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SEPARATION OF ORGANIC AND METAL IONS BY HIGH-VOLTAGE CAPILLARY ELECTROPHORESIS

TAKAO TSUDA*, KAZUHIRO NOMURA and GENKICHI NAKAGAWA
Laboratory of Analytical Chemistry, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya-shi 466 (Japan)
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SUMMARY

Electro-osmotic flows were measured in Pyrex glass capillary, fused-silica capillary and poly(fluoroethyl–propylene) tubings. The electro-osmotic flow exhibited a good linear relationship with electric current density. The factors which influence electro-osmosis and separations are discussed. Good separations of pyridinium salts, sulphonic acids and metal ions were demonstrated, eluting towards the negative or positive terminal at up to 16 kV in aqueous solution. Theoretical plate numbers for pyridinium salts were around $1.5 \cdot 10^5$–$2 \cdot 10^5$.

INTRODUCTION

Zone electrophoresis is generally carried out in stabilizing media, such as paper, cellulose acetate or gels[1]. If non-viscous media, such as water, are used instead, the migration path of a solute might become completely straight because it has no need to pass through a matrix, such as cellulose fibres, and the dissipation of the heat generated by the application of a voltage is more rapid, allowing the possibility to input high voltages. Although several geometrical designs would be possible, two are more obvious.

The first involves the use of a horizontal electrophoresis tube (3 mm I.D.) rotating around its longitudinal axis to counteract convective disturbances. This device was proposed by Hjertén[2], and he applied it to the separation of, for example, metal ions and nucleotides. The second possibility is to use a cell of very narrow cross-section, that is, a capillary tubing[3–6]. Mikkers *et al.*[3] used a poly(tetrafluoroethylene) tubing of 0.2 mm I.D. and succeeded in a good and rapid separation of carboxylic acids with a potential gradient detector in conductive mode. Jorgenson and Lukas[4,5] used a very narrow-bore glass capillary of 75 μm I.D. and applied voltages up to 30 kV, and obtained good separations of fluorescent derivatives of amino acids with a fluorescence detector.

In the present paper we use Pyrex glass capillary, fused-silica capillary and poly(fluoroethylene–propylene) (FEP) tubings. The conditions for zone electrophoresis in capillary tubings with on-column UV detection are discussed and applications to the separation of pyridinium salts, sulphonic acids and metal ions are described.

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EXPERIMENTAL

Straight Pyrex glass capillary (30–120 cm × 50–200 μm I.D.) was drawn by a glass drawing machine (GDM-1; Shimadzu, Kyoto, Japan). Fused-silica capillary (Scientific Glass Engineering, North Melbourne, Australia) and FEP were kindly supplied by Shimadzu. These tubings were used as columns for zone electrophoresis without any surface modification. In some cases, fused-silica capillary was connected with Pyrex glass capillary by the aid of a small piece of poly(tetrafluoroethylene) tubing for sensitive detection or the electro-osmosis experiment. At the point of UV detection, the Pyrex glass capillary and FEP were used as "cells" without any modification, but in the case of fused-silica capillary the resin on it was flamed off before use. The column parts of these tubings were regarded as the parts from the inlet to detector. High-voltage d.c. power supplies (IP-2A, Shimadzu; V-5, Toyo Kagaku-sangyo, Osaka, Japan) were operated in both constant-voltage and constant-current modes and delivered up to 25 kV. Platinum–iridium tubing was used as electrode. The solvents were aqueous solutions of Na_2HPO_4, KH_2PO_4, phosphate buffer (Na_2HPO_4–KH_2PO_4) and acetic acid, with or without 0.5% ethylene glycol.

Procedure

Solvent was degassed with helium gas or by the aid of an ultrasonic bath. Columns were filled with solvent under pressure or by dipping one end of the tube, which was kept 10 cm higher than the other end, in the solvent. After capillary had been filled, both ends were dipped in small beakers. Sample introduction was carried out by using electro-osmotic flow or by "downhill" flow in the same manner as used for filling a capillary with solvent[4–6]. The electric field was then applied.

RESULTS AND DISCUSSION

The linear velocity, u_{osm}, of a liquid under the influence of an applied field, E, is derived[6–9] as follows

$$u_{osm} \approx keE/Z\,\eta\,(C^s)^{\frac{1}{2}} \tag{1}$$

where k, e, η, Z and C^s are a constant, the amount of charge per unit surface area of the capillary tubing, the viscosity of the liquid, the number of valence electrons and the concentration of the electrolyte in water, respectively. In a previous paper[6] we discussed the effects of the electrolyte concentration and electric current, I, on the electro-osmotic flow. In the present study, the effects of the electric current density, i, tubing materials and pH on electro-osmotic flow are considered.

Relationship between electric current density and electro-osmosis

The relationship between applied voltage and electric current is, at first, linear and then becomes non-linear as shown in Fig. 1. This behaviour is supposed to be due to the increase in temperature in solution, the change in dielectric constant and/or a change in the kinetics of electrophoresis[7,10]. Also, there cannot be a linear relationship between electro-osmosis and applied voltage, E.

However, when the electric current, I, or current density, i, is used as the basic

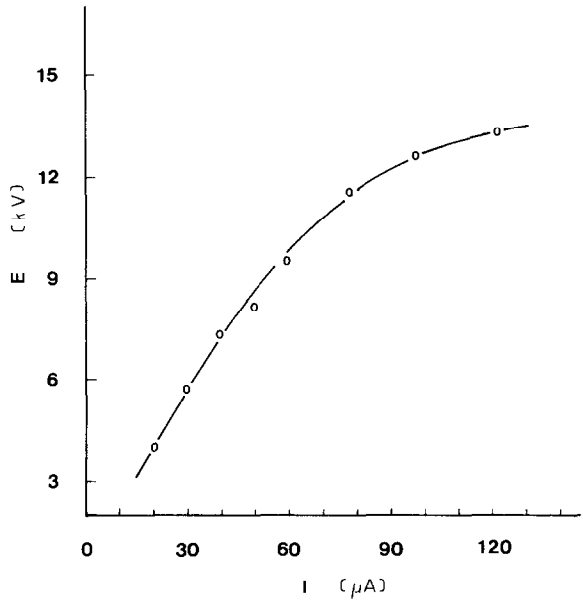

Fig. 1. Relationship between applied voltage, E, and electric current, I, under the conditions of zone electrophoresis. Capillary tubing: Pyrex glass capillary (422 mm × 85 μm I.D.) connected with fused-silica capillary (142 mm × 195 μm I.D.). Solvent: 0.02 M phosphate buffer, pH 7, with 0.5% ethylene glycol.

parameter instead of E, a good linear relationship between electro-osmotic flow, u_{osm}, and I or i is obtained as shown in Fig. 2. The parameter i is preferred to I, because the former includes the geometrical factor, namely, capillary radius, r. The parameter i is not affected by the increasing temperature in solution. From these considerations the following equation can be derived

$$U_{osm} = fi = fI\,(\pi r^2)^{-1} \tag{2}$$

where f is a constant calculated from Fig. 2 and is dependent on all parameters included in eqn. 1 except E, its dimensions being $cm^3\ A^{-1}\ sec^{-1}$. Current density is a more basic parameter than voltage in the study of electro-osmosis.

Effects of capillary materials and pH on electro-osmosis

The experimental values obtained by using Pyrex glass capillary columns of different I.D. can be plotted on the same lines, A and B in Fig. 2. The difference between the slopes of these lines is readily explained by the difference in concentration of each electrolyte in eqn. 1 (ref. 6). As the Pyrex glass capillaries were home-made, their surface conditions were quite similar in spite of the difference in their I.D. But this is not true for the fused-silica capillaries and FEPs, C–E and F, respectively, in Fig. 2. In these cases, each tubing has its own relationship between u_{osm} and i, even though for the same solution. This is due to the different manufacturing processes of fused-silica capillaries and FEPs which lead to different surface conditions with each column of different I.D.

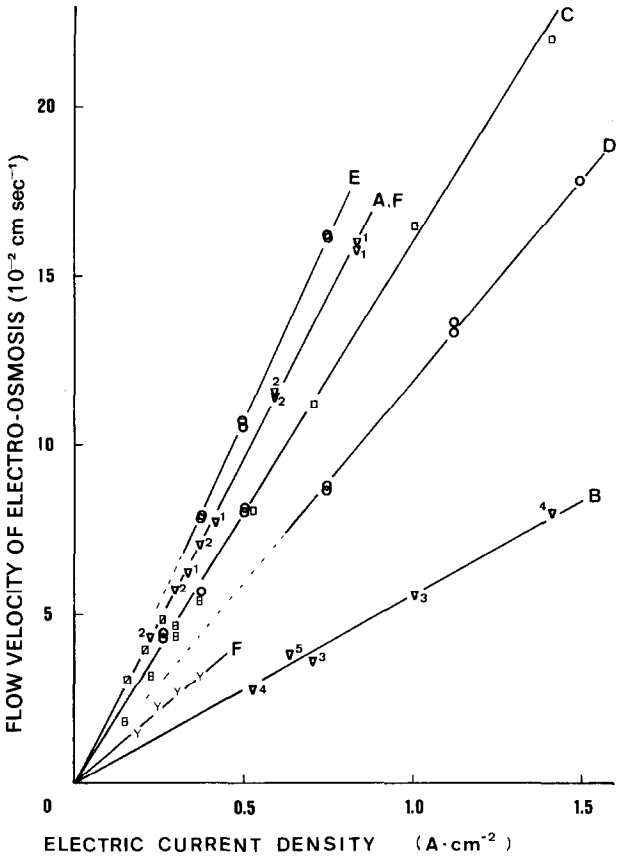

Fig. 2. The relationship between electro-osmotic flow and electric current density. A and B, Pyrex glass capillaries (▽) of 88 (1), 208 (2), 60 (3), 85 (4) and 200 μm I.D. (5); C–E, fused-silica capillaries (○) with 195, 92 and 72 μm, I.D., respectively, F, FEP with 323 (Y) and 500 μm I.D. (▨); C also shows (□ and ⊟) combined tubings of Pyrex glass and fused-silica capillary, 85 and 195 μm I.D. (□) and 208 and 195 μm I.D. (⊟), respectively. The solution was 0.02 *M* phosphate buffer, pH 7, with 0.5% ethylene glycol, except in the case of B, where it was 0.05 *M* Na_2HPO_4. Solutes were benzene or pyridine.

Another interesting result in Fig. 2 is the u_{osm}–i relationship for the combined Pyrex glass and fused-silica capillary tubings. These tubings were connected face to face with the aid of a small piece of poly(tetrafluoroethylene) tubing, 5 × 0.2 mm I.D. The relationship obtained is the same as that given by the fused silica capillary of 195 μm I.D., *i.e.*, C in Fig. 2. So it is clear that the total electro-osmotic flow in such a combination is controlled by the tubing which gives the lower electro-osmotic flow.

Electro-osmotic flow is slightly dependent on pH in the range shown in Fig. 3.

Elution time at different current densities and direction of electro-osmotic flow

Elution times, $T_{e,1}$ and $T_{e,2}$, for a given solute at different current densities, i_1

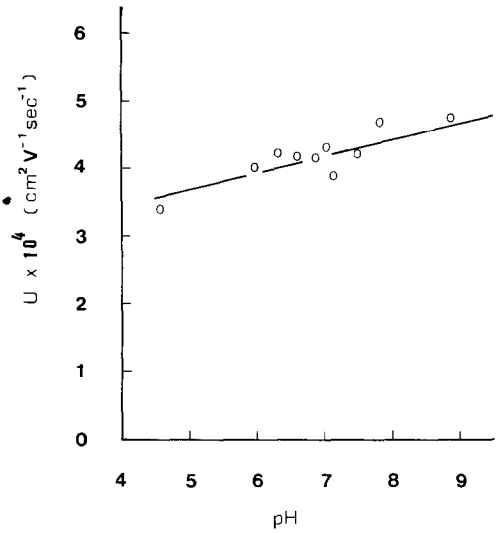

Fig. 3. Effect of pH on electro-osmotic flow. Pyrex glass tubing: 132 μm I.D., length 40 cm from inlet to detector and 10 cm from detector to electrode. Solute: benzene. Solvent: 0.05 *M* phosphate buffer, pH 7. Constant-voltage mode; 5000 V.

and i_2, in the same solvent are easily calculated from

$$\frac{T_{e,1}}{i_1} = \frac{T_{e,2}}{i_2} \tag{3}$$

where subscripts 1 and 2 correspond to the different experimental conditions. For example, with a Pyrex glass capillary (60 cm × 200 μm I.D.), N-methyl-2,4-dimethylpyridinium iodide was eluted at 17 min and 200 μA (case 1); using a Pyrex glass capillary of 60 cm × 60 μm I.D. it was eluted at 5.2 min and 60 μA (case 2). The solvent in each case was 0.05 *M* aqueous Na_2HPO_4, pH 8.9. The value of $T_{e,2}$ calculated by using eqn. 3 and the values of i_1, i_2 and $T_{e,1}$ is 5.1 min, in good agreement with the experimental value of 5.2 min.

When the present method is applied to separations, we should be careful to estimate values of the electrophoretic mobilities, u_{mob}, of the components of the sample and the electro-osmotic flow velocity, u_{osm}, in the capillary tubing, and also to determine their flow directions. In our case, u_{osm} was always in the direction towards the negative terminal in the aqueous solution used. However, Terada *et al.*[11] recently reported that the direction was towards the positive terminal when the solution contained cetyltrimethylammonium bromide. In that case the surface active agent was adsorbed on the inner wall, which thus was positively charged. In our case, the surface was always negatively charged under the applied electric field.

Effect of sample size on theoretical plate number, N

The relationship between theoretical plate number, *N*, and the injected amount of pyridine using a 72 μm I.D. fused-silica capillary is shown in Fig. 4. Smaller injected amounts give higher theoretical plate numbers. As the limit of detection of the sample in this experiment was around 1 ng, it was not clear by how much we should reduce the sample size in order to reach the region in which *N* was independent of the sample amount. In other words, if we used a detector with higher sensitivity

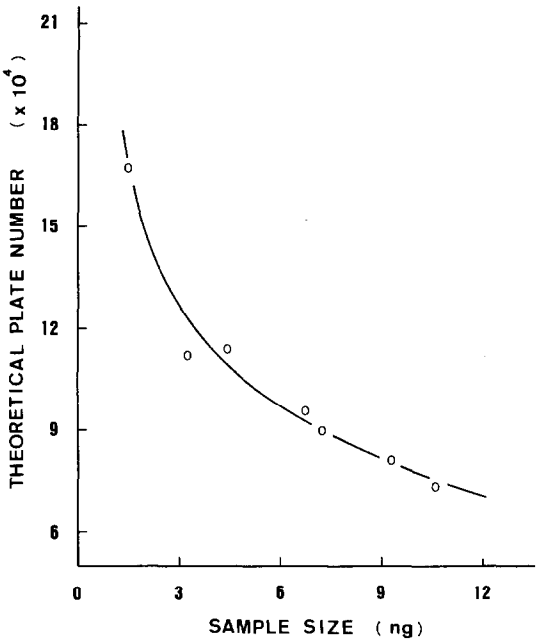

Fig. 4. Relationship between theoretical plate number and sample size. Fused-silica capillary; 72 μm I.D. × 780 mm, of which 610 mm was used as column. Solvent: 0.02 *M* phosphate buffer, pH 7, with 0.5% ethylene glycol. Sample: pyridine. Constant-voltage mode: 12.6 kV.

than the present one, a higher plate number should be obtained. In capillary electrophoresis, N is strongly dependent on the sample size. Therefore, one has to consider carefully the relationships which include the sample size; such as N and u_{osm} or $u_{osm} + u_{mob}$.

Separations of cations and anions

As the direction of u_{osm} is always towards the negative terminal under the present experimental conditions, cations move rapidly towards the negative terminal with the apparent velocity, u_{app};

$$u_{app} = u_{osm} + u_{mob} \tag{4}$$

Therefore, the separation of cations could be performed in a very short time.

However, in the case of anions, such as those of sulphonic acids, these flow towards the positive terminal and against the electro-osmotic flow. So the apparent flow velocity for anions is:

$$u_{app} = u_{osm} - u_{mob} \tag{5}$$

Here the positive sign of u_{app} means that the sample moves towards the negative terminal. Therefore the separation of anions usually requires longer times compared to the separation of cations.

Typical examples of separations are shown in Fig. 5–8. The u_{app} for adenosine 5'-monophosphate, shown in Fig. 7, is positive, but the u_{app} values for sulphonic

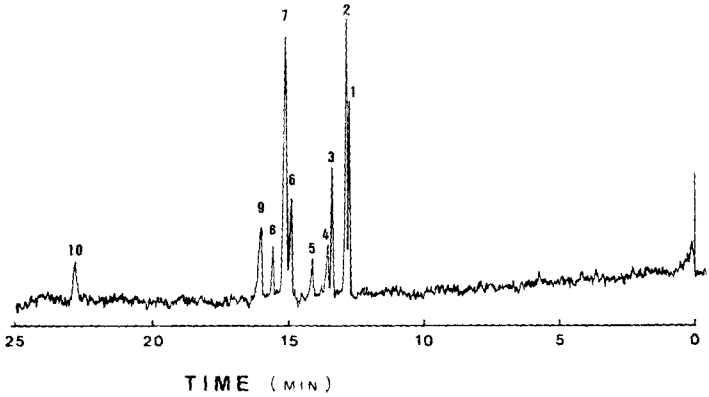

Fig. 5. Separation of pyridinium salts. Pyrex glass capillary: 85 μm I.D. × 105 cm, of which 90 cm was used as column. Solvent: 0.05 *M* Na_2HPO_4. Constant-current mode: 80 μA (*ca.* 14.2 kV). Each solute shows $1.5 \cdot 10^5$–$2 \cdot 10^5$ theoretical plates. Solutes: 1 = N-methyl-4-methyl-; 2 = N-methyl-3-methyl- or N-methyl-2-methyl-; 3 = N-methyl-2,4-dimethyl- or N-methyl-2,3-dimethyl-pyridinium salt; 4 = N-methyl-2-aminopyrimidinium salt; 5 = N-benzyl-; 6 = N-benzyl-4-cyano-; 7 = N-2,4-dinitrophenylpyridinium salt; 8 = unknown; 9 = 1,2,3-trimethyl-3,5-bis(ethoxycarboxyl)pyridinium salt; 10 = pyridine.

acids are negative (Fig. 8). Therefore, if we want to elute the sample at a given terminal, negative or positive, it is necessary to control the velocity of electro-osmosis. First, as u_{osm} is strongly dependent on the electrolyte concentration, we should select the concentration of the electrolyte. Secondly, we should choose the material of the capillary tubing. Thirdly, we should choose an additive to modify the surface of the capillary.

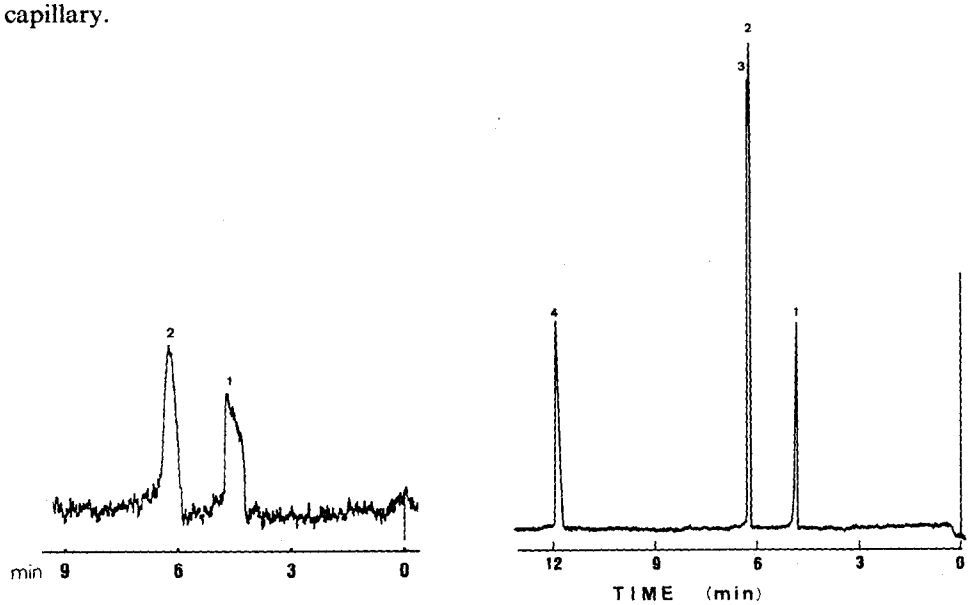

Fig. 6. Separation of cupric (1) and ferric (2) ions. Column: Pyrex glass tubing, 80 μm I.D. × 36 cm. Solvent: 0.05 *M* acetic acid. Constant-current mode: 10 μA (*ca.* 8.6 kV).

Fig. 7. Separation of a mixture including a cation and an anion. Column: fused-silica capillary, 610 cm × 72 μm I.D. Solvent: 0.02 *M* phosphate buffer, pH 7, with 0.05% ethylene glycol. Constant-current mode: 30 μA (*ca.* 16.6 kV). Solutes: 1 = pyridoxamine; 2 = pyridine; 3 = unknown; 4 = adenosine 5'-monophosphate.

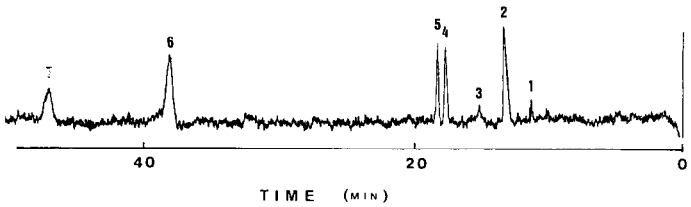

Fig. 8. Separation of sulphonic acids. Column: Pyrex glass capillary, 60 cm × 130 μm I.D. Solvent: 0.05 *M* KH_2PO_4. Constant-current mode: 200 μA (*ca.* 11 kV). Solutes: 1 = unknown from reagent 2; 2 = naphthalene-1,3,6-trisulphonic acid; 3 = unknown from reagent 2; 4 = 2,6-naphthalenedisulphonic acid; 5 = 3-(4-sulphophenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid; 6 = *m*-nitrobenzenesulphonic acid; 7 = 2-naphthalenesulphonic acid (all sodium salts).

Pyridine (10 in Fig. 5), which was neutral under the conditions of Fig. 5, was eluted only by the electro-osmotic flow. Peak 1 in Fig. 5 flowed to the negative terminal with an apparent velocity of which the mobility and electro-osmotic flow comprised 40 to 60%, respectively. Plate numbers for solutes in Fig. 5 are around $1.5 \cdot 10^5$–$2 \cdot 10^5$. Although metal cations in Fig. 6 were eluted within 8 min, it is better to use another detector because they have no strong absorbance at 254 nm. If we had used a detector with a high sensitivity for metal ions, we might have obtained a better electropherogram. Work along these lines is now proceeding in our laboratory.

Concerning the separation of metal ions, Hjérten[2] achieved a good separation between Bi^{3+} and Cu^{2+} within 3 min using his alternative method. As the solutes were detected by *in situ* UV scanning of the tube, their migration distance from the original spot was small compared to those in the present method.

From peaks 4 and 2 in Fig. 7, $-u_{mob}$ of adenosine 5'-monophosphate was about one-half of u_{osm}. Naphthalenedi- or -trisulphonic acids were eluted within 20 min owing to their high mobilities. The biomedical application of the present method to nucleotides in blood or liver will be discussed elsewhere[12].

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