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Determination of the isoelectric point of the capillary wall in capillary electrophoresis

Application to plastic capillaries

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Abstract

A refined method for measuring endosmotic flow in capillaries based on weighing the amount of liquid transferred is described. The method has been applied to the estimation of isoelectric points of two types of plastic capillaries (polytetrafluoroethylene and polytetrafluoroethylene–polyhexafluoropropylene copolymer). Contrary to expectation both plastic capillaries exhibited considerable electroosmotic flow and changes in hydrodynamic flow resistance without applied voltage. The nature of these phenomena is discussed.

1. Introduction

A crucial phenomenon in capillary electrophoresis is electroosmotic flow. This flow originates from the negative charges caused by the presence of silanol groups on the inner surface of the fused-silica capillary. Though widely used mainly because of their commercial availability, fused-silica capillaries exhibit some disadvantages caused mainly by sorption of solutes to the capillary wall. In our attempts to apply capillaries made of other UV-transparent materials the need of estimating their isoelectric point has arisen. In the present communication we describe a simple and precise approach for estimating isoelectric points.

Several methods have been described for measuring the electroosmotic flow in capillaries [1]. By weighing the solution emerging from the

capillary on an analytical microbalance the problem of adsorbing a neutral marker on the capillary wall can be circumvented [2,3]. Another method exploits the measurement of the electrophoresis current when a buffer with different ionic strength is introduced [4]. It is also possible to measure directly the ζ potential of the capillary wall as reported by Van de Goor *et al.* [5]. This measurement either uses weighing of the effluent (see also Altria and Simpson [2,3]) or involves measuring of the streaming potential when the solvent is pumped through the column. In this way the ζ potential and electroosmotic flow of polytetrafluoroethylene (PTFE) capillaries was measured.

A number of papers have been published on manipulating the electroosmotic flow [6,7]. In the simplest case the pH and the ionic strength of the electrolyte can be adjusted to give the optimum speed for a given separation. Another possibility is to vary the electroosmotic flow by

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introducing additives to the background buffer. By using surface active agents or organic solvents electroosmotic flow speed can be changed dramatically or reversed [8,9]. No matter what method of electroosmotic flow adjustment is used, the charge of the capillary wall plays an important role in these considerations.

2. Experimental

2.1. Material and methods

For capillary wall isoelectric point measurement a set of 5 mM disodium citrate buffers was used. The rather low buffer concentration was used to keep the current within reasonable limits (less than 80 μA) during the experiment. Buffer components were of analytical-reagent quality and were obtained from Lachema (Brno, Czech Republic). Runs with applied voltage were run at 4.0 kV per 70 cm \times 200 μm I.D. capillary. The applicability of the method was demonstrated with PTFE (Norton Performance Plastics, Willich, Germany) and polytetrafluoroethylene-polyhexafluoropropylene (Kablo, Vrchlabi, Czech Republic) capillaries. When purchased the outer diameter of the tested capillaries was 1 mm with an I.D. of 500 μm ; before measurement the capillaries were drawn to achieve I.D. of 200 μm . This rather large I.D. was used in order to obtain easily measurable volumes of transferred electrolyte. For stabilization after drawing the capillaries were left to rest for at least one day. Stabilization time was also necessary after filling the capillary with the electrolyte, because even modest filling pressure made the inner diameter expand and this causes additional flow upon shrinking. A stabilization time of 1 h was found sufficient.

2.2. Procedure

Estimation of the isoelectric point of the capillary was based on measuring the electroosmotic flow in a capillary inclined to give a gravity flow around of 8 mg per 5 min. The amounts of the background electrolyte that have

passed through the capillary were plotted against pH and the isoelectric point of the capillary was estimated as the intersection of lines obtained with and without applied voltage.

The determination of the isoelectric point of the capillary wall was done by weighing. The experimental set-up is shown in Fig. 1. There were two reasons for not using levelled electrode jars: (1) constant flow allowed for more precise weighing of the liquid that has passed through the capillary and (2) the constant flow through the capillary cooled the system and avoided bubble formation inside the capillary column. The capillary was for most of its part placed into a tube filled with circulating paraffin oil for cooling and temperature stabilization. The cathode jar was placed on a balance tray of a digital balance (Sartorius 2004 MP6; Sartorius, Göttingen, Germany). Because it was demanded that the accuracy of weighing was 0.1 mg at least, any influence on the jar mass had to be eliminated. There were four possible sources of error foreseen, namely (i) transmission of the small changes of the position of the capillary assembly, (ii) friction between the contact and the negative terminal of the high-potential source (HTS), (iii) electrostatic forces inside the balance room and (iv) evaporation of the fluid in the jar.

Fig. 2 shows this part of the arrangement in detail. At the passage of the capillary through the end of the paraffin oil bath the capillary is extended with a highly elastic silicon rubber tube. This tube passes tightly through the upper part of the jar. A thin Pt wire is placed into this tube (the path of the current is short-circuited in

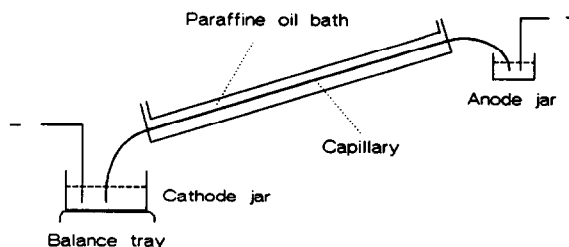


Fig. 1. Schematic representation of the experimental apparatus.

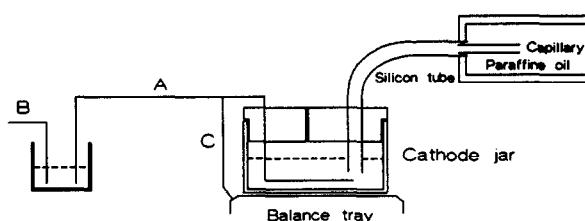


Fig. 2. Detailed representation of the cathode end arrangement. For details see Experimental.

this way) to abolish any electro-osmotic forces in it.

The connection to the negative terminal of the HTS was performed by a fluid contact materialized by wire A tightly passing the upper part of the jar. The other end of this wire was immersed in a vessel outside the balance tray filled with a 1% NaCl solution. Wire B connects the fluid in this vessel with the negative terminal of the HTS. An auxiliary wire C connects the metallic balance tray with the fluid contact. Because the metallic case of the balance was also connected with the negative HTS output any electrostatic field inside the balance was eliminated.

A small (0.4 mm) hole was drilled in the upper part of the jar to eliminate the influence of air pressure changes caused by the changing liquid height in the jar as well as possible changes in the atmospheric pressure. The diameter of the jar was 30 mm; in this way transferring of 10 μ l of the fluid during one measurement caused a fluid height difference in the jar of only 0.03 mm which can be neglected.

The upper electrode jar was connected to the positive output of the HTS source. Because of its elevated position hydrostatic flow from this jar through the capillary was generated. This arrangement was preferred to the "zero flow" mode (equilibrated jar levels) as in the set-up used no reversed flow due to pH changes could occur. Finally the ratio between the flow with and without applied potential was evaluated and the pH at which this ratio = 1 defined the isoelectric point. The measurements were done over a 5-min period at 4 kV and 0.1 mA. The mean value of 10 pairs of measurements was used for constructing the graph.

3. Results and discussion

As demonstrated in Fig. 3 both the PTFE and polytetrafluoroethylene–polyhexafluoropropylene capillaries exhibited distinct electroosmotic flow with isoelectric points at 3.25 and 3.0, respectively. The reliability of these measurements is evaluated in Table 1. Estimation of electroosmotic flow by the weighing method has been shown to be precise and uncomplicated confirming the previous results of Altria and Simpson [2,3]. There are, however, two phenomena which need some discussion. First, as the capillaries are made of tetrafluoroethylene and tetrafluoropropylene–hexafluoropropylene, respectively, they should be devoid of any chargeable functional groups on their inner surface. Consequently, from the strictly theoretical point of view they should be also devoid of any electroosmotic flow, which, however, is not the case. Investigations at the producers of these capillaries confirmed that no additives (softeners) of any kind that may be responsible for the inner surface charge are used during manufacturing of these products. As also dipole-caused charges are unlikely to occur in these capillaries, it is feasible to assume that charged buffer components may be sorbed on the capillary wall loading it with some (reproducible) charge that causes the electroosmotic flow when the capillary is attached to a high-voltage source. This conclusion is supported by the fact that electroosmotic flow at very low pH (below 2.5) ceases and the data obtained in this range are poorly reproducible. The decrease of the electroosmotic flow at very low pH values with imposed voltage as compared to the flow without voltage could be ascribed to the properties of the electric double layer.

The other phenomenon seen in Fig. 3 is the incline of the line corresponding to the flow at no imposed voltage. It has to be emphasized that practically no differences in buffer specific densities were observed (5 mM buffers were used) and therefore the phenomenon observed cannot be explained on this basis. Theoretically this dependence should be represented by a horizontal line parallel to the x -axis. However, if one

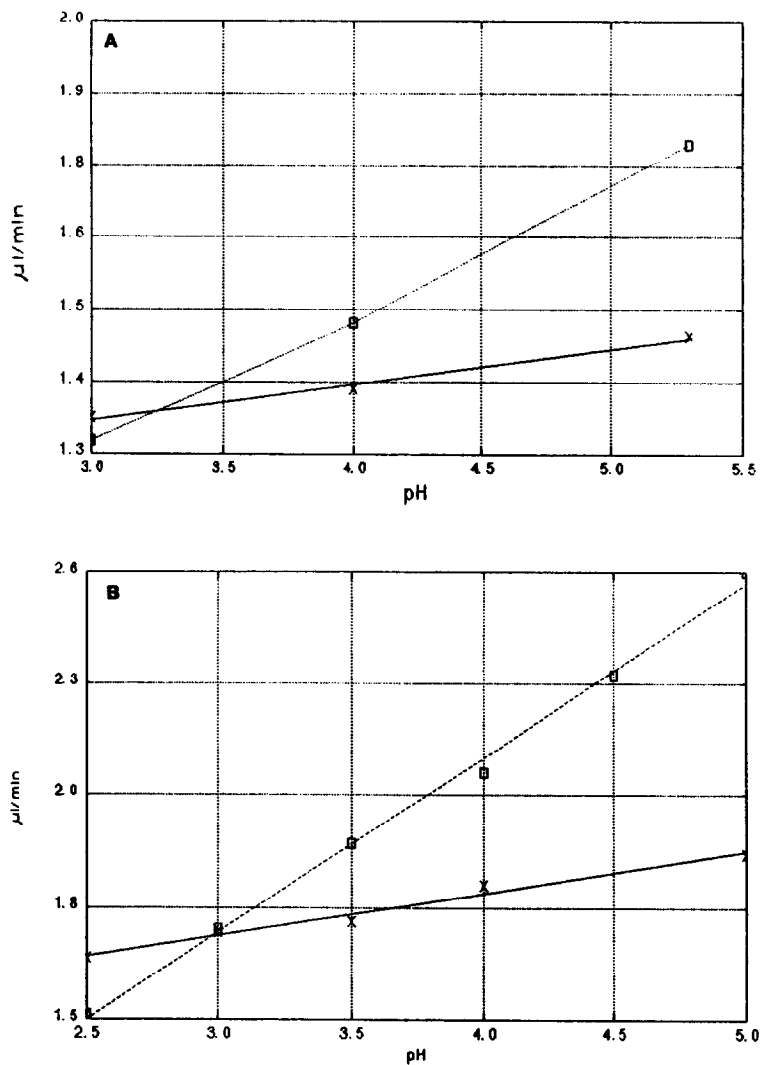


Fig. 3. Electroosmotic (dotted lines) and gravity (solid lines) flow vs. pH with (A) the PTFE capillary and (B) the polytetrafluoroethylene-polyhexafluoropropylene copolymer capillary. $\times = 0$ V; $\square = 4$ kV.

Table 1
Parameters of the regression lines (flow vs. pH) in Fig. 3

	PTFE		Polytetrafluoroethylene-polyhexafluoropropylene	
	0 kV	4 kV	0 kV	4 kV
Intercept	1.180273	0.684799	1.392216	0.426285
Slope	0.057819	0.209398	0.102162	0.426857
R^2	0.990419	0.998017	0.977752	0.996776

admits that the buffer components are sorbed on the inner capillary wall, then its properties are likely to change bringing about changed conditions for the hydrodynamic flow. From the nature of the experimental arrangement it is feasible to assume that the forces causing buffer components sorption should be hydrophobic by nature and rather independent on the pH of the running buffer used. Consequently the changes in the amount of the liquid flowing through the capillary should reflect its hydrodynamic flow resistance, for instance if the sorbed entities are more charged at higher pH being acidic by nature, then at higher pH the capillary should be more easily wettable, exhibit lower hydrodynamic resistance and offer a higher flow-rate. In this case, however one would expect that beyond a certain pH where practically all functional groups sorbed to the wall are charged, the line of the pH *versus* flow-rate dependence will bent to become parallel to the *x*-axis. Such an effect was, however, not observed. On the other hand it is necessary to keep in mind that as with fused-silica capillaries the sorbed charged entities will act in a similar way as the ionized silicic acid groups, *i.e.* they will be neutralized by hydronium ions (hydrated cations) forming a compact layer overlaid (due to the thermal motion) by a loosely held layer termed usually diffuse layer. Combination of these effects may lead to the linear dependences seen in Fig. 3. As a matter of fact the difference of the investigated capillaries to the commonly used fused silica may be that while the charged silanol groups in fused-silica capillaries are covalently bound to the capillary surface, with the plastic-type capillaries the charge observed stems from hydrophobic sorp-

tion forces. The flow-rate changes observed at different pH values are also unlikely to be caused by slow changes in capillary dimensions during use; both systematic experiments and random measurements were done with the same result.

Application of capillaries manufactured from other material than fused silica may broaden the possibilities of capillary electrophoresis and may help to overcome some well known problems *e.g.* irreproducible sorption of biopolymers to the capillary wall.

4. Acknowledgement

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