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

# Rheological evaluation of the influence of sterilisation on ocular gels using an experimental design

W. Weyenberg, V. Todorov, A. Ludwig

*Received July 4, 2003, accepted July 22, 2003*

*Annick Ludwig, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1,*
*B-2610 Antwerpen, Belgium*
*ludwig@uia.ua.uc.be*

*Pharmazie 59: 121–125 (2004)*

Different rheological characterisation methods are employed to investigate the influence of the sterilisation method (autoclaving), the polymer concentration (0.50, 0.75 and 1.00%) and the dispersing medium (i.e. isotonic phosphate buffer and mannitol solution) on Carbopol® 974 P NF dispersions, used as ocular gels. The evaluation of the different polyacrylic acid-based dispersions was performed using flow and oscillatory shear measurements, and herewith an experimental design was set up. The rheological data show that the choice of the dispersing medium has a significant influence on the rheological behaviour of the ocular gels prepared. The dispersions in mannitol exhibit higher elastic properties than those made in the phosphate buffer solution (PBS). Sterilisation increases only the elastic properties of the 0.50% (w/w) Carbopol® 974 P NF/ PBS dispersion but has no influence on the other dispersions prepared.

## 1. Introduction

Despite their widespread use due to their easy administration, eye drops are considered to be fairly primitive and ineffective dosage forms with considerable disadvantages, because the bioavailability of the drug instilled is quite low. Per day multiple applications of concentrated eye drops are often necessary to maintain a therapeutic concentration at the site of action. The frequent use of concentrated solutions may lead to toxic side-effects and cellular damage at the ocular surface (Baudouin 1996; Topalkara et al. 2000; Van Santvliet and Ludwig 1998). Several strategies can be followed to improve the bioavailability. A first strategy was to increase the viscosity of the vehicle of eye drops by viscolysers. However, a small increase of the retention time of the drug at the ocular surface was obtained (Ludwig and Van Ooteghem 1992). Another approach to optimise the dosage form was the implementation of the mucoadhesive concept. Suitable natural and synthetic polymers, interacting with the mucus layer coating the external surface of the eye were selected. By increasing the precorneal residence time of the preparation, some mucoadhesive polymers showed a good potential to increase the bioavailability of the drug instilled (Vadnere et al. 1984; Hui 1985; Saettone and Salminen 1995; Felt et al. 1999; Ceulemans et al. 2002a). A frequently used mucoadhesive polymer in ocular drug delivery systems is Carbopol®, a polyacrylic acid polymer, available in a wide range of molecular weights and being either linear, branched or cross-linked (Krishnamoorthy and Mitra 1993; Ceulemans and Ludwig 2002b). The acidic carboxyl groups dissociate in water, producing a flexible coil. Solvation, salt formation and electrostatic repulsion between the anionic groups cause gel formation, after addition of alkalines (Berry et al. 2000).

The aim of the present study was to investigate the influence of the sterilisation (autoclaving), the concentrations of the polymer, and the dispersing medium (an isotonic phosphate buffer solution pH 7.4 and 5.07% mannitol solution) on the rheological properties of the different Carbopol 974® P NF dispersions, using an experimental design. Steady shear flow rheology was used to compare the flow behaviour of the gels prepared in the different media. The influence of sterilisation, the polymer concentration and electrolytes was determined by oscillatory rheology.

## 2. Investigations, results and discussion

The advantage of the use of an experimental design is to obtain a maximum of information with a minimum of experiments. Based on a general formula and the use of a mixed $2^2 3^1$ full factorial design, the influences of the polymer concentrations, the sterilisation and the electrolytes on the rheological characteristics of the dispersions were studied (Table 1).

Twelve different dispersions are prepared to characterise all the points of the design. The elastic value in the linear viscoelastic region (LVER) in the Dynamic Stress Sweep

**Table 1: Factors and levels of the experimental design employed**

| Factor | Level | | |
|---|---|---|---|
| | −1 | 0 | +1 |
| A: Polymer concentration $x_p$ (%, w/w) | $x_{0.50}$ | $x_{0.75}$ | $x_{1.00}$ |
| B: Dispersing medium | PBS | | Mannitol |
| C: Sterilisation | No | | Yes |

curves (DSS) and the slope value of the Dynamic Frequency Sweep curves (DFS) are used as response factors. To calculate the effect of a factor, all measurements where the factor is at its lower level are substracted from all those where the factor is at its upper level and subsequently divided through half the number of measurements used in the calculation (Wiley 1987). The full factorial design enables to determine the main and the interaction effects. The main effect shows the influence of the factor on the response, and the interaction effects indicate the influence of the factors on each other. The design is analysed by the software Statistica® version 5.5 (Statsoft Inc, Tulsa, USA).

Flow measurements are used to study the relation between the stress (related to the force applied) and the shear rate on the samples, and to determine the viscosity and the flow characteristics. The flow curves are analysed using the mathematical Herschel-Bulkley model to calculate the consistency, the shear rate index and the yield stress. The shear rate index values are smaller than 1 and equal to 1 in the case of a pseudoplastic and a Newtonian behaviour, respectively.

The viscoelastic properties of the samples were studied using oscillatory measurements. During Dynamic Stress Sweep (DSS), the oscillation stress was increased logarithmically from 1 to 100 Pa to detect the linear viscoelastic region (LVER), which is the stress region characterised by a directly proportional relation between the stress applied on the sample and the strain of the sample. The polymer network stays intact, while applying an oscillation stress in this linear viscoelastic region (LVER). This relation is not more proportional after the LVER because of the destruction of the polymer network: a larger deformation of the sample is obtained due to the stress used, resulting in a decrease of the elastic values ($G'$). Thus, analyses of DSS measurements allow to characterise polymers, the force of their intermolecular bonds and their resistance towards the stress applied (Ceulemans and Ludwig 2002c; Weyenberg and Ludwig 2002).

Dynamic Frequency Sweep (DFS) measurements are performed to characterise the Carbopol® dispersions as a gel or sol state. The oscillation stress is maintained at a low constant value obtained from the DSS results to keep the structure intact, meanwhile the oscillation frequency is increased. In an entangled network the polymer chains can disentangle only if the time available is long enough (low frequency), while the bonds are fixed irrespective of the angular frequency applied in a network of secondary bonds. This structural behaviour results for an entangled solution in a limiting slope of 2 for $G'$ versus frequency in log-log plots. For a network with secondary bonds, a constant value of zero is observed for $G'$ over the whole frequency range (Ferry 1980; Ceulemans et al. 2002a).

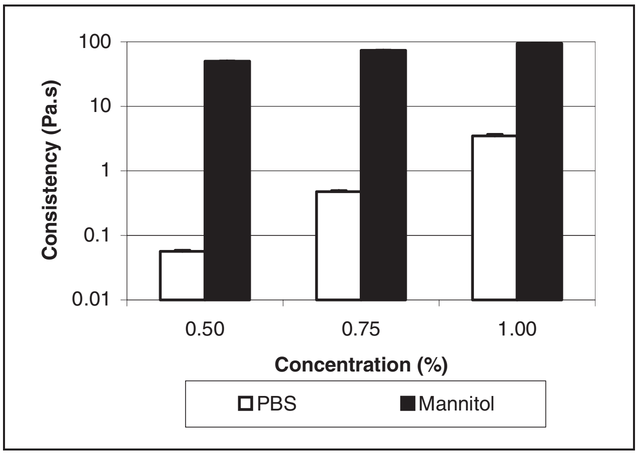

Fig 1: Influence of concentration and dispersing media on the consistency of the non-sterilised gels

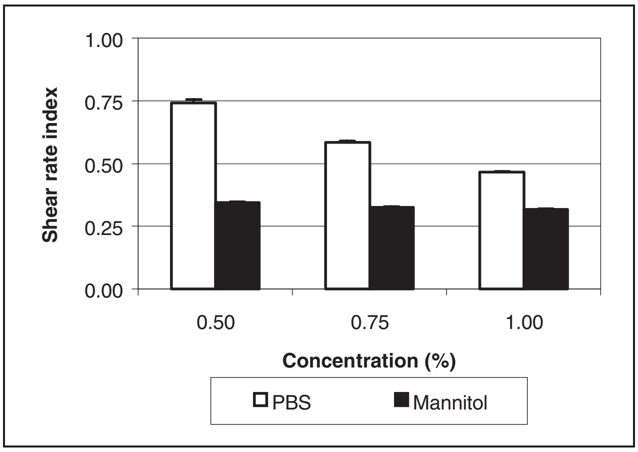

Fig 2: Influence of concentration and dispersing media on shear rate index of the gels prepared

The results of the flow procedures confirm that mannitol dispersions possess higher consistency properties than PBS dispersions, at the same concentration level of Carbopol® 974 P NF (Fig. 1). All the samples are characterised with a yield stress of zero and thus exhibit a pseudoplastic behaviour. The rate index values calculated are shown in Fig. 2. The degree of the pseudoplasic behaviour is much higher for the dispersions prepared in mannitol than in PBS. The effects observed are not due to the pH of the dispersions prepared, because it was kept constant at 7.4. At a low pH, the polymer chains are initially coiled into a spiral form, thus affording a relatively low viscosity. As neutralization progresses, the carboxyl groups of the acrylic acid become ionized, leading to an increasing repulsion of negative charges that causes the molecular structure to unwind thus inducing a gradual rise in viscosity. A reduction of the viscosity of the dispersions of Carbopol® 974 P NF prepared in PBS (Carb/PBS) in comparison to those prepared in mannitol (Carb/Man) is mainly due to the presence of sodium ions. This monovalent cation reduces the viscosity of the molecular network, because of

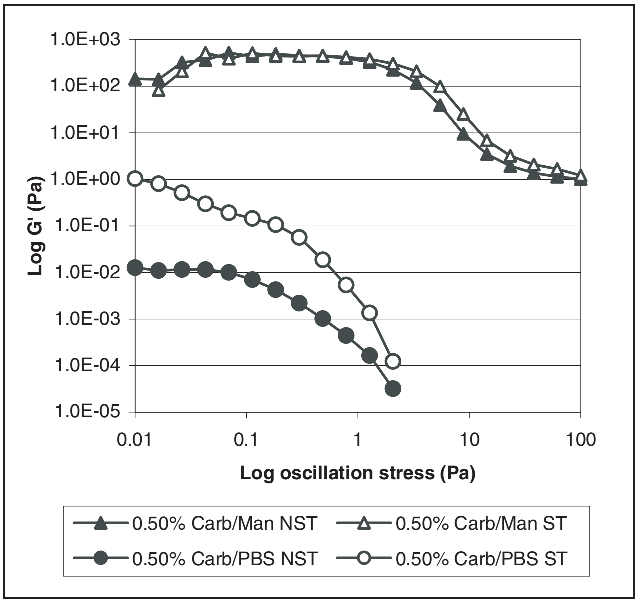

Fig 3: Influence of sterilissation on 0.50% (w/w) Carbopol® dispersion

Table 2: **The elastic ($G'$) values (Pa) of the dispersions prepared, at an oscillation stress value of 0.1 Pa ($n = 9$)**

| | 0.50% | 0.75% | 1.00% |
|---|---|---|---|
| Carb/Man NST | 440 ± 42 | 618 ± 54 | 786 ± 61 |
| Carb/PBS NST | **0.01** ± 0.001 | 7.64 ± 0.53 | 43.84 ± 3.72 |
| Carb/Man ST | 432 ± 38 | 597 ± 47 | 764 ± 56 |
| Carb/PBS ST | **0.144** ± 0.032 | 11.55 ± 1.03 | 42.56 ± 73.22 |

charge shielding, reducing the electrostatic repulsion between the polymer chains which result further in a decrease of the expansion of the network or the hydrodynamic volume.

The elastic storage modulus $G'$ is only shown in the oscillatory curves, because the polyacrylic acid dispersions prepared have mainly elastic properties. The Dynamic Stress Sweep results show that a concentration increase of Carbopol® 974 P NF from 0.50 to 1.00% (w/w) in the dispersion medium leads not only to an increase of the viscoelastic modulus $G'$ but also to a longer LVER. It is obvious that at higher polymer concentrations more resistance is formed against the increasing oscillation stress. The linear viscoelastic regions (LVER) are longer and the values of $G'$ for the Carbopol® 974 P NF dispersions in mannitol are higher in comparison to the dispersions in PBS, at the same concentration level (Fig. 3). Average $G'$ values at the oscillation stress value of 0.1 Pa for all the dispersions prepared are presented in Table 2. The $G'$ values obtained from the non-sterilised samples are only 0.01 Pa for the 0.50% (w/w) dispersion in PBS, while 440 Pa for the 0.50% (w/w) dispersion prepared in mannitol. The rheological behaviour of all the Carb/Man and the 0.75 and 1.00% (w/w) Carb/PBS dispersions was not influenced significantly by autoclaving. Only, a significant increase of $G'$ and the LVER after sterilisation was observed in the 0.50% (w/w) Carb/PBS dispersion (Fig. 3 and 4). The high temperature during the autoclaving procedure leads to a change in conformation of the Carbopol® polymers due to desaggregation of the polymer chains and a better hydration of the polymers during cooling, resulting in an increase of the elastic properties of the 0.50% (w/w) dispersion prepared in PBS.

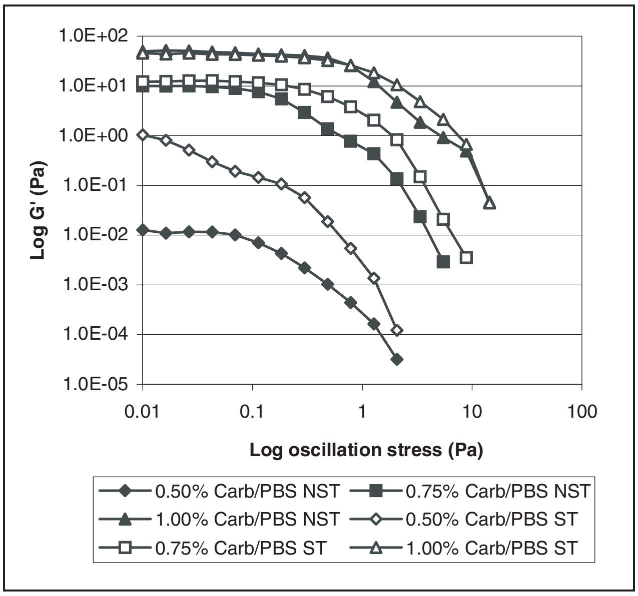

Fig 4: Influence of sterilisation on Carbopol® dispersions, prepared in PBS

In the other native dispersions (0.75% and 1.00% w/w, Fig. 4), a maximum formation of secondary bonds in the network is already obtained, because after autoclaving and cooling, when a better hydration of the polymers is expected, no extra formation of secondary bonds or higher $G'$ values are detected in the oscillatory curves of these dispersions. Only a small increase of the linear viscoelastic region (LVER) can be seen after sterilising the PBS-dispersions (0.75% and 1.00% w/w), indicating a stronger formation of the secondary bonds in the network or a better hydration of the polymers dispersed.

The DFS slope values for all the combinations of the investigated parameters, used in the factorial design, are shown in Fig. 5. Differences in the rheological properties of all the dispersions can be derived from the slope values calculated when log of the elastic parameter ($G'$) is recorded as a function of log of the angular frequency ($\omega$). The slope values (i.e. 0.225 and 1.467) of log $G'$/log $\omega$ on the level of 0.50% (w/w) Carb/PBS are significantly higher than at the other concentrations levels. The average slope value of the log $G'$/log $\omega$ curve in the sterilised 0.50% (w/w) Carb/PBS dispersion equals 0.225, which is significantly lower than 1.467 for the non-sterilised 0.50% (w/w) Carb/PBS dispersion ($p < 0.05$). This indicates that the structural behaviour of the non-sterilised dispersion is like an entangled dispersion. However, the slope value of the log $G'$/log $\omega$ curve in the dispersion made with 0.50% (w/w) Carbopol®974 P NF decreases significantly after autoclaving: the polymer chains are better hydrated and interact more with each other, forming secondary bonds. The low slope values of the log $G'$/log $\omega$ curves in all the Carb/Man gels and the 0.75 and 1.00% (w/w) Carb/PBS dispersions prove the network formation with a large presence of secondary bonds in the dispersions prepared. These secondary bonds are not influenced by the sterilisation performed.

The results obtained with factorial design show that the effect of the buffer solution (i.e. 1.467, 0.225, 0.047 and 0.045 versus 0.057, 0.071, 0.080 and 0.100) is significant on the concentration levels of 0.50 and 0.75% (w/w) ($p < 0.05$) (Figs. 5, 6). There is no significant influence of the sterilisation and concentration on the slope values of the log $G'$/log $\omega$ curves of the mannitol dispersions. Contrary, the sterilisation has only a significant influence (i.e. 1.467 versus 0.225) on the slope values of the log $G'$/log $\omega$ curves of the PBS dispersion, at the concentration level of 0.50% (w/w) ($p < 0.05$). An interaction between the factor dispersing medium and the sterilisation (i.e. 1.467, 0.057, 0.225 and 0.071) was only obtained on the level of 0.50% concentration.

Dispersing the lowest concentration of Carbopol® 974 P NF in mannitol (i.e. 0.50% w/w), results in a good hydra-

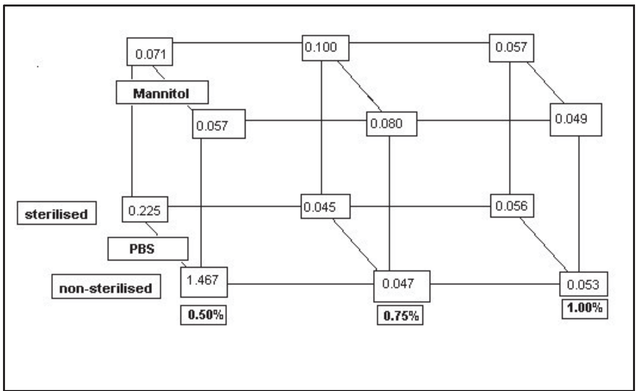

Fig 5: Cube plot (full factorial mixed design ($2^2 3^1$)) of average slopes of log $G'$/log $\omega$ for the dispersions prepared (w/w)

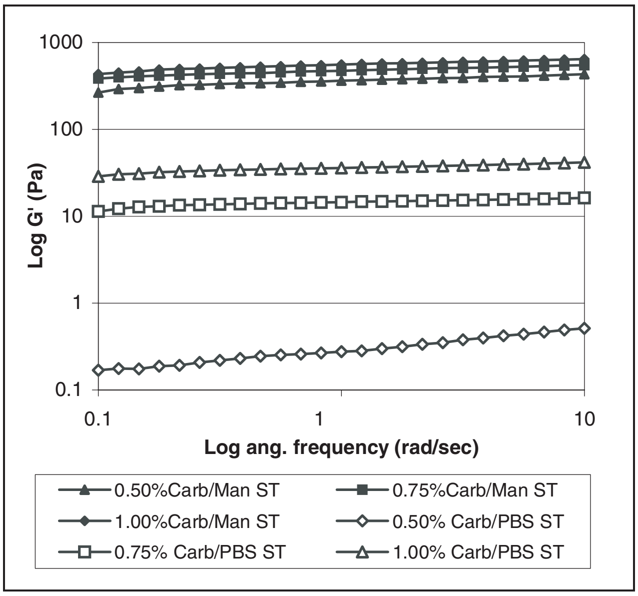

Fig 6: Influence of the dispersing media on the DFS slopes of the sterilised Carbopol® 975 P NF dispersions

tion of the polymers with as consequence the formation of a network containing mainly secondary bonds. Even when the sterilisation procedure is performed, no improvement of interactions is obtained. Although, when the same amount of Carbopol® 974 P NF is dispersed in PBS, the Carbopol® 974 P NF polymers have a smaller hydrodynamic volume, due to the charge shielding effect of sodium ions, resulting in a dispersion with almost no network formation, based on secondary bonds between these molecules.

Both the Dynamic Stress Sweep (DSS) and the Dynamic Frequency Sweep (DFS) data prove that autoclaving has only a significant influence on the rheological properties of the 0.50% (w/w) Carb/PBS dispersion. The elastic properties are increased after sterilisation because of the better polymer hydration of the polymers dispersed. The DSS measurements confirm that dispersions in mannitol (Carb/Man) have much more elastic properties than dispersions in the phosphate buffer solution (Carb/PBS) at the same concentration level (Table 2, Fig. 3). The differences observed for Carbopol® dispersions are mediated by the variation of the vehicle and can be attributed to the ionization of Carbopol® polymer. At a pH of 7.4, the mutual repulsion of ionized carboxyl groups produces more stretched Carbopol® backbones and those carboxyl groups form also stabile hydrogen bonds with water molecules through hydrophilic interactions. The presence of electrolytes in the dispersing medium influences the ionisation of the Carbopol® polymers and the stability of the hydrogen bonds.

The general conclusion that can be drawn is that sterilisation has no significant influence on Carb/Man dispersions and that these dispersions have much higher elastic properties in comparison to PBS dispersions. Consequently, an isotonic mannitol solution is a good dispersing medium to prepare Carbopol® gels without affecting its rheological behaviour by autoclaving.

## 3. Experimental

### 3.1. Materials

Purified water produced by Milli-Q (Millipore Co., USA), was used throughout the experiments. Carbopol® 974P NF was obtained as a gift from BF Goodrich (Brussels, Belgium). D-Mannitol was supplied by Ludeco (Brussels, Belgium). Sodium-dihydrogeno phosphate dihydrate ($NaH_2PO_4 \cdot 2\,H_2O$), disodium-hydrogeno phosphate dihydrate ($Na_2HPO_4 \cdot 2\,H_2O$) and sodium hydroxide (NaOH) are purchased from Merck (Darmstadt, Germany). An isotonic (pH 7.4) phosphate buffer solution (PBS) is prepared with sodium-dihydrogenophosphate dihydrate ($NaH_2PO_4 \cdot 2\,H_2O$) and disodium-hydrogenophosphate dihydrate ($Na_2HPO_4 \cdot 2\,H_2O$) in purified water.

### 3.2. Polymer dispersions

Carbopol®974 P NF dispersions of 0.50, 0.75 and 1.00% (w/w) were made by the addition of the polymer powder either to the isotonic 5.07% (w/v) mannitol solution or PBS with continuous stirring until completely dissolved (Thermolyne HP46820–26, Dubuque, IO, USA). A NaOH 1 N solution was used to adjust the pH to a value of 7.4 ± 0.1 (Benchtop 420 A pH meter, Orion, MA, USA). Afterwards the dispersions were stirred for 1 h, at room temperature. The dispersions were stored at 6 °C for a least 12 h. A part of the same dispersions were filled in glass vials (type I) and were sterilised by the autoclave (MELAG, TYP 23, Germany) during 1 h at 2 bar pressure.

### 3.3. Rheological characterisation

Rheological analyses were performed with a controlled stress rheometer (Carri-Med CSL$^2$ 100, TA Instruments, Brussels, Belgium) equipped with a 4 cm acrylic cone for high viscous samples (1.59 degrees acrylic cone, truncation 57 μm) or a double concentric cylinder for low viscous samples. The experiments were performed at 32.0 ± 0.1 °C, the temperature on the eye surface (Morgan et al. 1995). A pre-shear procedure was used to homogenise the samples. The test samples were equilibrated during 10 min allowing the polymers to recover from the destruction caused by the pre-shear procedure. During a flow procedure the shear rate was increased from 0 to 250 (1/s) and than decreased from 250 to 0 (1/s). The Herschel-Bulkley model, expressed by Eq. 1 was employed as mathematical model to fit the data obtained.

$$\sigma = \sigma_y + K \cdot \gamma^n \qquad (1)$$

$\sigma$ = shear stress (Pa)
$\sigma_y$ = yield stress (Pa)
K = consistency (Pa.s)
$\gamma$ = shear rate (1/s)
n = shear rate index

In the oscillation measurements the stress was increased logarithmically from 1 to 100 Pa at a constant frequency of 1 rad/s to detect the linear viscoelastic region (LVER), during a dynamic stress ramp. A stress value in the LVER was chosen to perform a frequency ramp, during which the oscillation frequency was increased logarithmically from 0.1 to 10 rad/s (Ceulemans and Ludwig 2002c; Weyenberg and Ludwig 2002). Three flow measurements, three DSS and two DFS oscillation procedures of the various dispersions prepared were performed in triplicate. Mean values and standard deviations were calculated.

### References

Baudouin C (1996) Side effects of antiglaucomatous drugs on the ocular surface. Curr Opin Ophthalmol 7: 80–86.
Berry M, Ellingham RB, Corfeld AP (2000) Membrane-associated mucins in normal human conjunctiva. Invest. Ophthalmol Vis Sci 41: 398–403.
Ceulemans J, Vinckier I, Ludwig A (2002a) The use of xanthan gum in an ophthalmic liquid dosage form: rheological characterization of the interaction with mucin. J Pharm Sci 91: 1117–1127.
Ceulemans J, Ludwig A (2002b) Optimisation of carbomer viscous eye drops: an in vitro experimental design approach using rheological techniques. Eur J Pharm Biopharm 54: 41–50.
Ceulemans J, Ludwig A (2002c) Development of a rheometric method to measure the mucoadhesive capacity of a dry powder formulation. Pharmazie 57: 181–185.
Felt O, Furrer P, Mayer JM, Plazonnet B, Buri P, Gurny R. (1999) Topical use of chitosan in ophthalmology: tolerance assessment and evaluation of precorneal retention. Int J Pharm 180: 185–193.
Ferry JD (1980) Viscoelastic Propeties of Polymers, Wiley, New York.
Hui HW, Robinson JR (1985) Ocular drug delivery of progesterone using a bioadhesive polymer. Int J Pharm 26: 203–213.
Krishnamoorthy R, Mitra AK (1993) Mucoadhesive polymers in ocular drug delivery. In: Mitra (Ed) Ophthalmic drug delivery systems, Marcel Dekker, New York (USA) 199–221.
Ludwig A, Van Ooteghem M (1992) Influence of viscolysers on the residence of ophthalmic solutions evaluated by slit lamp fluorophotometry. STP Pharma Sciences 2: 81–87.
Morgan PB, Tullo AB, Efron N (1995) Infrared thermography of the tear film in dry eye. Eye 9: 615–618.

Saettone MF, Salminen L (1995) Ocular inserts for topical delivery. Adv Drug Del Rev 16: 94–106.

Topalkara A, Güler C, Arici DS, Arici MK (2000) Adverse effects of topical antiglaucoma drugs on the ocular surface. Clinical and Experimental Ophthalmology 28: 113–117.

Vadnere M, Amidon G, Lindenbaum S, Haslam JL (1984) Thermodynamic studies on the gel-sol transition of some pluronic polyols. Int J Pharm 22: 207–218.

Van Santvliet L, Ludwig A (1998) The influence of penetration enhancers on the volume instilled of eye drops. Eur J Pharm Biopharm 45: 189–198.

Weyenberg W, Ludwig A (2002) In vitro assessment of the interaction mechanisms between mucin and a cationic cellulosic derivative by rheological methods using an experimental design. Pharmazie 57: 628–631.

Wiley J (1987) Measuring the effects of variables, Wiley, New York 291.