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Note

High-performance liquid chromatography of artificial tears

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The precorneal tear film (TF) is a very thin layer of fluid on the surface of the cornea. It is considered to possess a three-layer structure. The outermost lipid layer is in contact with the external medium, and covers the most voluminous aqueous layer which in turn overlies the deepest mucous layer[1–3].

The precorneal TF is important in preserving the integrity of the corneal epithelium[4,5]. The characteristic hypolacrimation, produced by deficiences in one or several components of the TF, results in dry eye syndromes. A very important treatment of these syndromes is the therapeutic usage of artificial tears[6–8].

In this work the molecular weights of the polymers constituting artificial and natural tears has been studied. The relative percentages of these polymers were also calculated.

EXPERIMENTAL

Apparatus

The chromatographic equipment comprised a Perkin-Elmer LC-25 liquid chromatograph, a Perkin-Elmer Type Shodex $S_{804/S}$ molecular-weight exclusion column, a refractive index detector and a Perkin-Elmer 56 plotter.

Reagents

Different types of artificial tears were examined (see Table I). The natural tears were taken from normal patients. The eluent was twice distilled water.

Standards

The following dextrans were obtained from Pharmacia (Uppsala, Sweden): 40T, 70T, 300T, 500T and 2000T. These have weight-average molecular weights of $40 \cdot 10^3$, $70 \cdot 10^3$, $300 \cdot 10^3$, $500 \cdot 10^3$ and $2000 \cdot 10^3$, respectively. Glucose was used to determine the permeated column volume, V_p, and Blue dextran 2000T was used to measure the exclusion volume, V_0.

Procedure

The normal procedure for the use of the liquid chromatograph was followed[9,10]. The determinations were carried out at 25°C and the volume injected was

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TABLE I

COMPOSITIONS AND MANUFACTURERS OF TEAR SUBSTITUTES

Abbreviations: DEX = dextran; HEC = hydroxyethyl-cellulose; HPMC = hydroxypropylmethyl-cellulose; PEO = poly(ethylene oxide); PVA = poly(vinyl alcohol); PVP = poly(vinyl pyrrolidone).

Trade-name	*Manufacturer*	*Polymers*
Adapettes	B&P (Alcon)	HEC, PVP
Bausch & Lomb (lubricant)	Bausch & Lomb	PEO, PVP
Confort	Dis-op	HEC, PVP
Contears	Allergan	PVA
Dacrolux	Cusí	HPMC, DEX
Liquifilm	Allergan	PVA
Tears naturale	Alcon	DEX, PVA

TABLE II

RETENTION TIMES, t_R, AND ELUTION VOLUMES, V_e, OF DEXTRAN STANDARDS

Standard	t_R *(min)*	V_e *(ml)*
Blue dextran 2000	0.2	0.2
Dextran K 500000	6.0	4.8
Dextran K 300000	7.0	5.6
Dextran K 70000	7.5	6.0
Dextran K 40000	8.0	6.4
Glucose	12.0	9.6

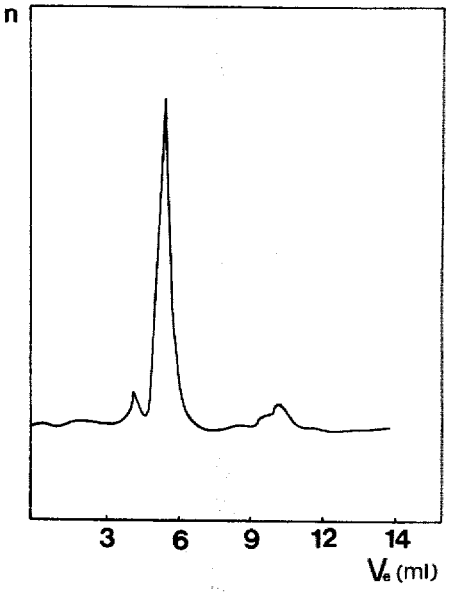

Fig. 1. Chromatogram of natural tears. n = Refractive index.

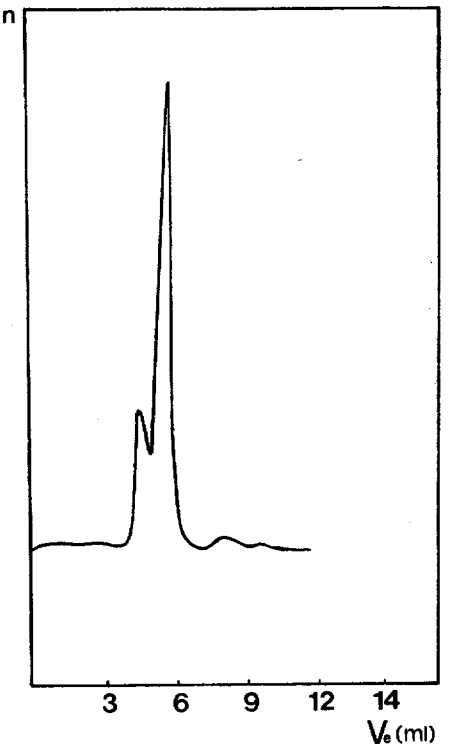

Fig. 2. Chromatogram of artificial tears, type "tears naturale". n = Refractive index.

TABLE III

RETENTION TIMES AND ELUTION VOLUMES OF ARTIFICIAL AND NATURAL TEARS

Artificial and natural tears	t_R *(min)*	V_e *(ml)*
Adapettes	7.0	5.6
Bausch & Lomb	7.2	5.9
Confort	5.8	4.6
	7.2	5.6
Contears	6.0	4.8
	7.4	5.9
Dacrolux	6.0	4.8
	7.4	5.9
Liquifilm	6.0	4.8
	7.3	5.8
	12.0	9.6
Tears naturale	5.8	4.6
	7.3	5.8
Natural (human) tears	5.2	4.2
(10 determinations)	7.0	5.6
	11.8	9.4

25 μl. The flow-velocity employed was 0.8 ml/min. The solutions were filtered through a 45-μm Millipore membrane filter.

RESULTS

The retention times, t_R and the elution volumes, V_e, of the dextran standards and the glucose are shown in Table II.

Figs. 1 and 2 show chromatograms of natural (human) and artificial tears, type "tears naturale", respectively.

The retention times and elution volumes of the different polymers constituting the natural (human) and artificial tears, are given in Table III.

Fig. 3 shows the relationship between the elution volumes and the molecular weights of the dextran standards.

DISCUSSION AND CONCLUSION

From the results in Table IV it is concluded that, based on the molecular weights of the component polymers and of their calculated distribution constants, K_{av}, the artificial tears of types "confort" and "tears naturale" simulate best the natural tears from the point of view of molecular weight.

The calculation of the relative area corresponding to each (Table V) makes it possible to establish another relationship between the natural and artificial tears. The artificial tears of types "liquifilm" and "tears naturale" give peak areas corresponding to the polymer constituents of 2.88, 6.90 and 0.80 and of 1.80 and 4.84 respectively, the relative percentages of polymers being 27.22, 65.22, 7.56 and 27.11 and 72.89,

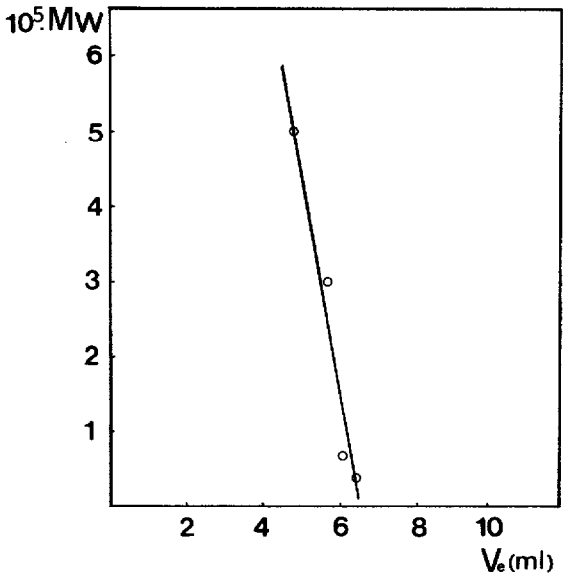

Fig. 3. Relationship between the elution volumes, V_e, and the macromolecular weights of dextran standards.

TABLE IV

DISTRIBUTION CONSTANTS, K_{av}, AND MOLECULAR WEIGHTS OF POLYMERS CONSTITUTING THE TEARS

$$K_{av} = \frac{V_e - V_0}{V_p - V_0}$$

Artificial and natural tears	K_{av}	$MW \cdot 10^{-5}$
Adapettes	0.57	2.4
Bausch & Lomb	0.61	2.1
Confort	0.43	5.5
	0.57	2.4
Contears	0.46	5.0
	0.60	1.65
Dacrolux	0.46	5.0
	0.61	1.65
Liquifilm	0.46	5.0
	0.60	1.9
Tears naturale	0.43	5.5
	0.59	1.95
Human (natural) tears	0.36	6.25
	0.57	2.4

TABLE V

POLYMER CONTENTS IN ARTIFICIAL AND NATURAL TEARS

Artificial and natural tears	*A* (cm^2)	*Relative percentage of polymers*
Adapettes	5.68	100
Bausch & Lomb	7.50	100
Confort	2.88	37.10
	3.88	50.00
Contears	2.10	31.34
	4.60	68.65
Dacrolux	3.78	37.57
	6.28	62.43
Liquifilm	2.88	27.22
	6.90	65.22
	0.80	7.56
Tears naturale	1.80	27.11
	4.84	72.89
Natural (human) tears	0.18	6.36
	2.61	92.20
	0.04	1.41

respectively. In the view of their polymer composition, these artificial tears resemble most closely the natural tears (6.36 and 92.2).

From this study it can be concluded that, among the artificial tears commercially available, that of type "tears naturale" will be the most adequate as a substitute for natural tears because of its distribution constant and polymeric percentage.

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