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International Journal of Pharmaceutics 119 (1995) 149–163

international journal of pharmaceutics

An in vitro technique for measuring contact angles on the corneal surface and its application to evaluate corneal wetting properties of water soluble polymers

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Received 4 August 1994; revised 22 November 1994; accepted 23 November 1994

Abstract

The development of an in vitro technique capable of measuring the extent and duration of corneal wetting by demulcent polymers is reported. The technique required modification of a contact angle goniometer to facilitate the measurement of contact angles of demulcent polymer solutions on freshly enucleated rabbit eyes. Contact angles of the test solutions on the corneal surface was measured under physiologically relevant conditions. Contact angle measurements were performed under conditions to ensure that the tissue was not dehydrated and the native surface characteristics of the tissue were unchanged. Additionally, experimental procedures for contact angle measurements were developed in order to provide a partial simulation of in vivo fluid dynamics that is typically observed upon topical instillation of a drop of a test polymer solution into the eye of a patient. This multistep experimental procedure was initiated by briefly dipping a freshly enucleated rabbit eye in the test polymer solution. The treated eye was then immersed upside-down in an oxygenated, lactated Ringer's solution and placed in the chamber of the goniometer. This medium was used to maintain ocular tissue viability and corneal surface integrity over the duration of the experiment. For measurement of contact angles, a well defined air bubble was slowly introduced into the chamber of the goniometer at close proximity to the enucleated eye. The air bubble was then entrapped on the corneal surface. Angle of contact of the air bubble against the corneal epithelial surface was visually measured with the goniometer. A new air bubble was entrapped at intervals of 5 min and the contact angle was measured as a function of time. The degree and duration of reduction of contact angle was observed to depend on the type and concentration of the water soluble polymer used. Most demulcents were found to rapidly desorb from the corneal surface. For these demulcent polymers the contact angle returned to pre-treatment values within 25 min of the start of the experiment. Hyaluronic acid showed the most sustained wetting of the cornea. The in vitro kinetic measurement of contact angle demonstrated the time dependent, marginally functional mucomimetic properties of demulcent polymers. The kinetics of desorption was considered to partially simulate the fluid dynamics in the eye. The validity of the inverted air bubble as well as this new technique for the kinetic measurement of contact angles was established on synthetic (polyethylene) surface prior to its utilization on freshly enucleated ocular surface. The measured contact angle and estimates of critical surface tension for polyethylene surface was in close agreement with values reported in the literature and with values obtained from conventional techniques of measurement of

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Elsevier Science B.V.
SSDI 0378-5173(94)00381-5

contact angle. The contact angle of lactated Ringer's solution on the rabbit corneal surface ranged from 45 to 55°. Removal of mucin from the corneal surface increased the contact angle of the cornea in the range of 55-65°. This increase in contact angle of the corneal epithelium devoid of mucin indicated decreased wetting and demonstrated its greater hydrophobicity than the mucin coated epithelium. Adsorption of demulcent polymers solutions decreased the contact angles to as low as 30° indicating enhanced wetting of the cornea. The measurement of contact angles of various demulcent polymer solutions possessing different surface tensions made it possible to estimate the critical surface tension of the rabbit corneal epithelium. The critical surface tension, representing the minimal surface tension of a formulation necessary to completely wet and spread over the corneal epithelium was estimated at 39 dyn/cm. Therefore, the reported air bubble trapping along with the kinetic technique for measurement of contact angles may be useful as an in vitro screening tool for the evaluation of new demulcent polymers. The technique may also be used for the identification of superior tear substitutes for the treatment of dry eyes.

Keywords: Demulcent polymer; Contact angle; Wettability; Ocular surface; Critical surface tension; Corneal epithelium; Desorption kinetics

1. Introduction

Ocular mucus membranes and cornea are covered by a thin film of tears (Wolff, 1954; Lamberts, 1983) that provides nutrients and is essential for maintaining good visual acuity. Even in normal eyes the tear film exists for only a short period of time before areas of 'break-up' are observed (Lemp and Hamill, 1973). In dry eye conditions there are qualitative as well as quantitative changes in tears, resulting in tear film instability and that eventually lead to ocular surface damage (Lemp, 1987). Artificial tear formulations containing demulcent polymers are extensively used for treating dry eye conditions (Bron, 1985; Lemp, 1987; Department of Health and Human Services, 1988). It is generally believed that for optimal performance the demulcent polymers should exhibit mucomimetic properties, i.e., act like ocular mucin (Lin and Brenner, 1982a,b; Sharma and Ruckenstein, 1985a,b, 1986a,b). It is speculated that mucomimetic polymers may help to stabilize the tear film and maintain hydration of the cornea, attributes which are believed to be important in the treatment of dry eyes. The nature of the precorneal tear film together with factors involved in wetting of ocular surface by tears has been a subject of extensive research. The importance of surface chemical properties of tear substitutes has been widely recognized (Holly and Lemp, 1971; Holly, 1974, 1978a,b,c, 1979, 1985; Tiffany et al., 1989). The influence of physicochemical properties of the vehicle on the retention of formulations in the eye has also been studied by several investigators (Krishna and Brow, 1964; Blaug and Canada, 1965; Krishna and Mitchell, 1965; Bach et al., 1970, 1972; Maurice, 1971; Benedetto et al., 1975; Hardberger et al., 1975; Lemp and Szymanski, 1975; Gilbard et al., 1978, Gilbard and Farris, 1983). Despite the importance of each measured property of the formulation, no definitive conclusions can be made regarding the influence of these properties in providing extended in vivo relief from dry eye symptoms. Preservatives, another essential component of artificial tear substitutes, have been reported to influence the surface chemical properties and in vivo performance of the products (Tripathi and Tripathi, 1989; Bernal and Ubels, 1991). Admittedly, most commonly used demulcent polymers are not mucomimetic, due in part to their poor corneal wetting and retention on the corneal surface. Hence, there is a need to identify new polymers or compositions with better mucomimetic properties. However, presently there are no in vitro models for assessing the mucomimetic properties of demulcent polymers. Furthermore, there are no good animal models for dry eye and conducting definitive human clinical studies for screening a large number of formulations is cost prohibitive.

The stability of a fluid film over a solid surface is governed by well established principles of interfacial science and surface chemistry. Sheludko (1967), outlined the following factors that influence the stability of thin films on solid surfaces:

(a) wetting property of the liquid on the solid surface, obtained by contact angle measurements; (b) surface excess free energy of the solid, obtained by calculating the critical surface tension; (c) spreading properties of the liquid on the solid; (d) interfacial tension between liquid and solid; and (e) surface tension, density and viscosity of liquid that forms the film. Recent investigations in bioadhesion (Robert et al., 1988) and mucoadhesion (Gu and Robinson, 1988) and their applications in the development of prosthetic and controlled drug delivery devices have evoked studies to provide a better understanding of the role of surface energetics in these phenomena (Lehr et al., 1992). Among the various muco-epithelial tissues of interest, the ocular surface has been a target tissue for achieving bioadhesion to enable controlled delivery of drug substances (Ueno and Refojo, 1983). Results obtained from different investigations are difficult to interrelate because of differences in experimental methodology used for preparation of tissue and also due to the differences in procedures used in measurement of the desired property. It is imperative that a suitable in vitro technique be available for appropriate and reproducible evaluation of surface properties of a tissue such that the properties of the tissue undergoes minimal changes during the in vitro measurements. Therefore, the objective of this study was to establish an in vitro model for rapidly assessing the wetting and retentive properties of demulcent polymers on the corneal surface and to demonstrate the utility of the technique for identifying polymers which are likely to have superior mucomimetic properties. Specifically, the objectives of this investigation were: (a) to determine the hydrophobicity of the rabbit ocular surface with and devoid of mucin; (b) to determine the wetting properties of water soluble polymers on the ocular surface; and (c) to evaluate the relative mucomimetic properties of these polymer solutions.

2. Materials and methods

2.1. Materials

The following chemicals were obtained from Fisher Scientific Co., Fairlawn, NJ and used without further purification: sodium chloride, calcium chloride dihydrate, magnesium chloride hexahydrate, sodium bicarbonate, sodium carbonate and potassium dihydrogen phosphate. Dextrose, methylcellulose (A15LV, E15LV and MC4000), carboxymethylcellulose, hydroxypropylmethylcellulose, polyvinyl alcohol, polyvinylpyrrolidone and hydroxyethylcellulose, hydroxypropylcellulose, polyethylene glycol 3350 were of USP grade. Sodium pentobarbital was obtained as a 6.5% solution from Butler Co., Columbus, OH. Acetylcysteine, bovine submaxillary mucin, bovine serum albumin and hyaluronic acid were obtained from Sigma Chemical Co., St. Louis, MO. Dulbecco's modified eagle medium (D-MEM; 320-1965) was obtained from Life Technologies Inc., Gaithersburg, MD.

2.2. Preparation of test solutions

Test solutions of commercially precedented demulcent polymers as well as investigational polymers were prepared fresh in deionized, distilled water at concentrations ranging from 1.5 to 0.01% by weight.

2.3. Preparation of physiological media

The physiological medium used for the investigations was a Ringer's solution of the following composition: 134 mM sodium chloride, 1.4 mM magnesium chloride hexahydrate, 1.5 mM calcium chloride dihydrate, 20 mM sodium bicarbonate, 5.0 mM sodium carbonate, 5.0 mM potassium dihydrogen phosphate and 10 mM dextrose. Dulbecco's modified eagle medium was used for rinsing tissues during the surgical procedure and for storage of the enucleated eye until the commencement of contact angle measurements.

2.4. Animals

All procedures using animals conformed to the ARVO Resolution on the Use of Animals in Research. The protocols describe were also approved by the Pfizer Animal Care and Use Committee. Female, New Zealand, albino rabbits weighing 2.5–3.0 kg were used in these investigations. Rabbits were placed in a restrainer and an area of fur over the marginal ear vein was clipped with a pair of scissors. A 10% tetracaine solution was applied to the exposed surface of the rabbit ear then the animals were killed with an overdose of sodium pentobarbital injected into the marginal ear vein.

2.5. Surgical procedure

Prior to initiating the surgical procedure the eyelids were closed and clamped using hemostat scissors. This prevented the ocular surface from drying as well as it protected the ocular surface from contamination. The eyes were enucleated extra-orbitally with the connective tissue holding the conjunctival sac in place and by cutting the optic nerve such that the ocular globe retained its shape. Next, all accessory glands and connective tissue was removed from the eye. Finally, the hemostat scissors were removed and the ocular surface was exposed by everting the eye lids. The conjunctival sac was clipped and the enucleated eye was placed in Dulbecco tissue culture medium until it was transferred into oxygenated, Ringer's solution maintained at 34°C. Eyes were used for surface-chemical characterization within a few hours of enucleation. The development of opacity in the cornea was considered as an indication of loss of tissue integrity, therefore, all measurements reported in this study were performed on eyes with only clear, transparent cornea.

2.6. Contact angle measurement: conventional technique

Contact angles were measured using an NRL contact angle goniometer (Rame-Hart, Inc.,

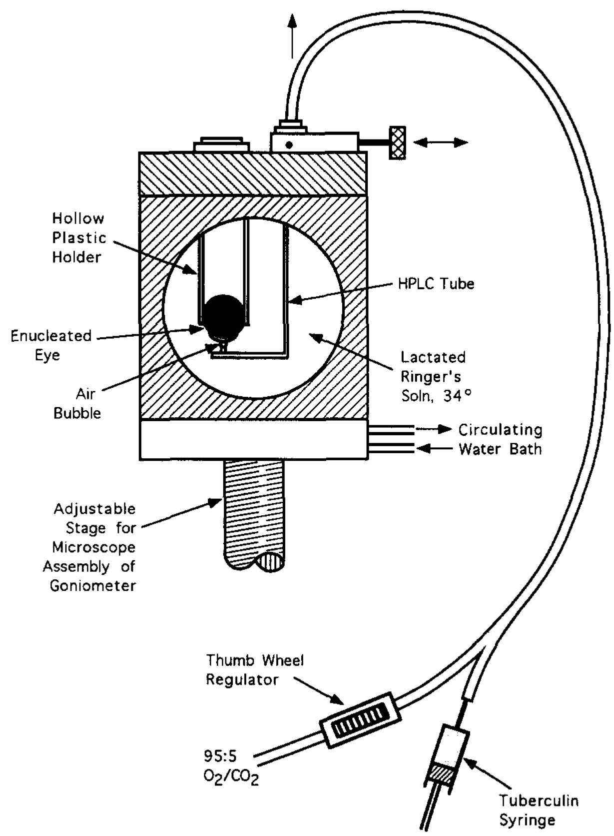

Fig. 1. Schematic diagram showing experimental set-up for measurement of contact angles of demulcent polymers on ocular surface by the captive air bubble technique.

Mountain Lakes, NJ), equipped with an environmental chamber. Temperature of the environmental chamber was maintained at 34°C by connecting it to a circulating water bath (Model Exacal EX-200β, Neslab Instruments Inc., Portsmouth, NH). Contact angles of test solutions on synthetic substrates was measured by the conventional technique as described in the instruction manual of the goniometer.

2.7. Contact angle measurement: air-bubble capture technique

Modifications were made on the goniometer to enable the measurement of contact angle of a solution on the surface of interest when the solid substrate was immersed in the test liquid (Fig. 1). Modifications were also made to the environmental chamber to reduce its volume to approx. 10 ml. As shown in Fig. 1, a custom-designed teflon holder was used to position the enucleated eye in the environmental chamber such that the corneal surface faced downwards. A stainless-steel tube with an internal diameter of 0.01 inches (Rainin Instrument Co., Inc., Woburn, MA) was bent into non-symmetrical U-shape and was placed inside the environmental chamber. A piece of Tygon tube was attached to the stainless-steel tube and the former was connected to a gas tank containing 95% oxygen and 5% carbon dioxide. The gaseous mixture was continuously bubbled at a slow rate to maintain ocular tissue viability. A tuberculin syringe was also attached to the Tygon tube via a y-connector. The syringe was utilized to generate uniform size air bubbles in a controlled manner to facilitate their disposition at the desired spot on the ocular surface. Contact angle of the air bubble was measured visually with the goniometer.

2.8. Desorption of demulcents: synthetic surface

Studies were conducted to assess the ability of tear substitutes to adsorb to synthetic substrates. The ability of the tear substitutes to resist desorption from the synthetic surfaces was also investigated. In a unique adaptation of the air-bubble capture technique, the kinetics of desorption of demulcents from a polyethylene test surface was determined by measuring contact angles at intervals of 5 min. The study was accomplished by following the following sequence of steps: (a) the polyethylene substrate was immersed in the test solution (0.05% w/w) for 30 min; (b) the coated substrate was submerged in the environmental chamber containing water; (c) contact angle of water was measured on the submerged surface; (d) after the lapse of 5 min, the air bubble was gently dislodged and a new air bubble was placed on the substrate at the same spot and its contact angle was measured.

2.9. Desorption of demulcents: ocular surface

The enucleated eye was placed in the custom designed teflon holder and then immersed in the test, isotonic demulcent solution for approx. 30 min at 34°C. The coated ocular surface was then placed in the environmental chamber of the goniometer containing Ringer's maintained at 34°C. During measurement of contact angles the flow of 95%/5% oxygen/carbon dioxide gas was stopped and all subsequent steps were identical to the procedure described in the previous section.

2.10. Histology

After completion of contact angle measurements the eyes were preserved in formalin. The cornea was removed from preserved eyes by cutting across its center and then cutting along the periphery. Each half of the cornea was processed separately, microtomed and stained with hematoxalin and eosin. Histological examination was conducted on all eyes and corneal tissue damage was compared with eyes that were preserved immediately after enucleation without contact angle measurements.

3. Results and discussion

The use of flat substrate surface is essential in contact angle measurement using the conventional technique. This requirement presented two limitations for the measurement of contact angle on corneal surface. First, the external surface of the cornea has significant curvature. Second, like most biological tissue, eyes are bathed in an extracellular fluid that is essential for maintaining its cellular viability. Removal of this fluid causes dehydration that results in loss of tissue viability and consequently there is irreversible changes in the native surface characteristics of the tissue. Therefore, to enable proper characterization of the wettability of the ocular surface it was essential for a technique to accommodate curved surfaces as well as maintain the viability of the enucleated tissue. Use of a captive bubble technique for measurement of contact angles on hydrogels immersed in water was described by Andrade et al. (1979). This method of contact angle determination was originally described by Hamilton (1972). In the current investigation, adaptation of the captive bubble technique along with

Table 1
Influence of different demulcent polymers on the wettability of synthetic substrates

Polymer concentration	% w/w	Contact angle (θ)(mean ± S.D.)		
		Polyethylene	Teflon	Silastic
Water		94 ± 2	145 ± 3	95 ± 3
HPMC	1.0	61 ± 2	119 ± 2	69 ± 1
	0.5	63 ± 1	121 ± 3	73 ± 3
	0.25	63 ± 2	125 ± 3	74 ± 1
	0.05	69 ± 2	131 ± 1	78 ± 2
HPC	1.5	70 ± 2	127 ± 3	79 ± 2
	1.0	75 ± 1	134 ± 3	90 ± 3
	0.5	77 ± 3	135 ± 3	90 ± 4
HEC	1.5	89 ± 3	144 ± 1	96 ± 1
	1.0	92 ± 1	145 ± 4	99 ± 1
	0.5	93 ± 3	145 ± 3	100 ± 1
	0.25	94 ± 2	147 ± 1	105 ± 2
CMC	1.0	99 ± 2	135 ± 2	90 ± 3
	0.5	103 ± 2	140 ± 4	109 ± 1
	0.2	105 ± 2	145 ± 1	111 ± 3
	0.05	105 ± 2	150 ± 4	111 ± 1
PVA	1.5	70 ± 3	135 ± 2	90 ± 3
	1.0	72 ± 1	142 ± 3	92 ± 3
	0.5	74 ± 3	141 ± 3	94 ± 1
	0.25	77 ± 3	145 ± 3	95 ± 2
BSA	0.5	89 ± 2	145 ± 3	101 ± 3
	0.1	92 ± 2	149 ± 3	103 ± 1
	0.05	92 ± 1	152 ± 3	104 ± 1
	0.01	94 ± 3	157 ± 3	105 ± 1
Dextran	1.5	97 ± 4	152 ± 1	105 ± 2
	1.0	96 ± 2	151 ± 4	106 ± 2
	0.5	99 ± 1	155 ± 3	108 ± 2
	0.25	102 ± 3	156 ± 4	110 ± 2
Fibronectin	0.1	85 ± 2	146 ± 2	95 ± 1
	0.05	103 ± 2	140 ± 1	106 ± 3
	0.025	100 ± 3	144 ± 1	108 ± 1
	0.001	108 ± 5	148 ± 3	109 ± 4
Hyaluronic acid	0.5	90 ± 1	155 ± 2	102 ± 2
	0.1	102 ± 1	147 ± 3	105 ± 1
	0.05	102 ± 1	150 ± 1	107 ± 3
	0.01	104 ± 1	147 ± 1	108 ± 3

HPMC, hydroxypropylmethylcellulose; HPC, hydroxypropylcellulose; HEC, hydroxyethylcellulose; CMC, carboxymethylcellulose; PVA, polyvinyl alcohol; BSA, bovine serum albumin.

providing a unique capability for investigating the kinetics of desorption of a polymer from a solid substrate has enabled a better assessment of the surface chemical properties of demulcent polymers as well as that of the ocular surface.

3.1. Contact angles on synthetic substrates

Initially, all in vitro evaluations of the wetting property of water soluble polymers were conducted by measuring their contact angles on synthetic substrates. This approach was undertaken: (a) to facilitate development of test methodology; (b) to minimize use of number of animals required for the studies; and (c) to corroborate results obtained by the modified technique with those reported by other investigators. Due to the lack of adequate information about the surface chemical properties of the corneal surface, three test substrates, polyethylene, teflon and silastic were chosen as model surfaces. The contact angles of various water soluble polymers at several concentrations of each of the polymers was determined by the conventional technique. Results from these investigations are summarized in Table 1. The data indicate that polyethylene was the only test substrate that provided some degree of differentiation among the various polymers. The data in Table 1 also show that the water soluble polymers generally provided better wetting, i.e., lower contact angles, with increasing concentration of the polymers particularly on polyethylene surface. However, only a marginal improvement in wettability was observed. Further increase in concentration of the polymer usually resulted in substantial increase in viscosity of the solution. Since solutions of high viscosity upon instillation into the eye impairs visual acuity, therefore, higher concentrations of the polymers were not investigated.

The need for a technique capable of measuring contact angles on submerged test substrates was previously enumerated. Modifications of the goniometer (Fig. 1) enabled measurement of contact angle of a solution on the test substrate when the substrate was immersed in a liquid. However, as illustrated in Fig. 2, in this procedure the method of measuring the contact angle was

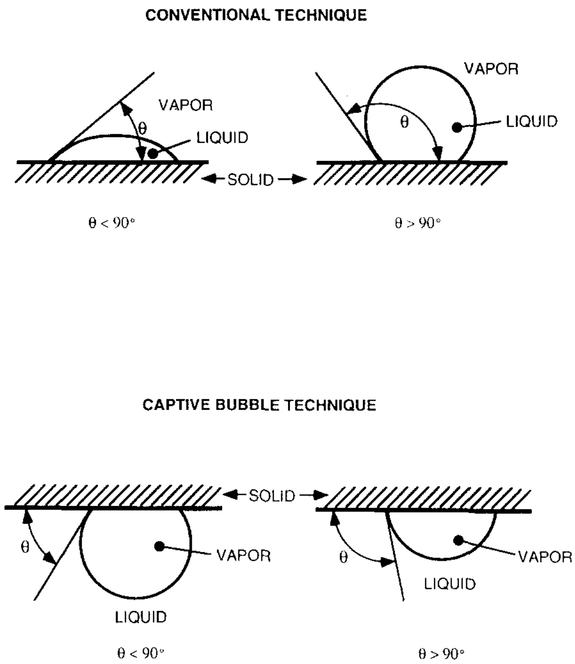

Fig. 2. Conventions used for contact angle measurements for the assessment of wettability of a substrate by the conventional and captive air bubble techniques.

slightly different from the conventional technique. Although, it was desirable to measure the wetting properties of demulcent polymers it was also important to ascertain the ability of these polymers to adsorb on to a test substrate and assess their capacity to resist desorption. It was believed that such investigations could provide information about the adhesive as well as the ocular retentive properties of the demulcent polymers. Therefore, in this study the modified goniometer was uniquely used to study the kinetics of desorption of polymers solutions from polyethylene surface. Results from these kinetic studies are shown in Fig. 3 and they demonstrate the utility and benefit of this technique in differentiating various demulcent polymers in their ability to adsorb and wet polyethylene surface. The data in Fig. 3 emphasize the fact that most demulcent polymers lack adsorptive capability and are rapidly desorbed from the surface of polyethylene. Inclusion of bovine submaxillary mucin in the desorption studies distinctly illustrates the

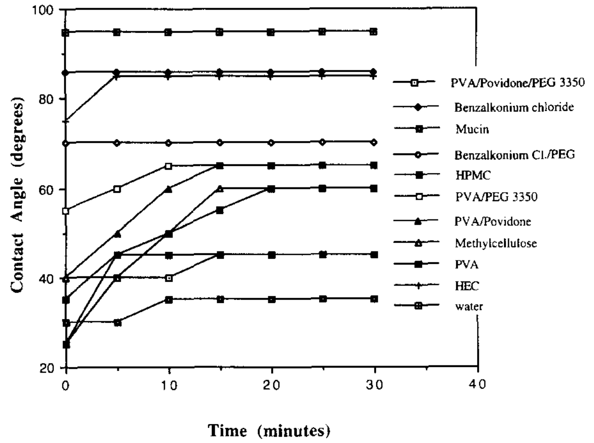

Fig. 3. Kinetics of desorption of demulcent polymers from polyethylene surface measured by captive air bubble technique.

ineffective mucomimetic property of the demulcent polymers.

Despite the uniqueness of the techniques for studying the desorption of demulcents these results do not facilitate in predicting the in vivo performance of the polymers when instilled into the eye. However, the results do provoke an interesting question. Why is there a time-dependent change in contact angle of water on polyethylene coated with demulcent polymer when polyethylene is immersed in water? The query is significant because when the demulcent polymers coats polyethylene then the contact angle of water would be lower and when the demulcent is desorbed, i.e., the original surface is re-exposed, then the contact angle is high. However, this all-or-none phenomenon of coating does not account for the observed intermediate values of contact angles. Since the demulcent solution from the substrate surface is diffusing into sink conditions (water), it is believed that the demulcent polymers exhibit properties similar to proteins at interfaces (Andrade and Haldy, 1986; Andrade et al., 1992). In a non-equilibrium process similar to the desorption of proteins, the 'binding site' or 'foot' of the demulcent polymer may be lifted and this may result in local diffusion, collision and reorientation of the polymer. The reorientation of polymer 'strands' could randomly expose hydrophobic portions of the polymer that usually facilitates its adsorption to the hydrophobic substrate. These dynamic processes may result in intermediate values for contact angles (Andrade, personal communication). The process of desorption may also be considered to be due only to convective flow of the demulcent into a sink condition in the environmental chamber of the goniometer. However, the existence of discrete, time dependent, contact angles intermediate between completely coated and nascent surface can not be completely explained on the basis of the available data. Detailed investigations with different polymers as well as the use of sophisticated reflectance spectroscopic techniques may provide insight about this interesting phenomenon of time dependent change in contact angles. It has also been suggested that the time dependency of contact angles may be due to the changes in the interfacial tension upon removal of the demulcent from the solid/air or the solid/water interface (anonymous reviewer of this paper). Confirmation of the latter hypothesis might be obtained by precise measurements of the interfacial tension as a function of time during the desorption of the demulcents from the ocular surface.

3.2. Contact angles on ocular surface

The successful demonstration of the ability to differentiate demulcent polymers on the basis of wetting and adhesive properties by kinetic measurement of contact angles provided impetus to investigate a similar approach for the ocular surface. However, studies with synthetic substrates were conducted on flat surfaces whereas the ocular surface has a distinct curvature. King (1980), based on geometrical considerations of the dimensions of a bubble provided a mathematical relationship to calculate the contact angle of a liquid on a substrate (Fig. 4). Once again, these relationships are valid only for flat surfaces. Using the principles of solid geometry the contact angle of bubbles on a convex surface may be calculated based on the knowledge of the dimensions of the bubble and radius of curvature of the test surface (Leah et al., 1985). The mathematical relationship shown in Fig. 4 illustrates that the flat surface approximation may be used for a bubble with a diameter significantly smaller than the radius of curvature of the ocular surface. Therefore, the use of a narrow bore HPLC plumbing enabled the use of this approximation.

The kinetic approach was used to investigate the ability of demulcent polymers to wet and desorb from enucleated rabbit eyes. All of the polymers shown in Fig. 3 were evaluated on each eye of at least six rabbits. For the purpose of brevity, data obtained from only one polymer, 0.5% solution of hydroxypropylmethylcellulose are shown in Fig. 5. Fig. 5 demonstrates two desorption curves obtained independently to illustrate the difference between ocular surfaces in the presence and absence of mucin. Acetylcysteine, a known mucolytic agent, was used for removing mucin from the ocular tissue by immersing the enucleated eye in 1% acetylcysteine solution for 15 min. Results shown in Fig. 5 indicate that the ocular surface with its mucin layer intact does not retain the demulcents for over 15 min. However, the acetylcysteine treated eyes exhibited a slower rate of desorption although the demulcents were all desorbed in about 20 min. This rapid desorption illustrates the inadequate mucomimetic property of the demulcent polymer. It is known that the phenomenon of continuous tear secretion and blinking imparts an extremely dynamic environment for the elimination of ophthalmic products from the external ocular surface. Therefore, a dilute solution of the demulcent (0.05%) was used for the kinetic measurement of contact angle in order to simulate this rapid ocular elimination process. Results from this investigation for hydroxypropylmethylcellulose are shown in Fig. 6. Once again the lack of mucomimetic property of the polymers was clearly demonstrated.

The contact angle of freshly enucleated eyes was measured to be $50 \pm 5°$ ($n = 12$) and that of mucin depleted eyes was determined as $60 \pm 5°$ ($n = 12$). The contact angle of water on polyethylene was $90 \pm 2°$, thus indicating that the ocular surface devoid of mucin was less hydrophobic

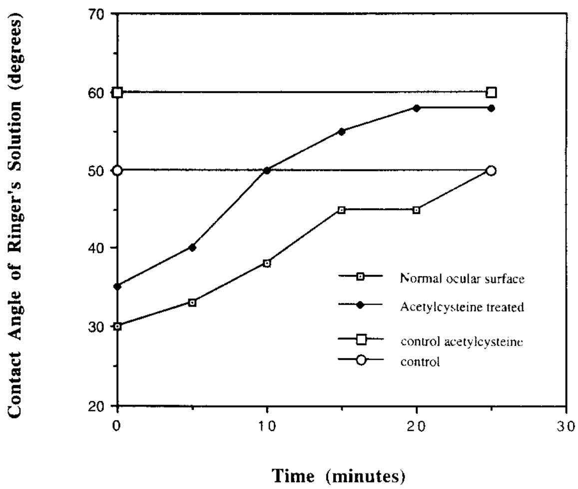

Fig. 5. Kinetics of desorption of hydroxypropylmethylcellulose (0.5%) from the ocular surface (a) with intact mucin and (b) devoid of mucin, i.e., treated with acetylcysteine.

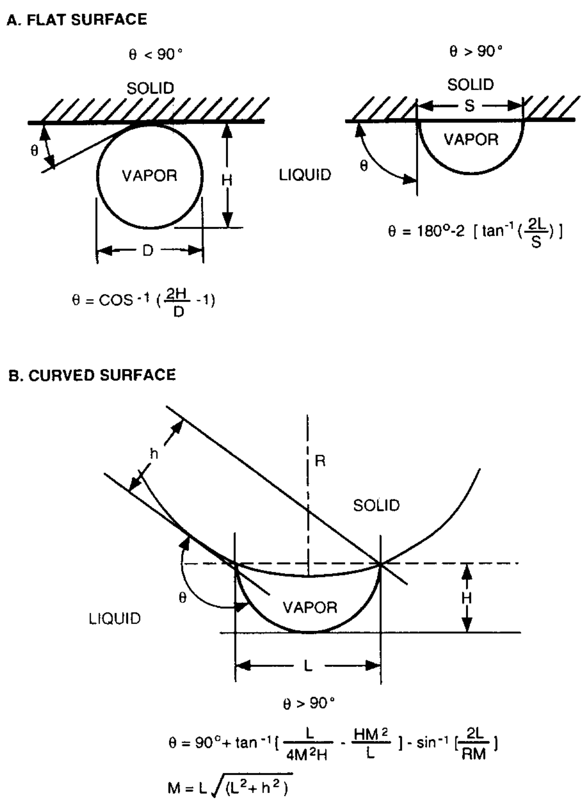

Fig. 4. Geometric considerations for calculation of contact angles of liquids on flat and curved substrate surfaces. Information on flat surfaces adapted from King (1980) and curved surface from Leah et al. (1985).

than polyethylene. However, Holly (1985) reported that the ocular surface devoid of mucin was similar to polyethylene in its hydrophobicity. Results obtained in this investigation are within the range of contact angle values reported by Spychal et al. (1989), for human gastrointestinal mucosal surface (70° for stomach, 62° for duodenum and 57° for the rectum). Hills (1985), reported an apparently contradictory behavior of mucin present on gastric mucosa when compared with mucin applied on a synthetic hydrophobic substrate. It was reported that hydrophobicity of mucosal surface remained the same in the presence and absence of mucin. However, when mucin was isolated from the stomach and applied to a hydrophobic surface, the surface was rendered hydrophilic. The latter observation is consistent with the results reported in this study. In the former case, dehydration of the gastric mucosal tissue during sample preparation could have resulted in modification of surface characteristics of the tissue. Therefore, no difference in hydrophobicity could be observed. Use of the captive bubble technique in the current investigation prevented changes in ocular tissue hydration, therefore, these measurements may be considered more representative of the in vivo environment.

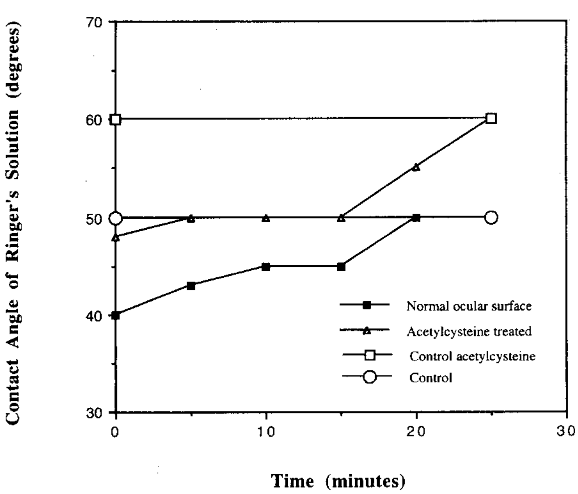

Fig. 6. Kinetics of desorption of hydroxypropylmethylcellulose (0.05%) from the ocular surface (a) with intact mucin and (b) devoid of mucin, i.e., treated with acetylcysteine.

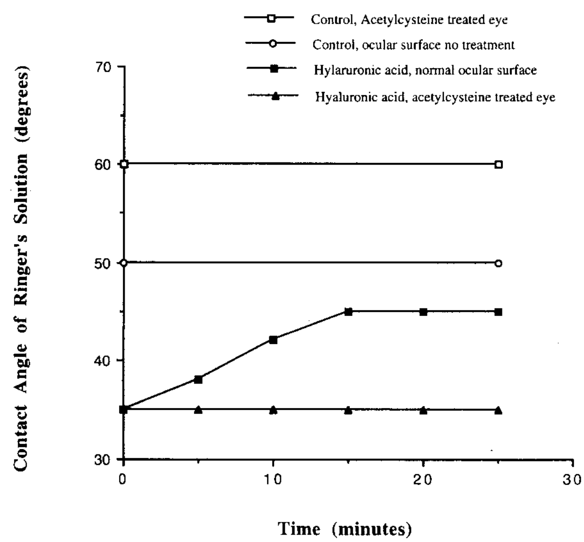

Fig. 7. Kinetics of desorption of hyaluronic acid (0.05%) from the ocular surface (a) with intact mucin and (b) devoid of mucin, i.e., treated with acetylcysteine.

Several new demulcent polymers were also investigated for their mucomimetic properties. Among all the polymers tested in this study, hyaluronic acid was found to be the most effective mucomimetic agent. Fig. 7 shows the kinetics of desorption of hyaluronic acid from the corneal surface of freshly excised rabbit eyes before and after treatment of the eyes with acetylcysteine. In the presence of mucin, hyaluronic acid exhibited good wetting properties, however, it was desorbed rapidly from the ocular surface. When applied to the ocular surface devoid of mucin, hyaluronic acid not only demonstrated improved wettability of the cornea it also was strongly adsorbed to the ocular surface and resisted desorption. The superior mucomimetic property of hyaluronic acid may be attributed to its structural similarity to mucin. Both mucin and hyaluronic acid are naturally occurring biopolymers that contain repetitive units of glycosaminoglycans. Consistent with these observations there are several clinical reports that highlight the beneficial effects of hyaluronic acid in the treatment of dry eye syndromes (Pollack and McNiece, 1982; Deluise and Peterson, 1984; Gold et al., 1986). The superior mucomimetic property of hyaluronic acid is unlikely to be attributed entirely to its highly negatively charged nature since other highly charged hydrophilic polymers, e.g., polycarbophil, polyglutamic acid, carboxymethylcellulose (Gu and Robinson, 1988) and polyacrylic acid (Saettone et al., 1989) exhibit less efficient mucomimetic properties. Macromolecules such as polysaccharides and glycoproteins have been purported to facilitate cellular adhesion via several different mechanisms that consider van der Waals forces, electrostatic forces, hydrogen bonding and macromolecular bridge formation (Bell et al., 1984).

3.3. Estimation of critical surface tension

The concept of critical surface tension was presented by Zisman (1964) as an empirical relationship between the cosine of the contact angle and the surface tensions of the wetting liquids for a given solid surface. This linear relationship represented by Eq. 1 was found to be applicable to low-energy surfaces, defined as solids with specific surface free energy values of less than 100 erg/cm^2:

$$\cos\theta = 1 - \beta(\gamma_w - \gamma_c) \quad (1)$$

In the above relationship, β is the slope of the straight line obtained by plotting cosine vs the surface tension of the liquids (γ_w), where θ is the contact angle of wetting liquids on the solid surface and γ_c denotes a parameter known as the critical surface tension. The value of γ_c is determined by extrapolating the line to $\cos\theta = 1$ (i.e., $\theta = 0°$) and obtaining the corresponding surface tension of the liquid on the abscissa. Although linear relationships with 'Zisman plots' have been obtained for several synthetic solid surfaces, there is no conclusive theoretical basis for the existence of such a relationship. However, critical surface tension values are used to distinguish between surface properties of different solids (Johnson and Dettre, 1969). The practical significance of the critical surface tension is that liquids or liquid mixtures with surface tensions greater than γ_c will not spread readily on the solid surface and that liquids with surface tensions lower than γ_c will most likely spread spontaneously. Any liquid or liquid mixture that may not have been used in

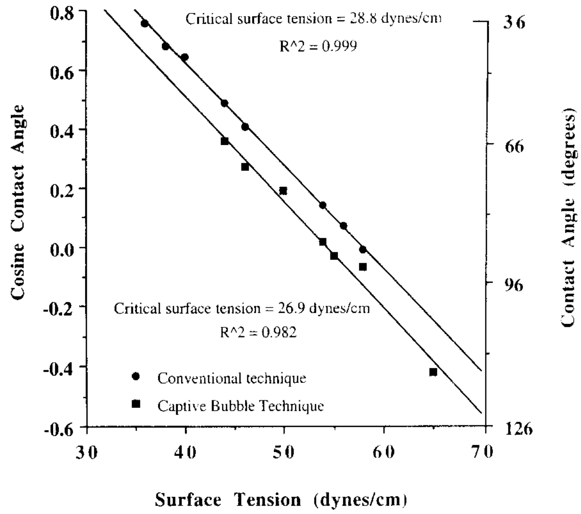

Fig. 8. Critical surface tension of polyethylene determined using demulcent solutions by conventional and captive bubble techniques.

the determination of the critical surface tension will be able to spread on the solid surface spontaneously if it meets the aforementioned criteria.

Traditionally, various organic solvents having different surface tensions are used to measure the contact angle on the test solid substrate. Results from these studies are used to calculate the critical surface tension by obtaining a Zisman plot. Since the surface tension and contact angles of various aqueous solutions of water soluble polymers were measured on polyethylene the data were utilized to estimate γ_c for polyethylene. The Zisman plot for contact angles measured by the conventional technique is shown in Fig. 8. The Zisman plot for polyethylene obtained using dilute solutions and the captive bubble contact angle measuring technique is also shown in Fig. 8. The critical surface tensions for polyethylene calculated by the two measurement techniques were 28.8 and 26.9 dyn/cm, respectively. This estimate was found to be in good agreement with a value of 31 dyn/cm reported by Wu (1982). The close agreement in values is particularly interesting because our estimate was based on data obtained with aqueous solutions of polymers, however, the literature reported value was estimated by using data obtained from organic solvents of different

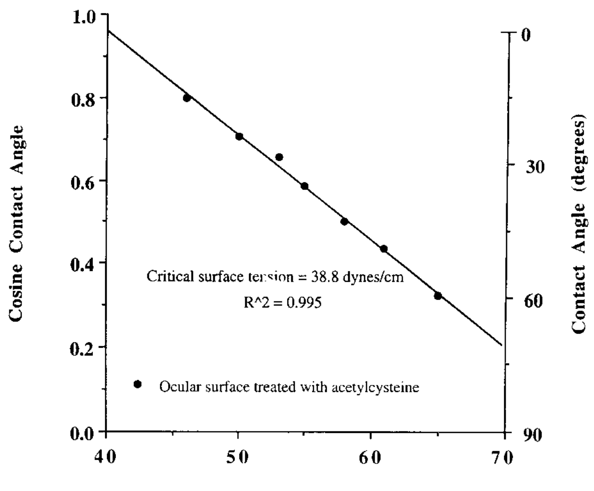

Fig. 9. Critical surface tension of ocular surface devoid of mucin determined by captive bubble technique.

surface tension. The observed difference in γ_c values between the current investigation and previous report may be attributed to the differences in the nature of test liquids used for measuring contact angles (Johnson and Dettre, 1969).

Based on the encouraging results obtained with polyethylene, the critical surface tension of the rabbit ocular surface was estimated. Fig. 9 is a Zisman plot for the corneal surface devoid of mucin obtained by plotting the contact angles of dilute solutions of aqueous soluble polymers by the captive bubble technique against the surface tension of the respective solutions. The critical surface tension was estimated to be 38.8 dyn/cm. This value of γ_c for the ocular surface was significantly different from 28 dyn/cm, the value reported by Holly and Lemp (1971). Based on this estimate of critical surface tension, Holly and Lemp (1971) reported that the ocular surface is similar to polyethylene in its hydrophobicity. However, the current investigation indicates that the corneal epithelium is significantly less hydrophobic than polyethylene. In the investigations conducted by Holly and Lemp (1971), the ocular surface was subjected to cleaning procedures that resulted in removal of the outermost cell layer of the corneal epithelium. According to the author, this loss in epithelial layer was considered inconsequential for surface evaluation of the cornea. Furthermore, in that study the ocular tissue was dehydrated and as a consequence it may have altered the physical nature of the biological surface and may also have undergone irreversible surface changes. Variation of contact angles of biological specimens due to changes in humidity has previously been reported (Mege et al., 1984). In addition, Holly and Lemp (1971), had used organic solvents for measurement of contact angles, however, the effect of these solvents on the ocular tissue was not reported. In the current investigation, use of the captive bubble technique and aqueous test solutions prevented changes in tissue hydration as well as maintained the integrity of the ocular tissue. Therefore, the current estimate of γ_c is more representative of the in vivo environment.

Although the linearity of the Zisman plots (Fig. 8 and 9) and the subsequent calculation of the critical surface tension is a measure of the surface free energy of the solid, it does not provide insight into the intermolecular forces responsible for the observed values of contact angles. Recent work on the contact angle titration in aqueous solution has facilitated in establishing the role of molecular interactions on contact angles and wettability (Whitesides and Laibinis, 1990). In addition, present understanding (Good and Chaudhury, 1991; Good et al., 1991; Van Oss and Good, 1991) of the interaction energies for large molecules has been demonstrated to consist of two major components: the apolar (or dispersive) and the acid-base (or non-dispersive). The Zisman plots shown in Fig. 8 are parallel indicating that the mechanism of interaction of the solvent molecules on polyethylene is independent of the contact angle measuring technique. However, the lower values of contact angle for the captive bubble technique may be attributed to the measurement of receding contact angles in contrast to the advancing contact angles measured by the conventional technique. The significant difference in the slopes of the plots of Fig. 8 and 9 suggests the differing mechanism of interactions between the aqueous media and the two solid substrates, i.e., polyethylene and ocular surface. In this report the more hydrophobic substrate (polyethylene) exhibited lower critical surface tension than the hydrophilic substrate (cornea) with aqueous solutions capable of hydrogen bonding, i.e., the surface tension of the solution has to be lowered to a greater extent to obtain spontaneous wetting of the hydrophobic surface. On the basis of various intermolecular interactions it can be concluded that the critical surface tensions reported in this study for polyethylene and the ocular surface are consistent with the current understanding of wetting processes (Good, 1992).

3.4. Histological examination of ocular surface

Light and electron microscopic investigations were performed on the preserved enucleated eyes to evaluate corneal epithelial damage after exposure to various test solutions during contact angle measurements. The extent of damage was compared with eyes that were not exposed to any test solution but were preserved and processed identically to the test specimens. Eyes treated only with acetylcysteine were used as control for ocular tissue damage assessment of mucin depleted eyes. In general, it was observed that exposure of the ocular surface to demulcent polymers while continuously maintaining tissue hydration resulted only in slight sloughing of epithelial cells at few spots of the cornea. The experimental procedures followed for contact angle measurements maintained the integrity of the ocular tissue. Eyes treated with acetylcysteine exhibited slight undulation of the epithelial surface without evidence of desquamation. Electron microscopic investigations of untreated corneal surface showed well defined microvilli. However, eyes treated with acetylcysteine showed flattened microvilli including some gaps between individual microvillus at some spots. Consistent with reports from other investigators (Tripathi and Tripathi, 1989; Bernal and Ubels, 1991), it was found in this investigation that prolonged exposure to solutions (greater than 1 h) containing benzalkonium chloride resulted in extensive desquamation of epithelial cells. This exfoliation with benzalkonium chloride was more extensive in eyes that were previously treated with acetylcysteine. Eyes that showed extensive tissue damage also showed the greatest variability in contact angle measurements. Low contact angles were observed with eyes exhibiting extensive desquamation of epithelial layer. The latter observation may be due to the exposure of the hydrophilic stroma after exfoliation of the epithelium. In summary, it may be concluded that the maintenance of surface cellular integrity was found to be of paramount importance in obtaining reproducible contact angle measurements.

4. Conclusions

This report has demonstrated that water soluble demulcent polymers commonly used in tear substitutes exhibit marginally functional mucomimetic properties. The in vitro technique described for the kinetic evaluation of desorption of polymers from the surface of a solid substrate provided a quantitative assessment and differentiation between wetting and adhesive properties of the demulcents. The captive bubble technique was shown to maintain the cellular integrity of the ocular tissue during the measurement of contact angles. Therefore, the measurements are believed to be more representative of the in vivo environment. The study has demonstrated that the corneal epithelium devoid of mucin is not as hydrophobic as polyethylene. Estimation of the critical surface tension of the ocular surface enabled the defining of a measurable criterion that needs to be achieved in order to obtain spontaneous wetting of the cornea. In this in vitro investigation it was shown that hyaluronic acid, a biopolymer similar in structure to mucin provided the most effective mucomimetic property among all of the substances investigated. The in vitro technique may be utilized to screen formulations or identify superior demulcents for the development of superior, long-acting and histologically non-disrupting artificial tear substitutes. However, final utilization of the results from the screening of demulcents to facilitate the advancement of superior formulations would only be possible when the in vitro screening studies are correlated to the in vivo clinical performance of these products.

Acknowledgements

The authors wish to acknowledge Mr Alan Curtiss and Mr John Goodrich for making modifications on the goniometer and for the design and fabrication of the ocular holder. Dr Jay Fortner is acknowledged for demonstrating a superior surgical procedure for excision of rabbit eyes. The authors also wish to thank Dr William Milisen, Ms Jeannie Carver, Ms Amy Jakowski, Ms Alanine Kennedy and Dr Alexander Walsh for help with the histological studies.

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