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In Vitro Evaluation of the Precorneal Residence of Water Soluble Polymers by Measuring Contact Angles

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The purpose of this investigation is to evaluate the capability of some water soluble polymers to adhere to the ocular surface and the effect of solution viscosity on precorneal residence. Captive bubble technique was used to study the desorption kinetics of those polymers from freshly enucleated rabbit eyes under physiological conditions. It was shown that the contact angle on mucin-coated corneal surface was 38.2±1.6° measured by sessile bubble method, and sodium hyaluronate (HA) had the most appropriate corneal wettability although not decreasing the surface tension. At a similar solution viscosity, the retentive capability of the five viscolyers was in the order Carbopol (residence time 15 to 25 min)>HA (15 to 22 min)>polyvinyl alcohol≅hydroxy-propylmethylcellulose (10 to 15 min)>sodium carboxymethylcellulose (10 min), which demonstrated that the viscosity had different effects on the residence time of various viscolyers.

Conventional ophthalmic solutions instilled into the cul-de-sac is rapidly eliminated due to tear turnover, resulting in low bioavailability; less than 5% of the drug permeates the cornea into the aqueous humour[1]. Since the drug concentration in lacrimal fluid acts as the driving force for passive diffusion, prolonging the contact time of drug on the corneal surface has been an effective approach to improve topical bioavailability. Moreover, sufficient contact time may be decisive for successful treatment of precorneal diseases, such as infections and inflammations.

Numerous studies have claimed enhancement of drug ocular retention using natural or synthetic water-soluble polymers. In these studies, the drainage rate decreased efficiently by moderately increasing viscosity[2-4]. After the pioneering work of Hui and Robinson[5], who reported that ocular bioavailability was significantly increased by a bioadhesive polymer, the physicochemical characteristics of different polymers and their capability of attaching to mucin on the cornea and conjuntival surface were considered as important factors by many researchers when screening for an appropriate viscosifying agent.

Based on a premise that the drainage of a drug, especially a less soluble drug, will be retarded by macromolecular entanglements, great efforts have been made to maximize ocular drug absorption by an optimum viscous solution. Therefore, rational estimation of various viscolyers *in vitro* should be useful in designing ophthalmic drug delivery systems. The aim of the present work is to evaluate the capability of polymers adhering to ocular surface and to observe the influence of viscosity on precorneal retention of different polymers.

MATERIALS AND METHODS

Carbopol 934P was received as a kind gift from BF Goodrich Co., USA. Sodium hyaluronate (ca. 200,000 MW), henceforth designated as HA, was obtained from Huayuan Biological Engineering Co., China;

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hydroxypropylmethylcellulose (HPMC K4M) from Colorcon Co., UK; Dulbecco's modified eagle medium (D-MEM) from Gibco Co., USA. Polyvinyl alcohol (PVA), sodium carboxymethylcellulose (CMC-Na) and sodium pentobarbital were from commercial sources. All other agents were of analytical grade.

Preparation of polymer solutions:

Carbopol was well dispersed in saline by magnetic stirring, then was neutralized by addition of 10% NaOH solution up to pH 7.4. This viscous solution was left standing for 1 d to hydrate and swell the polymer completely. HPMC, CMC-NA, PVA and HA solutions were prepared with isotonic phosphate buffered solution (PBS) pH 7.4.

Physicochemical measurements:

The apparent viscosity of each polymer solution was determined using a rotary viscometer (model NDJ-1, Shanghai, China) at a defined shear rate. All the measurements were carried out at 34° (corneal surface temperature). The effect of polymer macromolecules on solution osmolality was studied by measuring the freezing point depression[6]. The surface tension of polymer solutions was measured at 34° by maximum bubble pressure method[7].

Study of desorption kinetics from corneal surface:

Albino rabbits weighting 2.5-3.0 Kg were provided by the Animal Experimental Center of Shenyang Pharmaceutical University. The eyes were enucleated extra-orbitally after rabbits were killed with an overdose of sodium pentobarbital injected into the marginal ear vein, and were kept in Dulbecco tissue culture medium until the measurements began.

Using the captive bubble technique, the desorption kinetics of various polymers was determined by observing consecutive change of contact angle on the corneal surfaces of freshly enucleated rabbit eyes under physiological conditions as described by Shaker *et al*[8]. In the present study an air bubble was adapted instead of the mixed-gas bubble consisting of 95% O_2 and 5% CO_2 and the same bubble was kept on the down-facing cornea throughout one desorption process. Contact angles were recorded as soon as the enucleated eye, which was coated with a layer of polymer solution, was placed into the O_2 saturated Ringer's solution and then at interval of 5 min until no change can be observed. Contact

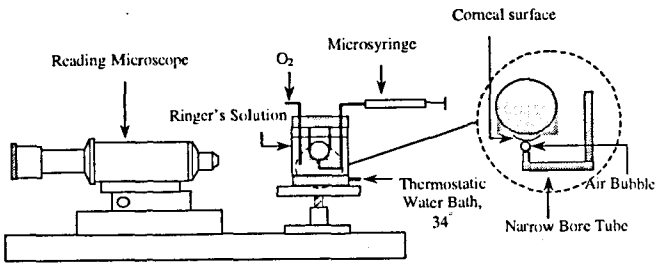

Fig. 1: Device used for the measurement of the contact angles on enucleated ocular

Uniform size air bubbles were generated on the ocular surface utilizing the microsyringe, then contact angle of Ringer's solution was measured visually with the microscope.

angles were calculated from measuring the dimensions of the bubbles using a reading microscope (model XPT-7, Nanjing, China) (fig. 1) according to the following equation:

$$\theta = \cos^{-1}\frac{2H}{W} - 1 \qquad \theta<90°$$

where H and W refer to the height and width of a bubble, respectively. The residence time was the mean of replicate determinations on each eye of at least four different rabbits. For some of the freshly enucleated eyes used in desorption study, the hydrophobicity of the corneal surface was studied by sessile bubble method[9]. After completion of contact angle measurements histological examination was conducted on the eyes using an optical microscopy, and corneal tissue damage was compared with eyes that were preserved immediately after enucleation.

RESULTS AND DISCUSSION

The concentrations of various polymer solutions with viscosity of approximately 12, 25 and 50 mPa·s are listed in Table 1. Solution viscosity was nonlinearly increased with polymer concentration. Polymers showed different viscosity-enhancing capabilities, at a similar viscosity, the concentrations of PVA were more than 10 times compared to that of HA. Because ionized carboxyl groups on the polymer chains generate electrolytic particles, Carbopol and CMC-NA solutions had lower freezing points compared to HPMC and PVA solutions. The ions introduced by addition of NaOH to neutralize Carbopol dispersion contributed to depress freezing point. HA solutions had an intermediate freezing point. The polymers,

TABLE 1: CHARACTERISTICS OF VISCOLYER SOLUTIONS

Composition (w/w)	Viscosity (mPa·s)	Freezing point (°)	Surface tension (mN/m)	pH
saline	0.98	-0.54	70.5	7.40
Carbopol 0.40%	12.2	-0.57	68.2	7.40
Carbopol 0.47%	24.2	-0.60	68.0	7.38
Carbopol 0.50%	53.4	-0.56	68.0	7.42
Phosphate buffer sol.	0.98	-0.55	70.5	7.39
HPMC 0.43%	12.0	-0.52	65.0	7.39
HPMC 0.55%	24.7	-0.52	63.8	7.38
HPMC 0.68%	50.3	-0.53	60.6	7.40
PVA 2.70%	12.0	-0.57	59.3	7.39
PVA 3.60%	25.3	-0.57	59.0	7.39
PVA 4.30%	51.7	-0.56	60.2	7.40
CMC-NA 0.50%	11.8	-0.59	68.2	7.39
CMC-NA 0.70%	25.1	-0.57	68.5	7.39
CMC-NA 0.91%	50.0	-0.64	64.7	7.39
HA 0.17%	12.0	-0.57	71.3	7.41
HA 0.23%	25.2	-0.57	72.7	7.32
HA 0.31%	49.2	-0.57	73.2	7.32

Carbopol powder was dispersed in saline and was neutralized by addition of NaOH up to pH 7.4. HPMC, CMC-Na, PVA and HA solution were prepared with isotonic phosphate buffered solution.

used at such low concentrations, had no significant effect on freezing point.

HPMC and PVA exhibited obvious surface activity, while Carbopol and CMC-Na decreased the surface tension of the vehicle slightly. HA did not reduce surface tension of its solutions, hence possessed no surface activity.

Contact angle measurements provide an appropriate method to characterize the hydrophilicity /hydrophobicity of a solid surface. Like other biological tissues, extracellular fluid is essential for maintaining ocular cellular viability. Exposure to the air causes dehydration of the enucleated ocular, resulting in loss of biological activity and consequent irreversible changes in the native ocular surface properties. The technique of measuring contact angles of a water-immiscible liquid or air on solid surfaces in water has the potential to probe surface properties of tissues under stimulated physiological conditions, so it is widely used in biological fields.

The corneal surface is coated with a layer of mucin, which hydrates, cleanses, lubricates, serves as a defense against pathogens, and determines the surface property of the cornea. The contact angle on freshly enucleated eyes measured by sessile bubble method was 38.2 ±1.6° (n=10). The smaller contact angle value obtained here than that in an earlier report using captive bubble method (50 ±5°)[8] may be predominantly due to the difference between the two experimental methods and the diminished polar forces of gas/solid and gas/liquid between the interface. A surprisingly similar contact angle of human retina (38.8°)[10] suggested that the properties of both tissues were alike. Lehr *et al.*[11] have measured the contact angle on pig small intestinal mucosa in various physiological media ranging from 48°

to 61°. These results imply that the cornea possesses a more appreciable hydrophilicity than the intestinal tract, and is more readily wetted by tears.

The hydrophobicity of a surface alters after absorption of a hydrophilic polymer resulting in lower contact angle, while when the original surface re-exposes, the contact angle becomes high again. The desorption processes can therefore be observed from the consecutive change of contact angles, as is seen in fig. 2.

The wetting property of a water-soluble polymer is related to the surface tension of its solution. Polymers showing surface activity, such as PVA, lowered initial contact angles (fig. 3). It is interesting that although HA decreased the contact angle as much as 17.3° yet did not reduce the surface tension measured by maximum bubble pressure method. This may be due to its molecular flexibility and the existence of various conformations in the solution[12]. When adsorbed onto the corneal surface, in order to decrease interface free energy by means of reorientation, the hydrophobic groups of HA may be attached to the corneal surface and the hydrophilic groups expose in the solution, so a small contact angle was obtained.

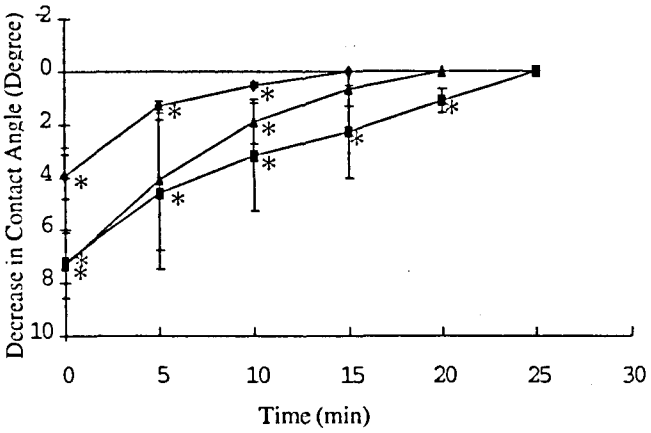

Fig. 2: Desorption kinetics of Carbopol at various viscosity from the freshly enucleated ocular surfaces (n=4).

Symbols (-◆-), (-▲-) and (-□-) represent carbopol solutions with low (12 mPa·s), medium (25 mPa·s) and high (50 mPa·s) viscosity, respectively. Vertical bars indicated ±SD of 4 observations. Statistically significant difference from the mucin coated cornea at * $P<0.05$ were tested using a paired *t*-test.

Polymers have different retentive properties on the corneal surface. The data listed in Table 2 illustrate that

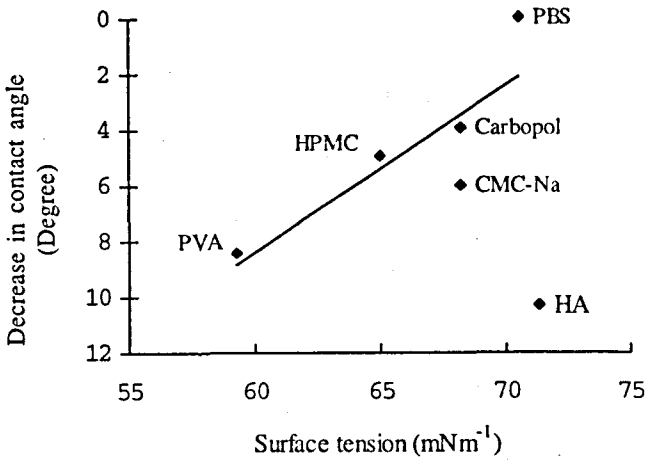

Fig. 3: Graph illustrating the relationship existing between the decrease in initial contact angle and surface tension of various polymer solutions

The viscosity of all the polymer solutions was approximately 12 mPa·s. The difference between the contact angles before and after coated with polymer was defined as decrease in contact angle. The surface tension of Ringer's solution was considered as same as that of PBS.

these polymers could be generally divided into three groups according to the relationship between viscosity and residence time. Under our experimental conditions, both Carbopol and HA could adhere to the freshly enucleated eye not less than 15 min, and their residence time tended to be lengthen with increasing viscosity. Compared to Carbopol solutions with higher viscosity, the shorter residence time of equiviscous HA solutions evidenced its inferior precorneal retentive capability (16 and 22 *vs* 20 and 25, respectively). HPMC and PVA are polymers traditionally used as ophthalmic viscosifying agents, however, neither of their residence times were as significantly effected by viscosity as mucoadhesive polymers. The precorneal residence time of the less viscous PVA solution was more sensitive to viscosity. This result is consistent with Ludwig's conclusion drawn by measuring the precorneal elimination rate of fluorescent substance *in vivo*[2]. Different from the other polymers, solution viscosity had no effect on the ocular retention of CMC-Na and no statistical differences were observed between the decrease in contact angle of each viscosity group.

The variance in residence times of equiviscous solutions implied different mechanisms of interaction between the polymers and the corneal mucin. For

TABLE 2: PRECORNEAL RESIDENCE OF VISCOLYERS

Composition	Viscosity (mPa·s)	Decrease in contact angle (degree) (mean±SD)	Residence time (minute) (mean±SD)	n
Carbopol	12.2	4.0±0.8	15	4
	24.2	7.4±1.2	20	4
	53.4	7.2±4.4	25	4
HA	12.0	10.3±2.4	15	4
	25.2	15.4±1.7	16 ±2	5
	49.2	17.3±5.1	22±3	4
HPMC	12.0	5.0±1.8	10	4
	24.7	4.8±2.3	10	4
	50.3	7.8±3.2	15	4
PVA	12.0	8.4±3.8	10	4
	25.3	6.7±1.7	12±2	7
	51.7	9.0±3.0	15	4
CMC-NA	11.8	6.0±3.2	10	4
	25.1	4.0±0.2	10	4
	50.0	4.9±2.3	10	5

Decrease in contact angle and residence time were the mean of replicate determinations and n is the number of measurements.

non-mucoadhesive polymers such as HPMC, PVA and CMC-Na, desorption process may be related to their diffusion rates. Viscosity and concentration of the polymer solution layer adjacent to the cornea play contrary roles during the passive diffusion, that is polymer diffusion into biological medium was retarded by the increased viscosity while was prompted by the raised concentration. Simultaneously subjected to both of the two actions, the residence time of these polymers could not be efficiently prolonged. However, the retention of CMC-Na was not affected by viscosity probably due to its rapid diffusion rate. Mucoadhesive polymers usually have numerous hydrophilic functional groups, such as carboxyl, amide and sulfate, which can establish electrostatic or hydrophobic interactions and hydrogen bonding with the underlying surface. The flexibility of the polymer will allow the chains to diffuse and penetrate into the mucin layer resulting in a loose network, so that the drainage of the incorporated drug from the precorneal area is sustained[13]. The sites of above interactions will increase with viscosity, which may explain the longer residence time of Carbopol and HA as well as the relationship between their retention and viscosity.

Optical microscopic investigations of cornea after exposure to various test solutions showed the outermost layer of corneal epithelium consisted of squamous superficial cells and no keratinization or papillae were observed. The cornea was kept intact during contact angle measurements.

In conclusion, the precorneal retentive capabilities of five viscolyers were distinguished by *in vitro* studying desorption kinetics from viable tissue, and Carbopol showed powerful potential for adhering to the cornea. Moreover, the excellent corneal wettability of HA is expected to help distribute the drug vehicle evenly on the eye. If the result is proven to be relevant to the *in vivo* situation by further tests and the observation interval is shortened, this inexpensive experimental method will be useful in screening many more ophthalmic viscosifying agents.

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