

Characterization of flow and voltage profiles in planar electrochromatography

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Abstract

Planar electrochromatography (PEC) is a new technology for thin layer chromatography (TLC) where the separation is driven by electroosmotic forces, not capillary action. This allows for much faster and more efficient chromatography in a planar format. Care needs to be taken when performing these experiments because voltage and flow characteristics can change through a single run, due to buffer gradients, temperature changes (Joule heating) and localized plate heterogeneity. We have designed a PEC instrument and cover grid to allow investigation of flow and voltage characteristics as solvent moves across a TLC plate. Our unique cover grid allows monitoring voltage at eight discrete points between the positive and negative reservoirs. A linear relationship between voltage and distance should be seen, giving a constant voltage drop across a plate, but this did not occur. This non-linear function changes over time, following the plate equilibration. Once a plate is equilibrated, voltage and flow characteristics remain fairly constant. Theoretical calculations support the physical observations. Larger plate widths (5 cm) were also briefly investigated and it is concluded that large width plates could be easily implemented to maintain multiple sample capability. © 2005 Elsevier B.V. All rights reserved.

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1. Introduction

Since the advent of high-performance liquid chromatography (HPLC), thin layer chromatography (or planar chromatography) has been a scarcely used analytical technique for chemists. This is due to faster analysis times, easier quantitative analysis, cost reduction and “push-button” operation of HPLC. The replacement of thin layer chromatography (TLC) was not only due to these tangible qualities required by industry, but also because chromatographic theory allowed for better peak capacities, efficiencies and other chromatographic figures of merit. Although conventional TLC is still widely used for “quick and dirty” analyses, newer TLC methodologies requiring expensive instrumentation and computer automation may not be cost competitive. According to Poole, “At the turn of the century modern TLC faces an uncertain future, while conventional TLC is likely to survive as a general laboratory tool in the same mold as precipitation,

crystallization and distillation, having survived as indispensable, low cost and low technology operations” [1]. The words “thin layer chromatography” haven’t escaped the chromatographer’s vernacular yet because of a few attributes that no other separation technique can easily provide. These include the use of post-chromatographic reactions, reduction of sample preparation and simultaneous multiple sample screening. Another less mentioned advantage is that a TLC plate can be a storage medium for a real separation not just electronic bytes lacking physical meaning [2]. Despite these unique advantages, there are fundamental limitations that restrict the separating power of TLC.

Solvent flow in TLC is driven by capillary action, which is not strong enough to create adequate mobile phase velocities and molecular diffusion becomes a limiting factor for the zone capacity of the system. Also, there is a decrease of mobile phase velocity with solvent front migration distance given by the equation:

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

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where v is the linear front velocity, κ is the velocity constant, Z_f is the solvent front migration distance. As the velocity decreases, a zone migrates less and less until it passes the point where zone broadening (molecular diffusion) surpasses zone migration. This limits the useful TLC plate length to about 8–10 cm for conventional plates and 5–6 cm for high-performance plates utilizing smaller particle diameters. This translates into a zone capacity of around ten, and less in real world applications. In order to take advantage of the unique attributes of TLC, the solvent flow must be constant and closer to the optimum rate.

To date there are three ways known to force the mobile phase through the separation media. The first forced-flow technology was rotational planar chromatography and is based on centrifugal forces. In this system a TLC plate is spun and the mobile phase dripped onto the center of the plate is drawn into the layer by capillary action and pulled outward by the centrifugal forces in combination with the capillary forces [3,4]. The second, over-pressured layer chromatography uses a common HPLC pump to force mobile phase through a plate. In this configuration the TLC layer is sandwiched on the top of the chromatographic phase, and the two sides are sealed so flow will only move in one direction [5–7].

The newest of the forced-flow technologies is planar electrochromatography (PEC). In this technique electrodes are attached to each end of a TLC plate and a potential is applied, creating electroosmotic flow (EOF) when the plate is wet. The idea of using electroosmosis as a mechanism for development of a planar medium can be traced back to Martin [8] and Synge [9]. In 1974, Pretorius was the first to report EOF on TLC plates (not gels) [10] originating what is now PEC. This concept seemed to go unnoticed for 20 more years, probably due to the lack of experimental details provided in that paper. In the mid-1990s papers by Pukl [11] and Poole and Wilson [7] seemed to re-initiate interest in this technique. Research into applying direct current (DC) voltage across wet TLC plates is of the greatest interest [12–18], but other research using initially dry plates [19–22] and alternating current (AC) fields are also being investigated [23–25]. Recently, the first review on the history and techniques of PEC was published [26], recognizing the growing interest in this mode of chromatography.

2. Experimental

2.1. Buffer

All chemicals were supplied by Fisher (Fisher Scientific, Fair lawn, NJ, USA) and water was purified with a Barnstead NANOPure II system (Barnstead International, Dubuque, IA, USA). Buffer was prepared at 100 mM by dissolving the correct amount of sodium acetate into purified water and adjusted to pH 4.7 with acetic acid using a Orion SA520 pH meter (Orion Research Inc., Beverly, MA, USA). This solution was then diluted to 10 mM without a change in pH

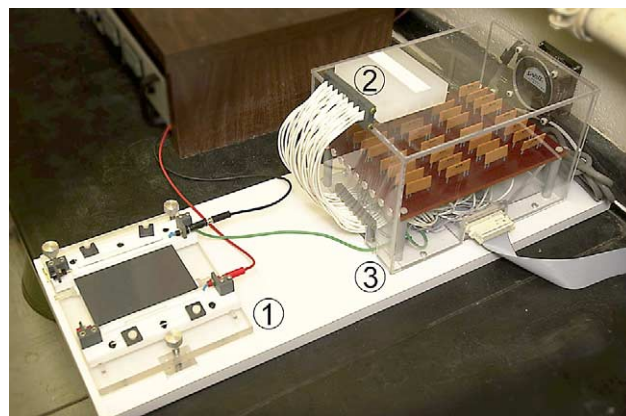


Fig. 1. Picture of planar electrochromatographic instrument. (1) Instrument base and solvent reservoirs, (2) cover grid, and (3) voltage divider box.

(4.7). The buffer was then mixed with methanol in a 50/50 proportion, by volume.

2.2. Instrument

The instrument used was designed and built in house. There are three main sections (Fig. 1), the first is the instrument base containing the power supply (source) electrodes and solvent reservoirs; the second is the cover grid containing the voltage reading (reader) electrodes, and the third section is the voltage divider box that allows transfer of information to the computer. The formica covered wood beneath, aligns the sections in the correct positions and distances.

The instrument base can be seen in closer view in Fig. 2. The base plate (1) is an 18 cm × 23 cm × 1 cm piece of acrylic with three aluminum level adjusters (2). Located on that are two 18 × 4 × 1 Teflon blocks (3) with solvent reservoirs

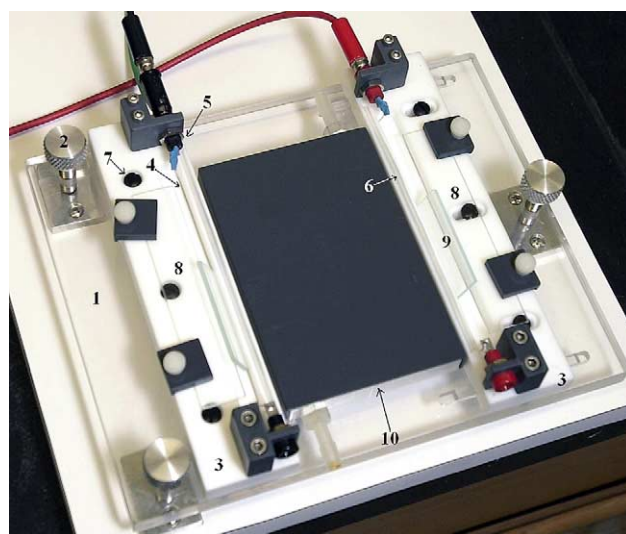


Fig. 2. Detailed picture of PEC base and solvent reservoirs. (1) Base plate, (2) levelers, (3) solvent reservoirs, (4) source electrode, (5) electrode plugs, (6) Tygon seal, (7) reservoir screws, (8) glass slide, (9) vertical glass plate, (10) PVC covered aluminum chiller base.

milled 10 mm wide and 8 mm deep, 5 mm from the inside edge. Placed in the solvent recess is a 0.051 cm (0.020 in.) platinum wire (4) attached at both ends to female banana clip receptacles (5). This is the “source” electrode. Source electrodes are connected directly to a Glassman EH10P10.0 high voltage (HV) power supply (Glassman High Voltage, Whitehouse Station, NJ, USA). This power supply can deliver a 10 kV maximum potential and up to 10 mA maximum current. Between the solvent trough and the inside edge of the reservoir is a length of 0.318 cm (0.125 in.) Tygon tubing (6) recessed into the Teflon, which provides a seal when the TLC plate is placed into position. Without this seal, solvent would weep under the TLC plate and short the system. On the back side of the solvent trough are nylon screws that hold the reservoirs in position (7). Horizontally on top of the Teflon reservoir lays a glass slide (8) and its two PVC hold-downs. When pushed inward, this allows the vertical glass plate (9) to start the solvent flow. This horizontal plate also acts as a solvent cover to reduce evaporation. Between the two reservoirs is a PVC covered aluminum chiller base (10), here, used only as a support for the weight placed on top of the TLC plate. It can also be seen that the left reservoir is stationary while the right is adjustable. This is because most 10 cm TLC plates are actually between 9.9 and 10.1 cm and the moveable reservoir corrects for these fluctuations.

The cover grid is shown in detail in Fig. 3. This picture shows the bottom side of the cover grid where the TLC plate is attached. This cover grid was designed to fit up to 10 cm × 10 cm TLC plates, and shown is a 2.5 cm plate attached to the cover grid such that the second column of reader electrodes is centered on the TLC plate. The cover grid is made from a 10 cm × 10 cm of Kel-F that is 2.85 cm (1.125 in.) thick. Inserted into it are thirty-two 0.051 cm (0.020 in.) platinum reader electrodes in a four column, eight row matrix. The columns are 2.0 cm from the edges and each other, and the reader electrodes within a column are 1.1 cm from the edges and each other (the center spacing is 1.2 cm for symmetry). Each reader electrode is flush mounted to the surface of the Kel-F on the bottom (shown) and the other end

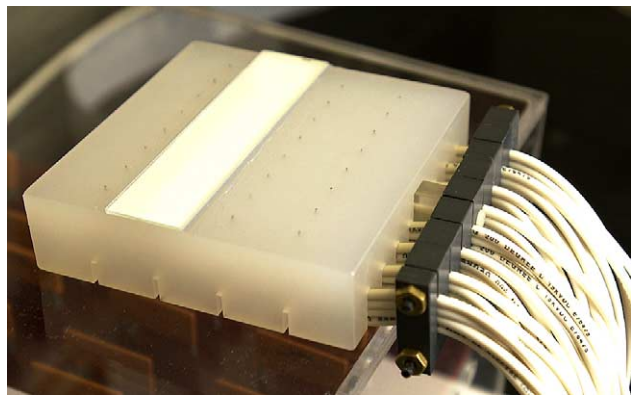


Fig. 3. Detailed picture of the cover grid with a 2.5 cm TLC plate attached. Shown is the bottom side where the reader electrodes are mounted. This is a 4 column-8 row matrix of readers (column 2 is under TLC plate).

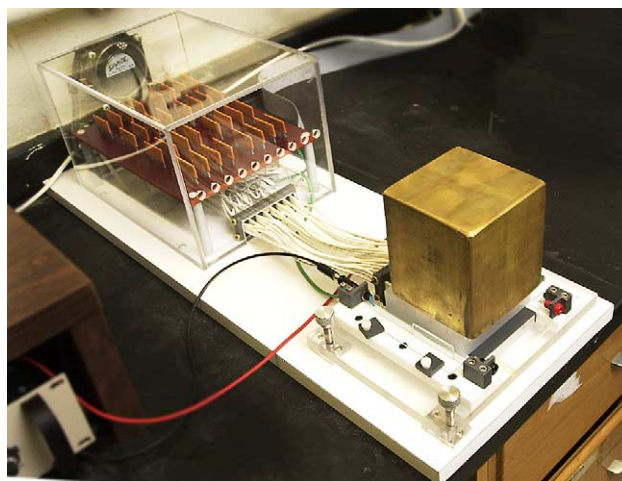


Fig. 4. Picture of the instrument ready for use. Here the cover grid (and TLC plate) is placed into the base and an external force is applied (brass weight).

side is soldered to a high voltage wire and routed to the voltage divider box (Fig. 1). The wiring is run through channels in the cover grid so the applied weight will lay flat on top (see Fig. 4) and routed through the wire guides to the right of the cover grid.

The voltage divider box (Fig. 1) is needed because the voltage from the reader electrodes is too high to route to a computer data acquisition card (DAQ). This is the acrylic box with vents and a fan. The dividers are mounted inside on a phenolic plate far enough apart to dissipate heat and have no arcing. High voltage from the reader electrode to the high side of the voltage divider is a 15 kV rated 22 AWG (7/30) SPC, 0.030 HV-Silicone wire (PN: 39X2215-9A) from Dearborn CDT, Wheeling, IL, USA. The voltage dividers are 10 kV rated 1000:1 dividers from Caddock Electronics Inc., Roseburg, OR, USA (PN: THV10-A100M-1.0-25). The low side of the divider is connected to the DAQ via common 37 pin ribbon cable. The DAQ is an Omega OME-PISO-813 32 channel (single ended) input card (Omega Engineering Inc., Stamford, CT, USA). The software used for monitoring and recording the data is LabView 7.0 (National Instruments, Austin, TX, USA). Data was evaluated with Microsoft Excel (Microsoft, Redmond, WA, USA).

Fig. 4 shows the instrument set-up for use. This illustrates how the cover grid lies on the instrument base and the weight is applied to the top. The pressure of the applied weight is distributed evenly across the TLC plate due to the PVC coated chiller block underneath it. A clear view of the chromatographic layer sandwiched between the glass backing and the Kel-F cover grid can be seen in Fig. 5. Also visible is the means by which the solvent is carried to the plate. The solvent in the reservoir will travel between the vertical glass plate and the wall of the reservoir by capillary action to the TLC plate. Once the plate is completely wet and the reservoirs are filled, sliding the vertical glass plates against the wall of the reservoir completes the electrical circuit that will drive the EOF.

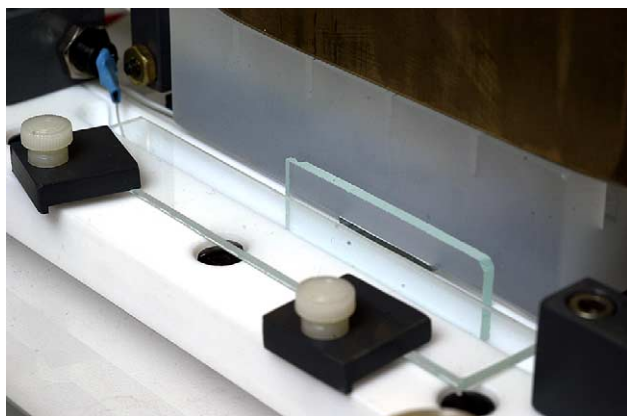


Fig. 5. A close up of how the TLC plate is mated to the instrument. The TLC plate is mounted with the chromatographic layer “face up”, so the glass backing is shown underneath the layer and the Kel-F is above the layer. Under the glass backing is the PVC covered aluminum block (not shown) supporting the applied pressure of the brass weights. The vertical glass plate lies in the bottom of the reservoir and stands vertically against the inside wall of the reservoir and contacts the TLC plate and Kel-F cover grid. The cover glass is lying horizontally on the outside of the vertical glass strip, to reduce solvent evaporation. The solvent in the reservoir will rise up to the TLC plate by capillary action induced between the vertical glass plate and the wall of the reservoir.

2.3. Experimental procedure

The TLC plates used were Analtech HPTLC-RPS plates (Analtech, Newark, DE, USA). These are a reversed-phase impregnated silica (coated, not bound) with a large chain hydrocarbon around C_{30} in size (actual structure is proprietary) that can be readily used in any proportion of methanol/water solvent system without any appreciable loss of the hydrocarbon [27]. Prior to experimentation, the 2.5 cm TLC plate was sealed to the cover grid by the sides using an RTV Silicone sealant (Permatex Inc., Solon, OH, USA) and was given at least 24 h to dry. Sealing the sides ensures that the solvent can only flow in one direction. Previous experiments on unsealed plates showed that the solvent (and voltage) will run off the sides, resulting in poor flow characteristics. Once the sealed plate was dry it was trimmed of any excess sealant and placed onto the instrument. The TLC plate would lay directly on top of the PVC covered aluminum block, and aligned square with the two solvent reservoirs. After it was assured the plate was in correct position, external weight (~ 27 kg) would be placed on top of the grid. This weight created an applied pressure of ~ 1.0 bar (~ 15 psi) inside the sealed chromatographic area and completed the assembly prior to experimentation. At this point buffer was placed into the positive reservoir and the vertical glass strip engaged, allowing buffer to be drawn into the TLC plate by capillary action. After complete wetting of the layer, as noticed by the translucence of wet versus dry silica, buffer was placed into the negative reservoir and that vertical glass strip would be engaged. Then the power supply could be turned on and data recorded.

Once set-up was complete, data would be obtained in the following sequence. The power supply was turned on for

60 min (equilibration run) then turned off for 1–2 h. During this time the solvent was not disconnected from the TLC plate, allowing the plate to stay completely saturated. In fact, the apparatus was not touched at all. After the allotted time, power was restored for 15 min, and then turned off again for another 1–2 h. Again power would be restored for another 15 min, and then turned off and at this point the apparatus would be disassembled and cleaned. The TLC plate would remain attached to the cover grid until it was deemed no longer useful (after the third experimentation day). Removing the plate could disturb the chromatographic bed, altering the flow characteristics for the next day’s experiments. These periods of time where voltage was not applied served two purposes. This enabled the ability to see “wet memory” effects, that is, how well the plate would restore (remember) the flow and voltage from the previous equilibration run. This also assured that no Joule heating was affecting equilibration of the TLC plate. If there were Joule heating, the memory runs would not have the same stable voltage characteristics as the end of the equilibration run. After the apparatus was cleaned, it would sit for 1–2 days, allowing the TLC plate to completely dry, then this procedure would be repeated for a second and third experiment day.

3. Results and discussion

This work is unique insofar as the ability to monitor and record voltage at different distances along an electrochromatographic experiment. To our knowledge this is the first time this has been reported. With this capability a better understanding of how voltage traverses a TLC plate and establishes EOF is possible. Unless otherwise stated, all experiments were performed with an applied field of 1000 VDC. This is a rather low field for an electrochromatographic experiment, but instrumental limitations impeded the increase in applied field. During the time this instrument was constructed it was assumed a small down force upon the TLC plate would be adequate, but our early results showed that we could not achieve voltages higher than 2000 VDC without significant Joule heating and solvent boiling. Since our initial experiments, Nurok’s group has published results with their “pressurized planar electrochromatography” (PPEC) instrument attaining good separations at fields up to 7000 VDC with applied pressures from 50 to 100 atmospheres [28]. This confirmed our belief that higher applied pressures were needed and we are now following their lead into higher pressures.

3.1. Plate equilibration and reusability

The first experiment was to investigate the reusability of a single plate. Although TLC plates are not designed for multiple uses due to the hindrance of solute elution off a plate under capillary mediated flow, a forced flow technique does allow for full elution and therefore could possibly be used for multi-

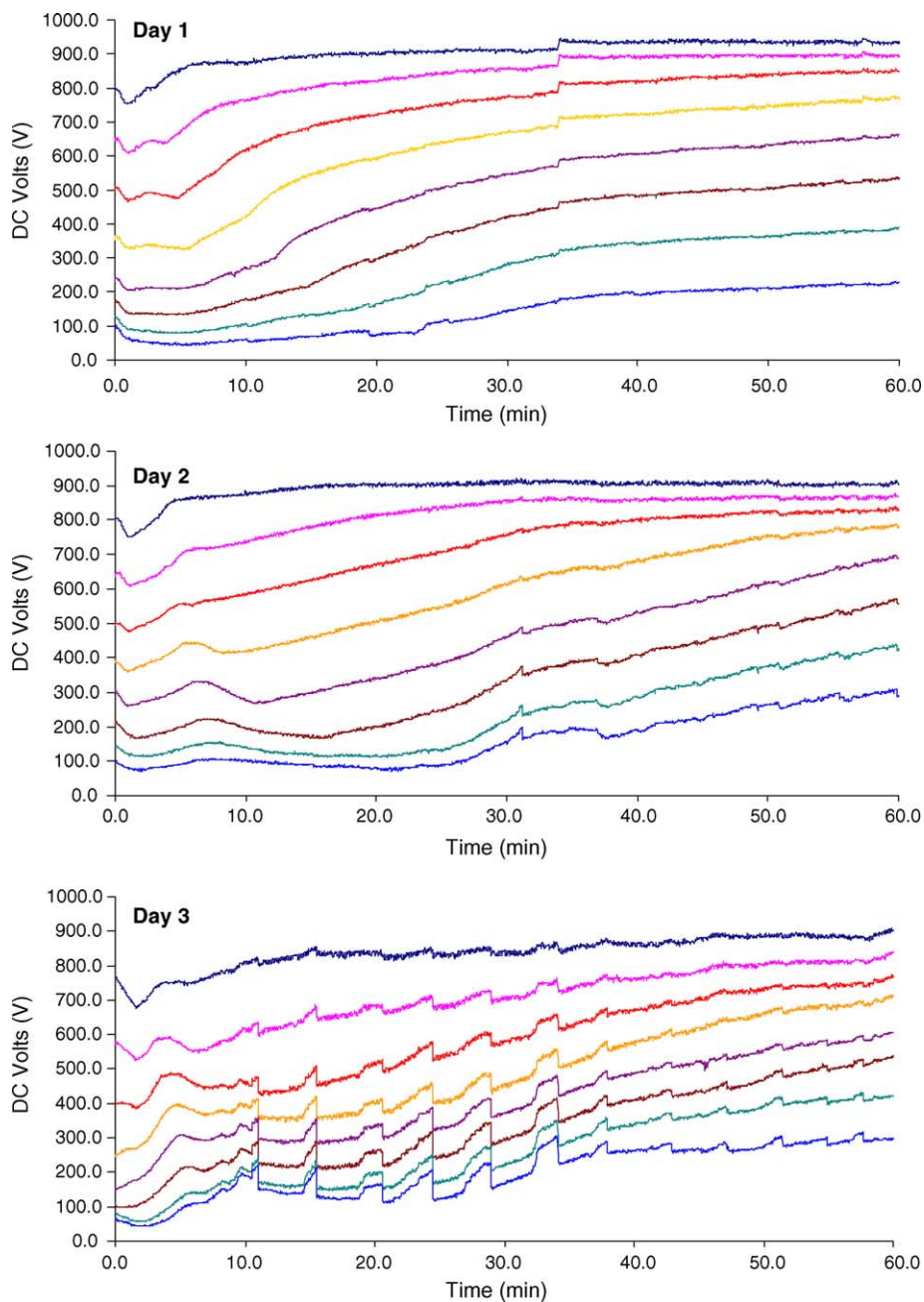


Fig. 6. Plate 1 equilibration effects with 1000 VDC applied. Each data series corresponds to an individual reader electrode. The data series are in sequential order, where the top series is 1.1 cm from the positive reservoir all the way to the bottom series representing 8.9 cm from the positive reservoir.

ple analyses. For these experiments a TLC plate was attached to the instrument, completely wetted with the run buffer and 1000 VDC was applied. It was previously determined that 60 min was sufficient for equilibration of the voltage characteristics across a plate. It should be noted here that “plate equilibration” here refers to the time it takes for a TLC plate to exhibit constant voltage at all reader electrode positions. This is not to be confused with the term used by other authors to describe “plate pre-equilibration” or “conditioning” of a TLC plate by thermal pre-treatment and/or pre-soaking the plate in the run buffer prior to PEC [13,16,18,26,29].

The day-to-day results of a single plate can be seen in Fig. 6. On the first day of experiments there are nice smooth “S” shaped curves for each reader electrode positioned on the TLC plate. The top data set represents the reader electrode closest to the positive reservoir (1.1 cm from positive) and follows in order to the bottom curve representing the reader closest to the negative reservoir (8.9 cm from positive). There is an equilibration time for each distance, the shortest equilibration time at the highest potential (~10 min) and longest at the lowest potential (~40 min). As the distance from positive source increases so does the time before the first inflection of

the “S” curvature, as well as decreasing slope of the curve. We believe this data represents a slow establishment of an EOF front. As solvent is allowed to initially wet the plate by capillary action, buffer ions move slower than the solvent front and there may be a buffer gradient along the TLC plate. When a field is applied the buffer starts to move and eventually enough buffer reaches the whole plate. This buffer movement would also bring with it a greater EOF velocity that could justify these inflection points. Also, in PEC there is a much greater solvent volume that could cause a slow response or lag time much like a steam locomotive trying to start with a heavy load where the steel wheels spin on the track before the train moves properly. Due to the large surface area and solvent volume, the first moment that voltage is applied to a plate buffer ions may take time to locate charged sites on the silica (and binder) to maintain a constant electrical double layer (and EOF) at that point. In reality the observed phenomena is most likely a combination of all the above effects (the last being small compared to the others). Therefore, the first point of inflection on each “S” curve may show the establishment of correct EOF at each position across the plate. This is not to say there is no EOF until this inflection is reached, but it may be the onset of a greater EOF velocity. The data also shows a small spike at around 35 min. Over the course of the experiments this was seen many times and has been found that hitting the benchtop can cause small perturbations in the instrument, most likely causing small movements in the vertical glass strips. Also if air bubbles form at the right (90°) angle between the vertical glass strip and TLC plate, hindering the intake of solvent, this can also cause small voltage perturbations.

The 60 min equilibration run for the second day of experiments illustrates a slightly different trend than seen in Day 1. The beginning looks the same for all readers and the top three data sets look alike for both days, but the bottom five do not level off as they did in Day 1. There is more noise in each trend and some slight fluctuations not seen before. This is believed to be from the residual buffer from Day 1 experiments. After Day 1 was completed the plate was removed from the instrument but remained attached to the cover grid by the silicon sealant. In this configuration the plate could not be removed for rinsing, and any internal rinsing by capillary action would have created another buffer gradient. The third day of experiments shows even more inconsistencies. All the curves have even more noise and greater fluctuations. Again this is attributed to the additional buffer from the prior experiments. Voltage spikes are common on this day and are believed to be caused by buffer precipitation, not instrumental shifting. These trends seen during the three experimentation days were reproducible from plate to plate. It is apparent that the first day gives the best results and each subsequent day provides less reliable information. Therefore, in this configuration the third experiment day was not used for further investigation. However, if a different configuration was adopted with the means of adequate rinsing, there might be a longer lifetime for each plate.

3.2. Voltage drop

According to the Smoluchowski equation, the EOF for a channel (with the assumption that the size of the double layer is small compared to the channel size) is given by:

$$u_{eo} = \frac{\varepsilon_r \varepsilon_0 \zeta E}{\eta} \quad (2)$$

where u_{eo} is the electroosmotic flow velocity; ε_r , the dielectric constant; ε_0 , the permittivity of vacuum; ζ , the zeta potential; E , the applied electric field and η is the viscosity of the mobile phase. Zeta potentials in different chromatographic systems have been estimated at 20–200 mV [30,31]. Under the assumption that the layer on a TLC plate is a network of intertwining channels, this equation has been the basis for fundamental understanding of EOF in PEC. The E term (Eq. (2)) is expressed as the linear voltage drop across the plate (or capillary) in V/cm where 1000 VDC applied over a 10 cm distance should show a 100 V/cm drop across the plate. The data shown in Fig. 7 shows a different trend. Instead of a constant 100 V/cm drop we see a curve that changes with time, at least during the equilibration period. The beginning of the equilibration run shows concave quadratic curvature, changing to sigmoidal during the middle and as plate equilibration finishes turns into a convex quadratic curve. During equili-

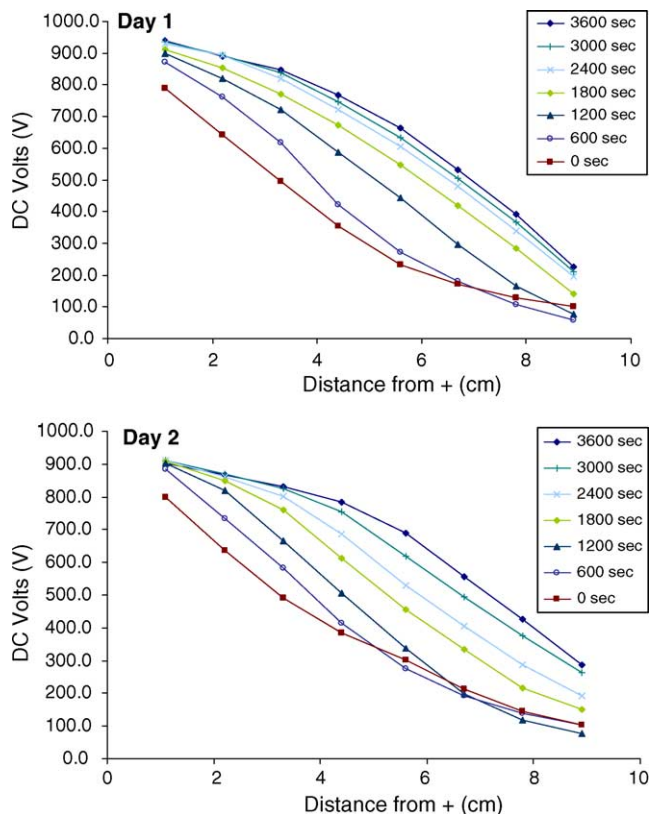


Fig. 7. Plots of voltage drop during the 60 min equilibration runs. Distance is measured from the positive reservoir to the reader electrode. These illustrate a nonlinear changing behavior over time, ending in a quadratic decreasing curve after plate equilibration.

bration the voltage drop can be localized because a buffer gradient exists, therefore there are more volt carriers at the positive (top) side of the plate and this changes over time, until equilibration and a constant EOF has been established across the whole plate. Once there is equilibrium across the plate there should exist the linear voltage drop provided by theory. It stands to reason that instrumental inadequacy has caused the observed curvature. There are not many PEC instruments being tested and developed, so it may still be some time before theory and experiment coincide. Nurok's group achieved constant EOF (and assumedly voltage drop) with their pressurized planar electrochromatographic instrument [28]. Their instrument was run at pressures greater than 50 atmospheres, which are not achievable with our current design. Consequently, higher pressures may be required for a better voltage profile.

To look at this data in a different dimension a linear regression equation was fit to voltage drop profiles at every 100 s and the data was graphed (Fig. 8). These graphs illustrate the linearity of the system and it is obvious that there is an ever-changing environment. This also defines more differences in experimentation day, where the first 2 days are quite similar in regression and correlation coefficients and the third day is not nearly the same. This reinforces the decision not to include the third day's data for further investigation. Also noticeable is how this data is compared to the ideal values. Overall the data does come close and further instrumental development is needed to assure a truly linear voltage drop where the regression slope and intercept are representative of the applied voltage.

3.3. Wet memory

On Day 1, after the 60 min equilibration run and sufficient time with no applied potential, the instrument was turned on again to see how the EOF would be re-established. These experiments can be compared with those that "pre-equilibrate" or "condition" a TLC plate prior to PEC as mentioned earlier, since any excess ions from manufacture and/or incomplete solvation of the stationary phase would have been eliminated in the prior 60 min runtime and subsequent standby time.

These results (Fig. 9) show a much more constant voltage versus time relationship at each reader electrode, which was expected since the plate was previously equilibrated. Also, the plots of voltage versus reader electrode distance (Fig. 10) holds the same convex curvature seen after equilibration during the 60 min run. What were interesting to see were the small "S" shape curves still present at each reader position (Fig. 9). This further reinforces the belief that this phenomenon mimicked the EOF. If the distance at which the reader electrode was positioned was plotted against the time of the onset of "S" curvature (from Fig. 9), the velocity of this front can be calculated. The graph of this (Fig. 11) shows a clear quadratic dependence ($R^2 = 0.9986$), but for the sake of calculation we are going to assume a bi-linear function,

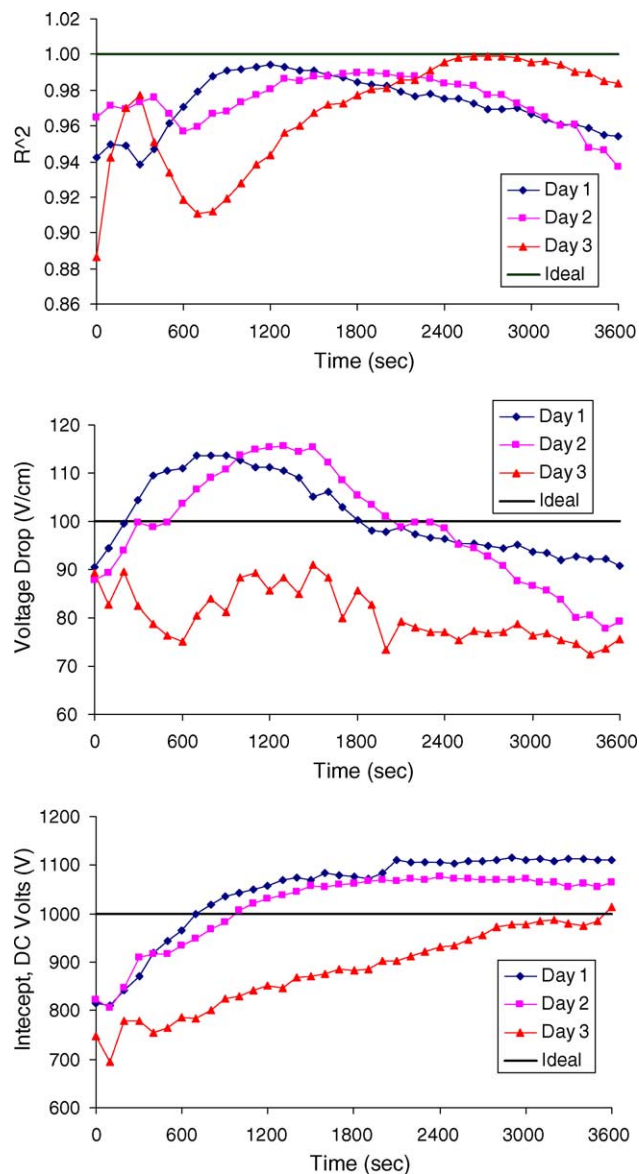


Fig. 8. Graphs of linear regression variables vs. time for 3 days of experiments, as well as the ideal values for each graph. Graph A represents R^2 vs. time, B represents voltage drop (slope) vs. time and C represents the intercept vs. time. Ideally a slope of 100 V/cm and intercept at 1000 V should be seen.

such that the first four and last four data points represent two individual linear functions (although it would be more accurate to take a tangent at every data point the meaning and purpose become lost). The linear correlation coefficients ($R^2 = 0.9986$ and 0.9964) do credit this assumption. Assuming linear functions, velocities can be calculated (represented by the slope of the line) and are 1.1 cm/min through the top half of the plate and 2.0 cm/min through the bottom half of the plate. For independent confirmation that this could be the correct EOF velocity, data after the front passed the whole plate (14 min) was used to find the voltage drop across the plate and used to solve Eq. (2) for EOF velocity. As with the previous graph, there was a clear quadratic curvature in

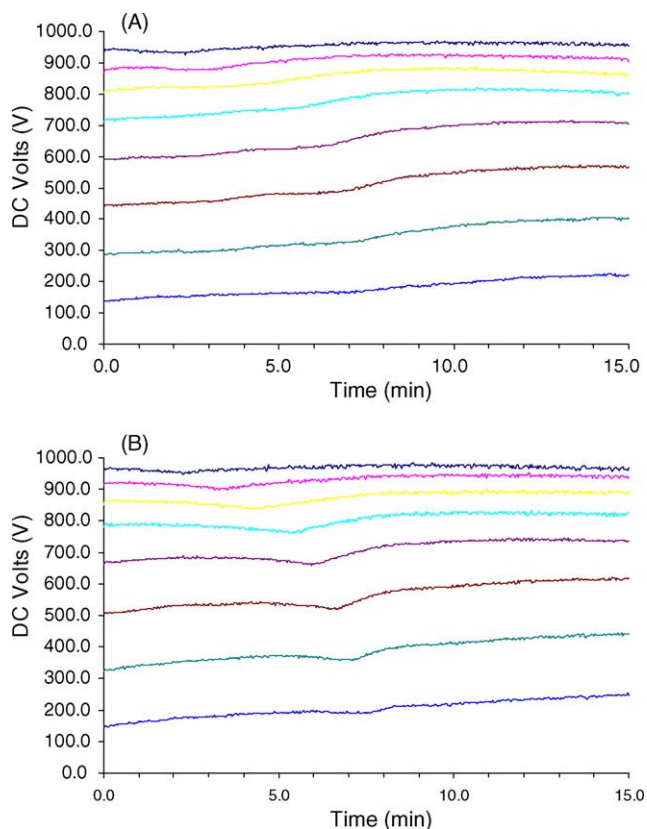


Fig. 9. Post equilibration voltage trends. Graph A represents the first post equilibration run and B the second. The data series in each graph are in sequential order, where the top series is 1.1 cm from the positive reservoir all the way to the bottom series representing 8.9 cm from the positive reservoir. These trends illustrate more stable voltage profiles at each distance across the plate. Also apparent are the inflections showing the EOF profile.

Table 1
EOF Velocities calculated by different means

	Top half of plate	Bottom half of plate
Day 1		
Velocity by voltage drop (cm/min)	0.7	2.0
Velocity by inflection (cm/min)	1.1	2.0
Day 3		
Velocity by voltage drop (cm/min)	0.8	2.2
Velocity by inflection (cm/min)	1.0	2.2

the relationship of voltage drop versus distance (Fig. 12) and again we assumed a bi-linear function. Again the correlation coefficients ($R^2 = 0.9987$ and 0.9699) do credit our assumptions. The top half of the plate shows a voltage drop of ~ 50 V/cm and the bottom half shows a drop of ~ 150 V/cm. Plugging these numbers into Eq. (2) (assuming $\zeta = 0.070$ V)¹ gives 0.7 cm/min at the top half of the plate and 2.0 cm/min for the bottom half of the plate (see Table 1). These post-equilibration experiments were performed on Day 2 and the

¹ Although the zeta potential is arbitrarily chosen it does fall within the limited range of accepted values noted earlier, which helps justify the assumption that EOF is mimicked by these inflections.

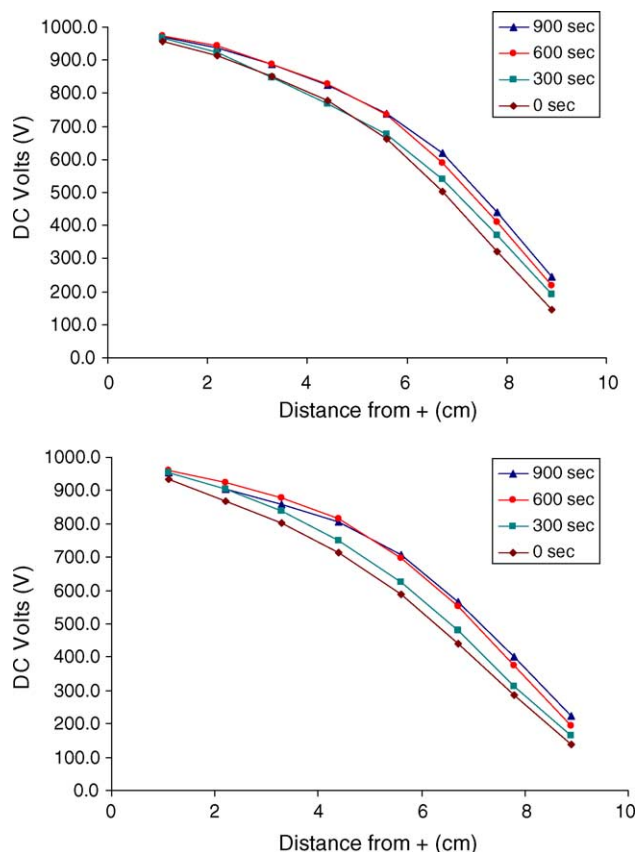


Fig. 10. Post equilibration trends in voltage drop. The top graph is the first post equilibration run and the bottom is the second. Distance is measured from the positive reservoir to the reader electrode. This illustrates the mostly constant concave quadratic curvature in voltage drop for up to 4 h after equilibration.

same quadratic curves for determining velocity by inflection and voltage drop were seen. Using the bi-linear assumption, these results are also listed in Table 1. The zeta potential (ζ) for Day 2 was assumed to be 0.100 V and this change can be correlated to the increase in buffer concentration on the second day. By calculating velocities of flow in two independent ways, one based on the time inflections were seen, the

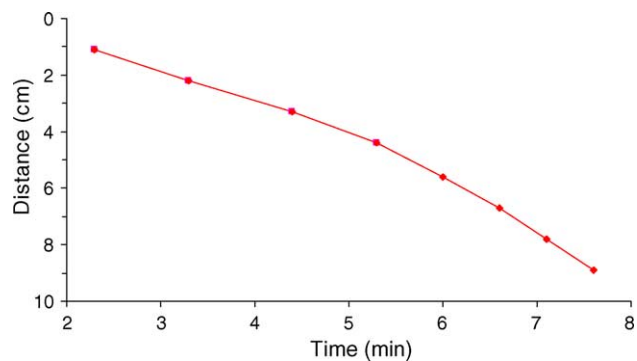


Fig. 11. Graph of distance (from positive reservoir) against time the inflection occurs (from Fig. 9 at each reader along the plate (Day 1)). Using a bi-linear fitting function, the two slopes (1.1 and 2.0 cm/min) represent the changing EOF velocity as it moves down the TLC plate.

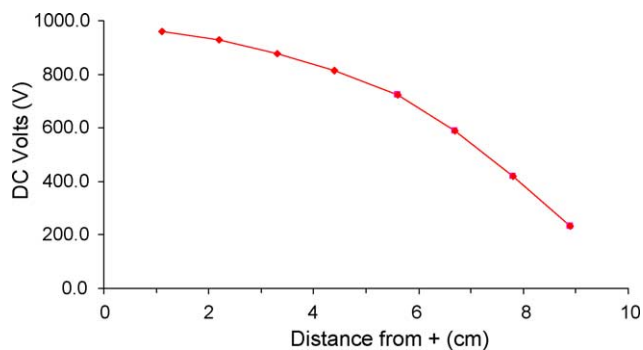


Fig. 12. Graph of voltage vs. distance (from positive reservoir) used to find the voltage drop across the plate (Day 1). Again a bi-linear function was used to obtain two slopes (50 and 150 V/cm) and these values were subsequently used to calculate the EOF velocity (0.7 and 2.0 cm/min).

other based on voltage drop across the plate at a fixed time lends proof that these inflections seen in the data are EOF markers as it progresses across the plate. Note that in the post-equilibration runs these inflections are mostly marking the EOF not establishing it, as it was in the 60 min equilibration runs. The difference is seen in the voltage versus time graphs. In the 60 min runs the inflection point marks the beginning of a great change in the voltage, significantly changing the EOF at that point. In the post-equilibration runs there are only minor voltage changes for each reader that alter the EOF slightly, but rather these small inflections are markers of EOF velocity as it crosses the plate.

3.4. Larger plate widths

One of the greatest attributes of TLC is its ability to perform simultaneous separations. In order to keep that attribute we are investigating the use of larger plate widths for use in PEC. As the plate width increases, the electrical resistance across it decreases requiring larger current to hold the same potential. As a result the current on a 5.0 cm TLC plate ranged from 2 to 4 mA on average, where a 2.5 cm plate would average about 1–2 mA for the same 1000 VDC potential. As current is increased, Joule heating can become a problem. Using a 5.0 cm plate with 1000 VDC applied; if the current surpassed ~ 4 mA the plate would become warm (or hot) to touch. This heating changes the properties of the binary solvent (viscosity, dielectric constant), and therefore changes the EOF. The current normally did not break this threshold on the first day of experiments, but by the third day it could become a problem (buffer accumulation).

Using the same instrumental conditions previously described we attached a 5.0 cm TLC plate to the cover grid. Here the plate was attached to the center of the grid having two columns of reader electrodes available for data acquisition. Reader electrodes were positioned 1.0 cm from the center of the TLC plate on each side and were 1.5 cm from the edges. In this configuration 50 kg of weight was added on top of the cover grid to try and keep the same pressure on the plate (~ 1.0 bar). During the equilibration hour the two

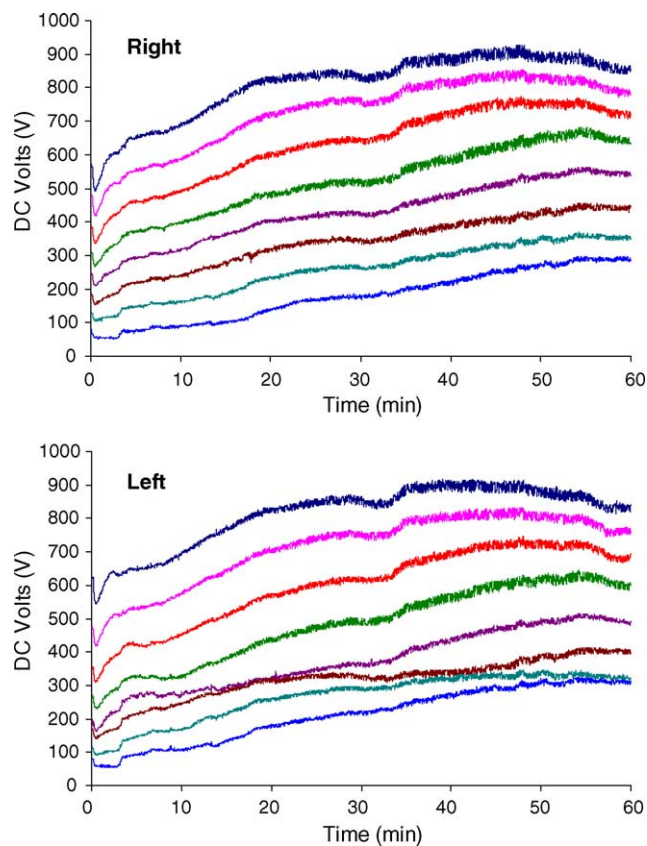


Fig. 13. Data shown for a 5.0 cm wide HPTLC plate. The top graph shows the voltage profiles for the right column of reader electrodes and the bottom shows the left. Note the similarities in most data series, except the fourth from the bottom (reader 5.6 cm from positive reservoir). Again, the data series are in sequential order, where the top series is 1.1 cm from the positive reservoir all the way to the bottom series representing 8.9 cm from the positive reservoir.

columns of reader electrodes displayed quite the same curves for most data sets (Fig. 13). There were some discrepancies in the reader positioned 5.6 cm from positive and looks incorrect only on the left column. This may have been caused by non-homogenous amounts of binder or residual ions in the chromatographic phase from manufacture or mishandling of the plate prior to use. Regardless, after the plate was equilibrated this anomaly disappeared and does not diminish the quality of data. This hour equilibration resembles those presented earlier with the general increase and leveling of the voltage curves. The post-equilibration runs also are similar to the 2.5 cm plate data with very steady voltage at each reader and the inflections within each set of data (Fig. 14). These inflections appear earlier in the data sets than with 2.5 cm plates but the 5.0 cm plates were more difficult to reproduce and it is not clear whether the flow was faster or if Joule heating affected these profiles. Trends between left and right columns are super-imposable assuring that there is only a voltage gradient between buffer reservoirs, and not between the sides of the plate. Unwanted voltage gradients would induce localized flow in the direction of greatest voltage drop, and solutes would not migrate in a straight line. The use of larger

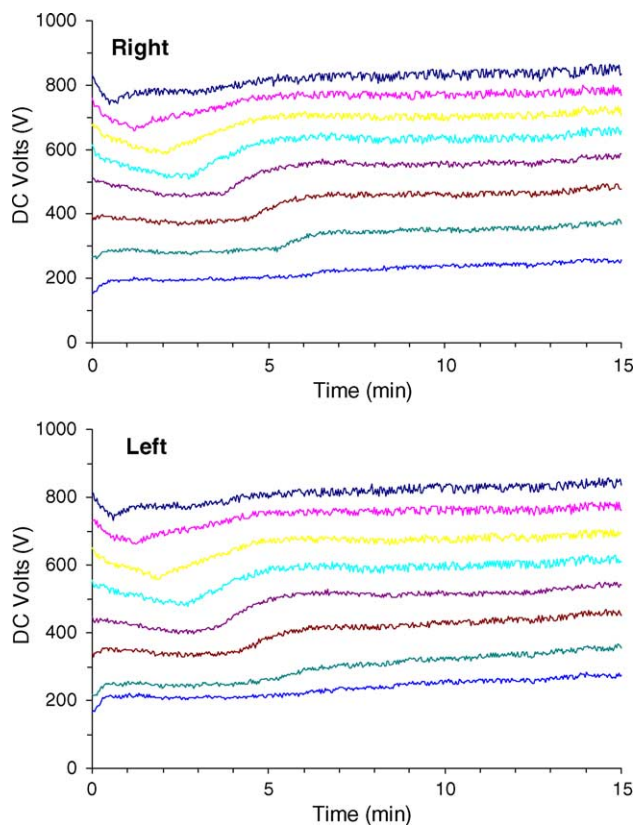


Fig. 14. Post equilibration run for 5.0 cm plate. The top graph is the right column of reader electrodes and the bottom graph is the left. Again, the data series are in sequential order, where the top series is 1.1 cm from the positive reservoir all the way to the bottom series representing 8.9 cm from the positive reservoir. This illustrates the ability of a plate to hold the same voltage profile regardless of path down the plate.

plate widths would also benefit from a higher-pressure apparatus by enabling larger applied fields without solvent boiling and reducing the amount of solvent in the chromatographic layer.

4. Conclusions

This work furthers understanding of the relationship between voltage and solvent flow in planar electrochromatographic experiments. The unique cover grid allows measurement of detailed information about voltage profiles and characteristics of a TLC plate under electroosmotic forces. It was observed that there is an equilibration time for these TLC plates in the instrument described above. This may be caused by both the buffer gradient established when initially wetting the chromatographic phase and the large volume of solvent needed to be moved (locomotive effect), with possible minor effects of double layer establishment lag time. Also observed was the voltage drop across the plate where the initial application of potential caused a concave quadratic decrease evolving into a sigmoidal curve and once equilibrated, sus-

taining a convex quadratic decrease. It was deduced that instrumental inadequacy was the cause of the non-linearity. Following equilibration, the plate was shown to be robust in re-establishment of the voltage drop and EOF, for at least 4 h after equilibration. Once a plate was dried (overnight) and re-wet, the hour-long equilibration would need to be performed again. After 2 days of experimentation the plate could not be used further because of buffer build up. A different experimental configuration may allow more replicates, but is not a disadvantage since most TLC analyses are not performed in an elution mode.

During post equilibration experiments an empirical relationship between EOF velocity and inflection point was determined and correlated to the theoretical EOF velocity by means of the Smoluchowski equation. Trends in this data are clearly quadratic ($R^2 > 0.99$) and unfortunately cannot be solved as such. Theory requires a linear voltage drop to solve the Smoluchowski equation and a linear relationship is necessary for distance versus time (giving a slope of velocity). Assuming a bi-linear relationship where the top and bottom halves of the TLC plate are individual linear functions, the equations can be solved. Although the data sets are small ($n=4$ for each) the results of these calculations show the intended relationship quite well. The calculated EOF velocity from the voltage drop (0.7 and 2.0 cm/min) was relatively the same as the observed velocity by means of inflection points (1.1 and 2.0 cm/min) (first number in parentheses is top half velocity, second is bottom half velocity). These inflections signify some front starting at the top of the plate when the power is turned on, like a wave riding the EOF.

Larger plate widths were briefly investigated here and determined that there is very realistic chance of attaining simultaneous separations on large width plates (10 cm and higher). The data presented illustrated two different voltage reader columns and, once plate equilibrium was established these data were virtually identical. This assures that the same EOF will be seen on either side of the plate as movement goes from top to bottom. Any voltage gradient from side to side would cause localized flow perturbations and send an ongoing separation off its straight-line path causing a chromatographic crash.

All data presented here gives useful information about solvent and voltage flow characteristics, as well as information on further design and capabilities of this type of instrument. Better understanding of instrument performance allows fixing those shortcomings and inadequacies. Some of the data presented here not only allowed us to see flow characteristics, but also see the instrumental flaws, that could not have been seen without the cover grid and reader electrodes mapping the pathway. We believe higher external pressure is the key to an instrument that will perform with more linear characteristics. Nurok et al. [28] have shown that higher pressures allow higher voltages, and hence better separations and our ability to see inside the separation will allow further information for designing an adequate instrument.

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