Separation using planar chromatography with electroosmotic flow

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Abstract

Planar chromatography with electroosmotic flow is used to separate either a mixture of dyes using 80% aqueous ethanol as the mobile phase or a mixture of miscellaneous compounds using 45% aqueous acetonitrile as the mobile phase. Both mobile phases are 1.0 mM in N-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid (TAPS) buffer. Separations using this technique are faster and more efficient than the same separations by conventional TLC. The respective relationships between migration velocity and applied potential, and between analysis time and distance migrated, are presented. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The use of electoosmotically driven flow is well established in both capillary electrophoresis and column electrochromatography. The first description in modern chromatography was by Pretorius et al. [1] who used it for separations in both column chromatography and thin-layer chromatography (TLC). The TLC separation was of a mixture of four steroids on a silica gel plate that was first treated with dichlorodimethylsilane. The report contains a diagram showing the TLC plate and electrodes, but provides no description of the solvent used as the mobile phase nor the quality of the resulting separation.

There have been surprisingly few reports on the performance of TLC under the influence of a large electric potential [1–5]. Two of these describe TLC in which the potential was applied to a plate that was initially dry [2,4]. These are not relevant to the current report and are not discussed further.

In 1998 our laboratory reported the separation of a mixture of dyes in the reversed-phase mode on a bonded octadecyl silica plate using aqueous ethanol as the mobile phase [3]. A vertical TLC plate was used with the bottom edge dipped into a solvent trough. The cathode was attached to the top of the plate and the anode dipped into the mobile phase in the trough. This arrangement is similar to that reported by Pretorius et al. [1]. The separation was faster by a factor of approximately two and sharper solute peaks were obtained, as compared to conventional TLC. It was noted that a mobile phase gradient caused by evaporation may have contributed to the sharper peaks of high R_F solutes.

Howard et al. [5] have recently described a TLC separation of a mixture of nitrogen-containing

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heterocyclic compounds in the normal-phase mode on a silica layer using electroosmotic flow. Either acetonitrile or ethanol was used as the mobile phase. The separation was performed on a horizontal TLC plate in apparatus machined from a solid block of teflon. The separation using acetonitrile was twelve-fold faster than the equivalent separation by conventional TLC.

Techniques in which a potential is applied to a TLC plate have been named high-speed TLC [1], planar electrochromatography (PEC) [2–4], thin-layer electrophoresis (TLE) [6], and electroosmotically driven thin-layer electrochromatography (TLE) [5]. In order to be consistent with Ref. [3] the technique is referred to as PEC in the report below.

The current report describes the relationship between solute migration and the voltage applied to the TLC plate. Small modifications in the technique that improve the quality of separation are discussed.

2. Experimental

Merck RP-18 F_{254}s plates (EM Science, Gibbstown, NJ, USA) were cut into 2.5×10 cm sections and conditioned at 120°C for 20 min. The solutes used were either a mixture of dyes or of miscellaneous compounds dissolved in acetone. The former is dye mixture No. IV (Analtech, Newark, DE, USA) and consists of Fat Red 7B, Sudan Orange G, Solvent Green 3, Sudan II, Solvent Blue 35. The Sudan Orange G is a mixture of two yellow compounds, of which one has the highest R_F of all compounds in the mixture when using aqueous ethanol as mobile phase. This is the Sudan Orange G referred to in the text below. The miscellaneous compounds are iopanoic acid, benzanalide, 3,4-dimethoxybenzoic acid, and *p*-hydroxybenzoic acid. These were a gift from Don Risley of Eli Lilly and Company. The mobile phase for the dye mixture was water–ethanol (1:4, v/v) and the mobile phase for the miscellaneous compounds was water–acetonitrile (4.5:5.5, v/v). An aqueous buffer solution was prepared at an appropriate concentration such that, when mixed with organic solvent, the mobile phase was 1.0 m*M* in *N*-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid (TAPS) buffer. It was adjusted to pH 10 before mixing with the organic

solvent. The water was filtered with a Milli-Q system and the ethanol, acetonitrile, and TAPS buffer were from Aldrich (Milwaukee, WI, USA).

The apparatus for holding the plate is shown in Fig. 1. For the sake of clarity, a glass counterplate and filter paper wick are not shown in this diagram. The apparatus is constructed of Delrin (for parts that came into contact with solvents) and Plexiglas (for parts that do not contact solvent). The plates used in this report were 2.5×10.0 cm. The design allows plates in the range of 1.5 to 3.3 cm width to be used. The cathode is a platinum foil (vide infra) that is placed on the layer. Mobile phase is wicked from the top of the plate during PEC by a strip (2.5×4 cm) of Whatman No. 1 Qualitative Filter Paper (Whatman, Clifton, NJ, USA). Fig. 2 shows the placement of the wick. It is folded such that about 1.5 mm is under the lower edge of the platinum electrode and is in
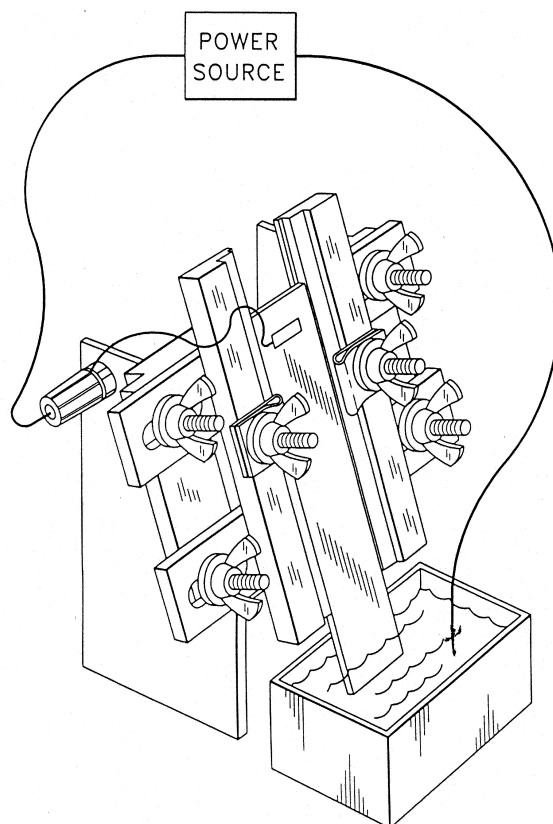

Fig. 1. Apparatus for performing planar electrochromatography. The glass counterplate and filter paper wick are not shown.
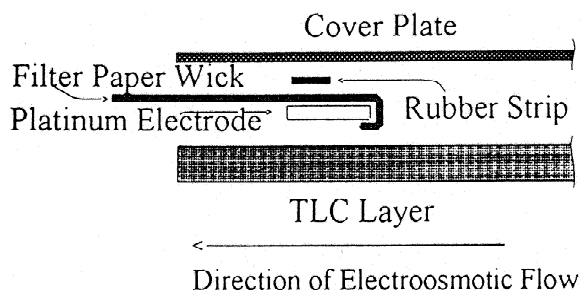
Fig. 2. Exploded view of the placement of the filter paper wick.

contact with the layer. The rest of the filter paper strip is folded over the electrode and is not in contact with the layer. In this arrangement mobile phase contacts the wick before reaching the electrode. A counterplate is clamped about 1 mm from the surface of the layer. A strip of rubber under the counterplate presses against the wick folded over the electrode. This forces the electrode, and the small portion of the wick under the electrode, into intimate contact with the layer. It was experimentally established that the pressure exerted is not sufficient to interfere with the wicking process.

The anode was 20-gauge platinum wire which was placed in the solvent trough. The cathode was a platinum foil (0.8×2 cm) spot welded to 20-gauge platinum wire. A Spellman high-voltage d.c. supply (Spellman High Voltage Electronics, Plainview, NY, USA) was used. The PEC procedure was performed in a Plexiglas cabinet, the door of which was fitted with two safety switches. When the door was opened, one switch disabled the power supply and the second activated a circuit that enabled any residual charge to drain to earth.

Before PEC the upper section of each plate was pre-wetted by dipping in 1 mM aqueous TAPS to within approximately 1 mm of the initial spot positions. Excess water was removed from the silica layer by blotting on a paper towel, before placing in the development apparatus.

A twin-trough chamber (Camag, Wilmington, NC, USA) was used for conventional chromatography. The plates were scanned with a Shimadzu (Kyoto, Japan) CS9000U dual wavelength flying-spot scanner in the reflectance mode. Both the dye mixture and the miscellaneous compounds were scanned at $\lambda=254$ nm.

3. Calculation of theoretical plates

The number of theoretical plates, N, were calculated using the formula:

$$N = 5.54 \cdot \left(\frac{M_{D}}{w_{1/2}} \right)^{2}$$

where M_{D} is the solute migration distance and $w_{1/2}$ is the peak width at half height. The above equation underestimates the efficiency of the system, as the initial width of a spot is not accounted for [7], but is adequate for comparison purposes.

4. Results

Since our first report, a few changes have been made to the apparatus and technique used for PEC. While these changes are small, they result in improved performance. In the initial report the cathode was a 20-gauge platinum wire, whereas in the current report a 0.8×2.0 cm platinum foil was used as the cathode. The method of placement of the wick has also been refined, as described in the Experimental section, and has been found to be important for obtaining a good separation. The wick was completely dry before starting each separation. The timing for the commencement of PEC is also important when using aqueous ethanol as the mobile phase. After the upper portion of the TLC plate is wetted, and the lower end of the TLC plate is placed in the solvent trough, the remaining dry area of the plate rapidly diminishes. The last few millimeters are wetted in two stages. During the first stage, the layer becomes more translucent as the small capillaries are filled with liquid, and during the second stage the layer becomes fully wet. PEC was commenced as soon as the translucent stage was reached, as preliminary results indicate that a poorer separation is obtained if the plate is fully wet before commencing PEC.

A good separation, illustrated in Fig. 3, can be obtained when the above precautions are taken. The potential across the TLC plate was 2.0 kV, and the mobile phase consisted of 80% aqueous ethanol containing 1.0 m*M* TAPS buffer. The value of the potential was selected in order to obtain a high flow-rate, even though the current drifts downwards
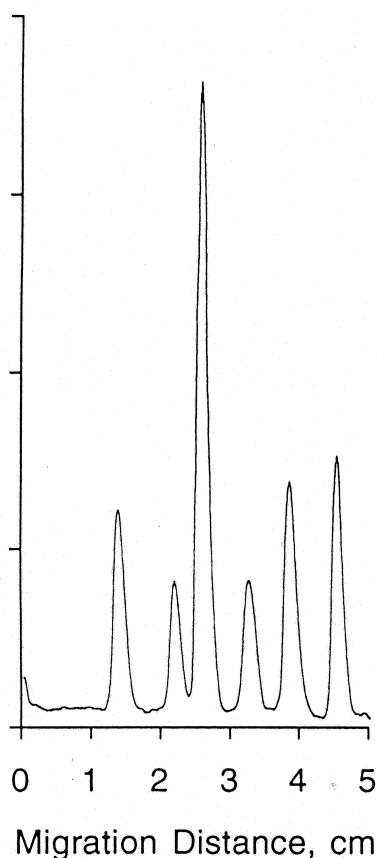
Fig. 3. The separation of a mixture of dyes on a C_{18} TLC plate using 80% aqueous ethanol containing 1.0 mM TAPS buffer, and an applied potential of 2.0 kV.

Table 1
Number of theoretical plates for the separation of six dyes by planar electrochromatography and conventional TLC

PEC			Conventional TLC[a]		
M_D[b] (cm)	$w_{1/2}$[c] (cm)	N	M_D[b] (cm)	$w_{1/2}$[c] (cm)	N
1.34	0.18	325	0.60	0.18	60
2.19	0.17	964	1.22	0.25	132
2.61	0.17	1370	1.67	0.27	218
3.22	0.18	1715	2.34	0.33	274
3.79	0.17	2888	3.08	0.38	374
4.49	0.18	3647	4.51	0.29	1331

[a] TLC was performed in a twin-trough chamber.
[b] M_D is the migration distance.
[c] $w_{1/2}$ is the peak width at half-height.

migration distance is lower by conventional TLC than by PEC. When the comparison is made on the basis of equal migration distances, the number of theoretical plates is between 2.5 and 4.5 times higher by PEC than by conventional TLC. The reproducibility of PEC is not as good as that of conventional TLC. Conditions that result in an improved reproducibility will be discussed elsewhere.

The current is reasonably stable at 1.0 kV, but at higher voltages there is downward drift during a run. This diminution of current becomes substantial at voltages that cause sufficient ohmic heating such that the layer becomes dry. This was only observed at the highest voltages used in this study, and after the highest R_F solute had migrated between 4 and 5 cm. Fig. 4 illustrates that there is a smooth relationship

as described in the following paragraph. The separation was terminated when the highest R_F solute had migrated 4.5 cm. This occurred after 18 min, compared to 37.5 min for the same separation by conventional TLC. It is possible that a focusing effect, due to evaporation of mobile phase, may have contributed to the quality of the separation. The efficiency of the separation, as measured by the number of theoretical plates listed in Table 1, is substantially higher for the PEC separation as compared to the same separation using conventional capillary driven TLC. The comparison between the efficiencies of the two techniques is approximate. The chamber design, and hence degree of vapor saturation, is different for the two techniques. For all solutes, apart from the solute of highest R_F, the
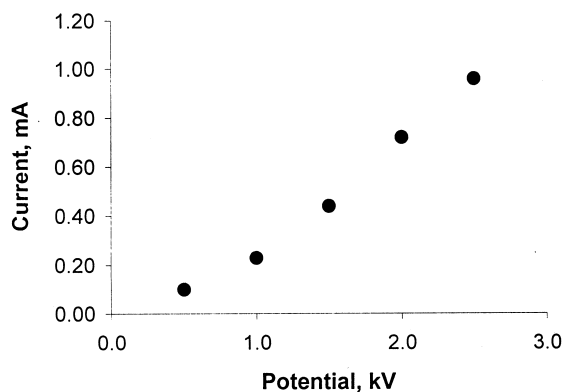

Fig. 4. A plot of current delivered by the power source versus the potential applied to the plate, at the time when Sudan Orange G had migrated 2.0 cm.

between current delivered by the power source and the voltage applied to the plate, at the time when the Sudan Orange G (the compound with highest R_F) had migrated 2.0 cm.

The counter plate becomes wet with condensation during the separation, and this could in principle lead to current leakage. It is assumed that any leakage was not substantial, as leakage would be expected to lead to a rise in current. The largest amount of condensation occurs towards the end of a separation at the highest voltages where the overall current drops due to drying of the layer. The vertical arrangement of the TLC plate, and counter plate, results in droplets of condensation running down the counter plate and not falling on the sorbent layer.

Fig. 5 is a plot of the rate of migration of Sudan Orange G versus distance, for a constant applied voltage of 2.0 kV. The rate is very high at the beginning of the run during the short period while the layer becomes fully wet. After this the fall in rate is gradual until the spot has migrated about 4 cm, after which the fall is substantial. The diminution of flow does not appear to be related to the ability of the wick to remove mobile phase. When a section of the filter paper used as the wick is placed in contact with a pool of aqueous 1 mM TAPS buffer (i.e. the solution used to pre-wet the upper section of the TLC plate) in a beaker, the solvent front advances about 4 cm in 3 min. This is faster than the rate of migration of the solvent front during PEC.

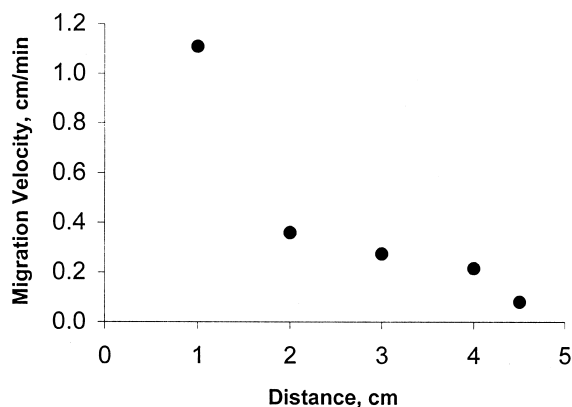The rate of migration of a solute increases with the applied potential, and it follows that the time required for a separation decreases with increasing potential. This decrease is more substantial for a separation requiring a longer migration distance than for a separation requiring a shorter distance, as illustrated in Fig. 6.

Fig. 7 shows the separation of iopanoic acid, benzanalide, 3,4-dimethoxybenzoic acid, and *p*-hydroxybenzoic acid in 45% aqueous acetonitrile containing 1.0 mM TAPS buffer and an applied voltage of 1.5 kV. The separation takes about 4 min, compared to about twelve min for the same separation by conventional TLC. There is no evidence of layer drying and it should be possible to use a longer migration path length than with the aqueous ethanol system. Further details of this system will be reported elsewhere.

It was noted in our first report that in addition to electroosmotic flow, solvent evaporation might also contribute to separation. Howard et al. [8] have demonstrated that under appropriate conditions mobile phase evaporation alone can cause solute migration. These authors have commented [5] that our report made no attempt to differentiate between "electroosmosis, electrophoresis and evaporation, any or all of which could account for the observed solute migration." The separation of alkanoic acids in the current report demonstrates that electrophoresis is not an issue. The compounds are present as anions in the run buffer and would migrate to the anode under electrophoretic conditions. While evapo-
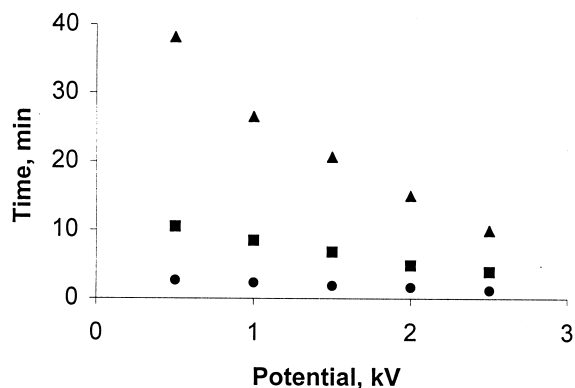

Fig. 5. A plot of the rate of migration of Sudan Orange G versus distance, for a constant applied voltage of 2.0 kV.

Fig. 6. Migration time of Sudan Orange G versus applied potential for three different migration distances; ● 1 cm, ■ 2 cm, ▲ 4 cm.
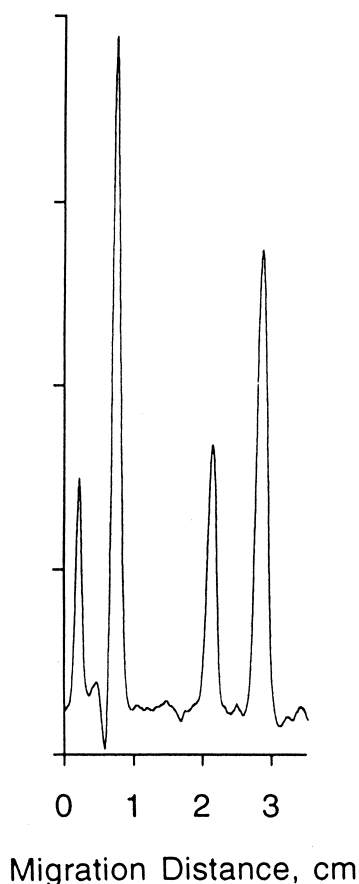
the separation proceeds. This would not occur if there was substantial evaporation from the TLC layer. There is a degradation in separation once the wick is fully wet. This may possibly be due to mobile phase being forced to the surface of the silica layer once migration in the forward direction is obstructed.

(iii) The most rapid migration occurs during approximately the first minutes of PEC during which the mobile phase enters the layer that is not yet fully wet.

(iv) PEC cannot be performed with the apparatus used in this report when the polarity of the electrodes is reversed. When 2.0 kV is applied to the plate with the electrodes reversed, there is no movement of the spots and within 1 min the layer near the mobile phase trough becomes dry.

The electric field across the plate is expected to vary in a complex manner during a separation, even when a constant voltage is applied to the TLC plate. This is because the predip buffer solution (of relatively high conductivity) is displaced by the run buffer (of lower conductivity) during the separation. Moreover the respective concentration, and hence conductivity, of both the predip and the run buffer will change due to evaporation as the separation proceeds. Some of this complexity can be avoided by using the run buffer for the predip, provided the solution is not too volatile.

Fig. 7. The separation of iopanoic acid, benzanalide, 3,4-dimethoxybenzoic acid, and p-hydroxybenzoic acid on a C_{18} TLC plate using 45% aqueous acetonitrile containing 1.0 mM TAPS buffer, and an applied potential of 1.5 kV.

ration clearly contributes to the separation, the primary cause of migration is interpreted as being due to electroosmotic flow for the following reasons.

(i) There is a gap of about 1 mm between the surface of the TLC plate and the glass counterplate. The atmosphere in the small volume above the TLC layer contains a high concentration of solvent vapor as evidenced by condensation of liquid on the surface of the counterplate. Thus the rate of evaporation is expected to be substantially less than in a tank of larger volume as used by Shafik et al. [8].

(ii) The wick becomes wet with mobile phase as

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