Drainage pattern of commercial ophthalmic gels: an innovative method of evaluation

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The feasibility of an innovative in vitro technique for providing information about the drainage pattern of certain commercially available ophthalmic gels has been investigated. The samples showed qualitatively and/or quantitatively different formulations in terms of either the gel-forming agents or the other auxiliary substances. Both torsional oscillation and sine-wave vibro viscometers have been employed during the experiments. The drainage behavior of the gels in systems that essentially differ in both probe adhesiveness and tonicity level of the dispersing media have been estimated. The results obtained revealed different patterns of drainage over time among the various samples. In conclusion, the technique developed offers a viable means of characterizing gels in terms of consistency, adhesion and clearance and it appears to be a useful tool for estimating the surface residence times of gels.

Key words: Ophthalmic gels – Carbomers – Torsional oscillation viscometer – Vibrational viscometer –Mathematical fitting.

Among the wide variety of ocular dosage forms, a relevant field of application of these preparations is represented by the tear replacement therapies for pathologies such as dry eye syndrome (also known as keratoconjunctivitis sicca - KCS). Such a disease is a common syndrome that affects approximately 10-20 % of the adult population, distressing the normal sight for which a moist and healthy ocular surface is required [1]. Human tears are composed of water, electrolytes, small molecules such as carbohydrates and lipids, and a variety of proteins, several of which have an enzymatic function. A sufficient quantity of tears, normal tear film composition, normal lid closure, and regular blinking are among the prerequisites for maintenance of a healthy ocular surface [2]. Lachrymal deficiency may lead to desiccation of the corneal epithelium, ulceration and perforation of the cornea, an increased incidence of infectious diseases, and, potentially, serious visual impairment and blindness. Correct diagnosis and adequate treatment are therefore essential [3]. The integrity of the three-layered lipid, aqueous and mucin structure that is vital to the effective functioning of the tear film is not easily reproduced by medical treatments [4]. Moreover, loss of water from the tear film is central to virtually all reduced aqueous tear outputs. As a consequence, the remaining tear solutes, such as sodium and potassium salts become more concentrated in the tear film. The concentration of electrolytes increases tear osmolarity above the normal limit of 311 mOsm/L [5]. The loss of water and increased osmolarity may also result from any event that either decreases tear production or increases tear evaporation [6]. The use of artificial tears has obvious limitations because they cannot completely substitute the complex composition of natural tears. Artificial tears act by adding volume to the tear film, but they can only do this while they adhere to the surface of the eye, and eye drops remain in contact with the ocular surface only for a few seconds. Viscosity-enhancing components are thus required to make artificial tear formulations effective. They are also added as mucoadhesive carriers for ocular drugs, due to their ability to coat the cornea and remain on the eye for a useful time period. However, modifications of hydrogel viscous properties over time, due to breaking or delay in the formation of intra- and inter-chain polymeric bonds, as well as alterations in the ocular surface/gel interaction as strongly affected by pH, ionic strength and presence of other substances are frequent drawbacks [7-9].

The following polymers have been regularly used in eye disorders: hydroxypropyl methylcellulose (HPMC) [10], dextran [11], carboxymethylcellulose (CMC) [12,13], polyvinyl alcohol (PVA) [14], polyvinylpyrrolidone (PVP) [15], polyethyleneglycol/propyleneglycol/hydroxypropyl-guar combination (PEG/PG/HP-guar) [16], hyaluronic acid (HA) [17, 18], Carbopol, also known as carbomer or polyacrylic acid (PAA) [19-22], and recently a polysaccharide derived by tamarind seed [23]. In particular, PAA is the most frequently used in the treatment of dry eye syndrome, either alone or in association. It is available in a wide range of molecular weights and with a linear, branched or cross-linked structure [24, 25]. Unfortunately, apart from the qualitative composition of formulations, the quantity of each excipient in such commercial gels is often omitted. Such an aspect is of major concern in some pathologies, suggesting that identification of any differences between nominally very similar products may provide interesting outcomes [26]. The rationale of the present study was to move beyond a purely subjective evaluation of the therapeutic use of ophthalmic gels by the development of an innovative technique, suitable for experimentally modelling the drainage pattern of different ophthalmic gels with the same therapeutic indications and, in some cases, the same nominal formulation. Such a goal was deemed to be both pertinent and appealing, and to achieve it two types of viscometer have been separately employed providing a precise and reproducible *in vitro* profile of the dispersion process of certain commercially available ophthalmic gels. Specifically, it appears to be possible to assess the drainage degree of commercial ophthalmic gels from the ocular surface mainly because of blinking, in relation to the different experimental parameters, such as probe geometry, probe surface material, mechanical solicitations, and medium salinity. On the other hand, a precise relation between the data obtained from the experiments and the physiological *in vivo* behavior of the various products available on the market, such as the quantification of the real contact time onto the ocular surface, has been considered beyond the aim of the present study.

The results obtained under identical experimental conditions show that the adopted technique is capable of both characterizing drainage patterns and revealing appreciable distinctions between ophthalmic gels at various media osmolarity, even in the presence of apparently negligible differences in their compositions.

I. MATERIALS AND METHODS

1. Materials

Seven commercially available ophthalmic gels were tested. Specifically, they were: Lacrinorm Farmigea S.p.A., Italy, hereinafter referred to as S1; Lacrigel Farmigea S.p.A., Italy, hereinafter referred to as S2; Viscotirs Gel, Novartis Farma S.p.A., Italy, hereinafter referred to as S3; Dacriogel, Alcon S.p.A., Italy, hereinafter referred to as S4; Lipovisc Gel Oftalmico, Bausch & Lomb Oftal S.p.A, Italy, hereinafter referred to as S5; GentealTM Gel, Novartis Farma S.p.A., hereinafter referred to as S6; Visine Dry Eyes, Pfizer Consumer Healthcare, United Kingdom, hereinafter referred to as S7. Their compositions are summarized in *Table I*. HA (pharmaceutical and medical device grade; weight-average of the molecular weight 1.2 MDa) and Carbopol 974P NF used for method validation purposes were provided by TRB Chemedica SA, (Vouvry, Switzerland) and BFGoodrich Co., (Cleveland, OH, United States), respectively. Hypotonic, isotonic and hypertonic phosphate buffers, hereinafter referred to as hypoPB, isoPB and hyperPB, respectively, have been classified in relation to the amount of NaCl. Milli-Q-water (Millipore, Watford, United Kingdom) was used in the dispersing media. All the other chemicals were purchased from Sigma-Aldrich and they were used without any further purification.

2. Instruments

To estimate the residence time of ophthalmic gels, an original method was developed using the following two instruments (*Figure 1*):
- Sine-wave vibro viscometer SV10 (A&D Company, Ltd.): such a device has been originally designed to measure viscosity by detecting the driving electric current necessary to resonate the two sensor plates at a constant frequency of 30 Hz and amplitude of 0.2 mm. Under these operating conditions, the sensor plates allow only non-significant deformation of the sample texture. The probe case is made of AISI 304 gold plated stainless steel. A quantitative evaluation of viscosity values is achieved by the current required to move the transducers placed in the sample [27]. For our purposes, the two-point viscosity calibration of the instrument has been used. Experimental values were recorded by a PC connection through an RS-232 port (data collection every 1 s);
- Viscomate VM-10AL (CBC Europe, Ltd.): this torsional oscillation viscometer is based on the evaluation of the absolute value of complex viscosity in the absence of mechanical solicitation. The basic operating principle is also schematized in *Figure 1*. The probe system is made of titanium and is a balanced structure in terms of inertia mass and vibration. The sensor and the actuator are driven by piezoelectric sources and the upper portion torsionally oscillates in exactly opposite angular momentum to the lower detector. The resonance frequency of the instrument is 1 kHz and the detector oscillation amplitude is 1 μm in the absence of resistance. The angular acceleration of the detector is measured and reported as the absolute value of the sample's complex viscosity [28]. Users cannot calibrate or adjust the viscosity values.

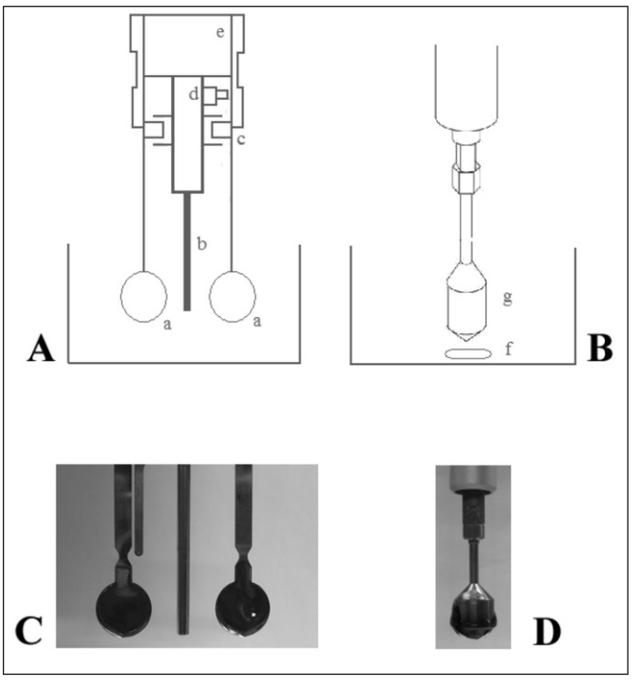

Figure 1 - Schematic drawing of the viscometers: A) SV10 (a: sensor plate; b: temperature sensor; c: electromagnetic drive; d: displacement sensor; e: spring plate). B) VM-10AL (f: magnetic stirring bar; g: detector). C) and D) show details of the experimental conditions.

During our experiments, the values were recorded by a PC connection through an RS-232 port (data collection every 2 s).

3. Experimental validation of the drainage pattern

A standard of a known viscosity, namely polydimethylsiloxane AK 1000 fluid from Bohlin (η = 1082 mPa.s at 20 °C, as confirmed by a Bohlin BV88 rotational viscometer), and purified water (η = 1.0 mPa.s at 20 °C) were adopted. An original procedure was selected to obtain consistent data with defined precision, reproducibility and sensitivity limits, using HA gel samples. The choice for this polymer is based on the information on physico-chemical characterization in both aqueous saline solutions and different film states [29] and on our recent study about its viscometric evaluation in terms of degradation pathway [30]. Nevertheless, HA is widely used in ocular discomfort treatment [17, 18, 31]. The desired amount of HA in order to obtain a final concentration of 1 % w/w was treated according to previous study [30]. The so-prepared HA hydrogels were poured into a 10 mL container and the viscometer probes were dipped into them at the required height. The corresponding values were expressed in terms of apparent viscosity

Table I - Formulations of the different commercial products tested.

Sample type	Composition
S1 (Lacrinorm)	Polyacrylic acid 0.2g[illegible]zalkonium chloride 0.01 g, sorbitol, water for injections
S2 (Lacrigel)	Carboxypolymethylene 0.2 g, thimerosal 0.002 g, sorbitol, water for injections
S3 (Viscotirs Gel)	Polyacrylic acid (Carbopol 980) 0.2 %, cetrimide, sorbitol, sodium hydroxide, water for injections
S4 (Dacriogel)	Carbomer 974P 0.3 %, sorbitol, benzalkonium chloride, sodium hydroxide, purified water
S5 (Lipovisc Gel Oftalmico)	Carbomer 0.2 %, cetrimide, sorbitol, middle-chain triglycerides, sodium hydroxide, purified water
S6 (Genteal Gel)	Hydroxypropilmethylcellulose 0.3 %, Carbopol 980, dequest 2060S, sorbitol, sodium perborate, water for injections
S7 (Visine)	Povidone K25, Carbomer 974P, middle-chain triglycerides, benzalkonium chloride, glycerol (85 %), sodium acetate trihydrate, trometamol, purified water

* The ingredients are nominally indicated as labeled and, where stated, quantitatively referred to 100 g of ophthalmic gel.

and recorded until a constant value was achieved (< 2 min). Following viscometric determination, the container was withdrawn. An amount of gel remained on the probes, giving a reproducible coating layer (about 1 mm, at the experimental conditions adopted). After a certain period of time (5 min for SV10, 2 min for VM-10AL), the drainage pattern of the residual gel on the probes was evaluated by dipping the coated probes into a vessel giving "infinite sea" conditions, in water [32]. With the development of proper handling methods for fresh HA samples, a level of precision defined by a coefficient of variation of less than or equal to ± 0.3 % was obtained. Based on this experience, similar tests have been also performed with Carbopol 974P gels at different concentrations (starting from 0.2 to 1 %) and freshly prepared. A comparable precision level was obtained.

4. Drainage pattern of ophthalmic gels

The drainage pattern of each ophthalmic gel that has been chosen for the present study was evaluated as described above by dipping the resulting coated probes into an "infinite sea" of media at different tonicity (water, hypoPB, isoPB and hyperPB). The initial value (ηstart) was the reproducible value as obtained either immediately or exactly 5 s after the probe dipping for SV10 and VM-10AL, respectively. Schematic representations of the systems used are shown in *Figure 1*. To maintain the reproducibility of the measurements, the devices were cleaned carefully with 0.9 % NaCl after each determination and subjected to viscosity tests using purified water as the standard to check effective cleaning (1.0 and 0.94 mPa.s for SV10 and VM-10AL, respectively).

At least five replicates were made for each commercial gel. They were stored at 4 °C until the measurements were performed. All the determinations were carried out within 28 days, at 20.0 ± 0.2 °C. In order to render the measurements obtained by the two instruments comparable, magnetic stirring (cylindrical magnetic bar 12 mm long, 5 mm in diameter, at 100 rpm) was also adopted in the case of VM-10AL determination, unless otherwise stated.

5. Mathematical fitting and statistical evaluation

The results reported represent the mean ± SD ($n \geq 5$). An iterative nonlinear least-square fitting method (NLLSQ) was applied to the data (GraphPad Prism 4.0 software) and the best-fit values ± SE were reported. One-way ANOVA was used with the Bonferroni post-test (InStat software, version 1.14 GraphPad Software Inc., San Diego, United States) for statistical analysis of the results. Significance was defined as a p value of less than 0.05.

II. RESULTS

1. Instruments characterization

Table II reports the viscosity values of the different native formulations as obtained by the two viscometers. Differences were detected in terms of apparent viscosities of the gels, with SV10 always leading to greater values, except for the S7 formulation. This discrepancy was also observed during the instrument validation procedure, when VM-10AL (for which, as already mentioned, user calibration is not possible) read the value of 942 mPa.s for the standard oil, instead of the declared value of 1082 mPa.s. However, for the practical purpose of this study on the dispersivity pattern of the gels, it was not deemed necessary to investigate this phenomenon any further. The presence of the same gelling agent was found to be not sufficient to predict the initial gel consistency: in fact, the experimental values obtained in the case of S2 were considerably lower than those obtained for the analogous formulations S1, S3 and S4.

Table II - Apparent viscosity values of the different ophthalmic gels as obtained by the two instruments.

Sample type	SV10	VM-10AL
	η (mPa·s)	
S1	1370 ± 42	290 ± 1.1
S2	126 ± 7.1	56 ± 0.5
S3	1117 ± 72	288 ± 14
S4	1698 ± 54	303 ± 2.1
S5	996 ± 10	291 ± 2.9
S6	946 ± 20	312 ± 4.1
S7	106 ± 2.1	150 ± 4.3

2. Drainage pattern in the presence of mechanical solicitation

Figures 2-4 illustrate the modification of in situ drainage behavior under the various experimental conditions adopted for the formulations S1, S6 and S7, respectively. Among others, they have been selected as being representative of the ingredients existing in the different formulations. As can be observed, although the starting values were very different, a similar exponential negative trend over time was observed in all cases. Moreover, the salinity led to a more rapid drainage profile, as shown in the insets, representative of both isoPB and hyperPB conditions, based on the gelling property variations of the polymers at level of the hydration shell, primarily in terms of modification of the hydrogen bond network [33]. According to these observations, the monoexponential model (1) was adopted to describe the data variation over time:

$$\eta_t = (\eta_{start} - \eta_{end})^{e^{-kt}} + \eta_{end} \qquad \textbf{Eq. 1}$$

Equation 1 provides good results in terms of fitting the mathematical description of almost all the patterns in the presence of intrinsic or

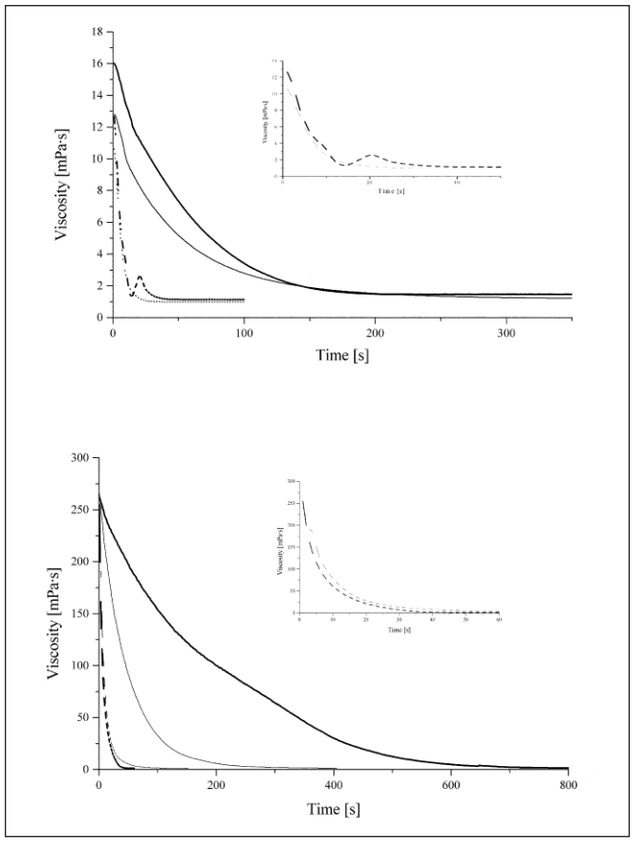

Figure 2 - Drainage evaluation of S1 by SV10 (top) and VM-10AL (bottom), obtained by dipping loaded probes into: ▬ water; —— hypo-PB; - - - iso-PB; – – – hyper-PB. The insets magnify the early drainage pattern in the latter two media.

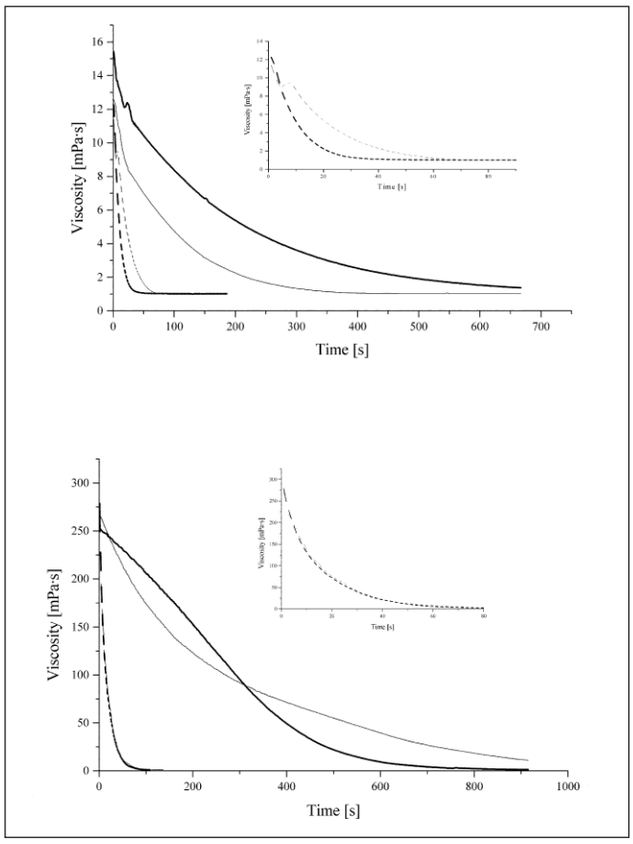

Figure 3 - Drainage evaluation of S6 by SV10 (top) and VM-10AL (bottom), obtained by dipping loaded probes into: ▬ water; — hypo-PB; - - - iso-PB; – – – hyper-PB. The insets magnify the early drainage pattern in the latter two media.

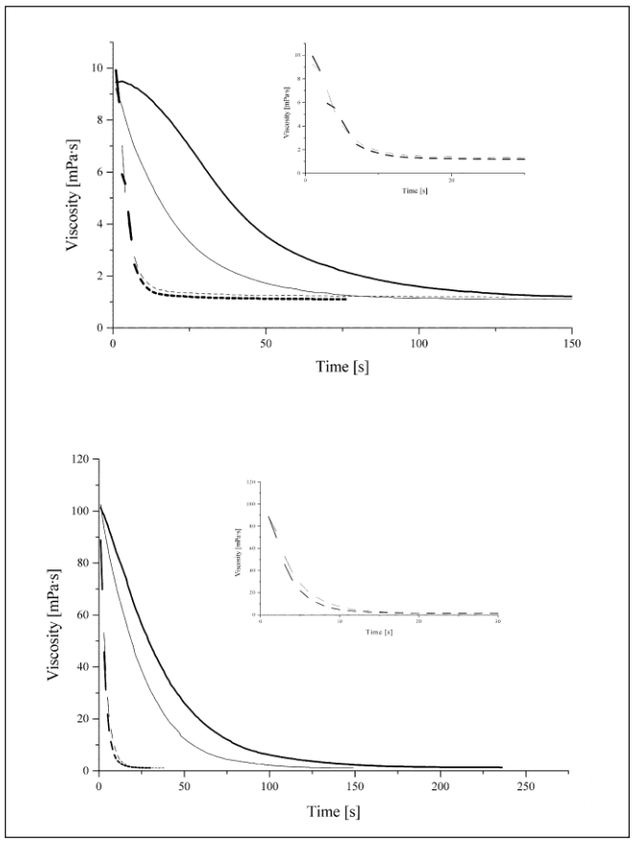

Figure 4 - Drainage evaluation of S7 by SV10 (top) and VM-10AL (bottom), obtained by dipping loaded probes into: ▬ water; — hypo-PB; - - - iso-PB; – – – hyper-PB. The insets magnify the early drainage pattern in the latter two media.

magnetic stirring for SV10 and VM-10AL, respectively. These results are reported in *Table III*, together with the k and r^2 values obtained. To validate the viscosity data obtained in the different sets of experiments, all the values within each set were also normalized with respect to the initial value (η_{start} = 100) and the mathematical model (*Equation 1*) was applied. No differences were observed among k value ratios (data not shown).

Figure 5 shows the k values and their SD: the greater the ion concentration in the environment, the greater the k values. A statistically significant correlation ($p < 0.001$) was observed for all water/hypoPB pairs. The only exception was S6 (no significance) in both the instruments adopted. In detail, a tendency to inversion was obtained in the case of SV10, with a statistically greater k value for water in comparison to hypoPB, whereas VM-10AL gave the same value of 3.6E-3. In contrast, concerning the isoPB/hyperPB pairs, SV10 gave similar k values for S1, S4 and S5 (no significance) and different values for S3 ($p < 0.05$), S2 and S7 ($p < 0.001$), while VM-10AL gave k values with $p < 0.001$ in all cases.

The correlation coefficients were always very good ($r^2 > 0.95$), except for S5 evaluation in isoPB ($r^2 = 0.916$) and hyperPB ($r^2 = 0.872$) using SV10. In the case of VM-10AL, $r^2 = 0.946$ and $r^2 = 0.941$ were observed for S6 in water and S7 in hyperPB, respectively.

In order to improve the description of these experimental patterns, the biexponential model indicated in *Equation 2* was also applied:

$$\eta_t = k_1 e^{-k2t} + (\eta_{start} - k_1 - \eta_{end}) e^{-k3t} + \eta_{end}\ \eta_t \qquad \textbf{Eq. 2}$$

Such an approach was also suggested by the presence of more than one component in the S5-S7 formulations, including the viscosity agents HPMC and PVP. However, it did not provide good results

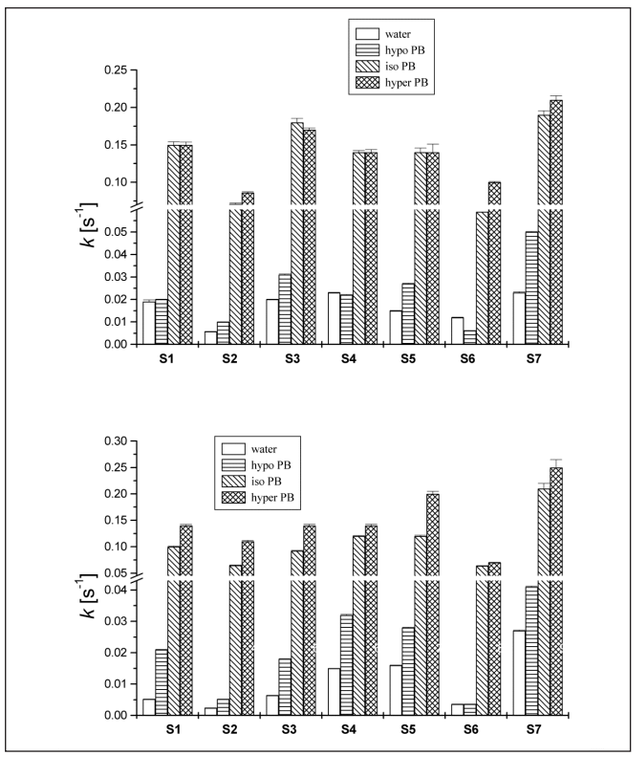

Figure 5 - Best-fit k values obtained by *Equation 1* in relation to the different dipping environments of the commercial gels using SV10 (top) and VM-10AL (bottom).

Table III - Experimental data, constant values and correlation coefficients of *Equation 1* describing viscosity patterns as obtained at the different conditions (see text for details).

Sample type	Environ-ment	SV10				VM-10AL			
		η_{start}	η_{end}	k (s^{-1})	r^2	η_{start}	η_{end}	k (s^{-1})	r^2
S1	water	16.0 ± 0.59	1.47 ± 0.11	1.9 E-2 ± 7.7 E-5	0.996	262 ± 1.00	1.35 ± 0.03	5.2 E-3 ± 1.8 E-5	0.992
	hypo PB	12.8 ± 0.06	1.18 ± 0.01	2.0 E-2 ± 8.2 E-	0.995	265 ± 0.70	1.33 ± 0.01	2.1 E-2 ± 1.2 E-	0.999
	iso PB	10.6 ± 0.13	1.00 ± 0.01	0.15 ± 3.9 E-3	0.966	240 ± 4.95	1.10 ± 0.02	0.10 ± 2.0 E-5	0.988
	hyper PB	12.7 ± 0.03	1.14 ± 0.01	0.15 ± 3.7 E-3	0.956	254 ± 4.24	1.13 ± 0.02	0.14 ± 2.4 E-3	0.987
S2	water	61.9 ± 3.14	1.31 ± 0.14	5.7 E-3 ± 2.4 E-5	0.984	54.6 ± 0.59	2.86 ± 0.60	2.4 E-3 ± 1.4 E-5	0.957
	hypo PB	48.5 ± 1.64	1.27 ± 0.27	1.0 E-2 ± 4.8 E-5	0.986	47.5 ± 1.89	2.28 ± 0.36	5.2 E-3 ± 2.6 E-5	0.985
	iso PB	26.2 ± 5.82	1.00 ± 0.02	7.1 E-2 ± 1.2 E-3	0.985	44.1 ± 0.72	1.10 ± 0.02	6.5 E-2 ± 4.9 E-4	0.996
	hyper PB	28.9 ± 3.79	1.11 ± 0.03	8.6 E-2 ± 1.2 E-3	0.986	47.9 ± 0.36	1.10 ± 0.02	0.11 ± 2.0 E-3	0.986
S3	water	15.5 ± 0.59	1.41 ± 0.02	2.0 E-2 ± 8.0 E-5	0.995	288 ± 2.08	1.38 ± 0.16	6.4 E-3 ± 2.5 E-5	0.991
	hypo PB	15.4 ± 1.63	1.17 ± 0.03	3.1 E-2 ± 3.1 E-4	0.976	223 ± 2.00	1.50 ± 0.36	1.8 E-2 ± 6.8 E-5	0.995
	iso PB	11.1 ± 1.74	1.10 ± 0.02	0.18 ± 5.5 E-3	0.961	236 ± 4.70	1.29 ± 0.14	9.2 E-2 ± 7.0 E-4	0.997
	hyper PB	12.1 ± 0.75	1.08 ± 0.01	0.17 ± 2.7 E-3	0.983	213 ± 3.20	1.15 ± 0.15	0.14 ± 2.6 E-3	0.987
S4	water	15.7 ± 0.93	1.27 ± 0.04	2.3 E-2 ± 1.4 E-4	0.994	295 ± 1.41	2.98 ± 0.03	1.5 E-2 ± 3.1 E-5	0.998
	hypo PB	12.9 ± 1.37	1.15 ± 0.02	2.2 E-2 ± 1.6 E-4	0.986	292 ± 1.41	1.99 ± 0.30	3.2 E-2 ± 1.9 E-4	0.991
	iso PB	11.9 ± 0.70	1.11 ± 0.02	0.14 ± 2.4 E-3	0.974	277 ± 4.24	1.08 ± 0.01	0.12 ± 1.2 E-3	0.993
	hyper PB	11.1 ± 0.40	1.08 ± 0.01	0.14 ± 3.6 E-3	0.963	295 ± 1.41	1.07 ± 0.01	0.14 ± 2.6 E-3	0.983
S5	water	14.8 ± 2.53	1.33 ± 0.13	1.5 E-2 ± 3.6 E-5	0.998	289 ± 2.82	1.18 ± 0.85	1.6 E-2 ± 4.7 E-5	0.997
	hypo PB	13.6 ± 0.88	1.16 ± 0.01	2.7 E-2 ± 2.2 E-4	0.981	292 ± 0.70	1.72 ± 0.33	2.8 E-2 ± 1.1 E-4	0.995
	iso PB	10.1 ± 0.16	1.07 ± 0.01	0.14 ± 5.7 E-3	0.916	288 ± 0.70	1.12 ± 0.01	0.12 ± 1.7 E-3	0.987
	hyper PB	11.3 ± 0.10	1.10 ± 0.01	0.14 ± 1.0 E-2	0.872	278 ± 1.41	1.08 ± 0.01	0.20 ± 5.0 E-3	0.975
S6	water	12.6 ± 1.86	1.04 ± 0.10	1.2 E-2 ± 5.7 E-5	0.989	253 ± 4.16	1.27 ± 0.32	3.6 E-3 ± 3.3 E-5	0.946
	hypo PB	15.4 ± 2.40	1.06 ± 0.08	6.2 E-3 ± 2.6 E-5	0.984	267 ± 15.6	2.04 ± 0.49	3.6 E-3 ± 1.0 E-5	0.990
	iso PB	14.4 ± 1.23	1.00 ± 0.04	5.4 E-2 ± 1.0 E-3	0.964	269 ± 2.08	1.05 ± 0.01	6.4 E-2 ± 3.0 E-4	0.998
	hyper PB	12.3 ± 1.05	1.02 ± 0.01	0.10 ± 1.0 E-3	0.988	279 ± 20.0	1.11 ± 0.01	7.0 E-2 ± 4.7 E-4	0.996
S7	water	9.47 ± 0.61	1.18 ± 0.18	2.3 E-2 ± 4.1 E-4	0.957	101 ± 2.12	1.33 ± 0.32	2.7 E-2 ± 2.2 E-4	0.990
	hypo PB	9.17 ± 1.43	1.10 ± 0.03	5.0 E-2 ± 2.4 E-4	0.997	102 ± 2.12	1.14 ± 0.13	4.1 E-2 ± 2.5 E-4	0.996
	iso PB	9.23 ± 0.21	1.19 ± 0.01	0.19 ± 5.7 E-3	0.944	88.6 ± 1.34	1.09 ± 0.01	0.21 ± 1.0 E-2	0.951
	hyper PB	9.92 ± 0.13	1.11 ± 0.01	0.21 ± 7.3 E-3	0.952	88.8 ± 10.1	1.14 ± 0.01	0.25 ± 1.5 E-2	0.941

from the point of view of the mathematical description of the residence time profile ($r^2 < 0.94$). It is interesting to note that the search for other mathematical fitting models, such as polynomial ones, improved best fitting ($r^2 < 0.97$) but the calculated k values gave abnormal SDs and could therefore lead to formally incorrect conclusions (data not shown). In order to extend the mechanism of the variations in terms of gel consistency in the different environments over time, instead of continuing to evaluate new mathematical functions, another set of experiments without mechanical solicitation was performed.

3. Drainage pattern in the absence of mechanical solicitation

For this purpose, experimental values obtained by VM-10AL without magnetic stirring were recorded, and they are summarized in *Table IV*. The initial and final viscosity values were generally superimposable, even if the SD values were greater with respect to previous sets where magnetic stirring was present. These differences can be justified by the time (> 5 s) taken to reach equilibria, as expected. Concerning the data fitting by *Equation 1*, with the exceptions of the water environment ($r^2 > 0.98$ for S1, S3, S4, S5 and S7), both S1 and S5 in iso- and hyper-PB, and S4 in all media ($r^2 > 0.98$), the other patterns are not generally well described ($r^2 < 0.940$). The k values were lower in comparison to those obtained in the presence of stirring, with a regular trend and very small SDs in all cases.

In *Table IV* the best fitting parameters resulting by *Equation 2* have been reported. With this function, $r^2 \geq 0.960$ values were obtained in all cases, prompting us to deem this approach very suitable to describe the phenomena. However, on close examination of the k values obtained, it appeared that k1 for S6 in water was not acceptable because of its abnormal SD, despite an $r^2 = 0.997$. For this reason, all the experimental curves were investigated in detail. An example is shown in *Figure 6*, which represents a comparison of the experimental data for S7, obtained by dipping loaded VM-10AL probes into isoPB and fitting curves using the two mathematical functions. The inset shows a detailed view of a peculiar and very reproducible end portion of the viscosity curve, together with its best fitting mathematical description, illustrating that the mathematical approach is not capable of describing the real behavior of the system.

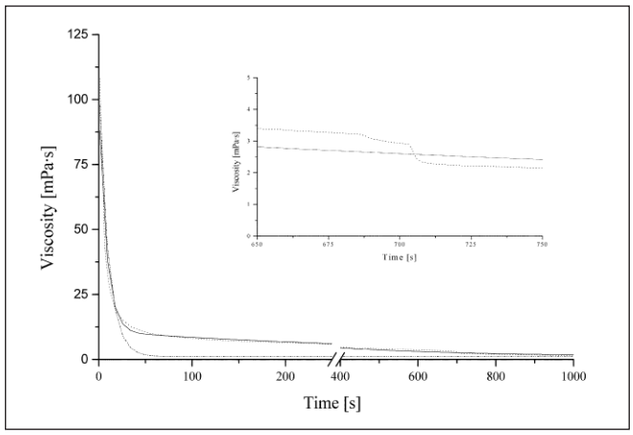

Figure 6 - Experimental data (·····) of S7, obtained by dipping VM-10AL loaded probes into isoPB in the absence of stirring, as well as fitting curves by functions 1 (– ·· –) and 2 (—), respectively. The inset shows some peculiar behavior of the drainage pattern.

Table IV - Experimental data, constant values and correlation coefficients of *Equations 1 and 2* describing viscosity patterns as obtained by VM-10AL in the absence of stirring (see text for details).

Sample type	Environment	VM-10AL no stirring							
		Monoexponential mode				Biexponential mode			
		η_{start}	η_{end}	k (s^{-1})	r^2	k_1 (mPa·s)	k_2 (s^{-1})	k_3 (s^{-1})	r^2
S1	water	274 ± 17.7	1.35 ± 0.77	2.2 E-3 ± 5.8 E-6	0.982	83.22 ± 0.85	7.1 E-3 ± 8.0 E-5	1.6 E-3 ± 4.6 E-6	0.999
	hypo PB	261 ± 5.65	1.33 ± 0.10	1.0 E-2 ± 9.6 E-5	0.983	200.9 ± 0.39	1.8 E-2 ± 6.2 E-5	2.4 E-3 ± 1.6 E-5	0.999
	iso PB	240 ± 6.52	1.10 ± 0.09	9.4 E-2 ± 1.3 E-3	0.979	87.37 ± 2.66	0.14 ± 4.1 E-3	3.6 E-2 ± 2.1 E-3	0.996
	hyper PB	254 ± 2.89	1.13 ± 0.01	7.7 E-2 ± 7.8 E-4	0.983	93.36 ± 1.82	0.11 ± 2.1 E-3	2.9 E-2 ± 1.2 E-3	0.997
S2	water	54.6 ± 0.03	2.86 ± 0.01	3.9 E-4 ± 1.5 E-6	0.898	0.38 ± [illegible]-2	7.8 E-4 ± 3.2 E-5	3.1 E-4 ± 2.9 E-6	0.962
	hypo PB	49.0 ± 2.12	2.28 ± 0.01	8.6E-4 ± 4.2 E-6	0.744	17.71 ± 0.10	6.9 E-3 ± 9.7 E-5	6.2 E-4 ± 2.4 E-6	0.987
	iso PB	47.2 ± 4.38	1.10 ± 0.06	2.7 E-2 ± 4.6 E-4	0.893	34.94 ± 0.23	5.6 E-2 ± 6.5 E-4	7.8 E-3 ± 1.1 E-4	0.997
	hyper PB	47.1 ± 1.13	1.10 ± 0.03	4.4 E-2 ± 6.8 E-4	0.953	37.31 ± 0.35	6.4 E-2 ± 9.0 E-4	1.1 E-2 ± 4.0 E-4	0.996
S3	water	277 ± 14.8	1.38 ± 0.47	3.1 E-3 ± 1.2 E-6	0.999	94.88 ± 1.4 E-7	3.2 [illegible]1.17	3.2 E-3 ± 0.67	0.999
	hypo PB	197 ± 36.1	1.50 ± 0.85	9.6 E-3 ± 9.1 E-5	0.849	105.2 ± 0.54	2.9 E[illegible]1 E-4	3.4 E-3 ± 2.7 E-5	0.992
	iso PB	218 ± 25.4	1.29 ± 0.01	0.12 ± 2.2 E-3	0.944	165.0 ± 2.11	0.17 ± 3.9 E-3	2.5 E-2 ± 1.3 E-3	0.989
	hyper PB	192 ± 29.7	1.15 ± 0.03	0.11 ± 1.7 E-3	0.968	131.3 ± 3.25	0.16 ± 4.8 E-3	3.6 E-2 ± 2.1 E-3	0.993
S4	water	280 ± 20.5	2.98 ± 0.06	2.5 E-3 ± 3.1 E-6	0.997	20.71 ± 0.60	1.3 E-2 ± 5.3 E-4	2.3 E-3 ± 4.3 E-6	0.999
	hypo PB	285 ± 9.19	1.99 ± 0.11	8.8 E-3 ± 3.9 E-5	0.971	230.9 ± 0.28	1.2 E-2 ± 2.1 E-5	2.2 E-3 ± 1.0 E-5	0.999
	iso PB	250 ± 37.5	1.08 ± 0.03	6.5 E-2 ± 4.7 E-4	0.992	186.2 ± 5.59	8.0 E-2 ± 2.1 E-3	2.6 E-2 ± 2.4 E-3	0.997
	hyper PB	258 ± 51.6	1.07 ± 0.11	1.0 E-1 ± 1.6 E-3	0.976	159.8 ± 6.56	0.16 ± 6.4 E-3	4.3 E-2 ± 2.8 E-3	0.995
S5	water	264 ± 34.6	1.18 ± 0.51	1.6 E-3 ± 9.7 E-7	0.999	173.1 ± 1.9 E-8	1.6 E-3 ± 1.97	1.6 E-3 ± 5.30	0.999
	hypo PB	278 ± 19.1	1.72 ± 0.90	1.2 E-2 ± 8.6 E-5	0.933	213.6 ± 0.33	1.8 E-2 ± 5.3 E-5	2.4 E-3 ± 1.5 E-5	0.999
	iso PB	274 ± 19.1	1.12 ± 0.02	7.9 E-2 ± 7.9 E-4	0.979	230.1 ± 1.40	9.9 E-2 ± 9.3 E-4	1.7 E-2 ± 6.3 E-4	0.997
	hyper PB	267 ± 15.5	1.08 ± 0.01	1.6 E-1 ± 3.1 E-3	0.963	237.6 ± 4.62	0.19 ± 6.5 E-3	2.6 E-2 ± 6.2 E-3	0.973
S6	water	265 ± 16.9	1.27 ± 0.10	4.7 E-4 ± 1.7 E-6	0.940	-4967 ± 16227	1.1 E-3 ± 1.1 E-4	1.0 E-3 ± 9.5 E-5	0.997
	hypo PB	247 ± 28.3	2.04 ± 0.10	1.7 E-3 ± 6.1 E-6	0.961	206.9 ± 1.05	1.7 E-4 ± 1.5 E-5	2.1 E-5 ± 3.1 E-5	0.960
	iso PB	278 ± 12.7	1.05 ± 0.10	2.0 E-2 ± 2.8 E-4	0.867	239.0 ± 0.53	3.2 E-2 ± 1.8 E-4	2.7 E-3 ± 3.4 E-5	0.995
	hyper PB	265 ± 19.8	1.11 ± 0.01	2.8 E-2 ± 4.4 E-4	0.912	211.7 ± 1.29	4.1 E-2 ± 5.2 E-4	4.4 E-3 ± 1.6 E-4	0.990
S7	water	122 ± 29.7	1.33 ± 0.93	5.2 E-3 ± 1.1 E-5	0.996	8.36 ± 0.34	7.4 E-2 ± 9.2 E-3	5.0 E-3 ± 1.4 E-5	0.998
	hypo PB	106 ± 5.65	1.14 ± 0.23	1.6 E-2 ± 2.4 E-4	0.857	69.75 ± 0.26	4.4 E-2 ± 3.1 E-4	5.5 E-3 ± 3.4 E-5	0.998
	iso PB	98.3 ± 13.7	1.09 ± 0.31	0.10 ± 2.2 E-3	0.684	97.07 ± 9.6 E-2	0.14 ± 9.3 E-4	2.6 E-3 ± 3.4 E-5	0.985
	hyper PB	94.9 ± 8.63	1.14 ± 0.44	0.12 ± 3.4 E-3	0.833	91.41 ± 0.33	0.15 ± 2.2 E-3	6.0 E-3 ± 3.6 E-4	0.981

III. DISCUSSION

The purpose of using two different devices to investigate the drainage pattern of certain ophthalmic gels can be summarized as follows: as there are no specifically dedicated analytic tools and no specific protocols for investigating the complex phenomenon of the diffusion/elimination of a gel in a medium, instruments that are normally only applied to determine viscosity have been used. The differences in the apparent viscosity values of the commercial ophthalmic gels, as determined by the two instruments and reported in *Table II*, derive from the different interactions with the system, which can be correlated with the peculiarities of each method and are characterized by the typical functioning principles of each device. These discrepancies were therefore judged to be of no significance for this study and an in-depth evaluation was considered beyond the aim of the present study. The different equilibration time and initial values during drainage evaluation were attributed to the different amounts of ophthalmic gels that adhered to the detector probes, in function of their thickness and their component materials. On the other hand, due to the different construction and functions of the two devices (e.g. mechanical solicitation by vibrational or torsional oscillations, differences in the forms and materials of the probes) a wider range of information has been possible to obtain. Such information, in some ways, can be related to the range of situations that *in vivo* administration can present (e.g. ocular mobility, closing of the lids, composition of lacrimal fluid), influencing the distribution and removal of tear substitutes.

As far as the trend of the experimental values shown in *Figures 2 to 4* is concerned, they can be fitted using numerous mathematical functions. The distribution of these values showed a clear tendency to decrease in time and the first function used was therefore the common decreasing exponential function, with the constants linked to the initial and final viscosity values, as experimentally evaluated.

Analysis of the data suggested that, with good r^2 values in most cases, the mathematical description did not appear to be entirely sufficient, especially for some particular samples. This is why an attempt was made at best-fitting with different functions.

The second function used in relation to the same data was a double exponential one, in which the independent (linear and nonlinear) parameters were again compared to the initial and final experimental values. Agreement with the data did not appear to be entirely satisfactory in this case either, thus suggesting that the various systems investigated have more complex physical behavior than can be described with a model that maintains its characteristics (e.g. solubility, viscosity, diffusivity) in time. The test carried out with non-exponential functions led to the same conclusions.

The investigation therefore aimed to verify the behavior of various samples in experimental conditions other than those in which mechanical disturbance was always present, in order to obtain comparable application information. To do this only the VM-10AL, in the absence of external mechanical stress (magnetic stirring), was used. As expected, the results obtained showed an increase in analysis time, i.e. a longer time between ηstart and ηend. The values of ηstart were also less reproducible and not always comparable to those obtained with magnetic stirring. Such a phenomenon may be explained by the different adaptation of the gel to the dispersion medium, which can lead to swelling that would not otherwise occur.

Concerning fitting, a monoexponential function was found to be

unable to describe some of the experimental profiles in an adequate manner, as judged by the r^2 values (the same or even lower than those obtained with stirring).

On the other hand, best fitting with double exponential functions appeared to be suitable for all samples, although a control of the statistical significance of the parameter values revealed intervals that were still too great. Lastly, even with the functions that offered acceptable fitting, it should be highlighted that the experimental data on the S7 samples showed marked differences in values at certain time points, which did not correspond to the graph of the function used.

For all the cases in which the mathematical representation can be considered adequate from the point of view of both the fitting and the significance of the parameters, it can be concluded that the investigation provides a satisfactory quantitative evaluation in the context of an original description of a highly complex process. To have brought to light different behaviors of compounds supplied for the clinical treatment of identical situations is in itself an interesting result. For the cases in which mathematical discussion has highlighted criticality, an initial hypothesis may be proposed based upon the presence of more than one component in the gel products on the market and the fact that significant discontinuity may occur during the dispersion process. Specifically, an initial evolution according to a homogeneous model, and a subsequent phase in which the different modes of interaction between the components and the solvent prevail have been supposed. The authors intend to continue the study of this phenomenon and expand the investigation to include substances that allow comparison between the behaviors of different components.

The selected mathematical function provided useful information about the mechanism of gel drainage in the different media, simulating both physiological and pathological environments, resulting in tear osmolarity variation, especially in case of dry eye [33]. The correlation coefficients generally showed inverse proportionality to the environmental salinity. The remarkably different behavior of the samples with respect to the environment was attributed to the very rapid decrease in gel consistency in the case of contact with gradually-increasing salinity of ionic buffers. In fact, with the presence of increasing amount of NaCl, the gel strength of the polymer will become progressively negligible because of polymer chains/hydrogen bonding interactions [4, 34]. In particular, k value for the monoexponential model, as well as both k_2 and k_3 values for the biexponential model, represent a quantitative estimation of the gel residence time under the experimental conditions adopted. These parameters give definite information for the evaluation of the clearance properties of gel products, in a manner that may be useful for selecting the appropriate treatment regimen. As an example, if it could be beneficial to use either different eye drops or ophthalmic gels interchangeably or sequentially. Further investigations are necessary for a correlation with the *in vivo* performance of the gels, but these are considered beyond the aim of the present study. In conclusion, the evaluation technique presented here could be a valuable tool to approach the clearance properties of ophthalmic gel products at different condition resembling both physiological and pathological conditions, primarily in terms of environmental salinity, helping the selection of the appropriate treatment regimen. We hope to have presented a possible methodology for a phenomenon that has not yet been described using any analytic tools and that can potentially be extended to the real behavior of formulation on the ocular surface, as well as to any other circumstances in which a viscous compound will tend to disperse itself in a medium.

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