an increase of the frequency and their slope values are almost zero.

The evaluation of the possible existence of mucoadhesive properties is based on the slope values calculated when log G' is recorded as function of log ω in the case of mucin (M), polymer (P) and mucin/polymer (M/P) dispersions. The formation of secondary bonds between mucin and the polymer should exist when the slope value of the log G'/log ω curve in the case of M/P mixture is significantly lower than in the case of P and M dispersions. For the interpretation of the results the slope data are used to calculate the smallest difference between the value obtained for the M/P mixture and M or P.

The results of Dynamic Frequency Sweep measurements indicate that the interaction between mucin and the polymer is limited to physical entanglement because an elastic slope value of 1 is obtained (Figs. 5 and 6). The chains have enough time at low frequencies to disentangle, thus a sol state is mainly present. The elastic properties of M/P are mainly achieved by mucin because the smallest difference between the slopes of the log G'/log ω curves are always observed in the case of M/P and M and not for M/P and P (Fig. 7). Increasing the mucin concentration has an influence on the elastic response parameter, so the elastic properties of the mixture polymer and mucin are mainly due to the elastic properties of mucin and not to the behavior of the cationic polymer JR-30M (Fig. 6). The differences of the Log G'/log ω value of M/P for (M12/P1.0 and M20/P1.0) and (M12/P1.4 and M20/P1.4) are larger than the values for (M12/P1.0 and M12/P1.4) and (M20/P1.0 and M20/P1.4). Mucoadhesion based on the elastic properties of the preparation will therefore depend on the mucin concentration in the tear film after instillation.

Both Dynamic Stress Sweep (DSS) and Dynamic Frequency Sweep (DFS) data prove that a dispersion of JR-30M in SLF has mainly viscous properties. The DSS measurements confirm that an interaction between JR-30M and mucin takes place. The formation of secondary elastic interactions between JR-30M and mucin does not occur in a large amount. According to Hoffman et al. the good lubrication properties of mucin are due to entanglement of the polymer chains [11]. Strong interactions of cationic polymers and mucin based on electrostatic interactions and secondary bonds could, from a theoretical point of view, be necessary to obtain a cohesive network. Strong interactions however should favour the formation of mucin threads or precipitation at the eye surface resulting in discomfort for the patients. Thus a physical entangled network with a small amount of secondary bonds should be favourable to relieve dry eye discomfort.

As general conclusion can be stated that the mucoadhesive effect of JR-30M is mainly due to physical entanglements after mixing with mucin. The formation of secondary elastic interactions between the cationic derivatives and mucin does not occur in a large amount.

The use of JR-30M as treatment of dry eyes should be further investigated *in vivo*.

3. Experimental

3.1. Materials

Purified water was used throughout the experiments. Mucin type II (Crude from Porcine stomach) was purchased from Sigma Chemicals (Bornem, Belgium). The use of commercial mucins is justified by Rossi et al. [12], who demonstrated the lower batch-to-batch variability shown by commercial samples with respect to those freshly prepared. However, the rheological behavior of ocular mucin is predominantly determined by the gel-forming MUC5AC mucin, which is also responsible for the rheological behavior of gastric mucin [13, 14].

The Ucare polymer (polyquaternium-10: JR-30M) was supplied by Amerchol (Vilvoorde, Belgium). Salts, supplied by Federa (Brussels, Belgium) were used to prepare Simulated Lacrimal Fluid (SLF) and an isotonic phosphate buffer solution (pH 7.4). Simulated Lacrimal Fluid (or SLF) is an electrolyte solution composed of 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 $MgCl_2$ [15]. The solution obtained has a pH of about 8.00; by adding 0.1 N HCl, a physiological pH of 7.40 $\pm$ 0.05 is reached.

3.2. Polymer solutions

Four different polymer dispersions were made to investigate the mucoadhesive properties of the cationic cellulosic derivate (Fig. 8).

A weighed amount of mucin is dispersed in the SLF to prepare M. This dispersion is stirred during 24 hours at room temperature. Following samples were prepared in this way: 12, 16 and 20% mucin (w/w). Dispersions of 1, 1.2 and 1.4% (w/w) JR-30M were made by the addition of powder to the phosphate buffer (P/Pb). Isotonic phosphate buffer pH 7.4 is selected as vehicle, because of its use in many commercial preparations. Preparation of the dispersion P/Pb in SLF, called P is based on the mixing of one part SLF and one part of polymer dispersion in phosphate buffer solution (P/Pb). The dilution of P/Pb with SLF is made because eye drops are diluted in vivo by the tear fluid after instillation. The tear film amounts about 10 µl and from a biopharmaceutical point of view the ideal eye drop should be 10 µl. Consequently a 1/1 dilution is then obtained [1, 16].

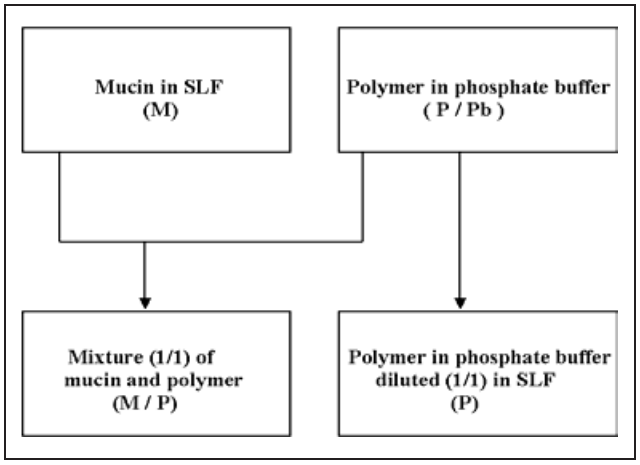

Fig. 8: Four different dispersions used for the in vitro assessment of the interaction between mucin and the native polymer tested

One part P/Pb is mixed with one part M to prepare M/P. The concentrations used of mucin in SLF and polymer for the preparation of M/P were reduced to 6, 8 and 10% and 0.5, 0.6 and 0.7% (w/w) respectively in the 1/1-dilution.

3.3. Rheological characterization

Rheological analyses were performed with a controlled stress rheometer (Carri-Med CSL^2 100, TA Instruments-Waters, Brussels Belgium) using the plate-plate geometry with gap of 500 µm. The experiments are performed at 32.0 $\pm$ 0.1 °C, the temperature on the eye surface [17]. A pre-shear procedure is used to spread homogeneously the samples between the plates. The test samples were equilibrated during 10 minutes allowing the polymers to recover from the destruction caused by the pre-shear procedure. During a dynamic stress ramp, oscillation stress was increased logarithmically from 1 to 10 Pa at a constant frequency of 1 rad/sec to detect the linear viscoelastic region. A stress value in the linear viscoelastic region (LVER) was chosen to perform a frequency ramp, during which the oscillation frequency was increased logarithmically from 0.1 to 10 rad/s [18, 19]. Three DSS and two DFS oscillation procedures of the various dispersions prepared were performed and mean values and standard deviations were calculated.

References

1 Lee, V. H. L.; Robinson, J. R.: J. Ocul. Pharmacol. **2** (1), 67 (1986)
2 Ludwig, A.; Van Ooteghem, M.: J. Pharm. Belg. **42**, 259 (1987)
3 Gu, J. M.; Robinson J. R.; Leung S. H. S.: CRC Crit. Rev. Ther. Drug Carrier Systems **5**, 21 (1988)
4 Peppas, N. A.; Buri, P. A.: J. Contr. Rel. **2**, 257 (1985)
5 Calonge, M.: Surv. Ophthalmol. **45**, 227 (2001)
6 Denick, J.; Heiler, D.; Hu, Z.; Salamone, J.; Smerbeck, R.: United States Patent 6.348.508, april 4 (2000)
7 Hassan, E. E.; Gallo, J. M.: Pharm. Res. **7**, 491 (1990)
8 Mortazavi, S. A.; Smart, J. D.: Int. J. Pharm. **116**, 223 (1995)
9 Dalton, P. D.; Chirila, T. V.; Hong, Y.; Jefferson, A.: Polym. Gel. Netw. **3**, 429 (1995)
10 Ferry, J. D.: Viscoelastic properties of polymers, Wiley, New York 1970
11 Hoffman, W.; Hauser, F.: Comp. Biochem. Physiol. **105B**, 465 (1993)
12 Rossi, S.; Ferrari, F.; Bonferoni, M. C.; Caramella, C.: Eur. J. Pharm. Sci. **10**, 251 (2000)
13 Argüeso, P.; Gipson, I. K.: Exp. Eye Res. **73**, 281 (2001)
14 Nagyova, B.; Tiffany, J. M.: Curr. Eye Res. **19**, 4 (1999)
15 Van Haeringen, N. J.: Surv. Ophthalmol. **5**, 84 (1981)
16 Ludwig, A.; Van Ooteghem, M.: Pharm. Acta Helv. **62**, 56 (1987)
17 Morgan, P. B.; Tullo, A. B.; Efron, N.: Eye **9**, 615 (1995)
18 Ceulemans, J.; Ludwig, A.: Pharmazie **57**, 181 (2002)
19 Ceulemans, J.; Vinckier, I.; Ludwig, A.: J. Pharm. Sci. **91**, 1117 (2002)

Received March 18, 2002
Accepted April 4, 2002

Annick Ludwig
Department of Pharmaceutical Sciences
University of Antwerp
B-2610 Antwerpen
Belgium
ludwig@uia.ua.ac.be