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INFLUENCE OF THE VISCOSITY AND THE SURFACE TENSION OF OPHTHALMIC VEHICLES ON THE RETENTION OF A TRACER IN THE PRECORNEAL AREA OF HUMAN EYES.

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ABSTRACT

The influence of the viscosity and the surface active characteristics of dextran and polyvinylalcohol (PVA) solutions on the retention of fluorescein in the precorneal area of human eyes is investigated. A non-invasive method using a slit lamp fluorophotometer is employed. The tear elimination coefficient and the AUC value of the recorded fluorescence decay curve are calculated.

The addition of dextran and polyvinylalcohol (PVA) to an iso-osmotic buffer solution retards the precorneal loss of fluorescein. The instillation of a 4.2% PVA solution, however, causes discomfort, inducing in some cases rapid blinking and elimination of the tracer.

The comparison of vehicles of similar viscosity but different surface tension shows no significant difference of AUC values. The significant difference of the tear elimination coefficients, however, suggests a different behaviour of both polymers.

The viscous effect of the vehicle influences the retention of the tracer. But the physicochemical properties of the polymers and their influence on the tear film structure and on the blinking dynamics seem also to play an important role.

INTRODUCTION

To prolonge the ocular contact time and in particular to enhance the bioavailability of drugs, suitable water - soluble polymers are added to ophthalmic vehicles [1-3].

But some viscosity-enhancers also exhibit surface activity. Sharma and Ruckenstein demonstrate that surface active agents could interact with the components of the lacrimal film. The overall effect may be stabilizing or destabilizing [4,5]. Therefore surface active polymers could influence the spreading of the precorneal lacrimal film, the break up time, the blinking rate and consequently the elimination of the drug instilled.

The aim of the present work is to examine the influence of the viscosity and the surface tension of ophthalmic solutions on the retention of a fluorescent tracer in the precorneal area of human

eyes. The elimination of the tracer is measured by monitoring the fluorescence decay in the tear film with a slit lamp fluorophotometer.

MATERIALS

1. Tracer

The tracer selected is sodium fluorescein, because of its low toxicity and high fluorescent efficiency. The test substance is not irritating and is not resorbed in significant amounts through the intact corneal and conjunctival epithelium, but is lost from the eye via the drainage apparatus [6,7].

2. Polymers

The following polymers at the indicated % (w/w) concentration are used, without further purification :

- 5% and 10% dextran, clinical grade average MW 78500 (Sigma Chemical Company, St Louis MO, USA).
- 1.4%, 2.8% and 4.2% polyvinylalcohol (PVA) Polyviol W40/140 (Wacker Chemie GmbH, München, FRG)

3. Ophthalmic solutions

The solutions are prepared by adding the appropriate amount of polymer to an aqueous iso-osmotic phosphate buffer solution pH 7.4 (Ph Helv V), containing 0.05% sodium fluorescein. The solutions are sterilized by aseptic filtration . The viscosity, the surface tension and the osmolality of the various solutions are determined.

METHODS

1. Viscosity determination

The viscosity determination on the sterile polymer solutions are made at 32°C (corneal surface temperature) using a capillary Ostwald viscosimeter (KPB Viskosimeter Schott Geräte, Mainz, FRG), because of their approximating newtonian behaviour [8,9].

2. Surface tension measurement

The surface tension is measured by the Wilhelmy plate method, using a Cahn electrobalance.

Since the equilibrium surface tension of viscous solution is reached only after several hours, the measurement is performed 20 seconds after pouring the solution in the petri dish. This time period is chosen in relation to the blinking dynamics in humans and corresponds to the mean BUT (tear break up time) of the volunteers.

3. Osmolality measurement

The osmolality of each solution is determined with a vapor pressure osmometer (model 5500 Wescor, Logan UT, USA).

4. Slit lamp fluorophotometry

The fluorescence of the tracer in the precorneal tear film is measured with a slit lamp fluorophotometer, as described in previous communications [10,11].

5. Instillation procedure and fluorescence monitoring

The instillation of the various ophthalmic solutions is done to volunteers without ocular

disorders, aged from 25 to 42 years. Informed consent was obtained after the nature of the procedure was fully explained.

A volume of 10ul of the solution is instilled carefully in the lower conjunctival sac of the left eye. Immediately following instillation, the subjects are asked to close and roll their eyes in order to mix the tracer with the resident lacrimal fluid. Afterwards they behave naturally and blink as they feel necessary during the fluorenscene monitoring of the tear film. The fluorescence decay curve is recorded and analyzed [10,11].

RESULTS

1. Physical characterization of the ophthalmic solutions

The results of the different physical determinations carried out on the different ophthalmic solutions under investigation are listed in table 1.

All solutions are almost iso-osmotic. The polyvinylalcohol solutions show a reduced surface tension with respect to the phosphate buffer solution.

2. Fluorescence decay curves

Schematical plots of the fluorescence signal of the tracer as a function of time are drawn in fig 1 and 2. The output of the fluorescence signal is expressed in arbitrary units. Those plots originate from experiments with volunteer 1.

TABLE 1. Characteristics of the Ophthalmic Solutions

composition	viscosity mPa.s	surface tension mN/m	osmolality mosm/kg
phosphate buffer solution	0.98	71.9	280
+ dextran 5%	2.9	71.4	283
+ dextran 10%	6.7	71.7	289
+ PVA 1.4%	2.3	44.2	287
+ PVA 2.8%	7.4	43.2	300
+ PVA 4.2%	24.9	43.9	308

As shown in figure 1, the addition of dextran has only a slight effect on the retention of the tracer in the case of volunteer 1. It is noted that 4 minutes after application more than 90% of the dose instilled is lost.

The decay curves from the other volunteers, however, indicate that the rapid fluorescence decay seen after instillation of the phosphate buffer solution is slow down by the addition of dextran to the vehicle. In the case of volunteers 2,3 and 4, only 75% of the dose is eliminated after 4 minutes.

Both dextran solutions are well tolerated by each volunteer. In general the viscosity increase

of the solution by dextran reduces the loss of the tracer from the precorneal area of human eyes.

The 1.4% PVA solution has a similar decay profile compared to the phosphate buffer solution and the dextran solutions tested. An important fluorescence increase is observed after instillation of a 2.8% PVA solution compared to the application of the phosphate buffer solution. Moreover the decay curve of the 2.8% PVA solution shows an example of increase of fluorescence intensity due to an involontary forceful blinking. When a volunteer blinks forcefully, lacrimal fluid is squeezed out of the conjunctival sac, resulting in a higher volume available for spreading over the precorneal surface. Concomitantly the tear film thickness is increased and an greater amount of fluorescein is in contact with the cornea.

As seen from figure 2. the instillation of a 4.2% PVA solution causes about a 2.5 fold higher fluorescence signal. This indicates an increase of the tear film thickness, since the fluorescence intensity approximates thickness x tracer concentration. The visualy larger marginal tear strip is maintained only for 60 to 90 seconds, because the discomfort of this "sticky" solution induces rapid blinking.

The 1.4% and 2.8 PVA solutions are as well tolerated as the 5% and 10% dextran solutions by each volunteer. After instillation of the 4.2% PVA solution the volunteers complain about a "sticky" sensation and sometimes about blurred vision at

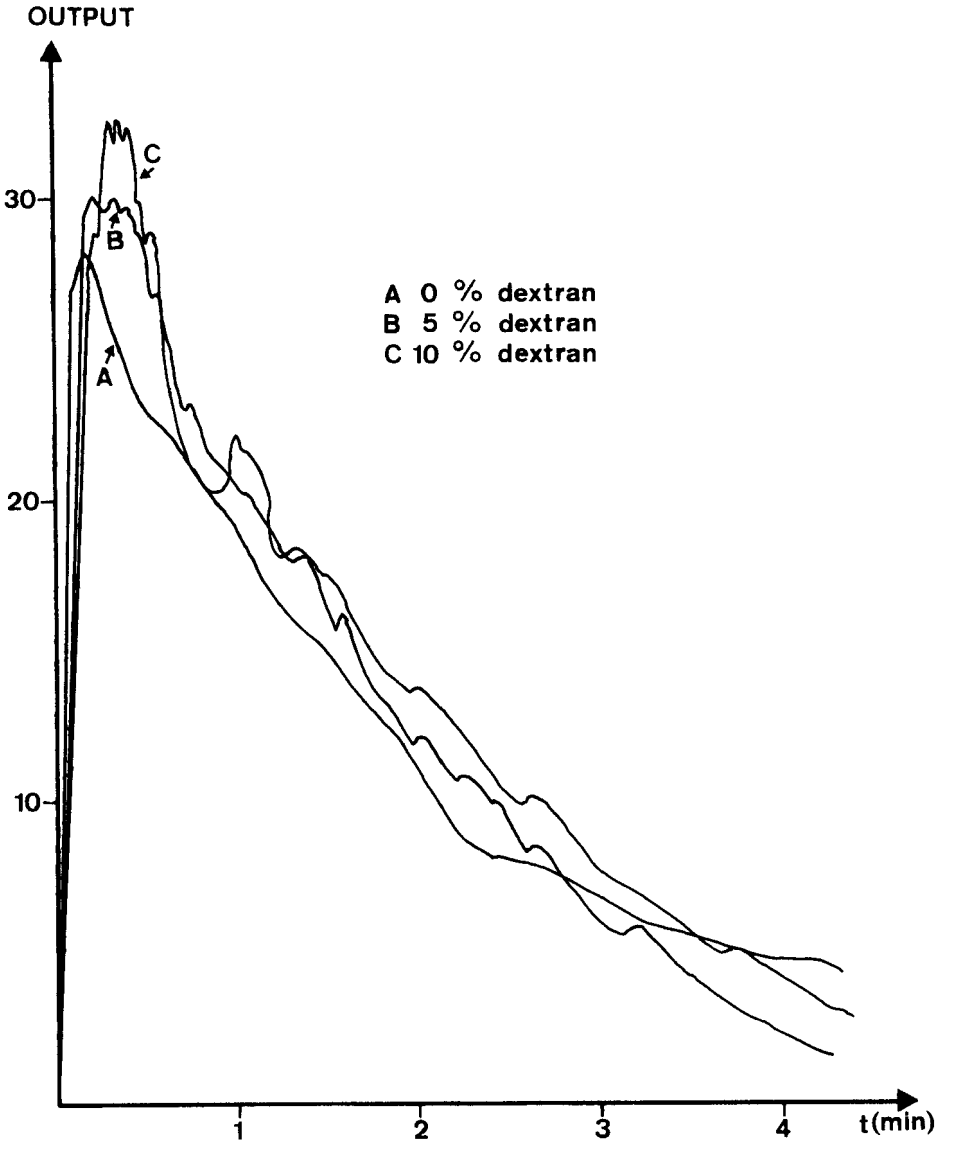

FIGURE 1. Fluorescence decay curves (volunteer1)
A = 0% dextran (phosphate buffer sol.) ;
B = 5% dextran ; C = 10% dextran

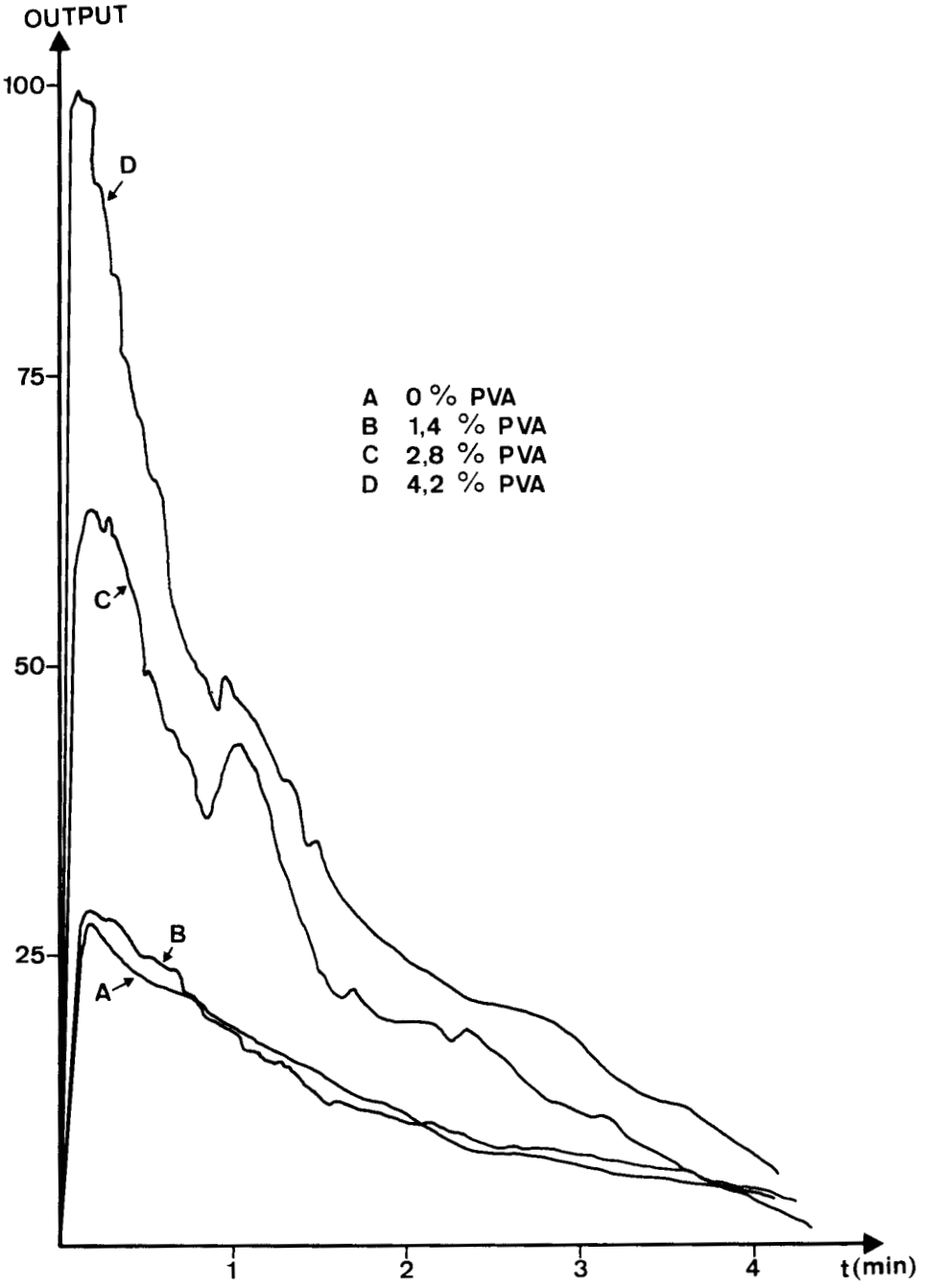

FIGURE 2. Fluorescence decay curves (volunteer 1)
A = O% PVA (phosphate buffer sol.) ;
B = 1.4% PVA ; C = 2.8% PVA ; D = 4.2% PVA

the beginning of the test and about discomfort. The 4.2% PVA solution does not form immediately a homogenous film on the surface of the eye. The enlarged marginal tear strip decreases to its normal dimensions at about 90 seconds or 3 minutes depending on the volunteer.

3. Tear elimination coefficient and AUC of the decay curve

The decay profile recorded after instillation of the phosphate buffer solution and the dextran solutions obeyes fairly well first order kinetics. The data are subjected to linear regression analysis and the first order rate constant, called tear elimination coefficient (k), is determined for each experiment. The mean of six k values of each series of ophthalmic solutions tested and the SD values are given in table 2.

The areas under the decay curves (AUC) are also determined. Their values should reflect the amount of tracer present in the tear film. The summary of the data is presented in table 2.

The data reported in table 2. show that generallly the addition of dextran prolonges the retention of the tracer in the precorneal area of the eye. The increase of the AUC, in the case of volunteer 3 and 4, corresponds to the reduction of the elimination rate.

The addition of polyvinylalcohol to the phosphate buffer solution reduces the elimination rate of the tracer, except for volunteer 1. The increase of the PVA concentration of the ophthalmic ve-

TABLE 2. Tear elimination coefficient k ($10^{-3}.sec^{-1}$) and AUC of the decay profile

vehicle	phosphate sol.		5% dextran		10% dextran	
	k (± SD)	AUC (± SD)	k (± SD)	AUC (± SD)	k (± SD)	AUC (± SD)
volunteer						
1	10.80±4.11	25.8±7.5	13.93±2.58	24.4±9.2	15.36±4.22	29.7±9.7
2	8.56±1.21	13.7±2.4	7.46±1.15	16.5±3.6	7.81±1.77	17.0±6.0
3	6.40±1.52	18.9±4.3	3.40± 0.89	23.0±7.9	3.03±1.11	34.8±11.7
4	16.06±2.40	11.8±1.7	7.83±1.88	18.6±3.4	4.37±1.57	34.6±14.8
	1.4% PVA		2.8% PVA		4.2% PVA	
	k (± SD)	AUC (± SD)	k (± SD)	AUC (± SD)	k (± SD)	AUC (± SD)
volunteer						
1	7.40±1.38	27.9±7.0	12.76±3.89	46.1±20.6	13.87±4.11	49.8±13.4
2	6.47±1.44	14.7±3.3	7.50±2.15	16.3±3.5	6.10±1.26	11.5±3.4
3	5.59±2.86	20.8±8.3	3.79±1.49	28.0±6.8	3.05±1.48	34.0±11.2
4	11.29±2.97	20.8±5.6	8.89±2.46	24.9±10.0	6.44±3.04	36.0±15.5

hicle results in a moderate or important AUC increase.

The data from volunteer 1 show a high AUC value associated with a high k value. This paradoxical phenomenon results probably from the discomfort due to the a thick and "sticky" tear film inducing a higher blinking rate. The tear strip volume decreases rapidly occasioning a high k value.

But accumulation of the tracer is observed at the inner canthus, probably due to a slower drainage of the viscous solution compared to the non-viscous buffer solution, through the naso lacrimal duct. Thus, the overall effect of the enlarged marginal tear strip, the increased tear film thickness and the collecting of the tracer at the inner canthus, is a high AUC value in comparison with the phosphate buffer solution.

DISCUSSION

1. Influence of the viscosity

The increase of the viscosity of ophthalmic vehicles in general prolonge the retention of the instilled tracer and the amount of tracer present at the eye surface.

The Kruskal Wallis analysis of variance shows no significant difference of k values of the dextran solutions with respect to the non-viscous phosphate buffer solution at the 95% level of confidence, in the case of volunteers 1 and 2. The data from volunteers 3 and 4, on the other hand, indicate a significant difference for the k values

of the dextran solutions compared to the k value of the buffer solution at the 99% confidence level.

By increasing the PVA concentration of the ophthalmic vehicle a general trend of a decrease of the k values and an increase of AUC is noted. But as mentionned earlier, paradoxical results of high k values associated with high AUC are observed in the case of volunteer 1.

Statistical analysis of the PVA data reveals significant difference for the k values of those solutions compared to the buffer solution at the 95% or 99% confidence level as far as volunteers 1 or 3 and 4 are concerned. No significant difference is observed with volunteer 2.

The marked inter subject variations observed are due to individual variations in reaction to the discomfort occassioned by the viscosity of the eye drop. The acceptance of a viscous vehicle seems to be related to the change of the marginal tear strip volume and the blurring of the vision.

2. The influence of the nature of the polymer added.

The good acceptance of the dextran solutions, which do not reduce the surface tension of the vehicle, is probably due to the adsorption of the dextran molecule to the corneal surface and lubrification of the ocular surface [12].

Although the addition of PVA to the vehicle increases the corneal contact time, the period over which the eye is exposed to high concentra-

tions of the tracer is still a few minutes. The strong fluorescence, as observed with volunteer 1, is indicative of a greater tear film thickness. Benedetto and coworkers demonstrate that polyvinylalcohol produce a thicker tear film, because of its water dragging capacity [13].

The instillation of the surface active PVA solutions causes only discomfort at the highest concentration examined. The reduced surface tension of the vehicle does not seem to facilitate the mixing of the solution with the resident tear fluid and the immediate formation of an homogeneous film.

Some of the present observations are contrary to the results of a previous study concerning the influence of the non-ionic surfactant polysorbate 80 on the retention of fluorescein [14]. The volunteers complain about mild irritation when a polysorbate 80 solution of 0.1% with a surface tension of mN/m is applied, while the 2.8% PVA, with a surface tension of 43.2 mN/m, is well tolerated by each volunteer. Consequently of discomfort of a 4.2% PVA solution, having a surface tension of 43.9 mN/m, could be attributed to its viscosity or some interactions of the polymer with the tear film components.

The reported data of volunteers 3 and 4 are compared. It should be mentioned that volunteers 3 and 4 have very different mean BUT values (tear break up time), under normal conditions, 20 and 5 seconds respectively. The date show that a 10%

TABLE 3. Statistical analysis

Comparison 1.4% PVA vs 5% dextran	k value		AUC
volunteer 1	P	O.O1	NS
volunteer 2	NS		NS
volunteer 4	P	O.O5	NS
Comparison 2.8% PVA vs 1O% dextran			
volunteer 1	NS		NS
volunteer 2	NS		NS
volunteer 3	NS		NS
volunteer 4	P	O.O1	NS

dextran solution produces a 1.8 fold and 2.9 fold AUC increase over the phosphate buffer solution, respectively. The instillation of the 2.8% PVA solution with the same viscosity causes only a 1.5 and 2.1 fold AUC increase. But similar results to the 1O% dextran solution are exhibit by the more viscous 4.2% PVA solution. This solution produces a 1.8 and 3 fold AUC increase over the polymer - free solution. The volunteer exhibiting the highest blinking rate seems to be more sensitive to vehicle viscosity effects.

The k values and AUC differences among isoviscous vehicles are also analyzed with a non-parametric test. The results of the statistical ana-

lysis with the Mann Withney test is summarized in table 3.

The statistical analysis reveals, however, that the AUC differences are not significant, when solution of similar viscosity are compared. Although differences in k values, thus in elimination, seem to indicate that the volunteers react differently.

The instillation of water - soluble polymers could alter the physicochemical parameters gover ning the tear film stability or could change physiological processes, such as drainage. Also interactions could occur in the tear film or with the mucous layer at the surface of the cornea and the conjunctiva.

CONCLUSIONS

In conclusion, the addition of viscous polymers to ophthalmic vehicles prolonge the retention of the tracer in the precorneal area of human eyes, when they do not cause discomfort. Not only the viscous effect, but also the nature of the polymer seems to play an important role.

It seems from the results obtained, that the use of dextran should be preferred to polyvinylalcohol for the formulation of ophthalmic solutions containing drugs for the external eye.

Further investigations are needed to clarify the possible interaction between the physiochemical characteristics of the polymers and the physiological processes of the eye.

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