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Electrochromatography with continuous sample introduction

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ABSTRACT

The flow profile and height equivalent to a theoretical plate in electromatography are discussed. Apparatus for continuous injection is proposed. Continuous injection of sample onto the column was accomplished for a period of up to 5 min, and charged solutes were in the column. Typical examples are presented.

INTRODUCTION

Electrochromatography, in which two functions (mobility and sorptive interaction) are used at the same time, is a highly effective separation method[1–4]. Otsuka and Listowsky[1] and O'Farrell[2] separated ferritin subunits by applyling a voltage along a column. As the applied voltages in these experiments were relatively low, such as 12 V/cm, the separation process took more than 10 h. In previous studies[3,4] we demonstrated electrochromatography with a high voltage along a column and obtained effective separations within a few minutes. A theory of band broadening in a column was proposed and the effect of an electric field on the nature of the column support was examined[4].

In this paper we discuss several examples of variations in flow profiles with respect to the time axis due to different values of the linear velocity of pressurized flow, v(pres), and mobility, v(mob).

In electrochromatography, it is possible to retain a solute in a column during operation under pressurized flow if the velocity due to its mobility toward the inlet of the column is higher than that due to pressurized flow toward the outlet of the column. Therefore, we can concentrate a solute in a column during a period of several injections and then elute it after stopping the voltage applied to the column[4]. In this work, we have developed a technique that allows a solute or group of compounds to be concentrated in a column under an applied voltage with continuous injection of the samples.

EXPERIMENTAL

The apparatus is shown in Fig. 1. The glass column (5 cm × 4 mm I.D.) (Kusano Kagaku Seisakusho, Tokyo, Japan) was slurry packed with silica gel bonded with

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octadecylsilane of particle diameter 7 μm (Capcell C_{18}; Shiseido, Yokohama, Japan). The injector and connectors (2, 4, 5 and 7 in Fig. 1) were made of PTFE resin (Kusano Kagaku Seisakusho). To exclude bubbles generated at the terminals, fused-silica capillary tubing (20–30 cm × 50 μm I.D.) (9 and 9′ in Fig. 1) were used. As there was a high voltage gap between the outlet of 9 in Fig. 1 and the air, a side flow of the effluent was electrically sprayed into the air. This side flow was estimated to be less than 0.1 ml/min. Sample was injected by a pump (Microfeeder MF-2; Azuma Denki Kogyo, Tokyo, Japan) at 4–10 μl/min. The sample was stored in the pump in a microsyringe (250 or 500 μl), with the end of the needle directly connected to a fused-silica capillary tube (8′ in Fig. 1) by a short PTFE tip (10 mm × 0.25 mm I.D.). A UV absorbance detector operated at 254 nm (Uvidec II; Jasco, Tokyo, Japan), a pump for supplying effluent (LC-6A; Shimadzu, Kyoto, Japan) and a high-voltage d.c. power supply (Matsusada Precision, Yokaichi, Shiga, Japan) were used. To avoid high voltages, the pumps (12 and 13 in Fig. 1) were connected to that part of the apparatus which carried the high voltage by long fused-silica capillary tubes (ca. 60 cm × 50 μm I.D.; 8 and 8′ in Fig. 1), which offered high electrical resistance. In this work, the inlet of the column was kept at high voltage. A positive high voltage means that a positive high voltage is applied at the inlet part of the column.

Reagents of guaranteed grade (Wako, Osaka, Japan) were used. *cis*-N-Methyl-4-β-styrylpyridinium iodide was a gift from K. Takagi, Nagoya University.

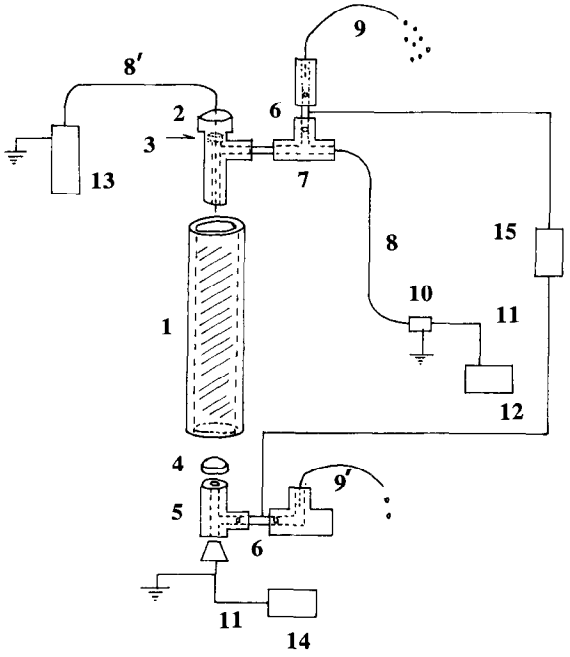

Fig. 1. Schematic diagram of apparatus for continuous injection. 1 = Column (5 cm × 4 mm I.D.) made of Pyrex glass, packed with silica-ODS; 2 = injector; 3 = silicone-rubber septum; 4 = frit; 5 = connector; 6 = platinum tubing (I.D. 0.5 mm) for terminal; 7 = three-way connector; 8, 8′, 9 and 9′ = fused-silica capillary tubing; 10 = connector; 11 = stainless-steel tubing (O.D. 1/16 in.); 12 = pump for effluent; 13 = pump for sample injection; 14 = UV detector (254 nm); 15 = high-voltage d.c. power supply.

THEORY OF CHROMATOGRAPHIC BEHAVIOUR

In electrochromatography there are two factors, electrophoretic mobility and electroosmotic flow, that do not exist in ordinary liquid chromatography. Therefore, the band broading of a solute in electrochromatography is different from that in ordinary chromatography. The apparent mean linear flow velocity of a solute, v(app), is

$$v(\text{app}) = v(\text{pres}) + v(\text{mob}) + v(\text{osm}) \tag{1}$$

where v(pres), v(mob) and v(osm) are mean linear flow velocities due to pressurized flow, mobility and electroosmosis, respectively. Generally, v(osm) in a slurry-packed capillary column is 10–20% of that in an open-tubular capillary column[5,6]. If v(osm) in a slurry-packed capillary column is considerably lower than v(mob), eqn. 1 becomes

$$v(\text{app}) = v(\text{pres}) + v(\text{mob}) \tag{2}$$

In the process of flow in electrochromatography, the flow of v(pres) is operated with a Poiseuille flow profile, and we assume that the flow pattern of v(mob) would be a plug flow profile. Therefore, the velocity inequality in the radial direction is due only to laminar flow, namely v(pres). The flow pattern in electrochromatography is assumed to be mixed Poiseuille and plug flow.

The assumed flow profiles are shown in Fig. 2; x_0 is the point of injection and the region $x \geqslant 0$ corresponds to the column, but the region $x < 0$ before the column inlet is assumed to exist. The region $x < 0$ is necessary to figure out the retarded solute at the column inlet. At time t_0, solute was introduced as a plug (Fig. 2a). Fig. 2b shows the flow profile at time t_1 under the conditions v(mob) $= 0$ and v(pres) > 0. Fig. 2c shows the flow profile at time t_1 under the condition v(pres) $= 0$. Fig. 2d shows the actual net profiles under the condition v(pres) > 0 and $|v(\text{mob})| > 0$.

The sample will remain in the column under the condition v(pres) $\leqslant -2v$(mob), shown in Fig. 2d-1-2 and d-1-3, because the highest velocity in laminar flow is twice the mean linear flow velocity. When the inside diameter of the connecting tubing between the injector and the column is extremely small compared with that of the column, v(pres) in the connecting tube will be considerably larger than $-2v$(mob). Under this condition the solute will be forced to remain just at the inlet of the column when there is an applied voltage.

The plate height, H in general chromatography is[7]

$$H = B/v + C_s v + C_m v + (1/A + 1/C_m v)^{-1} \tag{3}$$

and H in electrochromatography is

$$H = B/v(\text{app}) + C_s v(\text{app}) + H_3 + (1/A + 1/H_3)^{-1} \tag{4}$$

where

$$H_3 = w_\alpha^2 w_\beta^2 \cdot \frac{d_p^2 v(\text{pres})}{2D_m v(\text{app})} \cdot v(\text{pres}) = C_m \cdot \frac{v(\text{pres})^2}{v(\text{app})} \tag{5}$$

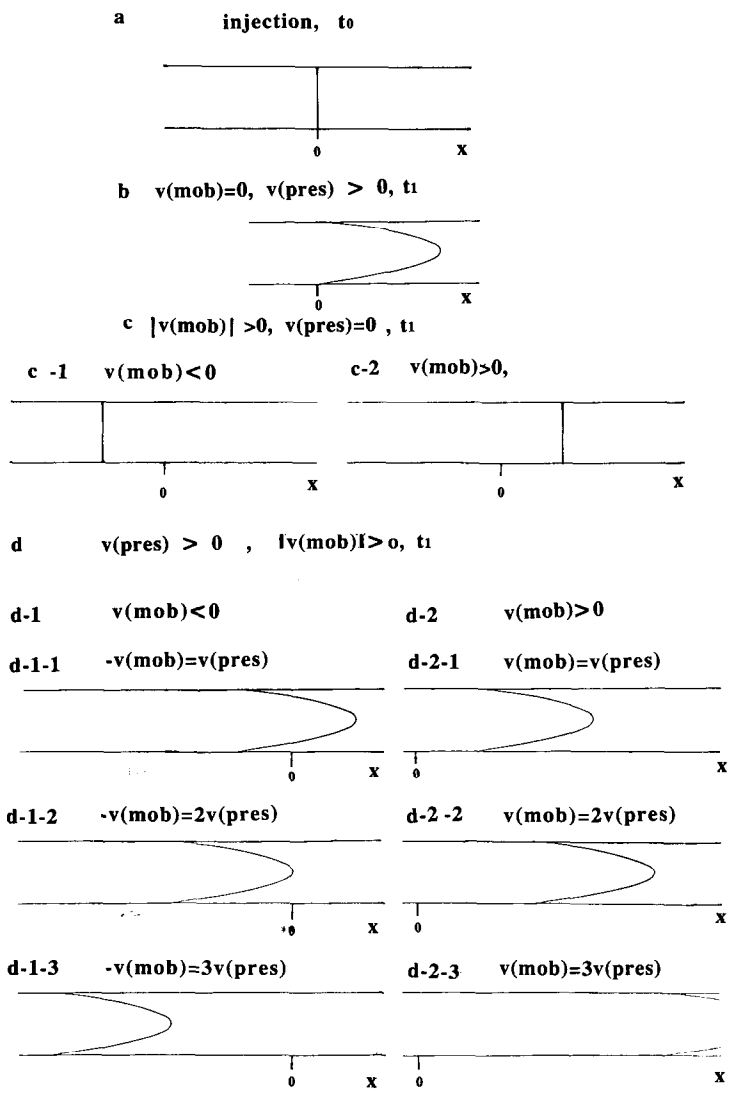

Fig. 2. Assumed flow profile in electrochromatography. t_0, t_1, Time; x, direction of pressurized flow.

In these equations A, B, C_s and C_m are coefficients for eddy diffusion, axial molecular diffusion, resistance to mass transfer in the stationary phase and resistance to mass transfer in the mobile phase, respectively, w_α is a coefficient of a step in molecular diffusion, w_p is equal to $\Delta v(\text{pres})/v(\text{pres})$ and Δv is the difference between the extreme and the mean velocities[4].

Eqn. 4 is valid in the region $v(\text{app}) > 0$. H as given by eqn. 4 is different from H in general chromatography, given by eqn. 3, in the following respects. First, the flow velocity of a solute is equal to $v(\text{pres}) + v(\text{mob})$. Second, H_3 in electrochromatography is completely different from H_3 in general chromatography, given by

$C_m v$(pres). When v(mob) $>$ v(pres), the contribution of v(mob) to H makes the value of each term less than 1 in general chromatography. When v(app) $<$ v(pres), owing to the negative value of v(mob) the solute will remain in the column longer than in ordinary chromatography. Hence the second term in eqn. 4 becomes smaller and the other terms larger compared with the corresponding terms when v(mob) $= 0$.

The variation of H_3 *with* v(app) for several values of v(mob) is shown in Fig. 3. The H_3 term under the condition v(mob) > 0 is always smaller than that when v(mob) $= 0$. Conversely, H_3 under the condition $-v$(mob) $< v$(pres) becomes larger than that when v(mob) $= 0$. It is understandable that H become larger, as the solute is retained in the column for a long period of time.

RESULTS AND DISCUSSION

The apparatus for continuous injection is shown in Fig. 1. The sample is injected via a fused-silica capillary tube, the extremity of which is placed on or just into the head of the column packing material. The reproducibility of the sample injection is shown in Fig. 4. The amount of sample injected was proportional to the duration period of injection by the pump (13 in Fig. 1). The amount of sample injected per second was adjusted by setting the flow-rate of the pump. This device may be a very good system for electrochromatography. Because the fused-silica capillary works as an electric insulator, the process of injection is safe. Also, from Fig. 4, no tailing of the peaks is observed. Therefore, the electroosmotic flow in the fused-silica capillary due to applied voltage[8] is not sufficient to cause continuous leakage of sample from sample storage in the pump.

The chromatographic behaviour with continuous injection of sample solution into the column inlet is demonstrated in Figs. 5 and 6. The chromatograms in Figs. 4 and 5 were obtained with continuous operation. In Fig. 5, continuous injection of *cis*-N-methyl-4-*β*-styrylpyridinium iodide (CIS) for 90 s gave a strong solvent peak (c) and an unknown peak (b). The latter peak has a negative charge. Just after stopping the

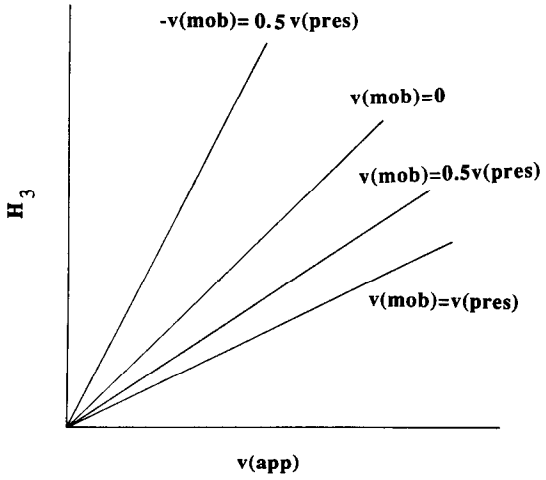

Fig. 3. Relationship between H_3 and v(app) at different values of v(mob).

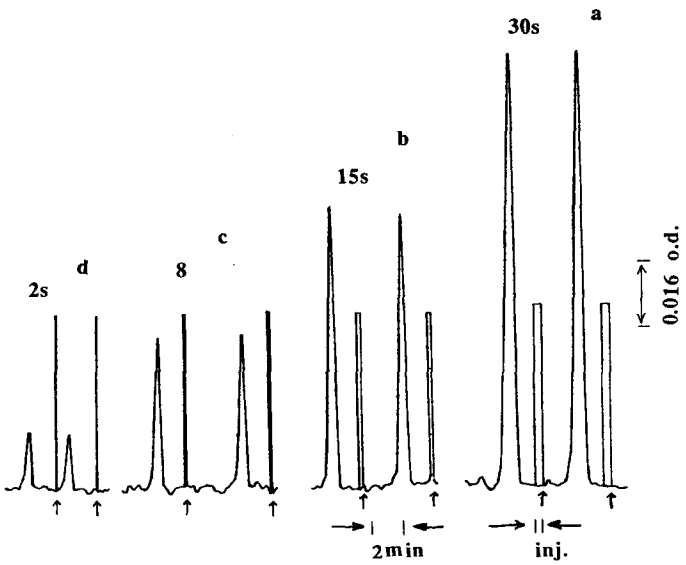

Fig. 4. Reproducibility of continuous injection. Sample, 1% benzene–methanol solution; applied voltage, −5.1 kV (58 μA); detection, UV 254 nm, 0.16 a.u.f.s.; duration of continuous injection for a, b, c and d, 30, 15, 8 and 2 s, respectively; sample injection rate, 10 μl/min; eluent, 2 m*M* phosphate buffer–methanol (5:95) at a flow-rate of 0.4 ml/min.

electric field at time zero in chromatogram D, two peaks were eluted, which were isomers of CIS. These isomers, having a positive charge, had been retained in the column while the applied voltage was −8.1 kV. Continuous injection for 5 min (total sample amount 50 μl) is shown in Fig. 6. During this period a positive electric field was applied in order to retain sample components of negative charge. In chromatogram c in Fig. 6 there is a large solvent peak due to the relatively long period of sample injection. After stopping the applied voltage, sulphonic acids that had been retained in the column were eluted. As the total length between the two terminals was about 12 cm, the real voltage applied to the column was *ca.* 40% of the actual applied voltage.

We used samples of relatively high concentration, and the sample injection rates were 4–10 μl/min. The latter rate was also increased up to 10% of the flow-rate of effluent. In this instance, we kept the total flow-rate of the eluent and sample solution in the column constant and avoided a reverse flow to the pump at the time when injection began.

Even if a solute has a high mobility, such as $-v(\text{mob}) \geqslant 2v(\text{pres})$, it is possible to retain it in the column. Hence it is possible to concentrate a very minor component of the mixture solution by passing an amount of the mixture through the column under an applied high voltage. Electrochromatography might be a good method for concentrating some types of compounds.

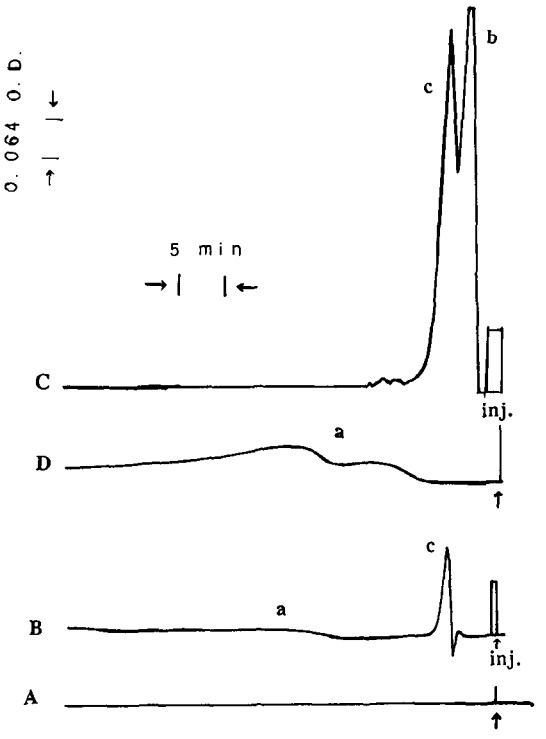

Fig. 5. Chromatogram with continuous injection of a solute having a positive charge. Sample, 10^{-2} *M* *cis*-N-methyl-4-*β*-styrylpyridinium iodide (CIS); sample injection rate, 10 *μ*l/min; eluent, 2 m*M* phosphate buffer (pH 7)–methanol (5:95); applied voltage, −8.1 kV (80 *μ*A). Peaks a and b belong to the sample and peak c is the solvent peak. A, Applied voltage on, without injection; B, applied voltage off, 0.5-min injection; C, applied voltage on, 1.5-min injection; D, chromatogram obtained just after stopping the applied voltage at time zero.

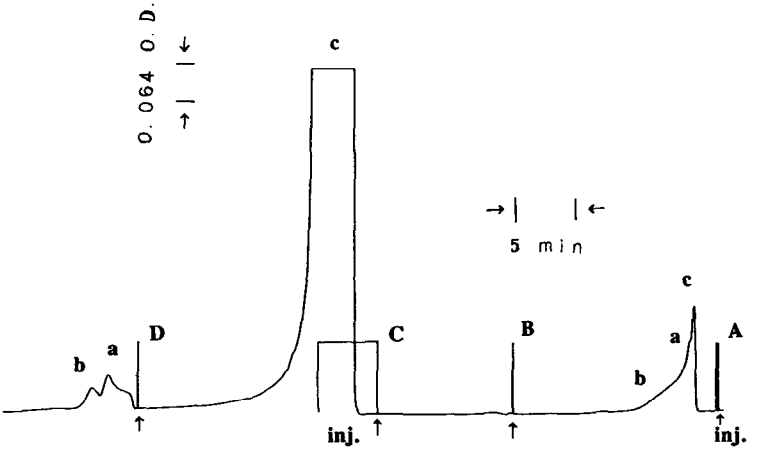

Fig. 6. Chromatogram with continuous injection of a solute having a negative charge. Sample: 10^{-2} *M* aqueous 1,2,6-naphthalenetrisulphonic acid solution; sample injection rate, 10 *μ*l/min; eluent, 0.1 m*M* phosphate buffer (pH 7)–methanol (40:60); applied voltage, +5 kV (75 *μ*A). Peaks a and b belong to the sample and peak c is the solvent peak. A, Applied voltage off, 15-s injection; B, applied voltage on, without injection; C, applied voltage on, 5-min injection; D, chromatogram obtained just after stopping the applied voltage at time zero.

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