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Physico-chemical, *in vitro* and *in vivo* characterisation of polymers for ocular use

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The influence of artificial tear fluid (AT) on ionic and nonionic ophthalmic polymer excipients was rheologically established. In usual concentrations polyvinylalcohol, polyvinylpyrrolidone, dextran, hydroxypropylmethylcellulose, hydroxyethylcellulose and methylcellulose did not show any changes. In contrast, solutions of polyacrylic acid, sodium hyaluronate (S-Hya), sodium alginate (S-Alg) and chitosan decrease the apparent viscosity in contact with AT, while gellan solution increases the viscosity and shows thixotropy. The adhesion of selected polymers (polysaccharides) on mucin was evaluated using a rheological method and resulted in the order S-Hya > Gellan > S-Alg > dextran. Miosis testing of Gellan containing pilocarpine HCl formulations in rabbits shows a possible reduction of drug concentration from 2% to 0.5% obtaining the same bioavailability.

1. Introduction

Because of the high drainage rate, the bioavailability of ocular administered drugs is rarely above 1–3% [1]. Also systemic resorption of drug occurs, which causes side effects [2–5].

Established but still discussed is the concept of increasing the ocular bioavailability of ophthalmics using macromolecules as excipients [6]. An increase in viscosity leads to a prolongation of contact time on the cornea [7]. Furthermore polymer containing formulations were used in the treatment of Keratoconjunctivitis sicca [8]. Next to viscosity, mucoadhesion is an important criterion in this indication [9].

In contact with the eye, formulations are exposed to the physiological environment, causing changes in temperature, pH, and electrolyte concentration. Some of these effects were used for the development of in situ-gelling systems [10–12]. Solutions of Gellan, an ion-sensitive polymer, enhance their viscosity in contact with tear fluid [13]. The effect leads to an increase in bioavailability of topically applied ophthalmics [14–16].

The influence of artificial tear fluid (AT) on common ophthalmic polymer excipients was studied *in vitro*. AT equals human tear fluid in content of cations. Mucoadhesion of selected polymers was detected by a method established by Hassan and Gallo [17]. By means of rheology the polymer adhesion on mucin was measured. Buffers, based on AT, were used to simulate the environment of the eye. Furthermore the effect of Gellan was quantified by miosis test of a Gellan-pilocarpine formulation (GPF) on rabbit eyes. Finally GPF was compared with an EDTA-pilocarpine-formulation (EPF) in an *in vitro* permeation model using bovine cornea described by Siefert and Keipert [18]. The aim of this experiment was to evaluate a possible enhancer effect of the polymer.

2. Investigations, results and discussion

2.1. Rheological examination

Maximally 10 µl of an applicated drop of about 50 µl remain in the eye. The human as well as the rabbit eye contains a volume of about 8 µl liquid [19, 20]. Due to the fact, that every blinking of the eye pumps another 2 µl of tear fluid in the conjunctival sac, the mixture between applicated formulation and tears can be estimated as an 1 : 1 ratio. Therefore several polymers commonly used in ophthalmics were investigated purely and mixed 1 : 1 with AT.

Nonionic polymers like polyvinylalcohol (PVA), polyvinylpyrrolidone (PVP) K25 and K90, dextran, hydroxypropylmethylcellulose, hydroxyethylcellulose and methylcellulose did not show, as expected, significant rheological changes in contact with artificial tears in a concentration range commonly in ophthalmics. Solutions of these polymers not represented can be evaluated as Newtonian fluids.

In contrast, solutions of 0.05 to 0.3% polyacrylic acid (PAA) if mixed with AT (1 : 1) show a significant decrease in viscosity more than that expected by dilution (Fig. 1). The character is close to be ideal viscous one. Likewise, sodium hyaluronate (S-Hya) (Fig. 2) from 0.01 to 0.8% shows a decrease in viscosity in contact with AT, but this decrease is not as big as in the case of PAA (Fig. 1). The flow properties are pseudoplastic. Also, 2–4% chitosan solutions show a decrease of viscosity in contact with AT (Fig. 3). Moreover a decrease in viscosity in contact with AT can be detected for sodium alginate (S-Alg) (Fig. 4).

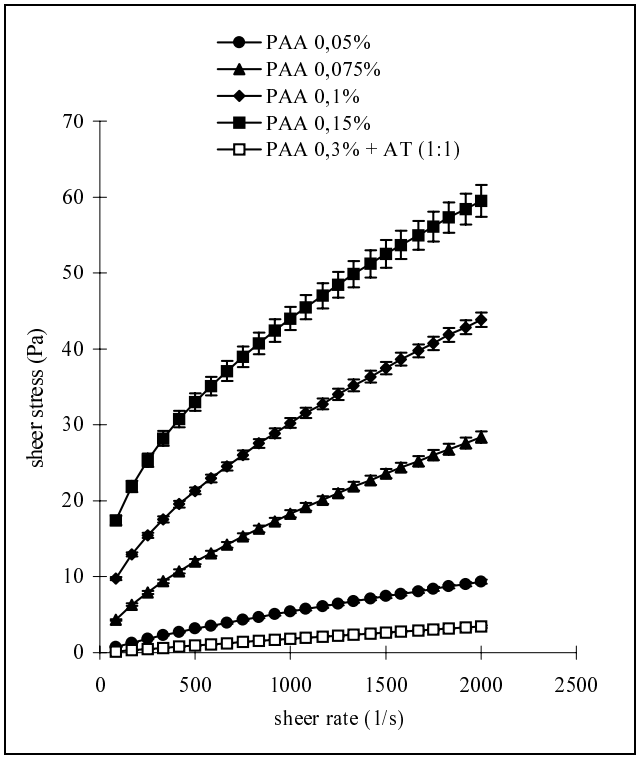

Fig. 1: Rheological effect of AT on polyacrylic acid (PAA)

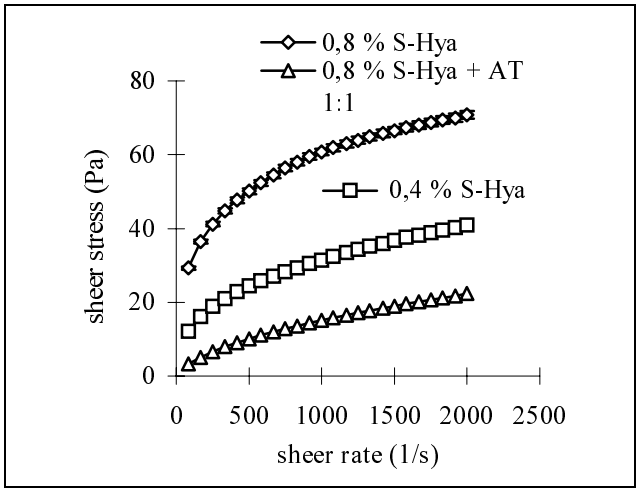

Fig. 2: Rheological effect of AT on sodium hyaluronate (S-Hya)

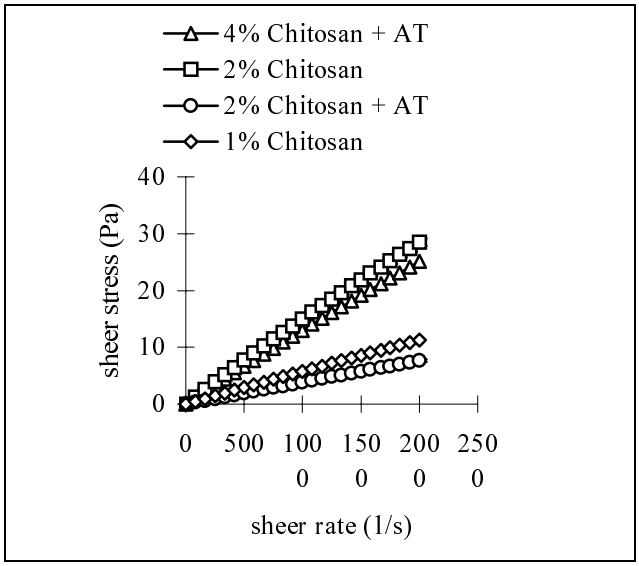

Fig. 3: Rheological effect of AT on Chitosan

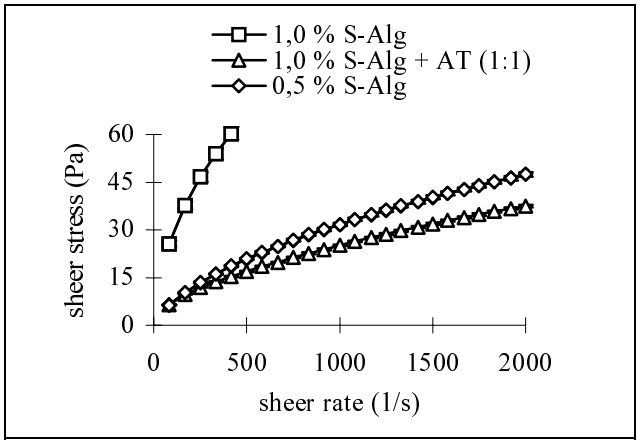

Fig. 4: Rheological effect of AT on sodium alginate (S-Alg)

Furthermore mixtures of S-Alg and AT (1 : 1) show thixotropy. Hysteresis areas of these solutions increase with raising the concentration of the polymer.

In contrast to the polymers mentioned above, Gellan shows an increase in viscosity by contact with AT (Fig. 5). Similar to S-Alg, a concentration dependent thixotropy can be detected, which even reaches about ten times higher values in comparison to S-Alg.

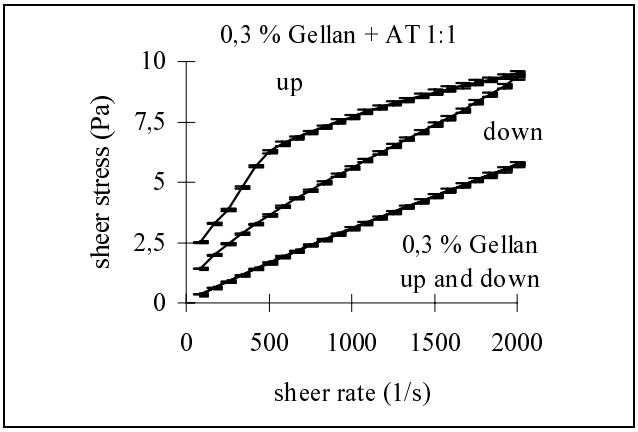

Fig. 5: Rheological effect of AT on gellan

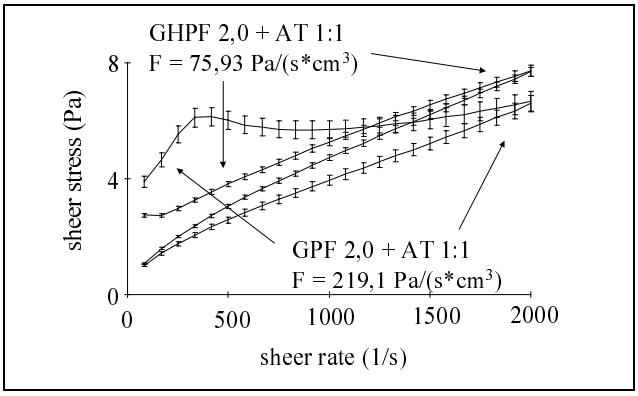

Fig. 6: Hysteresis areas of gellan/pilocarpine-formulation with (GHPF 2.0) and without (GPF 2.0) addition of 0.2% S-Hya

In contrast to nonionic polymers, ionic polymers like PAA, S-Hya, Chitosan and S-Alg show a decrease whereas Gellan shows an increase in viscosity in contact with AT. The effect should be taken into consideration during the development of ophthalmic formulations containing these polymers. However, the thixotropy measured for S-Alg and Gellan has been discussed to be probably not a desirable situation in the human eye since the system becames thinner with time [21]. It is possible to avoid thixotropy by combining these polymers with other, non-thixotropic polymers like hyaluronic acid in ophthalmic formulations.

The effect of an addition of 0.2% S-Hya to a 0.6% Gellan/2.0% pilocarpine formulation (GHPF 2.0) is demonstrated in Fig. 6. The hysteresis area is dimished from 219.1 Pa/(s · cm^3) to 75.93 Pa/(s · cm^3).

2.2. Mucoadhesion

Mucoadhesion was detected by evaluating the adhesion of polymer on mucin. The testing was carried out at pH 5.5, a compromise pH value for optimal stability of the model drug pilocarpine [22] and physiological conditions in the eye. In addition, mucoadhesion was also measured at physiological pH 7.4.

At pH 5.5 and a polymer concentration of 0.15% mucoadhesion was found in the range of Gellan > S-Hya > PAA > S-Alg > dextran. At pH 7.4 the range was Gellan > S-Hya > S-Alg > PAA > dextran (Fig. 7). The index of mucoadhesion (Pa) increases with the concentration (0.15 – 0.9%) of the tested macromolecule. For concentrations higher than 0.15% the following range results: S-Hya > Gellan > S-Alg > dextran both at pH 5.5 and

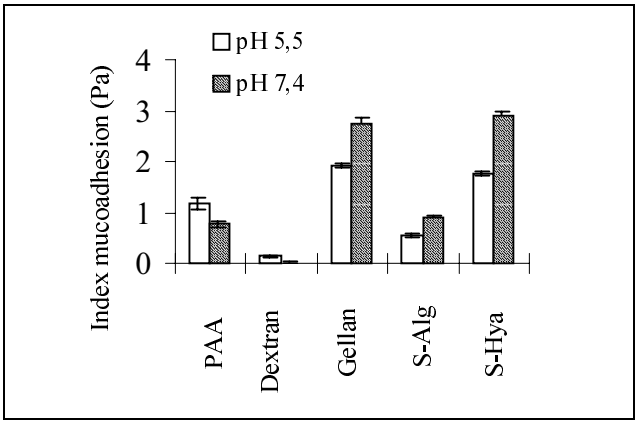

Fig. 7: Index of mucoadhesion of polymers (0.15%) at pH 5.5 and 7.4

7.4. The calculation of regression gradients for the effective mucoadhesion enhancers S-Hya, Gellan and S-Alg tendentially provides a ratio like 1 to 1.5 to 3 at pH 5.5 and 1 to 1 to 1.5 at pH 7.4, respectively.

2.3. Miosis testing

Changes of pupil diameter were measured for different formulations containing 2.0, 0.5 and 0.1% pilocarpine HCl in Gellan (GPF). Reference was a 2.0% aqueous formulation of pilocarpine hydrochloride (APF). The physiological compatibility of the vehicle gellan has been verified by the registration of Uniget® XE.

A 2% pilocarpine and 0.6% gellan containing preparation (GPF 2.0) shows, compared with an aqueous 2% pilocarpine preparation (APF), both larger AUC, higher maximum (Δc_{max}) and a longer duration of miosis (M-dur.) (Fig. 8; Table 1).

A 0.1% pilocarpine and 0.6% gellan containing formulation (GPF 0.1) shows a smaller AUC and duration of miosis compared with APF (Table 1). Ten minutes after application of GPF 0.1 a larger miosis was measured compared with APF. Also, the higher concentrated gellan preparations GPF 0.5 and GPF 2.0 (Fig. 8) show a larger miosis 10 min after application. A possible reason for this rapid reaction of rabbit eyes, even in the presence of a very small amount of pilocarpine in the formulation may be on the one hand the in situ-gelling effect of the polymer,

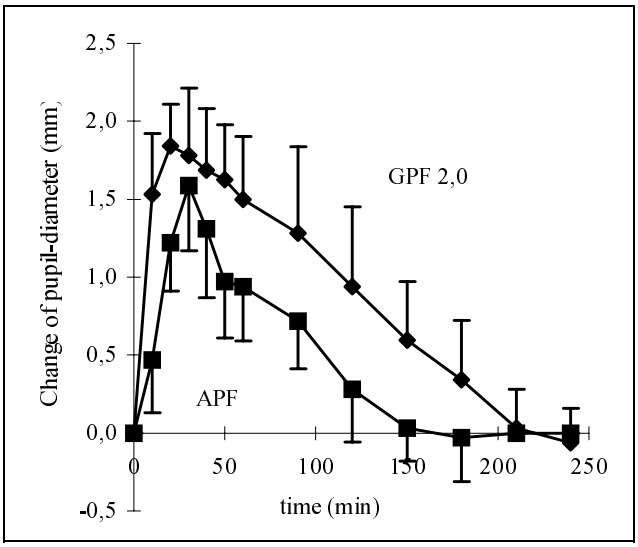

Fig. 8: Change of pupil diameter rabbits after application of GFP 2.0 and APF

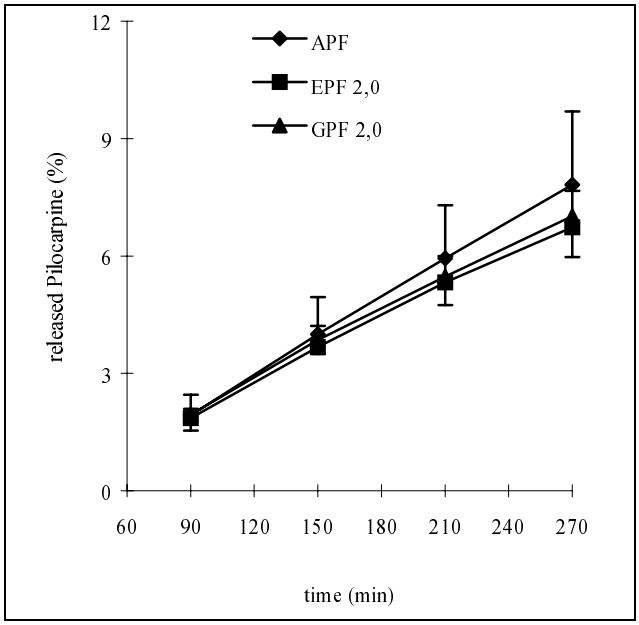

Fig. 9: Permeation of pilocarpine out of APF, EPF 2.0 and GPF 2.0 through isolated bovine cornea

Table 1: Change of pupil diameter in rabbits after application of APF, GPF 2.0, 0.5 and 0.1.

Preparation	AUC (mm · min)	t_{max} (min)	Δc_{max} (mm)	M-dur. (min)
APF	104.84	30	1.59	150
GPF 2.0	209.84	20	1.84	210
GPF 0.5	119.47	20	1.47	150
GPF 0.1	61.88	20	1.16	90

Table 2: Artificial tears and buffers

substances [g]	AT	AT-buffer	
		pH 5.5	pH 7.4
NaCl	8.415	8.415	8.415
KCl	1.640	1.640	1.640
$CaCl_2 \cdot 6\ H_2O$	0.262	0.262	0.262
$MgCl_2 \cdot 6\ H_2O$	0.142	0.142	0.142
Tris	–	q.s.	6.075
Acetic acid	–	3.003	q.s.
Bidist. water	ad 1000	ad 1000	ad 1000

Table 3: Preparations

substances [g]	GPF 2.0	GPF 0.5	GPF 0.1	GHPF 2.0	APF	EPF
Gellan	0.3	0.3	0.3	0.3	–	–
S-Hya	–	–	–	0.1	–	–
Pilocarpine	1.0	0.25	0.05	1.0	1.0	1.0
Tris	0.496	0.125	0.025	0.496	0.496	–
Mannitol	0.650	2.065	2.495	0.650	0.650	–
EDTA	–	–	–	–	–	1.0
Bidist. water	ad 50.0	ad 50.0	ad 50.0	ad 50.0	ad 50.0	ad 50.0

which should diminish drainage after application. Comparable results were found by *in vitro* liberation tests [23]. On the other hand an origin of the effect may also be an enhancer effect of Gellan. This might be comparable to the enhancer effect of EDTA for permeation of glycerol in the rabbit eye reported by Grass and Robinson [24] which was also mentioned by Lee [25] for atenolol.

For further investigation of a possible enhancer effect of Gellan [26] a corneal permeation model [18] was used. As membran between donor and receiver served bovine cornea. It was not possible to detect any enhancer effect for Gellan (GPF 2.0) or EDTA (EPF 2.0) with the used model (Fig. 9).

3. Experimental

3.1. Materials

Pilocarpine hydrochloride and EDTA were obtained from Merck, Darmstadt, Germany. Gellan (Gelrite®) was purchased from Kelco, San Diego, USA, whereas methylcellulose (Metocel 25 mPas), hydroxyethylcellulose (medium viscosity) and PVP K 90 were obtained from Fluka AG, Buchs, Switzerland. S-Alg (medium viscosity), S-Hya (from rooster comb) and mucin type II (from porcine stomach) were obtained from Sigma Chemicals St. Louis, USA. Hydroxypropylmethylcellulose (Metolose 65 SH-4000) was purchased from Shin-Etsu, Tokio, Japan, PAA (Carbopol® C 980) from Goodrich, Cleveland, USA, and chitosan (Seacure CL 110) from Pronova, Drammen, Norway.

Solids were dissolved in water. In case of buffers pH was ajusted with acetic acid or tris. The composition of artificial tears and buffers is presented in Table 2.

In the case of GPF 0.1, 0.5 and 2.0 gellan, tris and mannitol were dissolved by autoclaving for 40 min. After allowing the solution to cool down to room temperature pilocarpine was added under aseptic conditions. Sterility was obtained by means of filtration (0.22 μm Sartorius® membran filter). In the case of APF and EPF, solids were dissolved in bidistilled water under aseptic conditions. Again sterility was obtained by filtration (0.22 μm Sartorius® membran filter). The composition of the test preparations is documented in Table 3.

3.2. Methods

3.2.1. Rheological characterisation

For rheological characterisation AT was mixed 1 : 1 with polymer solution to simulate the situation of application before rheological parameters were measured. Thixotropy was determined by increasing the sheer rate from 0 to 2000 (1/s) and back to 0 (1/s) during 200 s and measuring the sheer stress each 4.17 s. All data were measured with a Rheolab® MC 100 universal measuring drive system using the concentric cylinders double gap measuring system MS-Z1 DIN (Physica GmbH, Stuttgart, Germany) at 32 °C.

3.2.2. Mucoadhesion

Mucoadhesion was determined by investigating the rheological changes of a polymer in contact with mucin by the following equation:

$$\mu MA = \mu MP - (\mu MB + \mu PP)$$

where μMA is the index of mucoadhesion (mPas), μMP is the apparent viscosity (mPas) of mixture of polymer in bidist. water with a solution of 20% mucin in AT-buffer 5.5 or 7.4, μMB is the apparent viscosity (mPas) of a mixture of 20% mucin solution in AT-buffer 5.5 or 7.4 mixed with bidist. water, and PP is the apparent viscosity (mPas) of polymer solution in AT-buffer 5.5 or 7.4 mixed with bidist. water 1 : 1.

3.2.3. Miosis test

The miotic effect of GPF was evaluated in eight female white New Zealand rabbits wheighing about 2.0 – 2.5 kg. The rabbits were housed separately in cages under standard laboratory conditions: 12 h dark/12 h light cycle. The rabbits had free access to food and water. The experiments conformed to the German law for the prevention of cruelty to animals. Samples 50 μl were administered into the conjunctival sac of one eye, while the other eye served as control. Measurements were carried out under standardized light conditions. The pupillary diameter was measured with a stencil. Wells of the stencil varied 0.5 mm in diameter.

3.2.4. Permeation study

The permeation study was carried out with bovine cornea on a diffusion apparatus described by Siefert and Keipert [18]. The donor is filled with 1.0 ml of each formulation, the receiver, with the same capacity, is constructed in a way that 30 ml Soerensen phosphat buffer 7.4 could be pumped in a circle. The two compartments are separated by bovine cornea with the epithelial site facing the donor and covering an area of 0.95 cm^2.

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