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Capillary electrophoresis instrumentation as a bench-top viscometer

Michael S. Bello☆, Roberta Rezzonico and Pier Giorgio Righetti*

Faculty of Pharmacy and Department of Biomedical Sciences and Technologies, University of Milan, Via Celoria 2, Milan 20133 (Italy)

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ABSTRACT

A simple and reliable method is proposed for measuring the viscosities of different solutions by using standard capillary zone electrophoresis equipment. The capillary is first filled with, *e.g.*, a liquid of known viscosity and then the solution under analysis is pumped through it at a constant pressure drop and constant temperature. The migration time of the boundary between the two liquids from the injection port to the detector is carefully measured and the experimental data are entered in a derived modified Poiseuille equation for calculating the unknown viscosity. Viscosities of small analytes (*e.g.*, sucrose solutions) and of macromolecular solutions (*e.g.*, methylcellulose) could be assessed with a precision of the order of 3%. The boundary between the two liquids is usually detected by refractive index gradients, even in the presence of non-UV-absorbing species. With very minute refractive index variations, the boundary is easily detected by spiking one of the two solutions with traces of a strongly UV-absorbing compound (*e.g.*, riboflavin).

INTRODUCTION

One of the most recent trends in the mass separation of macromolecules (notably proteins and nucleic acids) in capillary electrophoresis (CE) is the use of capillaries filled with viscous polymer solutions, according to an original idea first proposed by Bode [1] and De Gennes [2]. Such polymer solutions can be extremely viscous, and the separation (especially with nucleic acids) seems to be directly correlated with the viscosity of such solutions [3,4]. Therefore, simple and reliable methods allowing the rapid assessment of the viscosity of a given solution used in biochemical separations are highly desirable. Whereas sophisticated (and very expensive) viscometers are commonly found in laboratories dealing with polymer science, they are rarely to be seen in a biochemical laboratory. We report here a simple and efficient procedure by which a standard instrument for capillary electrophoresis can be used as a bench-top viscometer.

The possibility of measuring the viscosity of a liquid by measuring its velocity in a tube of known diameter is well known and follows from the classical Poiseuille equation [5,6]. However, to put this method into practice one needs tubing, a pump with connections, a marker and a detector. Fortunately, a CE unit contains all these components and hence can be used for viscosity measurements. In the Theory section we derive an approximate equation for calculating the time taken by the boundary between two liquids to migrate from one end of the capillary

* Corresponding author.
☆ Permanent address: Institute of Macromolecular Compounds, Bolshoi 31, 199004 St. Petersburg, Russian Federation.

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to the UV window. The Experimental section describes a procedure developed to measure the viscosity of a liquid by means of a Waters Quanta 4000 CE unit. Measured viscosities were compared with those given in tables and measured by a standard technique.

THEORY

Consider a capillary of total length L, distance between the entrance end and the UV window l and inner diameter d (Fig. 1). Assume that the capillary was initially filled with a liquid having viscosity η_1 and that this liquid is replaced with another liquid with a viscosity η_2 under a pressure drop Δp. Obviously, the time it takes to replace the first liquid in the capillary by the second one depends on the viscosities of both liquids. In order to obtain a quantitative relationship between the time and the viscosities we have to solve the following problems: (1) what is the velocity of the liquid inside the capillary as a function of time?; and (2) how long does it take the boundary between the liquids to reach the UV window and how is this time related to the viscosity of the liquids?

As an approximation, we assume that the boundary between the two liquids is flat and does not disperse when flowing down the capillary. Let the boundary be located at a distance x from the entrance end of the capillary (see Fig. 1) at time t. The velocity of the boundary is, obviously, equal to the average cross-sectional velocity, which must be the same at any cross-section of the capillary but can vary in time. The average cross-sectional velocity of the viscous

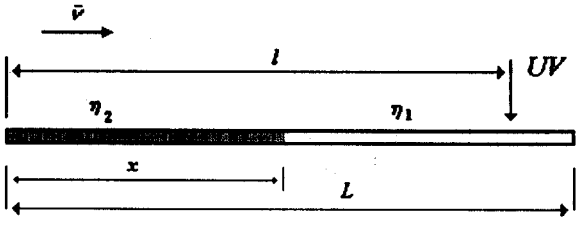

Fig. 1. Scheme of the experimental set-up for measuring viscosities in a CZE unit. L = total capillary length; l = distance between capillary entrance and detector (UV); x = coordinate of the boundary between the two liquids of viscosities; $\bar{v}$ = average cross-sectional velocity.

fluid flowing in a tube is given by the Poiseuille equation [5,6].

In accordance with the assumptions made above, we apply the Poiseuille law separately to both parts of the capillary filled with the two liquids:

$$\bar{v} = \frac{\Delta p_1 d^2}{32(L-x)\eta_1} \qquad (1)$$

$$\bar{v} = \frac{\Delta p_2 d^2}{32x\eta_2}$$

where $\bar{v}$ is the average cross-sectional velocity, x is the coordinate of the boundary between the liquids and Δp_1 and Δ_{p2} are the pressure drops on the parts of the capillary occupied by the first and the second liquid, respectively. Eqn. 1 transforms to

$$\bar{v} = \frac{\Delta p d^2}{32[\eta_2 x + \eta_1(L-x)]} \qquad (2)$$

where Δp is the total pressure drop, equal to the sum of the partial pressure drops. This equation relates the average velocity of the liquid in the capillary with the instantaneous position of the boundary.

The velocity $\bar{v}$ is also the velocity of the boundary between liquids and is given by $\bar{v} = dx/dt$. After substituting this expression into eqn. 2 one obtains a differential equation, integration of which gives the following relationship between the boundary migration time and its position along the capillary axis:

$$t = \frac{32Lx\eta_1}{\Delta p d^2}\left[\frac{x}{2L}\left(\frac{\eta_2}{\eta_1} - 1\right) + 1\right] \qquad (3)$$

This equation can be used for estimating the time necessary to replace one liquid in the capillary with another. Setting $x = L$ in eqn. 3 gives

$$t_s = \frac{16L^2}{\Delta p d^2}(\eta_2 + \eta_1) \qquad (4)$$

where t_s is the time during which the boundary is moving within the capillary.

For $x = l$, eqn. 4 easily transforms to

$$\frac{l}{2L}\cdot\eta_2+\left(1-\frac{l}{2L}\right)\eta_1=\frac{\Delta t\,\Delta p d^2}{32Ll} \quad (5)$$

where Δt is the time during which the boundary is migrating from the entrance of the capillary to the detector window.

Eqn. 5 allows one to calculate the viscosity of one of the liquids if the viscosity of the other liquid, time Δt and other parameters are known. The capillary length L and the distance between the capillary entrance and the UV window can be easily measured. The pressure drop can be read from a display and the capillary diameter is usually given by the manufacturer or can be measured as suggested in ref. 7. The only unknown parameter to be determined experimentally is the time Δt.

Often the boundary between two liquids can be registered by a UV detector, as the latter is also sensitive to the difference in refractive index. Thus, for example, boundaries between distilled water and the buffer or water and sucrose solution are visible. If the boundary is invisible, a small amount of a light-adsorbing substance should be added.

EXPERIMENTAL

A Waters Quanta 4000 capillary electrophoresis system (Millipore, Milford, MA, USA) was used. Capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA). The inner diameter of the capillary was determined according to ref. 7 by using a software package [8] and found to be $d = 75.4 \pm 0.4$ μm. The temperature was read from a Digiterm Quartz 1505 thermometer (Hanhart, Schwenningen, Germany) installed in the vicinity of the capillary.

In order to demonstrate the possibility of using the CE unit for viscosity measurements, three solutions of different viscosities were used. A 0.5% methylcellulose (Methocel A4-M; Dow Chemical, Midland, MI, USA) solution was prepared by a hot-water method and centrifuged for 45 min in a Biofuge (Heraus Sepatech, Am Kalkberg, Germany) at 51 000 g. A 30% sucrose (Merck, Darmstadt, Germany) solution was prepared. Riboflavin-5′-phosphate (Bio-Rad, Richmond, CA, USA) was used to reveal the boundaries between distilled water and Methocel solution.

Viscosity measurements on the polymer solution were performed on a Bohlin VOR Rheometer (Bohlin Rheologi, Lund, Sweden).

In order to measure the viscosity of a liquid, a capillary of $L = 0.982$ m and $l = 0.909$ m was installed in the Waters Quanta 4000 unit. The entrance end of the capillary was positioned in the vial with the first liquid and the capillary was filled with a liquid by applying a pressure (purge on) of $\Delta p = 5.53 \cdot 10^4$ N m^{-2}. After a certain time sufficient to fully replace the liquid in the capillary, the vial was changed and the pressure was applied again. The moment of turning on the pump and the moment when the boundary passed by the UV window were monitored. The vial was then replaced with the first one and the procedure was repeated several times.

RESULTS

Fig. 2 illustrates the described procedure for the measurement of the time Δt necessary for the boundary between the sucrose solution and distilled water to pass the distance between the entrance of the capillary and the UV window. The capillary was initially filled with distilled water. Then the capillary entrance was placed in the vial with the sucrose solution and the pressure was applied at time t_p. The upper meander-type line represents the light absorbance signal received from detector 1 during pumping. At the beginning the light absorbance is constant and corresponds to that of the water. The transition to a low level of the UV signal shows the boundary between distilled water and the sucrose solution. The difference in the amplitude of the detector signal is probably caused by the difference in the refractive indices of water and the sucrose solution. The inflection point of the curve is associated with the position of the boundary and by measuring its coordinate one finds the time t_b at which the boundary passes the UV window. The time t_p of the start of purging can be assessed by either a chronometer or by recording the current signal. This signal is shown below the UV signal in Fig. 2. The peculiarity of the CE unit we used is that the

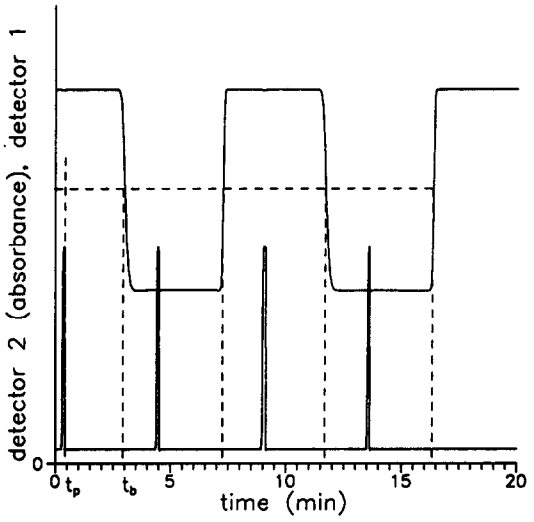

Fig. 2. Procedure for measurement of the boundary migration time. The upper meandering curve is given by the UV detector as the water–sucrose boundary passes through it (probably caused by differences in refractive indices between the two liquids; detector 1). The lower peaks (detector 2) are produced as spikes in the current signal when the lid of the box containing the capillary is opened or closed (this signal is taken as the start of boundary migration, *i.e.*, zero time). The graph shows the passage of four consecutive starting signals and of four consecutive boundaries.

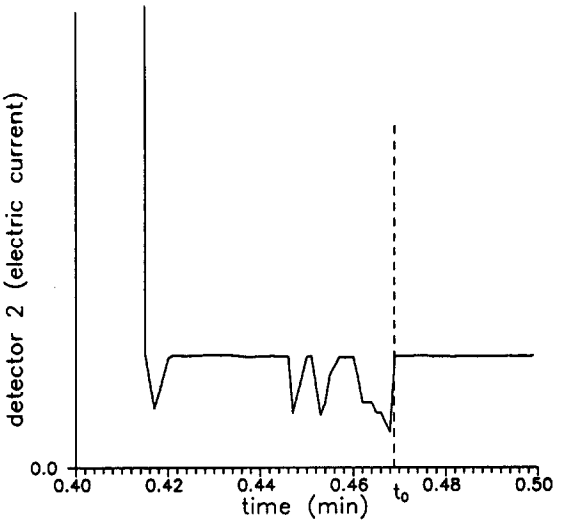

Fig. 3. Example of the spikes in the current signal produced by the up and down movement of the loading carousel. The last of this train of spikes is used as a marker for the start of the pumping of the viscous solution inside the capillary (*i.e.*, zero migration time).

current signal (detector 2) gives spikes when the box containing the capillary is opened and closed and when the carousel moves up and down. Spikes corresponding to the opening of the box are shown in Fig. 2. Spikes corresponding to the motion of the carousel have a lower amplitude, as shown in Fig. 3. The latest of these spikes was used as a marker for the moment when the pump had been turned on. In our experiments the button "Purge" had been pressed immediately after the carousel had moved to its normal position. Thus, t_b was found as a coordinate of the inflection point of the UV signal curve (detector 1) and t_p was found as a coordinate of the last spike on the detector 2 signal. Finally, we obtain the time interval during which the boundary was migrating between the capillary entrance and the UV window as $\Delta t = t_b - t_p$.

Table I presents times Δt and relative viscosities for the sucrose solution calculated from the run shown in Fig. 2. The value of the relative viscosity measured by means of a rotating viscometer is 3.0 and agrees well with the data presented in Table I. The error of the measurements depends on the accuracy of the pressure readings, edge effects, especially for viscous liquids, and on the precision with which time Δt is determined.

In our case we cannot expect the experimental error to be less than 3–4% and therefore we include only two valid decimal digits for the measured viscosities.

Viscosity measurements on distilled water were performed in order to compare the experimental data with those presented in ref. 9. We used two vials, one of which contained

TABLE I

RELATIVE VISCOSITY OF THE 30% SUCROSE SOLUTION AT 27.5°C MEASURED BY MEANS OF THE CE UNIT

Substitution	Δt (min)	η/η_w
Water–solution	2.55	3.1
Solution–water	2.72	3.0
Water–solution	2.57	3.1
Solution–water	2.7	3.0

TABLE II

MEASUREMENTS OF THE VISCOSITY OF WATER AT DIFFERENT TEMPERATURES OF THE AMBIENT AIR

T (°C)	Δt (min)	$\sigma_{n-1} \cdot 10^3$ (min)	$\eta_m \cdot 10^3$ (kg m^{-1} s^{-1})	$\eta_w \cdot 10^3$ (kg m^{-1} s^{-1})	ε (%)
27.5	1.26	3	0.83	0.84	1.2
30.3	1.2	11	0.79	0.8	1.3
26	1.32	7.7	0.87	0.87	0

distilled water and the other a 0.01% solution of riboflavin. It was assumed that such a low concentration of riboflavin would not change the solution viscosity. Table II presents times Δt representing average values for three series of measurements. The series of measurements were performed on different days and at different temperatures T. Table II also gives the standard deviations, σ_{n-1}, for the times Δt, the measured viscosities, η_m, the viscosity of water, η_w, taken directly from ref. 9 or obtained by linear interpolation and the percentile difference, ε, between the measured and standard values. It is seen that the agreement between measured and standard values is fairly good.

Table III presents the results for viscosity measurements on the polymer solution. Δt_1 denotes the time Δt measured when water was replaced with methylcellulose solution and Δt_2 that when water replaced methylcellulose. The

TABLE III

VISCOSITY MEASUREMENTS ON THE METHYLCELLULOSE (MC) SOLUTION

Substitution	Δt_1 (min)	Δt_2 (min)
Water–MC	13.4	
MC–water		15.6
Water–MC	13.44	
Water–MC	13.6	
MC–water		15.96
Water–MC	14.1	
MC–water		16.9
Δt (min)	13.6	16.2
σ (min)	0.32	0.67

calculated viscosities are $21 \cdot 10^{-3}$ and $22 \cdot 10^{-3}$ kg m^{-1} s^{-1}, respectively. These data are in good agreement, but the standard deviation of the boundary migration time is three times lower when the more viscous liquid replaces the less viscous liquid. The agreement with the viscosity obtained by using the standard viscometer is not so good: the measured viscosity was $16 \cdot 10^{-3}$ kg m^{-1} s^{-1}. However, this discrepancy can be attributed to the non-Newtonian properties of the polymer solution.

DISCUSSION

The precise assessment of the migration of a boundary was adopted long ago in separation science for measuring the physico-chemical properties of a solute. An example is the analytical ultracentrifuge, in which the velocity of a boundary of a sedimenting protein was used to assess the sedimentation coefficient and, in turn, the molecular mass of such a macromolecule. It should be noted that the spreading of such a boundary with time (seen as a sedimenting peak, obtained as the first derivative of a sigmoidal transition from a zone of pure solvent to a solute zone) was also used for the precise measurement of the diffusion coefficient of the macromolecular analyte. In an analogous fashion, measurement of the velocity of protein boundaries in an electric field was used for the determination of the free mobilities of proteins in the Tiselius moving boundary unit. In both techniques (centrifugal and electric fields), perhaps the most complex part of the instrumentation was the bulky optical bench used for revealing (and photographing) such boundaries. While present-day analysts might be unaware of such a discipline, the observation of boundaries was a complex problem and developed into an almost independent science. In the early days, Svedberg and Jette [10] made use of the fluorescence of colourless proteins when irradiated from the side by UV radiation. Subsequently, Svedberg and Tiselius [11] adopted a direct UV absorption method for tracking sedimenting protein zones. The method finally adopted, however, was based on the deviation of light at the boundary, due to sharp refractive index variations, as proposed in

1902 by Abegg and Gaus [12]. The methodology finally adopted was the Schlieren observation technique, introduced in 1937 by Tiselius [13] in electrokinetic processes. Soon after, Philpot [14] reported a self-registering Schlieren optics arrangement for the ultracentrifuge, which was later improved by Longsworth [15] and Svensson [16]. Just to give an idea of the complexity of such systems, in the analytical ultracentrifuge the sedimenting peak is obtained by the synchronous mechanical movement of a horizontal edge and a vertical plate placed on the optical bench, which transform the boundary (a sigmoidal curve) into a Gaussian profile.

In the present method also a precise assessment of the transit time of a boundary between two liquids is used for a physico-chemical measurement, namely the viscosity of a solution. Fortuitously we did not have to spend considerable time in implementing complex Schlieren optics for observing such boundaries, as the miniaturized, yet very powerful, UV detection system normally present in CE units was found to be adequate for recording such signals. We have noted that, even when both liquids are UV transparent, the refractive index variation at the boundary if often sufficient for generating a signal at the detector (see the meandering pattern in Fig. 2). Even when the signal tends to vanish (due to minute increments in refractive index) it is still possible to detect the boundary by adding traces of a strongly UV-absorbing substance (*e.g.*, riboflavin) to one of the two liquids. This is a simple, yet effective, method proposed as early as 1899 by Masson [17].

An additional advantage of the suggested procedure is that only very small volumes of the analyte liquid are necessary for a satisfactory viscosity measurement.

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REFERENCES

1 H.J. Bode, *Anal. Biochem.*, 83 (1977) 204–210 and 364–371.
2 P.G. De Gennes, *Scaling Concepts in Polymer Physics*, Cornell University Press, Ithaca, NY, 1979.
3 M. Chiari, M. Nesi, M. Fazio and P.G. Righetti, *Electrophoresis*, 13 (1992) 690–697.
4 J.L. Viovy and T. Duke, *Electrophoresis*, 14 (1993) 322–329.
5 R.B. Bird, W.E. Stewart and E.N. Lightfoot, *Transport Phenomena*, Wiley, New York, 1960.
6 F.M. White, *Viscous Fluid Flow*, McGraw-Hill, San Francisco, 1974.
7 M.S. Bello, M. Chiari, M. Nesi, P.G. Righetti and M. Saracchi, *J. Chromatogr.*, 625 (1992) 323–330.
8 M.S. Bello, E.I. Levin and P.G. Righetti, *J. Chromatogr.*, 652 (1993) 329–336.
9 R. Weast (Editor), *CRC Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL, 67th ed., 1986–1987, p. F-37.
10 T. Svedberg and E.R. Jette, *J. Am. Chem. Soc.*, 45 (1923) 954–960.
11 T. Svedberg and A. Tiselius, *J. Am. Chem. Soc.*, 48 (1926) 2272–2276.
12 R. Abegg and W. Gaus, *Z. Phys. Chem.*, 40 (1902) 737–745.
13 A. Tiselius, *Trans. Faraday Soc.*, 33 (1937) 524–531.
14 J.S.L. Philpot, *Nature*, 141 (1938) 283–285.
15 L.G. Longsworth, *Chem. Rev.*, 30 (1942) 323–343.
16 H. Svensson, *Ark. Kemi Mineral. Geol.*, 22A (1946) 1–156.
17 O. Masson, *Philos. Trans. Roy. Soc. London, Ser. A*, 192 (1899) 331–340.