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Electroosmotically driven thin-layer electrochromatography on silica media

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Abstract

Electroosmotic solvent flow has been demonstrated in planar chromatography media. The application of a 800 V cm^{-1} field along conventional silica thin-layer chromatography plates has been shown to give solvent migration rates of 0.039 and 0.210 cm s^{-1} with ethanol and acetonitrile, respectively. Electroosmotic elution has been applied to the separation of pirimicarb and a number of related compounds by planar electrochromatography. The elution characteristics of these compounds were similar to those obtained by conventional thin-layer chromatography, yielding the same elution sequence, but more rapidly. Whilst these compounds are separated by using conventional thin-layer chromatography, they require an elution time of ca. 18 min and comparable electrochromatographic separations can be achieved electroosmotically in only 90 s; this corresponds to a 12-fold increase in elution rate. Reduced band-broadening is also evident with the electrochromatographic elution. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Electrically driven separation techniques have a long history. Whilst electrophoresis has become a crucial technique in most biochemical laboratories, until recently few electrically driven separation techniques had gained popularity in the separation of smaller molecules in the chemistry laboratory. In the past two decades, however, we have seen the development of a number of new techniques, most notably in the form of capillary electrophoresis, capillary electrochromatography, and their many variants. These have introduced modes of separation which were not previously available. Much of this growth can be traced to the development of inert capillary columns produced as a spin-off from fibre optic technology and the commercialization of electrophoretic instrumentation for isotachophoresis.

In electrically driven chromatography the electric field can be employed to induce both solute and solvent migration. Electroosmotic solvent flow (EOF) arises from the formation of an electrical double layer at a solid–liquid interface, an effect first described and explained in 1910 [1]. Under the influence of an electric field parallel to the solid surface, the diffuse layer moves due to the electrostatic force on the excess ions, dragging with it the solvent near the solid surface.

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The origins of electrically driven chromatography can be attributed to the work of Pretorius et al. [2], who in 1974 reported the use of high potential gradients to effect very rapid separations on reversed-phase chromatographic media. Whilst results were reported for separations both on TLC plates and in open columns, only the column work was subsequently fully developed, resulting in modern capillary electrochromatography [3–6]. Attempts to reproduce and further develop the work based on planar media have been few and far between. This is probably attributable, at least in part, to the limited experimental detail in the original publication.

The paper by Pretorius et al. [2] described an experimental set-up utilising vertically mounted silanized (presumably largely reversed-phase) TLC plates, with a solvent reservoir at the base. They were pre-wetted with solvents such as benzene, toluene and hexane [7]. An electric field strength of around 1–2 kV cm^{-1} was applied between the top and bottom of the plate. Separations that would normally take 60 min were reported to occur in 4 min and this enhanced solvent migration rate was believed, by the investigators, to be due to electroosmotic flow.

Very recent work by Nurok et al. [8] has employed a similar vertical experimental set-up to that used by Pretorius et al. The reversed-phase chromatography plates were pre-wetted with water–ethanol, a solvent combination which is more likely to exhibit electroosmotic flow than the original non-polar solvents employed by Pretorius et al. Migration rates of up to 2 cm min^{-1} were obtained, which were again attributed to electroosmotic flow. No attempt was made, however, to differentiate between migration due to electroosmosis, electrophoresis and evaporation, any or all of which could account for the observed solute migration.

Our recent work [9] has demonstrated that evaporation-induced solvent migration is a major cause of solvent flow in vertically mounted thin-layer electrochromatography (TLE). This has cast doubt on whether the early planar separations were ever due to electroosmotic solvent migration.

In view of the ambiguities that have arisen from previous studies of electroosmotic solvent migration in planar chromatography, a new study was undertaken, which employed both normal phase chromatography media and solvents that had the capability of supporting electroosmotic flow. Here we describe a method of generating high, sustainable rates of genuine electroosmotic flow through normal phase TLC plates and demonstrate the technique of planar electroosmotic chromatography by the study of the migration characteristics of the pesticide pirimicarb and a set of related compounds.

2. Experimental

2.1. Apparatus and materials

2.1.1. Development chamber

The experiments were carried out in a specially constructed development chamber machined from a solid block of PTFE, and covered with a PTFE lid incorporating a glass viewing window (Fig. 1). Plates were supported horizontally in the chamber, with a solvent reservoir at each end of the plate. Solvent was transported to and from the plate by two

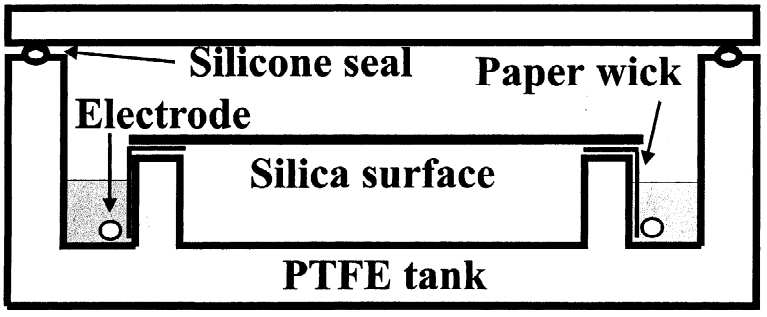


Fig. 1. Horizontal development chamber.

filter paper solvent wicks (Whatman No 1, Whatman International Ltd., Maidstone, UK). Electrical contact to the solvent was made via two stainless-steel electrodes, 3 mm in diameter, which were submerged in the solvent.

2.1.2. Power supply

A Brandenberg Alpha 807R power supply was employed in the work (0–30 kV, 1 mA, Brandenberg, West Midlands, UK). A safety interlock was constructed to disable the power supply if the chamber lid was removed and an ammeter was included in series with the supply for monitoring current flow. Current could be continuously recorded during a run by employing the voltage drop across a 100 Ω resistor to drive a plotter (Fig. 2).

2.1.3. Chemicals

Reagent grade ethanol, diethyl ether and acetonitrile (Aldrich Chemicals, Gillingham, UK) were redistilled before use. The deionized water had a specific resistance of ca. 18 MΩ cm. β-Carotene (Fluka Chemicals, Gillingham, UK) was used as an electroosmotic flow marker and was prepared as a 2 mg ml^{-1} solution in diethyl ether. Pirimicarb and its related compounds (Fig. 3) (Zeneca Agrochemicals, Berkshire, UK) were prepared as saturated solutions in ethanol.

2.1.4. Chromatographic media

Unmodified silica-gel TLC plates (glass backed, 4 nm pore size, 250 μm layer thickness, Phase Separations Ltd., Deeside, UK) were used in the experiment. They were cut into 25×75 mm strips, washed in deionized water for 1 h, rinsed in deionized water for a further 1 h, and then oven dried overnight at 80°C.

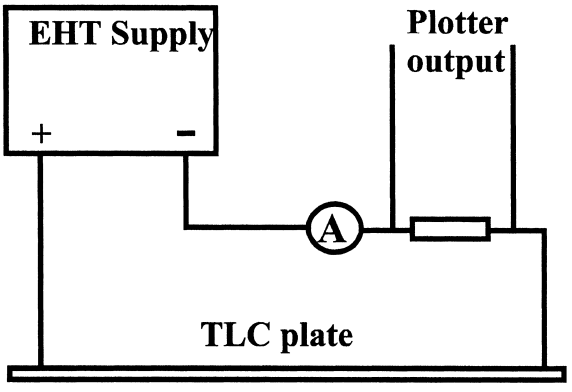
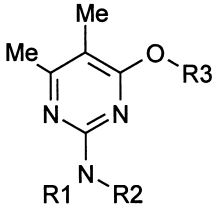


Fig. 2. Electrical arrangement for thin-layer electrochromatography.



	R1	R2	R3
Pirimicarb	Me	Me	$CO.N(Me)_2$
Compound 1	CHO	Me	$CO.N(Me)_2$
Compound 2	H	Me	$CO.N(Me)_2$
Compound 3	Me	Me	H
Compound 4	H	Me	H
Compound 5	H	H	H

Fig. 3. Structures of pirimicarb and related pyrimidines.

2.1.5. Solvent wicks

Solvent wicks constructed from Whatman No.1 filter paper (Whatman International Ltd.) were oven dried at 80°C overnight. New solvent and solvent wicks were used with each plate.

3. Methods

The plates were manually spotted, the sample being applied in a row of spots across the plate. Sample spots were applied across the centre of the plates used in determining flow–voltage relationships and 15 mm from the positive end of the plates used

in the separation of pirimicarb analogues. After the application of a sample to the plate it was left to dry for 2 min, then dipped briefly in the elution solvent. The plate was then placed in the development chamber with the silica surface facing downwards, making contact with the paper wicks at the solvent reservoirs. A potential difference was applied across the plate. At the end of the run, the power supply was switched off, the plate was removed from the chamber, allowed to dry, and the position of the spot was noted by viewing under a UV lamp. Controls were run, with no applied potential, and with the polarity of the potential reversed.

For the determination of the migration velocity–applied potential relationship, a neutral coloured marker, β-carotene, was used. It was necessary to run different plates for different lengths of time, in order to obtain measurable migration distances whilst not eluting the sample off the end of the plate. In experiments using acetonitrile, plates run at 1–3 kV runs were completed in 20 s, while the 4–6 kV runs were carried out for 10 s. Plates eluted with ethanol were all run for 1 min. Conventional thin-layer chromatography of the compounds was also carried out in order to establish the retention behaviours of β-carotene and the pirimicarb analogues when eluted with ethanol or acetonitrile.

In order to determine the optimum potential for separating pirimicarb and its related compounds, two series of plates were run with both ethanol and acetonitrile. Optimum performance was obtained with ethanol at 7 kV (930 V cm^{-1}) giving a run time of 90 s.

4. Results

4.1. Effect of plate conditioning on plate resistance

Current flow through the chromatographic media is a fundamental aspect controlling both solvent flow and heating effects. Not only can localised heating effects drive solvent flow, but the Joule heating of the plate results in the loss of the solvent from the plate. It is therefore important in thin-layer electrochromatography that plates are freed of ionic contaminants that would prevent a constant current flow through the adsorbent layer. In early experiments it was noticed that not only were currents excessively large, but also that they changed with time. As this would deleteriously effect the electroosmotic solvent flow, experiments were carried out to both assess the nature of this effect and to explore ways by which the current could be better controlled. When an ethanol wetted plate was investigated in greater detail it was found that within a minute of the application of the applied potential the current rose to a maximum of ca. 1 mA but that the current then fell off to less than 500 μA within another minute (Fig. 4).

This current rise did not re-occur when the plate was re-used, implying that contaminant material had migrated off the plate under the influence of the applied potential. Washing the plate in deionized water prior to use was found to have the effect of removing these contaminants, resulting in a greatly improved current–time profile (Fig. 4).

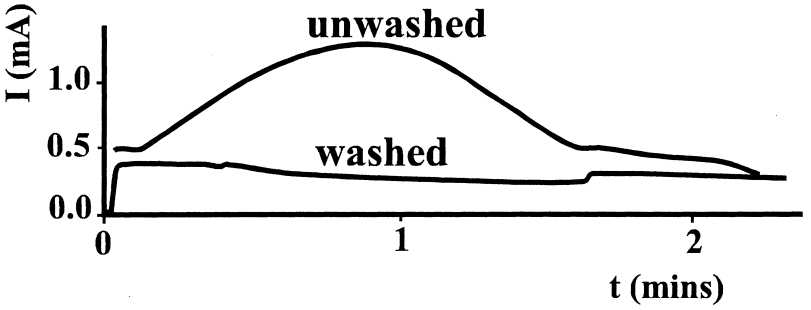


Fig. 4. Current variation using untreated and washed plates.

When assessing the solvent migration velocity by measuring the migration of a neutral marker, the R_f of that marker for the solvent–support system must be known. This was determined for both ethanol and acetonitrile, and was found to be 0.95 and 1.00 ± 0.02, respectively. In order to correct for chromatographic retention the measured spot migration velocities were divided by the appropriate R_f, giving the 'unretained' migration velocity. The R_f-corrected velocities are shown plotted against applied potential for plates run with acetonitrile and ethanol (Figs. 5 and 6).

Migration velocity was found to increase in an approximately linear fashion with increasing potential, for both ethanol and acetonitrile. The migration velocity achieved with acetonitrile was approximately 5–6 times that obtained with ethanol.

4.2. Separation of pirimicarb analogues

In order to demonstrate the potential of electroosmotic solvent pumping in planar chromatography a group of compounds related to the pesticide pirimicarb was investigated. These compounds were eluted with ethanol on identical silica-gel TLC plates using both conventional vertical thin-layer chomatography using capillary solvent flow and also

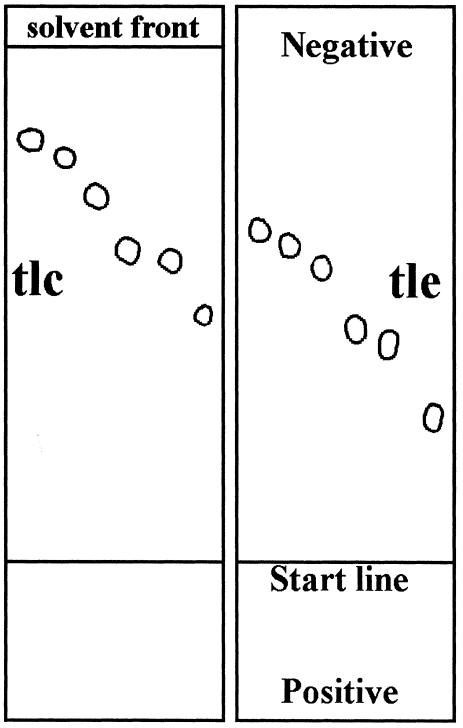


Fig. 6. Comparison of standard TLC (left) and electroosmotically driven TLC of pirimicarb (far left of each plate) and related compounds (1–5, to the right of pirimicarb).

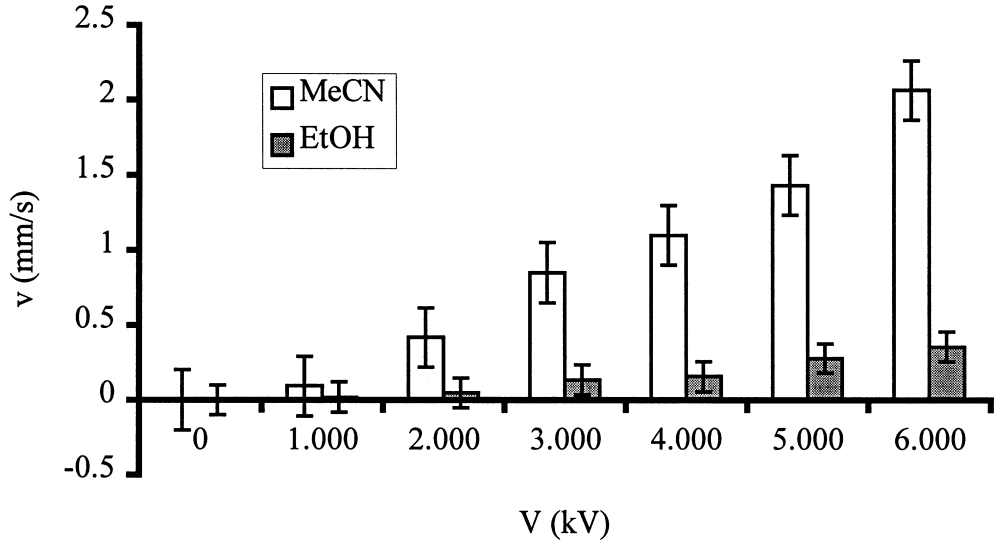


Fig. 5. Acetonitrile and ethanol migration velocities vs. applied potential.

using electrically driven elution by electroosomotic flow under the influence of a 7 kV potential (Fig. 6).

Comparable results were obtained by the two methods but the times required to achieve each run were very different. Whereas the standard TLC took 18 min, the electrically driven elution was possible within 90 s.

5. Discussion

The separation efficiency that can be achieved by most modern TLC is limited by the inadequate mobile phase flow under capillary action [10,11]. This capillary-induced solvent flow is neither fast enough nor constant through the chromatographic run. The use of electroosmosis to pump mobile phase through the stationary phase bed has the potential to generate optimum flow velocities. Considerable benefits are to be gained from the solvent adopting a plug flow profile, resulting in improved efficiency and resolution together with faster analysis times.

The application of an electric field across the length of a solvent-wetted thin-layer plate can give rise to a number of different effects. Four classical electrokinetic phenomena have been known for over a century: electrophoresis, electroosmosis, streaming potential and sedimentation potential [12]. Electroosmosis has been observed in a wide range of solid–liquid systems [13]. It is generally accepted that the solvent used must be able to support ions in solution in order for a double layer to form and for this reason polar solvents, such as methanol and acetonitrile, are commonly employed for EOF generation in capillary electrochromatography (CEC).

5.1. Choice of solvent and support

Preliminary thin-layer electrochromatography experiments clearly demonstrated the desirability of applying the highest potential possible across the plate, in order to induce high migration rates. The electrical resistance of the plates is therefore very important, since the power transferred to the plate increases with the square of the voltage ($P=V^2/R$) and consequently a small increase in potential can lead to a large increase in power. Power levels in excess of 1 Watt on a 75×25 mm plate lead to rapid solvent evaporation, and the drying of the plate in under 1 min. Evaporative solvent loss can be minimized by the selection of high boiling point solvents [14] but this can lead to subsequent problems in the detection of the separated solutes. Maintaining the resistance at the highest level possible allows the application of larger potentials whilst minimising the power input to the plate.

The resistance of a wetted TLC plate is largely determined by the density of mobile ions in the solvent layer. These ions may originate from the solvent itself, if it contains dissolved ions or undergoes autodissociation. They may also originate from the plate surface, which can have water and various ionic species adsorbed onto it. Irrespective of the source of the ions, it is desirable to reduce the ion density in order to achieve high plate resistance. However, sufficient ions must remain to allow the formation of a double layer. Non-polar, non-ionic solvents, such as hexane, which do not readily support ionic species in solution are therefore unlikely to provide a sufficient ion density to allow double layer formation. Whilst both ethanol and acetonitrile have been widely employed in CEC, acetonitrile should not autoionize and the mechanism by which EOF is generated with this solvent has yet to be established. Investigations are currently being carried out to investigate the role of residual water in this solvent migration process.

The use of non-aqueous solvents with low dissociation constants can reduce the contribution of the solvent to the density of mobile ions, while washing and oven drying prior to use can reduce the contribution arising from the plate itself. Drying the plate at over 120°C would result in the loss of most of the surface water. This heating step may provide a useful increase in plate resistance, and reproducibility but can have the undesirable effect of increasing the activity of the silica, causing it to strongly retain ionic and polar sample molecules.

5.2. Plate pre-conditioning

Early experiments encountered considerable difficulties in generating sustainable electroosmotic flow. These arose largely due to the evaporation of solvent from the plate surface due to Joule heating [9], which was influenced by both solvent and plate

choice. Measurement of the current flow characteristics of the solvent-wetted plate under the influence of an applied potential indicated that the resistance was changing in a manner that could not be wholly explained by solvent evaporation. The current flow indicated that ionic impurities were being eluted from the plate during the run, resulting in a temporary increase in current flow. Not only would this result shorten potential run times by increased plate heating but reproducibility would be compromised by the inconsistent plate resistance and co-elution of plate-derived impurities with sample components.

The plate impurities causing the temporary current increase were readily removed by washing in water. Pre-washing of the plates resulted in a higher plate resistance by the removal of readily soluble ionic impurities and led to the establishment of a consistent population of surface silanol groups. This led to both reduced current flow, and reduced solvent evaporation.

5.3. Evaporative, electrophoretic or electroosmotic migration?

The compounds and solvent systems employed in this work were chosen to largely exclude the possibility of electrophoretic migration effects. β-carotene in particular was selected to be non-ionizable, non-polar, coloured and to exhibit minimal stationary phase–solute interactions on silica. The pyrimidines are similarly expected not to be susceptible to significant electrophoretic effects in ethanol. Their movement under the influence of an applied potential should therefore be governed by the flow of the eluting solvent and not due to solute migration in the electric field. When the thin layer and TLE separations of the pyrimidines are compared not only is the elution order unchanged, but there are great similarities between the relative migration distances. As any electrophoretic behaviour would differ between the compounds this tends to support the migration being as a result of solvent flow and not solute migration in the applied field. The solvent flow must therefore be due to either capillary flow arising from gravitational or thermal effects, or from electroosmotic flow.

While the use of low conductivity solvents minimises evaporative effects, evaporation can never be totally eliminated and it is an inevitable consequence of applying a potential to the plate. In systems exhibiting high rates of electroosmotic flow, the continuous flow of solvent through the silica bed is an effective means of heat removal from the plate, considerably reducing evaporation. This results in a sustainable system, where solvent evaporation is continuous, but evaporating solvent is replaced by fresh solvent flowing onto the plate.

If the observed migration of β-carotene were to have been due to evaporative solvent migration it would be necessary for there to exist a mechanism by which differential solvent evaporation could occur. By carrying out the separation using a horizontally mounted plate, solvent drainage to the base of the plate, a problem encountered with vertically mounted plates, can be avoided. In the first analysis, the horizontally mounted plate is symmetrically fed by solvent at its two ends and the solvent depleted region of the plate is therefore at its centre. There is no fundamental difference between the two ends of the plate and evaporation-based solvent migration should therefore be towards the centre of the plate. By reversing the electrical polarity of the system it was possible to demonstrate that electroosmotic solvent flow was independent of cell connection and in a consistent polarity direction. The application of sample spots at the centre point removes the possibility that migration is thermally induced. Indeed it can be argued that any thermally or capillary induced migration of the test compounds will be opposed to any electroosmotic flow, reducing observed solvent flows.

It is therefore believed that the major mechanism responsible for the chromatographic migration observed under the application of an applied potential is electroosmotic, a conclusion supported by the observation of bulk solvent migration above the surface of overly wetted plates. Electroosmotically driven planar electrochromatography offers high resolution rapid separations and is at present the subject of active further development.

6. Conclusions

Electroosmotically driven thin-layer electrochromatography (TLE) has been carried out using con-

ventional silica thin-layer chromatography media and polar solvents such as ethanol and acetonitrile. Significantly reduced elution times can be achieved by electroosmotic TLE. Ethanol and acetonitrile develop solvent migration rates of 0.039 and 0.210 $cm\ s^{-1}$, respectively, in a field of 800 $V\ cm^{-1}$ which corresponds to an order of magnitude increase in elution rate and reduced band-broadening. A significant difference was observed in the EOF flow rates achieved with acetonitrile and ethanol and the contributions of factors such as viscosity and dielectric strength in determining these differences have yet to be studied in planar systems.

In a study of the separation of a number of pyrimidines, comparable separations were achieved by both electroosmotically driven TLE and by conventional thin-layer chromatography. Essentially identical separations were achieved by the two methods but the TLC separation took ca. 18 min whilst the comparable electrochromatographic separation take only 90 s. Electroosmotically driven TLE offers a significant time saving over conventional thin-layer chromatography and some improvement in band-broadening due to the more rapid elution. Further work is currently underway to further develop the new possibilities of this method and to investigate the mechanisms of electroosmotic flow in ethanol and acetonitrile. These include both the identification of potential applications of the techniques and its enhancement through, for example, the incorporation of plate cooling to further reduce band broadening and solvent evaporation.

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