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Review

Planar electrochromatography

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Abstract

Recent developments in planar electrochromatography (PEC) in both the normal-phase and the reversed-phase modes, and at both atmospheric and elevated pressure, are reviewed. Other forced-flow techniques in planar chromatography are also briefly covered. Mobile phase migration in PEC is primarily due to electroosmotic flow, which is controlled by the applied electric field. Capillary mediated flow is an important secondary contributor to migration, and occurs because the layer is unsaturated as a consequence of liquid evaporating from the layer due to Joule heating. The magnitude of the electric field and the concentration of ions in solution are important variables that control both electroosmotic flow and Joule heating. Separations are faster and more efficient than those obtained by conventional planar chromatography, provided appropriate experimental conditions are selected. With inappropriate conditions, either mobile phase accumulates on the surface of the sorbent layer, or Joule heating causes excessive evaporation. The former results in poor spot shape, and the latter can cause the layer to dry. Good separations are obtained when there is a balance between these two effects. The problems associated with mobile phase accumulating on the surface of the sorbent layer, and with excessive evaporation of mobile phase, do not occur with pressurized planar electrochromatography. This technique is performed at high pressure, under conditions that allow heat to be removed form the sorbent layer. This allows the use of a substantially higher electric field than in PEC, and results in a high mobile phase flow rate.

Keywords: Reviews; Electrochromatography; Planar electrochromatography

Contents

1. Introduction

1.1. Attractive and unattractive features of classical thin-layer chromatography (TLC)

Planar chromatography, also called thin-layer chromatography, is a widely used separation technique that is over 65 years old [1]. The most important features, apart from the simplicity of the technique, are the ability to separate multiple samples simultaneously and the fact that separations can be evaluated without transporting the separated components to a detector. Other attractive features include the large variety of post-separation visualization reagents that are available [2] and the ease with which these may be applied, the ability to perform true two-dimensional separations [3], and the ability to perform bioautography [4] in which the biological activity of separated compounds is directly tested.

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Quality scanning densitometers are available for TLC [5], and with proper care both precision and accuracy in the range 0.6–1.5% are attainable [6], but compared to HPLC, the technique is not widely used for quantitative analysis. Video densitometers are useful for rapid data acquisition but quantitative analysis with these instruments is not a good as with a scanning densitometer.

The chromatographic efficiency of TLC is usually substantially worse than that of HPLC due to a poor mobile phase flow profile, and this is the dominant reason why TLC is not more widely used for quantitative analysis. Good efficiency is attainable using high performance layers but only with very short migration distances, which limits such separations to typically six or seven compounds. Peak sharpening techniques that use multiple developments are available, but are time consuming and not widely used.

The poor flow profile in TLC with capillary mediated flow is due to the inverse relationship between the linear velocity of the solvent front and its migration distance, given by:

$$u = \frac{\kappa}{2Z_f} \quad (1)$$

where u is the linear velocity, κ the solvent velocity constant and Z_f is the distance migrated by the solvent front. The progressive diminution of mobile phase velocity with the distance migrated by the solvent front can result in lengthy separations, especially when these are performed in the reversed-phase mode. Moreover, it is impossible to perform a separation at an optimum flow rate, and this often results in poor chromatographic efficiency. These effects are exacerbated when using layers consisting of very small particles, as the solvent velocity constant is directly proportional to particle diameter [7].

1.2. Forced-flow techniques in planar chromatography

Forced-flow (or more correctly non-capillary mediated) techniques have been introduced to overcome the poor flow profile of classical TLC. Of these, rotational planar chromatography (RPC) [8], and overpressured layer chromatography (OPLC) [9] are established techniques for which apparatus is commercially available. In RPC, the TLC plate is rapidly spun and the mobile phase flows due to the centrifugal force. An improved flow profile is obtained, but the flow velocity diminishes as the mobile phase migrates from the center of the plate to the circumference. In OPLC, the sorbent layer is covered by a flexible membrane, which is subjected to high pressure. This allows mobile phase to be pumped through the layer. In contrast to RPC, a linear flow profile is obtained, and this results in substantially higher chromatographic efficiency than obtainable with classical TLC. This is especially evident when using high performance TLC plates, which can be used at an optimum flow rate and at longer migration distance than possible with classical TLC. The newest forced-flow technique is shear-driven liquid chromatography [10] in which a layer of sorbent a few microns in thickness is coated on the lower wall of a channel, of which the top wall is moveable. The channel is filled with mobile phase and when the upper wall is moved in the axial direction, the viscous drag causes the mobile phase to flow in the desired direction. Separations are fast and chromatographic efficiency is high, but only separations of two components, using very short migration distances (<2 cm), have been reported. When fully developed, this shear-driven method has the potential to be a powerful separation technique.

The remaining forced-flow techniques are those where the mobile phase is driven by an electrical field and comprise planar electrochromatography (PEC), with either pre-wetted plates [11–19] or with initially dry plates [20–25], and planar dielectrochromatography [26]. Other names that have been used for PEC are high-speed thin-layer chromatography [11] and thin-layer electrochromatography [13].

Planar electrochromatography with an initially dry layer is performed with a horizontal TLC plate in contact with a mobile phase reservoir at each end of the plate, and with the electric field applied through an electrode in each of the reservoirs. It is also performed in an alternative mode that does not involve electroosmotic flow because the electric field is perpendicular to the sorbent layer. The enhancement in migration velocity in either mode is generally modest. The subject is included in a recent book chapter [27] and, apart from noting its historical contribution in the section on the early work, is not discussed further in this review.

In planar dielectrochromatography an alternating electric field is applied to the TLC plate. Theory predicts that such a field acts on the meniscus of a liquid in a capillary tube [26], and this is extrapolated to explain an enhancement of flow when an alternating field is applied to a chromatographic bed. The technique is complex because the alternating field can also cause an increased interaction with the stationary phase and a diminution of solute mobility. Results have been presented which show a modest enhancement of mobile phase flow and either a small diminution or enhancement of solute migration velocity.

1.3. Electroosmotic flow

The velocity, u, of electroosmotic flow is given by:

$$u = \frac{\varepsilon\zeta E}{4\pi\eta} \quad (2)$$

where ζ is the zeta potential, E the electric field and η is the viscosity. Comparison of Eqs. (1) and (2) indicate that there are substantial advantages to using the electroosmotic force rather than capillary forces to drive the mobile phase in planar chromatography. In theory electroosmotic flow should have a flat flow profile in contrast to the profiles in either capillary mediated or pressure driven flow. It is important to note that the derivation of Eq. (2) assumes that there is no overlap of the electrical double layer in the capillary channels within a sorbent bed [29], and this is not always the

case. The latter can lead to anomalous behavior that is discussed in a different section of this review.

The simplified theory predicts that the flow velocity should be independent of migration distance and sorbent particle diameter. A recent report [28], however, considers both interstitial flow and flow within the pores of particles and suggests that for capillary electrochromatography (CEC) the optimal particle diameter should be in the range 0.5–1.0 μm. This is substantially smaller than the diameter of particles in the layers currently used in TLC, and suggests that particle size in layers for PEC can be substantially reduced without any sacrifice in the magnitude of the electroosmotic flow. Such a reduction in particle size should result in a substantial increase in chromatographic efficiency.

Electroosmotic flow can be controlled by adjusting the magnitude of the electric field, and this should allow PEC to be performed at an optimum flow velocity. In many of the papers reviewed, voltage rather than electric field is reported, for the reason that the voltage gradient across the sorbent layer may not be linear. For those reports where electric field is used, this review also uses electric field.

Electrophoresis must also be considered when charged analytes are separated by PEC. Electrophoretic mobility will either increase or decrease the distance migrated by such solutes, depending on the sign of the charge.

2. Planar electrochromatography

2.1. The early work

The earliest report of using an electrical field to effect a separation on a TLC layer was for thin-layer electrophoresis [30]. The first report on planar electrochromatography was by Pretorius et al. in 1974 [11], who called the technique high-speed thin-layer chromatography. The separation of four steroids fully justified this name, as the separation, on pre-wetted plates, was fifteen times faster than an equivalent separation by conventional TLC. The report unfortunately contains little information as to the experimental conditions used, and it is not clear whether the separation was in the normal-phase or reversed-phase mode. The separation was on a silica plate treated with dichlorodimethylsilane but the identity of the mobile phase was not reported. Shafik et al. reported [32], on the basis of a personal communication from Hopkins, that the solvents investigated were *n*-hexane and toluene, but this is an unusual choice of solvents for a separation on a silanized layer. The very fast separation does, however, suggest the use of a mobile phase of low viscosity.

Shafik et al. questioned whether “the early planar separations were due to electroosmotic solvent migration” and have suggested that the migration of mobile phase in the 1974 report can be “accounted for wholly by evaporation of solvent from the upper portion of the TLC plate”. This would allow enhanced capillary flow due to the layer being unsaturated. These authors [32] demonstrated that when TLC was performed for 4 min, with a potential of 10 kV across a 6.5 cm plate, there was an increase in migration velocity compared to the equivalent separation without an applied voltage. The increase in migration distance was 40% with hexane, and 86% with toluene, as the mobile phase. A 20 μA current was attributed to the presence of dissolved impurities, and the increase in migration velocity was attributed to increased evaporation of mobile phase due to Joule heating and coronal discharge. The increase in migration velocity was not due to electroosmotic flow because the increase was independent of the polarity of the applied voltage.

Joule heating will always occur during PEC, and will cause evaporation of mobile phase unless the system is efficiently cooled or the atmosphere in contact with the sorbent layer is fully saturated with vapor. The magnitude of the resultant ‘thermally induced solvent flow’ will depend on a number of variables including the magnitude of the Joule heating, the volatility of the mobile phase and the volume of the space in contact with the sorbent layer.

Thermally induced solvent flow undoubtedly contributed to the mobile phase flow in the ‘high-speed’ separation reported by Pretorius et al. This contribution was almost certainly small, as compared to the contribution of electroosmotic flow, and cannot explain the 15-fold increase in the speed of the separation on a 12 cm TLC plate (the plate length is estimated from the reported voltage and electric field applied). Moreover, the 1974 report states that “solvent accumulating at the top of the plate was removed by suction”. Such accumulation is consistent with electroosmotic flow but would not be found if thermally induced solvent flow was the major cause of the increased migration velocity.

It is surprising that the 1974 report did not initially spark any interest in applying electroosmotic flow to planar chromatography. This may be due to the lack of detail in the report or may be due to the fact that separations by PEC can be of low quality when inappropriate experimental conditions are selected.

After a hiatus of 20 years Pukl et al. [20] reported the separation of a mixture of six dyes on initially dry layers using the experimental setup described earlier in this review. This was the first report to use the name ‘Planar Electrochromatography’ for a separation driven by electroosmotic flow on a planar surface. Separations on a variety of layers and using different mobile phases were investigated. On a silica gel layer, with ethyl acetate as mobile phase, the migration velocity of the mobile phase is enhanced on the anode side of the plate and diminished on the cathode side, which is consistent with electroosmotic flow towards the cathode. The maximum enhancement or diminution of flow was about 14%, and it is assumed that this was at the maximum reported field of 2000 V/cm. The relative solute migration distances were different on the two sides of the plate, and these were in turn different from the migration distances by conventional TLC. These effects can at least

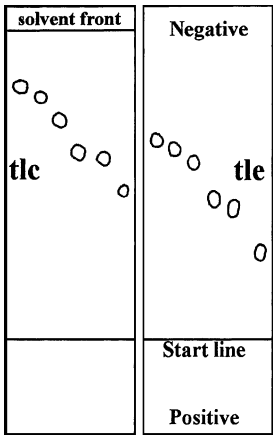

Fig. 1. Comparison of the separation of pirimicarb and related pesticides by TLC and PEC (referred to as thin-layer electrochromatography—tle) on a silica gel layer using ethanol as mobile phase for both separations. A potential of 7 kV was used for PEC. From ref. [13] with permission.

partially be accounted for by a combination of electroosmotic and electrophoretic effects. The report stated that significant flow was observed only on silica gel and polyamide layers. Electroosmotic flow was not observed on RP_8, RP_{18}, or diol modified layers, or on cellulose or alumina layers.

In 1997, Poole and Wilson [31] wrote a paper titled 'Planar electrophoresis and electrochromatography: time to revisit these techniques?' in which the authors outlined the various advantages of using electroosmotic flow in planar chromatography, and discussed why this appeared to have more promise than pressure driven flow. The authors predicted that 'separation performance should be considerably enhanced over conventional development methods with a significant reduction in separation time', and stated that there was an 'overwhelming' argument for research into this technique.

2.2. Planar electrochromatography in the normal-phase mode

Howard et al. [13] have demonstrated that very fast separations may be obtained by PEC in the normal-phase mode. The authors call the technique thin-layer electrochromatography (TLE). Both pure acetonitrile and pure ethanol were investigated as mobile phases. At 800 V/cm, the migration velocity for ethanol was 0.39 mm/s and for acetonitrile was 0.21 mm/s. The migration velocity increased with applied potential in an "approximately linear fashion" for both ethanol and acetonitrile, and the highest velocity reported was about 2 mm/s for acetonitrile.

Fig. 1 shows the separation of six pyrimidine insecticides by both TLC and PEC (referred to as tle) on a 7.5 cm long silica layer using pure ethanol as mobile phase. The plates were wet with the mobile phase before commencing PEC with an electric field of 930 V/cm. The separation was in a special horizontal development chamber, constructed from PTFE, and designed to minimize any evaporation of mobile phase from the layer. The same separation order and a very similar pattern of spots is found for the PEC and TLC separation, but the latter separation requires 18 min, compared to 90 s for PEC. The authors note that the enhanced flow rate results in reduced band broadening, but it is not possible to judge the magnitude of this effect because circles were used to indicate the position of the separated spots on the TLC plate. Electrophoresis does not contribute to the separation mechanism, as the analytes do not have an electrical charge under the conditions used.

The TLC plates were washed with deionized water and then dried before use in order to remove soluble ionic material from the layer. This resulted in a substantially lower and steadier electrical current during the separation, and lower Joule heating.

Both ethanol and acetonitrile are strong solvents and are suitable only for separating polar compounds. Normal-phase separations have been successfully performed in CEC using mobile phases other than pure ethanol or acetonitrile, and it is reasonable to expect that these systems will be applicable to PEC.

2.3. Planar electrochromatography in the reversed-phase mode

The first report of a reversed-phase separation was of a mixture of six dyes on a bonded C_{18} plate [12]. The experimental design was similar to that used by Pretorius et al. with a 2.5 cm × 10 cm plate supported in a nearly vertical position in a Plexiglas frame, with the bottom of the plate dipping into a pool of mobile phase in contact with a platinum wire anode. The cathode was a platinum wire pressed against the sorbent layer near the top of the plate. A wick was present at the top of the plate to prevent accumulation of liquid. A counter plate was used such that there was a 1 mm gap between it and the layer surface. The apparatus is very similar to that shown in Fig. 2, which was used in a later report.

Before commencing PEC, the top section of the plate was dipped into a pool of 1 mM TAPS buffer such that the layer was wet to within 3 mm of the spot position. Ethanol was a component of the mobile phase but was not included in the dip solution in order to minimize evaporation from the layer during PEC. Excess liquid was removed from the surface of the layer by blotting on filter paper (this procedure to remove excess liquid appears to have been used in all reports on PEC at atmospheric pressure in the reversed-phase mode). The plate was inserted into its holder, and PEC was commenced once the mobile phase had reached the sample spot. The interface between the mobile phase and the aqueous dip solution was visible, and this enabled the migration of the solvent front to be observed.

The mobile phase was 80% (v/v) aqueous ethanol containing 1 mM TAPS buffer. The pH of the aqueous component was 10.2 before adding ethanol, and in this review such a mobile phase is said to have a nominal pH of 10.2. Three

of the dyes contain phenolic groups that would have been at least partially ionized with a negative charge at this pH. Electrophoresis may also contribute to the separation mechanism for these three solutes. The electrophoretic mobility would be in the opposite direction to electroosmotic flow and thermally induced solvent flow. In spite of any electrophoretic contribution, all solutes migrated in the forward direction.

Sharper peaks were obtained by PEC than by TLC, and this was interpreted as being due either to a flow rate closer to the optimum or due to a focusing effect caused by solvent evaporation. Depending on the conditions used, the separation by PEC was 1.3–2.1 times faster than a similar separation by TLC. The migration velocity, however, diminished substantially once the spots had migrated about 4 cm.

The same apparatus, with two small modifications, was used for a second report [14]. The apparatus is shown in Fig. 2. A strip of platinum foil was substituted for the wire cathode, and the filter paper wick was wrapped around the leading edge of the cathode as shown in Fig. 3. In this arrangement the mobile phase contacts the filter paper before the platinum foil, and this prevents accumulation of liquid at the cathode. The procedure for pre-wetting the layer was the same as in the previous report.

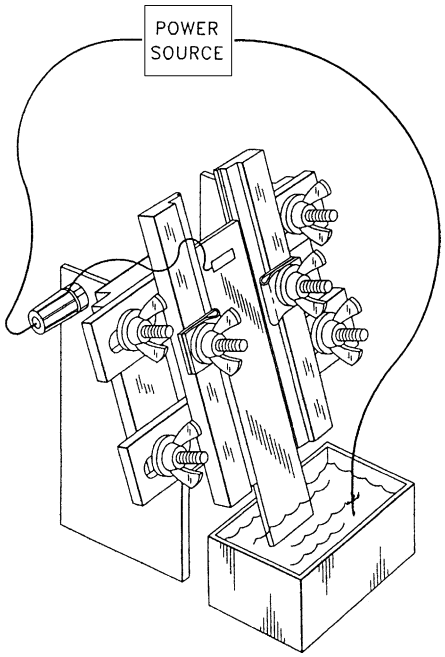

Fig. 2. Apparatus for performing PEC in the vertical mode. The glass counter plate and filter paper wick are not shown. From ref. [14] with permission.

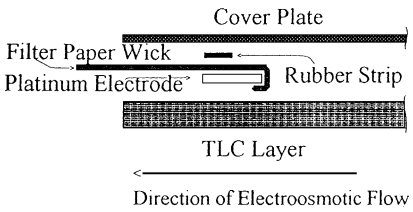

Fig. 3. Exploded view of the placement of the filter paper wick. From ref. [14] with permission.

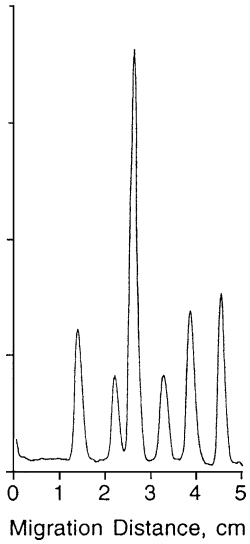

Fig. 4. The separation of a mixture of dyes on a C_{18} TLC plate using 80% aqueous ethanol containing 1.0 mm TAPS buffer, and an applied potential of 2.0 kV. From ref. [14] with permission.

Fig. 4 is a chromatogram showing the separation of the same dye mixture as used in the previous report on a bonded C_{18} layer at an applied voltage of 2.0 kV, using 80% aqueous ethanol containing 1.0 mM TAPS buffer as the mobile phase. The separation required 18 min compared to 37 min by TLC. Depending on which solute is considered, the number of theoretical plates generated is between 2.5 and 4.5 times higher for PEC than for the corresponding separation by TLC. The layer eventually dries during PEC with 80% aqueous ethanol and this is accompanied by a diminution of the electric current. Evaporation is assumed to cause a solvent focusing effect but also limits the maximum migration distance attainable to about 5 cm with this mobile phase.

Included in the report was the separation of iopanoic acid, benzanalide, 3,4-dihydroxybenzoic acid, and *p*-hydroxybenzoic acid on a bonded C_{18} phase with 45% aqueous acetonitrile containing 1.0 mM TAPS buffer as mobile phase. PEC was at an applied voltage of 1.5 kV and required 4 min for a baseline separation of all solutes, compared to 12 min for the corresponding separation by TLC. There was no evidence of layer drying during the separation.

It was suggested in ref. [13] that 'any or all' of electroosmosis, electrophoresis, and evaporation' could account for the separations reported in references [11,12]. The reasons why electroosmotic flow is considered the primary mechanism of mobile phase flow in reference [11] were discussed earlier in this review. The same apparatus was used in both references [12,14], and in the latter report reasons were given why electroosmotic flow is considered the major contributor to the separations. The filter paper wick at the top of the TLC plate becomes wet during PEC, which is consistent

with electroosmotic flow. When a voltage of 2.0 kV is applied with the polarity of the electrodes reversed, the layer near the mobile phase reservoir dries within 1 min. The rapid drying is also consistent with electroosmotic flow. There is no reservoir at the top of the plate, and with reversed polarity, the mobile phase is flowing from the top of the plate towards the reservoir at the bottom. Neither of these effects would occur if the migration of the mobile phase were primarily due to thermally induced solvent flow. The reason that the latter does not make the major contribution to separations in this apparatus, in contrast to its major contribution in the report by Shafik et al. [32], is that the channel above the plate is very narrow. This results in a high concentration of vapor above the layer as evidenced by substantial condensation on the glass counter plate. Thermally induced solvent flow may nevertheless be an important secondary contributor to the separation process. Any contribution due to electrophoresis is likely to be small. Three of the dyes in ref. [12] and the three organic acids in ref. [14] will be present as anions in the buffer solution used as mobile phase. In all the separations these analytes migrated towards the cathode, and not towards the anode as would have been the case if electrophoresis were a major influence.

The same apparatus was used to investigate the effect of changing experimental variables [15]. The solutes in the test mixture were 17-α-acetoxy-progesterone, benzanilide, 4-pregnen-11β, 17α,21-triol-3,30-dione, and benzamide. All separations were on bonded C_{18} layers with aqueous acetonitrile containing acetate buffer as the mobile phase.

Chromatograms were used to illustrate the effect of changing a single variable while keeping the other variables constant. The reference chromatogram was for a separation using a mobile phase consisting of 55% aqueous acetonitrile containing 0.25 mM acetate at a nominal pH of 4.5, and an applied potential of 1.5 kV. The single variables that were considered were the applied potential, the percentage of acetonitrile, the buffer concentration and the pH. All separations, including the reference, were for 6.5 min. The migration distance increased with an increase in each of the variables. There was a small increase in the quality of separation with an increase in the applied voltage, or an increase in the concentration or pH of the acetate buffer. An increase in the concentration of acetonitrile resulted in overlap of the two fastest migrating peaks. The changes in the chromatograms are difficult to interpret. The dimensions of the double layer and the magnitude of the zeta potential will be affected by changes in the concentration of acetonitrile, and in changes of both pH and concentration of the buffer. Electroosmotic flow is proportional to the magnitude of the zeta potential (see Eq. (2)). The effect of a change in the size of the double layer is complex and is discussed in a later section of this review. An increase in the magnitude of the applied voltage, or in either the pH or the concentration of the acetate buffer, will result in increased Joule heating. The resulting increase in temperature will increase the rate of mobile phase evaporation, which will cause both a solvent focusing effect and an increase in thermally induced solvent flow. The increase in temperature will cause an increase in the migration distance of each of the solutes due to a diminution of retention factors. It will also cause an increase in electroosmotic flow through a diminution of the mobile phase viscosity (see Eq. (2)), but will also increase the size of the electrical double layer [33], which has an effect on electroosmotic flow that is difficult to predict.

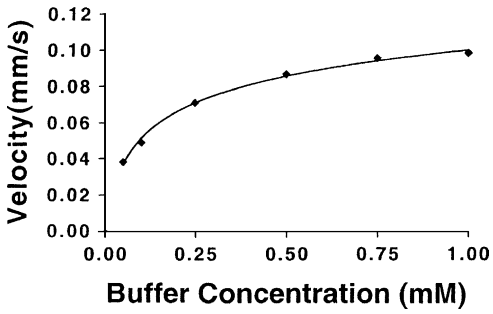

Fig. 5. Plot of migration velocity of xylene cyanol versus acetate buffer concentration. Separations were on a bonded C_{18} TLC plate at 1.0 kV using 55% aqueous acetonitrile as the mobile phase. From ref. [15] with permission.

The effect of buffer concentration on the velocity of mobile phase flow was measured using xylene cyanol as a marker for the position of the solvent front. This is a strongly colored compound with an R_f close to unity in this mobile phase. The buffer concentrations were limited to a range 0.05–1.0 mM, because the power source had a maximum output of 2.0 mA.

At each buffer concentration the migration velocity of xylene cyanol was constant for a distance of 2.5 cm, and a plot of migration distance versus time yielded a straight line for each of the different buffer concentrations. The corresponding migration velocities were calculated from the slopes of the lines and used to construct Fig. 5, which shows an increase in migration velocity with increasing buffer concentration. This increase in electroosmotic flow with increasing buffer concentration is not predicted by theory [34,35]. It may be due to a reduction in the overlap of the electrical double layer, as discussed below, as well as an increase in thermally induced solvent flow due to increased Joule heating.

For CEC there are two reports [34,35] showing that electroosmotic flow diminishes with the square root of buffer concentration, and also a report [36] where electroosmotic flow initially increases, and then decreases, with increasing buffer concentration. The size of the electrical double layer diminishes with increasing buffer concentration, and this should be accompanied by a diminution of electroosmotic flow. This assumes that the size of the channels is large as compared to the size of the electrical double layer. Wan [29] has attributed the anomalous increase of electroosmotic flow with increasing buffer concentration as being due to a reduction in the overlap of the electrical double layer in narrow channels. In other words, when a low concentration

of buffer leads to overlap of the double layer in narrow channels, the electroosmotic flow will be lower than might be expected from the usual relationship between electroosmotic flow and buffer concentration. When this occurs, an increase in buffer concentration will diminish the overlap and increase the magnitude of the electroosmotic flow. Once there is no overlap, any further increase in buffer concentration should result in the diminished flow predicted by theory. Thus, it is possible that if PEC were performed over a larger range of buffer concentrations, there would be an initial increase in migration velocity followed by a diminution of velocity at the higher end of the buffer concentration range.

The separations in refs. [12,14,15] were performed in the vertical mode with a single mobile phase reservoir at the bottom of the apparatus. The following four reports are for PEC in the horizontal mode, using the commercially available DS II chamber, which, while designed for TLC, is easily modified for PEC. The TLC plate is used in a face down position over a shallow trough on the floor of the apparatus. The latter was either filled with mobile phase or contained filter paper wetted with mobile phase, in order to partially control evaporation of mobile phase from the surface of the layer. The DS chamber is supplied with a 5 mm thick glass plate covering the top of the apparatus, which is not airtight, as there are holes for 'eluent distributors', that move 'reservoir cover plates'. The latter action is taken before commencing TLC, and results in a capillary channel that allows mobile phase to flow from the reservoir to the sorbent layer. The apparatus was modified [16] by substituting a 1 mm thick glass plate for the 5 mm thick plate. The holes for the 'eluent distributors' were slightly larger in the modified plate than in the original, and this may have contributed to lower vapor saturation during PEC. The anode and cathode were platinum wires cemented into holes in the glass plate, such that each dipped into one of the mobile phase reservoirs that are at each end of the chamber. It was subsequent reported [17] that PEC could be performed using a glass cover without holes for the eluent distributors. In this mode the 'reservoir cover plates' were moved into position before placing the cover plate in position and commencing PEC. In a further modification of the apparatus [19], the glass cover sheet did not have any holes and the two electrodes were introduced into the mobile phase reservoirs through the wall of the chamber. This final modification is shown in Fig. 6. The counter plate and clip shown in Fig. 6 were used only in refs. [18,19].

In the first report [16] on the use of this chamber, the role of evaporation was investigated by performing replicate PEC separations on TLC plates that were 10 cm long and either 1, 2, or 4 cm wide. The best results were found for the 2 cm wide plate for which three replicate separations are shown in Fig. 7, which also lists the conditions used. This reproducibility illustrated in the figure was not always obtained and it was noted that 'there was an occasional outlier'.

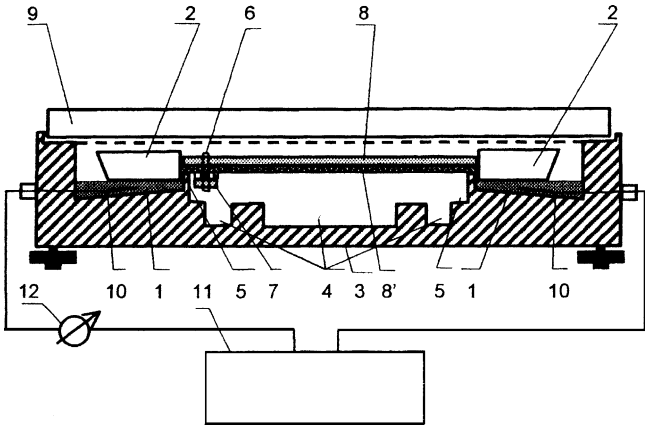

Fig. 6. Horizontal DS Chamber as used in references [18,19]. (1) Mobile phase reservoir (the gray areas are the mobile phase), (2) reservoir cover plate, (3) body of the chamber, (4) troughs for the mobile phase, (5) an edge, (6) the clip holding the counter plate, (7) the 1 cm × 2 cm counter plate, (8) TLC plate, (8′) sorbent layer, (9) cover plate, (10) platinum wire electrodes, (11) power supply, and (12) ammeter. From ref. [19] with permission.

Both the separation quality and the reproducibility were slightly lower for the other two plate widths, with the 4 cm wide plate yielding better results than the 1 cm wide plate. The chamber is designed for a 5 cm wide plate, and with the 1 cm wide plate there is a large gap on either side of the plate. This results in lower vapor saturation of the atmosphere in contact with the sorbent layer, and this results in excessive evaporation and eventual drying of the layer. The separation can be completed by allowing the layer to rewet by capillary action in the absence of an electric field, and then restarting PEC.

There is only a small gap on the sides of the 4 cm wide plate, and the poorer results than with the 2 cm wide plate

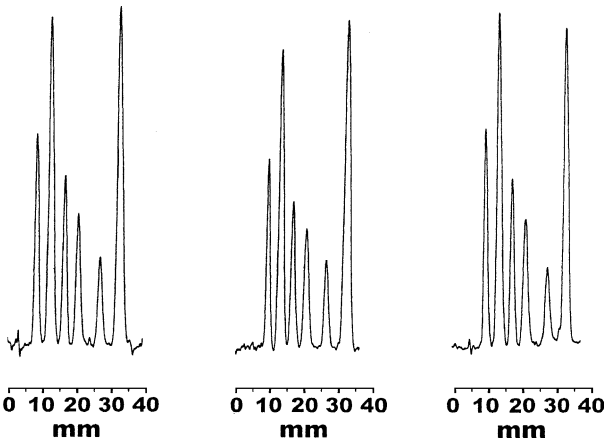

Fig. 7. Replicate separations of a six-component mixture on 2.0 × 10 cm bonded C_{18} TLC plates. PEC was for 10.0 min at 1 kV using 55% aqueous acetonitrile containing 25 mM acetate buffer (nominal pH of 4.5) as the mobile phase. The solutes in order of increasing R_f are: 17α-acetoxy-progesterone, 2′-acetonaphthone, benzanilide, *o*-nitroaniline, 3,4-dimethoxybenzoic acid, *p*-hydroxybenzoic acid. From ref. [16] with permission.

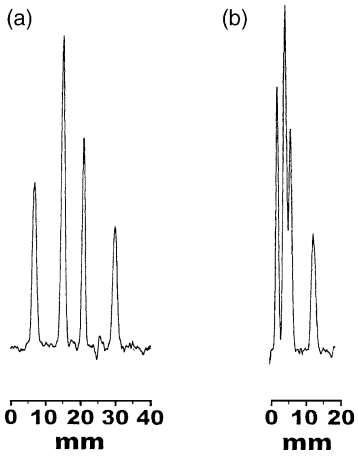

Fig. 8. Chromatograms showing the effect of changing the electrode polarity when separating a four-component mixture on 2.0 × 10.0 cm bonded C_{18} TLC plates. PEC was for 6.5 min at 1 kV using 55% aqueous acetonitrile containing 25 mM acetate buffer (nominal pH of 4.5) as the mobile phase. The usual electrode polarity was used for chromatogram (a), and the reversed polarity was used for chromatogram (b). The solutes in order of increasing R_f are: 17α-acetoxy-progesterone, benzanilide, 4-pregnen-11β,17α, 21-triol 3,20-dione, benzamide. From ref. [16] with permission.

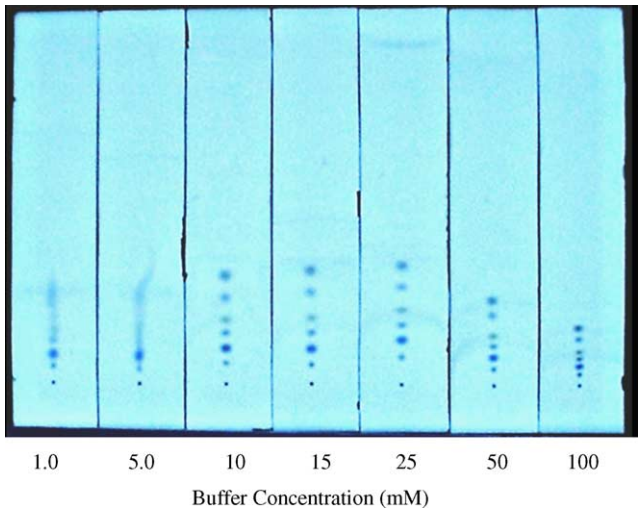

Fig. 9. Separations of a seven component mixture on a bonded C_{18} layer at 1 kV using 55% aqueous acetonitrile containing acetate buffer (nominal pH of 4.5) as the mobile phase. The buffer concentrations are as indicated. The compounds listed in order of increasing R_f are: 4-cholesten-3-one, 17-∝-acetoxy-progesterone, 2′-acetonapthone, benzanilide, *o*-nitroaniline, 3,4-dimethoxybenzoic acid, *p*-hydroxybenzoic acid. From ref. [17] with permission.

may be due to a sufficiently high level of vapor saturation, such that the peak focusing effect is either reduced or eliminated.

The above separations were for 10 min. A substantial diminution of migration velocity was encountered when PEC was performed for longer periods of time, even when the layer did not dry. This was interpreted as being due to selective evaporation of acetonitrile and the accompanying diminution of mobile phase strength.

Reversing the polarity of the electrodes results in a substantially reduced migration distance as illustrated in Fig. 8. The fact that there is some small migration is most probably due to the layer being unsaturated due to blotting before PEC and due to thermally induced solvent flow.

While PEC can produce separations that are both faster and of higher quality than those obtainable by TLC, inappropriate selection of experimental conditions results in poor separation due to either drying of the layer—caused by excess Joule heating—or accumulation of excess liquid on the layer surface. This accumulation, which was first observed by Frost [37], has been explained in two different ways. The first explanation [17] is that in the sorbent bed there is a distribution in the size of the channels through which the mobile phase flows, and the flux of liquid from one channel to the next may be substantially different. When a channel of higher flux leads to a channel of lower flux, the excess liquid may migrate to the surface. If the magnitude of this migration is large enough, and the system is open as in PEC, it will lead to accumulation of liquid on the surface. The second explanation [19] is that accumulation is due to liquid migrating along the surface by electroosmotic flow.

It is important to select a combination of applied voltage and buffer concentration such that separation occurs without accumulation of liquid on the surface or drying of the layer. This can be accomplished by the correct combination of buffer concentration and applied voltage [17]. This is illustrated in Fig. 9, which shows the separation of a mixture of solutes on seven different TLC plates. Five of the runs were for 10 min, and the remaining two were terminated early because the layer dried. The concentration of acetate buffer was different for each run, but other experimental conditions are the same and are listed in the figure caption.

The results are interpreted in terms of an increase in evaporation of liquid from the surface with increased Joule heating. The latter increases with the concentration of ions in solution, i.e. with the buffer concentration at a constant pH. A secondary effect of increasing buffer concentration may be a diminution of the overlap of the electrical double layer, and this may lower the tendency for the mobile phase to migrate to the surface of the layer.

There is streaking at buffer concentrations of 1 and 5 mM, due to insufficient evaporation of liquid from the layer surface. At buffer concentrations of 10, 15, and 25 mM there is a balance between evaporation and liquid being driven to the surface, and good separations are obtained. Excessive Joule heating occurs at buffer concentrations of 50 and 100 mM and the layer dries after approximately 4 and 2 min, respectively.

The magnitude of the Joule heating and accompanying evaporation may be either increased or decreased by adjusting the pH of the solution while maintaining a constant buffer concentration. Examples are given where streaking is eliminated in a previously poor separation by raising the pH

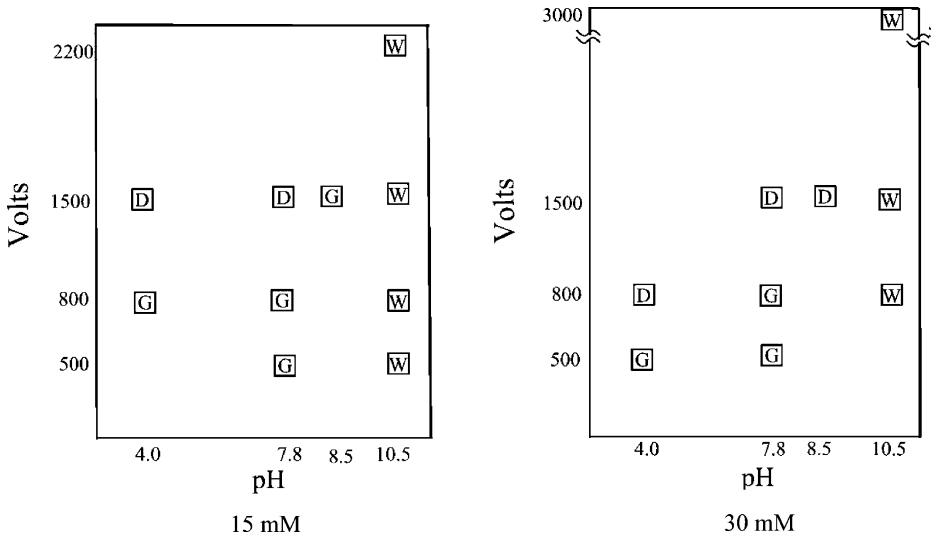

Fig. 10. Plots indicating the separation quality obtained for different combinations of applied voltage and pH using 65% aqueous acetonitrile, containing either 15 or 30 mM Tris buffer, as the mobile phase. The solutes separated were the same as those used for Fig. 9. (D) Indicates that layer drying was observed, (G) indicates that a good separation, without any evidence of either streaking or drying, was observed, and (W) indicates that layer wetting or streaking of higher R_f spots was observed. From ref. [17] with permission.

of the acetate buffer, or streaking is induced in a previously good separation by decreasing the pH of the acetate buffer.

The interplay between buffer concentration, pH and applied voltage was also discussed in ref. [17]. The buffer used in the study was Tris, which is basic and has a positive charge at lower pH and is neutral at higher pH. For Tris, in contrast to acetate buffer, lowering the pH from the pK_a value increases the concentration of ions in solution, and raising the pH has the opposite effect. The results are summarized in Fig. 10 where the letter D indicates that drying of the layer was observed, G indicates a good separation was obtained, without evidence of either drying or streaking, and W indicates that layer wetting and spot streaking was observed. The results were presented as plots of separation quality (D, G, or W) as a function of applied voltage and nominal pH, using 65% aqueous acetonitrile, which contained Tris buffer at two different concentrations.

The separation varies from ‘dry’ to ‘good’ to ‘wet’ as the nominal pH is increased from 4.0 to 10.5, at 1500 V for the 15 mM buffer concentration, and at 800 V for the 30 mM buffer concentration. The increase in pH causes a diminution of Joule heating due to a reduction in the concentration of the charged form of the buffer. This, in turn, leads to progressively less evaporation from the layer, which explains the above transition from ‘dry’ to ‘good’ to ‘wet’. The reason that the layer dries either at 1500 V (for 15 mM Tris) or at 800 V (for 30 mM Tris) at pH 4.0 is that the buffer is in an ionic form at low pH, and this results in substantial Joule heating. Inspection of the figure indicates that a good separation may be obtained at pH 4.0 when Joule heating is reduced through a reduction in the applied voltage.

There is a diminution of electrical current with an increase in pH, due to a reduction in the concentration of charged species, and this results in diminished Joule heating, and accumulation of liquid on the surface. This explains why, at a pH of 10.5, wetting of the layer is observed for all combinations of buffer concentration and voltage.

The electric current typically diminishes during PEC when it is performed at a constant voltage, and this is accompanied by a diminution of Joule heating. There are two reports [13,19] where the current first rises and then falls. This has been attributed to the presence of impurities, and in normal-phase PEC [13] a lower and more constant current was found when the plates were pre-washed with deionized water. An alternative approach to using constant voltage is to perform PEC at constant power, and this has been briefly discussed [17]. Both voltage and current may fluctuate, but the amount of power dissipated, and the magnitude of the Joule heating, remains constant during a separation. A chromatogram obtained at a constant power of 3.0 W is shown in Fig. 11. The plate dries after about 4 min, and the conditions used do not yield good reproducibility. Nevertheless very sharp peaks are obtained due to evaporative effects, and this approach may be worth investigating under conditions that do not lead to drying of the layer.

A report by Dzido et al. [19] used the modified DS development chamber, shown in Fig. 6, which has the platinum electrodes inserted through the PTFE walls into the two mobile phase reservoirs. A similar configuration had been used by Malinowska and Rozylo [21] for a study of PEC on initially dry layers.

At first separations were not reproducible and ‘enormously increased dispersion of sample bands’ was observed. This was attributed to the flow of mobile phase from the anode reservoir over the surface of the sorbent layer. This flow was prevented by clipping a 1 cm × 2 cm section of TLC plate, coated with paraffin oil, to the surface of the

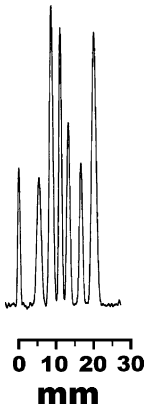

Fig. 11. Separation of the same solute mixture as in Fig. 9 on a bonded C_{18} layer at 3.0 W, using 55% aqueous acetonitrile containing 100 mM acetate buffer (nominal pH 4.5) as the mobile phase. From ref. [17] with permission.

TLC layer. The position of the plate was between the anode reservoir and the point of sample application. Most of the separations were on 2 cm × 10 cm plates, with a 0.5 cm section of the sorbent layer, along the two longer edges, coated with paraffin oil, i.e. the untreated sorbent layer was 1.0 cm wide. Samples were applied either 15 or 20 mm from the mobile phase origin.

In an initial set of experiments a stock solution that was 50 mM in boric acid, adjusted to pH 10.0 with sodium hydroxide, was used. The solution was also 50 mM in potassium chloride. PEC was performed on bonded C_{18} plates with 90% acetonitrile, in which the aqueous component was either pure water, or various dilutions of the stock solution. The electric current first rises, and then falls to zero within 3 min, which indicates drying of the layer, for mobile phases in which the aqueous component was a 1:1 or lesser dilution of the stock solution. At greater dilutions the drop in current was more gradual, and PEC could be performed for more than 6 min.

A series of experiments was performed on the bonded C_{18} plates with 90% aqueous acetonitrile, in which the aqueous component was a 1:3 (stock solution:water) dilution, and, in which the applied voltage was varied between 0.5 and 2.5 kV. Apart from the separation at 0.5 kV, the number of theoretical plates generated was in the range of two to four times higher than the corresponding separation by conventional TLC (with HPTLC plates). Each of the solutes separated exhibited the highest number of theoretical plates at the applied voltage of 2.5 kV. The speed of separation was approximately proportional to the applied voltage, and at 2.5 kV PEC was about two times faster than the corresponding separation by TLC. The analysis time for the 1 kV separation was approximately the same as that for the separation by TLC, but the number of theoretical plates generated was about two times higher, reflecting a better velocity profile for the mobile phase.

The TLC layer was pre-wetted by dipping into a pool of the mobile phase immediately before each PEC separation.

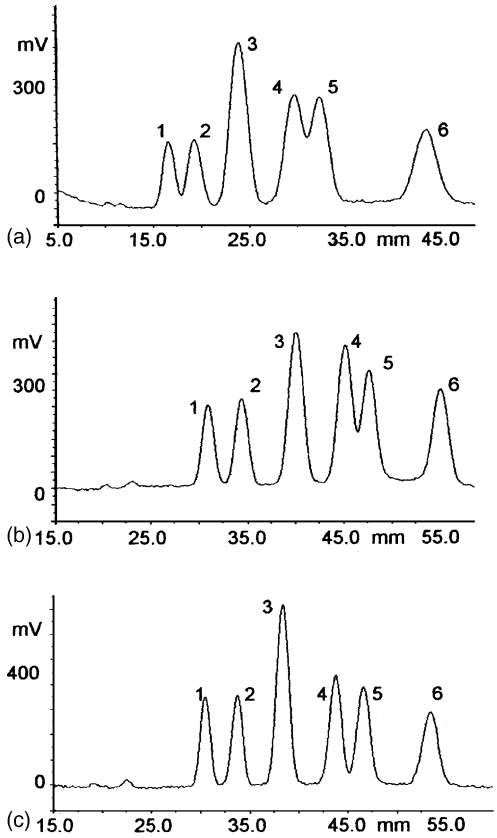

Fig. 12. Separation of (1) 1-aminoanthraquinone, (2) 4-(4-ethoxyphenyazo)-1-naphthol, (3) 4-(diethylamino)azobenzene, (4) 1-(4-hydroxyphenylazo)-2-naphthol, (5) 5-[4-(*N*-ethyl-*N*-2-hydroxyethylamino)-phenylazo]-*N*-methylphthalimide, (6) 4-nitroanaline on bonded C_{18} plates using 90% aqueous acetonitrile, containing a pH 10 buffer, as mobile phase. The boric acid buffer, described in the text, was diluted with three parts of water. Chromatogram (a) is for conventional TLC, chromatogram (b) is for PEC at 2.0 kV with the plate pre-wetted by dipping, and chromatogram (c) for PEC at 2.0 kV with the plate pre-wetted by soaking for 18 h. From ref. [19] with permission.

The authors found that this did not allow sufficient time for liquid to fill all the pores in the sorbent layer. The quality of separation was substantially improved by allowing the plate to soak in the mobile phase for 18 h before commencing PEC. This is illustrated in Fig. 12, which shows the separation of a mixture of six dyes by TLC, by PEC with a conventional dip, and PEC after soaking the plate in the mobile phase for 18 h.

The above report also contains a chromatogram showing the separation of the enantiomers of phenylalanine on a Merck CHIR plate using 60% aqueous acetonitrile, in which the aqueous component was 2 mM in copper(II) acetate. PEC was at 2 kV and yielded a separation that was more efficient and about twice as fast as the corresponding separation by TLC.

As noted earlier in this review, the migration velocity is enhanced by evaporation of mobile phase from the layer surface due to Joule heating. This effect depends on the degree of vapor saturation in a chamber and also on the

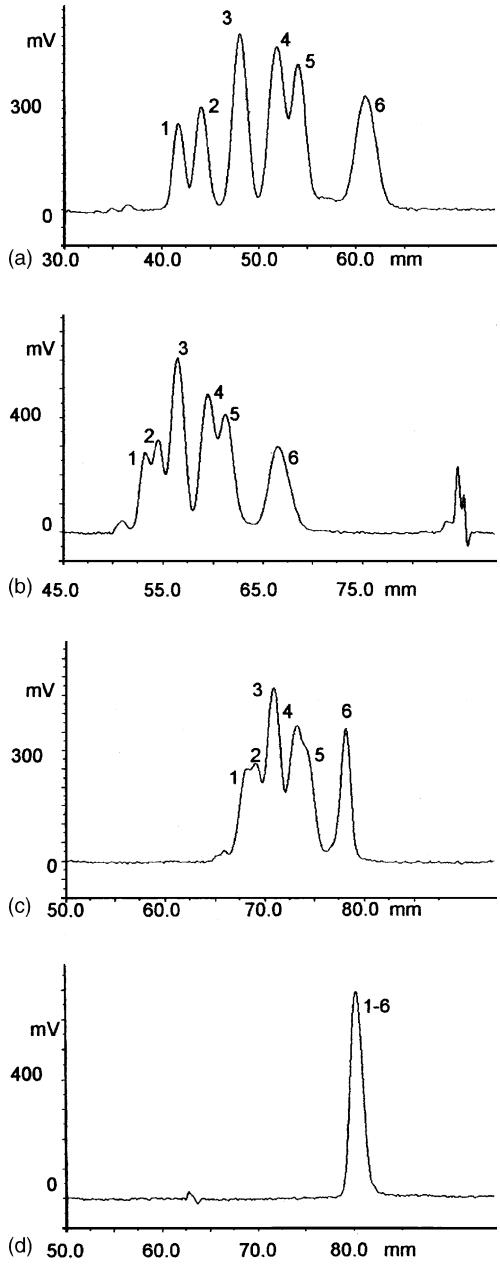

Fig. 13. Four chromatograms showing that the distance of the spotting position from the mobile phase origin affects separation quality. Spotting was at the following distances from the origin; (a) 35 mm, (b) 50 mm, (c) 65 mm, (d) 80 mm. All other experimental details are as in Fig. 12b. From reference [19] with permission.

concentration of the buffer (or more precisely of the electrolytes) in the mobile phase. Dzido et al. [19] report that at higher buffer concentration (i.e. where there is more Joule heating) drying occurs near the center of the plate, whereas at lower buffer concentration drying will occur closer to the cathode. This is illustrated in Fig. 13, which shows chromatograms where the sample mixture was applied at 35, 50, 65, and 80 mm, respectively, from the mobile phase origin. For the experimental conditions used, the layer eventually dries at 80 mm from the mobile phase origin. The quality of separation degrades as the samples are applied closer to this position, and there is a complete lack of separation for the sample applied at the 80 mm position. Even before the layer dries there is no net movement of mobile phase across this position. Mobile phase travels from the origin by a combination of electroosmotic flow and thermally induced solvent flow. Mobile phase travels in the reverse direction (i.e. from the cathode compartment) by thermally induced solvent flow. The latter effect becomes more substantial as the buffer concentration increases, and this explains why at high buffer concentration drying occurs at a position nearer the middle of the plate.

Evaporation of mobile phase from the layer can be prevented by cooling. This has been performed [18] by placing a container with water at 5 °C in contact with the glass backing of a TLC plate in the DS chamber, and this results in a more stable electric current. When a mixture of polar aromatic compounds was separated with cooling there was some improvement in the separation quality and the distance migrated by the fastest moving solute was 55 mm, compared to a 44 mm migration without cooling. The same mobile phase was used as for the chromatograms in Fig. 12. The challenge with cooling the layer is to prevent excess evaporation while still maintaining sufficient evaporation for a degree of solvent focusing.

The above reports show that PEC at atmospheric pressure, in either the normal-phase or the reversed-phase modes, can be both a faster and a more efficient technique than classical TLC. The most significant limitations of the technique are that the sorbent layer dries when there is excessive Joule heating, and that spot streaking can occur when liquid accumulates on the surface of the layer. These two shortcomings do not apply to the technique that is discussed below.

3. Pressurized planar electrochromatography

Pressurized planar electrochromatography is performed by pressurizing the sorbent layer while it is covered by a sheet of material, such as aluminum nitride ceramic that is both an electrical insulator, and a thermal conductor. This allows the pressurizing medium to act as a heat sink, and also prevents accumulation of liquid on the surface of the layer.

A prototype apparatus [38] uses a hydraulic ram to press a die block, covered by a 1 mm thick sheet of aluminum nitride ceramic, onto the surface of the layer, which is covered by a 0.25 mm-thick sheet of PTFE. The plates used are 3.3 cm × 12 cm and the pressurized region is 2.5 cm × 10 cm. Sorbent is scraped from 4.0 mm of each of the long edges of the plate, and the scraped area is coated with a silicone rubber sealant. This coating, together with the PTFE sheet, prevents liquid from being forced from the layer. An exploded view of the Delrin frame that holds the TLC plate is shown in Fig. 14. The filter paper wick removes liquid that migrates to the top of the plate. The assembled frame is placed between

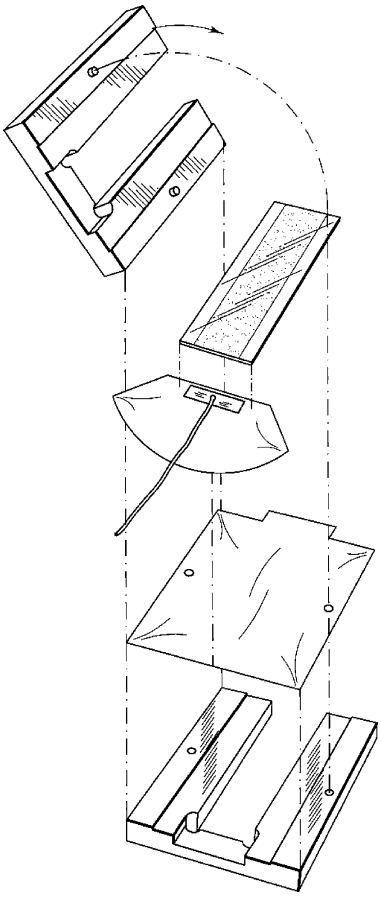

Fig. 14. An exploded view of the Delrin frame and components. From bottom to top the diagram shows a section of the Delrin frame, the PTFE sheet, the filter paper wick, the electrode, the TLC plate facedown, and the second section of the Delrin frame. From ref. [38] with permission.

the two die blocks that are shown in Fig. 15. The apparatus, with the Delrin frame in place, is shown in Fig. 16. The hydraulic press that is used to apply pressure is in the foreground.

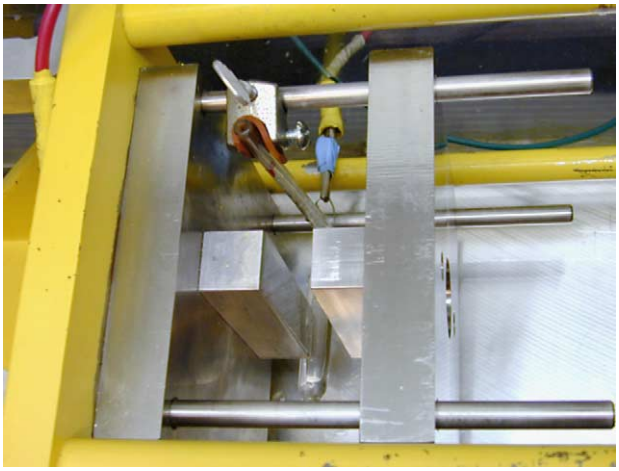

Fig. 15. View of die blocks that pressurize the layer. From ref. [38] with permission.

Fig. 16. Apparatus for PPEC with Delrin frame in place. From ref. [38] with permission.

Before the plate is placed in the holder the sample spot is applied and allowed to dry. The plate is then dipped in the mobile phase, and the excess liquid allowed to drain along the long axis of the plate for about 5 s. The plate is then placed in the frame without blotting the sorbent layer.

Both regular and high performance (LiChrospher) Merck bonded C_{18} plates were used. The former consist of particles of irregular shape with a wider size distribution, an average size of ∼11 μm, and a carbon load of ∼14%. The LiChrospher plates consist of spherical particles with a narrower size distribution, an average size of ∼7 μm, and a carbon load of ∼6%. Both types of plates require activation in an oven before use, and it is found that solute migration velocity increases with an increase in the temperature at which the plates are activated.

The model solute mixture used in the study, in order of increasing migration distance, was 4-cholesten-3-one, 17α-acetoxyprogesterone, 2′-acetonaphthone, benzanilide, and *o*-nitroaniline. The 4-cholesten-3-one does not migrate under the conditions used, and acts as a marker for the spotting position. The first set of separations were performed on LiChrospher C_{18} plates at 118 atm and 6 kV, using 55% aqueous acetonitrile, containing 50 mM acetate buffer at a nominal pH of 4.7 (1 atm = 101,325 Pa). Under these conditions the migration velocity is independent of the distance migrated, as evidenced by linear plots of distance versus time for each of the four solutes that are mobile. This indicates that, at least under these conditions, the temperature of the layer remains constant during the separations, and that the die block that pressurizes the sorbent layer, acts as an efficient heat sink. Under some experimental conditions the die block becomes noticeably warm, and this should result in an acceleration of solute migration.

In a similar set of experiments, performed at a pressure of 59 atm, the migration velocity was found to be directly proportional to the applied voltage, as would be expected from Eq. (2).

Separation is substantially faster in PPEC as compared to TLC. Fig. 17 shows a 1 min separation of the test mixture on LiChrospher plates at three different pressures, with otherwise identical conditions for each separation. A baseline separation is obtained at a pressure of 19.7 atm. A good

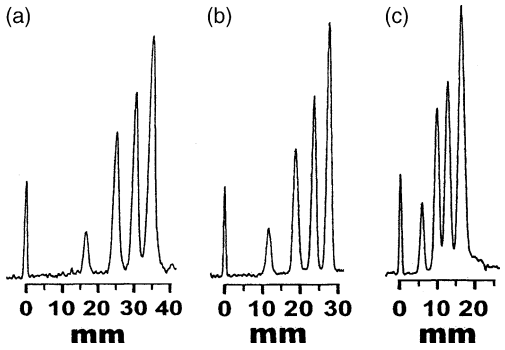

Fig. 17. A 1 min separation on a LiChrospher C_{18} plate at 9 kV, and a pressure of (a) 11.8 atm, (b) 19.7 atm, or (c) 118 atm. The compounds separated, in order of increasing migration distance, are 4-cholesten-3-one, 17α-acetoxyprogesterone, 2′-acetonaphthone, benzanilide, and *o*-nitroaniline. The mobile phase was 55% aqueous acetonitrile containing 25 mM acetate buffer at a nominal pH of 4.7. From ref. [38] with permission.

separation is obtained on either the LiChrospher or the regular plate by TLC, but the time required is about 24 min.

There is an improvement in the separation quality as the pressure is increased from 11.8 to 19.7 atm, and this is attributed to an improvement in the quality of the sorbent bed due to a diminution of the interstitial spaces between particles. A similar improvement in efficiency has been observed in OPLC [39].

There is a diminution of migration velocity as the pressure is increased, and at 118 atm the migration velocity is slower, and the separation quality worse, than the separations at the two lower pressures shown in Fig. 17. This diminution of migration velocity may be due to an increasing overlap of the electrical double layer as the channels between particles become smaller. A substantially better separation, shown in Fig. 18a, is obtained in 3 min under the same experimental conditions, apart from an increase in the voltage from 9 to 10 kV. Fig. 18b shows the separation on regular Merck plates under identical conditions as those used with the LiChrospher plates. The quality of the separation is substantially worse, due in part to the slower migration on the regular plates. The differences in migration velocity may be due to

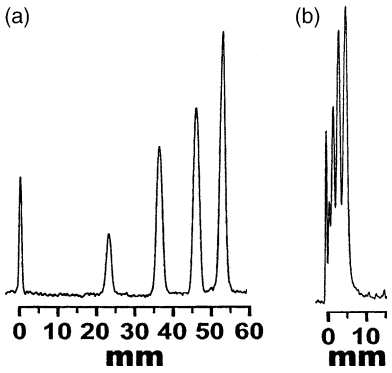

Fig. 18. PPEC for 3 min at 118 atm and 10 kV on (a) a LiChrospher C_{18} plate and (b) a regular Merck C_{18} plate. The solutes and mobile phase are the same as those in Fig. 17. From ref. [38] with permission.

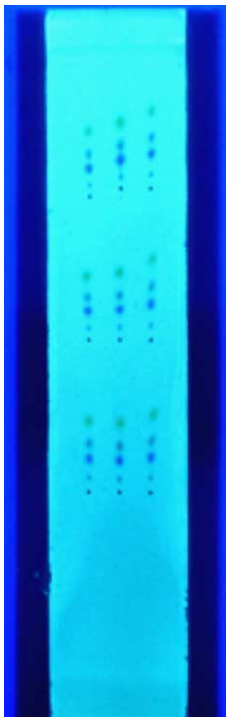

Fig. 19. Separation of nine samples by PPEC in 1 min on a LiChrospher C_{18} plate at 59 atm and 7 kV. The solutes and mobile phase are the same as those in Fig. 17. From ref. [38] with permission.

the different carbon loads of the sorbents in the two different layers. The LiChrospher plates have a lower carbon load, and this should expose more of the silica surface to the mobile phase, and may result in a higher concentration of ionized silanol groups, which should enhance the electroosmotic flow. The excellent peak shape may be due to the bed consisting of spherical particles of a narrow size distribution.

There appear to be a number of advantages to working at a higher pressure. By analogy to OPLC [39], the quality of the sorbent bed should increase with increasing pressure. This will be accompanied by a diminution of migration velocity that can be overcome by increasing the magnitude of the electroosmotic flow by working at a higher voltage. For a given applied voltage the electrical current was found to drop with increasing pressure, and this should be accompanied by a drop in Joule heating. While preliminary results are promising, further work is required to establish the advantage of working at very high pressure.

An advantage of PPEC over other forms of planar chromatography is that an array of samples can be applied to the TLC plate. This is possible because the plate is prewetted and the separation of all samples commences simultaneously. Fig. 19 shows the separation of nine samples of the five-component test mixture in 1 min.

The reproducibility of the method was evaluated at 6 kV for 4 min on LiChrospher plates and at 7 kV for 10 min on the regular plates. Six replicate separations were performed with each type of plate using 55% aqueous acetonitrile containing 25 mM acetate buffer as the mobile phase. The

relative standard deviation in retention for the four solutes varied from 4.0 to 9.1% for the regular plates and from 2.6 to 4.6% for the LiChrospher plates. The average height of a theoretical plate diminishes, with increasing migration distance, from 55 to 27 μm for the regular plates and from 29 to 21 μm for the LiChrospher plates.

The report concluded with a short discussion of how to improve the reproducibility of the method. Better temperature control of the layer is required, a better method is required of connecting the power source to the cathode, there is a drift in pressure during PPEC that should be eliminated, a higher quality oven should be used for preconditioning the plates, and an automatic device should be used for prewetting the plates.

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