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Journal of Chromatography A, 841 (1999) 127–132

JOURNAL OF
CHROMATOGRAPHY A

Short communication

Evaporation-induced solvent migration in electrically-driven thin layer chromatography

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Received 17 December 1998; received in revised form 12 February 1999; accepted 23 February 1999

Abstract

When high potential electric fields are applied to thin layer chromatography media solvent migration is observed that in early work was assigned to electroosmosis. Whilst these early experiments can be reproduced using modern chromatographic media, this migration has been shown to be independent of the field direction, and cannot therefore be electroosmotic in origin. This migration has been shown to be dominated by solvent evaporation resulting from Joule heating and coronal discharge, which is concentrated in a restricted region of the chromatographic plate. Similar migration rates can be achieved in the absence of the electric field by the application of localised heating. Localised heat application is discussed as a possible means of inducing solvent migration in planar chromatography. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Electrochromatography; Electroosmotic flow; Thermal effects; Electrically driven chromatography; Heating

1. Introduction

Over the past two decades there has been an explosion in the use of separation techniques based on the application of electric fields. Of particular significance has been the development of the many variants of capillary electrophoresis (CE) and capillary electrochromatography (CEC). Much of this interest can be traced to early work carried out by Pretorius et al. [1], who in 1974 reported the use of high potential gradients (ca. 1000–2000 V cm⁻¹) to effect very rapid separations. These studies investigated separations both on thin layer chromatography (TLC) plates and in unpacked glass tubes. Whilst the latter tubular medium became the forerunner of

modern CEC [2–5] little interest was shown in the work that had been performed on TLC plates.

The separation efficiency that can be achieved by most modern TLC is limited by the inadequate mobile phase flow under capillary action [6,7]. This capillary-induced solvent flow is neither fast enough nor constant throughout the chromatographic run. The observation that solvent could be induced to flow through planar chromatographic media under the influence of an electric field should have been taken up at that time as an important development offering both increased resolution and much faster separations.

The application of an electric field along a solvent-wetted thin layer plate can give rise to a number of electrokinetic phenomena, most importantly in separation science, electrophoresis and electroosmosis

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[8]. In their initial paper Pretorius et al. assigned the movement of solvent through a TLC plate under the influence of an electric field to electroosmotic flow (EOF). This solvent migration is a consequence of the formation of an electrical double layer at a solid–liquid interface; an effect first described and explained in 1910 [9]. Electroosmosis has been observed in a wide range of solid–liquid systems [10]. It is generally accepted that the solvent used must be able to support ions in solution in order for a double layer to form. Polar solvents, such as methanol and acetonitrile, are commonly employed for EOF generation in CEC.

The maximum velocity reached during conventional TLC development is significantly lower than that required for optimum kinetic behaviour, and minimum plate height. A number of approaches have been taken to enhance the elution rate, such as overpressured chromatography [11,12] and rotational chromatography [13]. The use of electroosmosis to pump mobile phase through the stationary phase bed may also allow the optimum flow velocity to be approached or reached, with considerable benefits to efficiency and resolution. A recent attempt to reproduce the planar electrochromatography results obtained by Pretorius et al. has led us to an alternative thermal explanation for the solvent migration behaviour observed in some experiments carried out under the application of an electric field.

2. Experimental

2.1. Apparatus and materials

2.1.1. Development chambers

The experiments were carried out in two specially constructed development chambers, one for vertical development and the other for horizontal development. The vertical development chamber closely resembles the experimental set-up used in the original work carried out by Pretorius et al. [1]. The main body of the chamber was a cylindrical glass tank, 110 mm in height and 120 mm in diameter. It was covered with a PTFE lid (150×150 mm) which incorporated a clip for securing the TLC plates at the top. The top electrode was a platinum wire (0.5 mm in diameter) running along the inside face of the clip

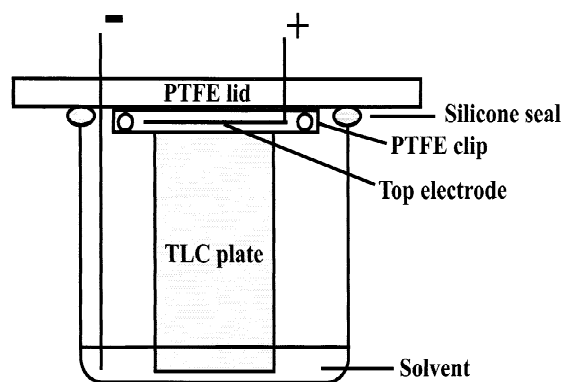


Fig. 1. Vertical development chamber.

and compressed onto the silica surface by the clip. A second platinum wire, sheathed in PTFE but with an unsheathed solvent contact area, dipped into solvent at the bottom of the tank, forming the bottom electrode (Fig. 1).

The horizontal development chamber was machined from a solid block of PTFE, with a solvent reservoir at each end of the chamber. Electrical contact was made via two stainless steel electrodes, 3 mm in diameter, which were submerged in the solvent. A PTFE lid with a glass viewing window covered the chamber (Fig. 2).

2.1.2. Power supply

The power supply used was a Brandenburg Alpha 807R (0–30 kV, 1 mA, Brandenburg, West Midlands, UK). A safety interlock was included on both chambers to disable the power supply when the lid was removed.

2.1.3. Chemicals

Reagent grade methanol, ethanol, hexane and toluene (Aldrich, Gillingham, UK) were redistilled in

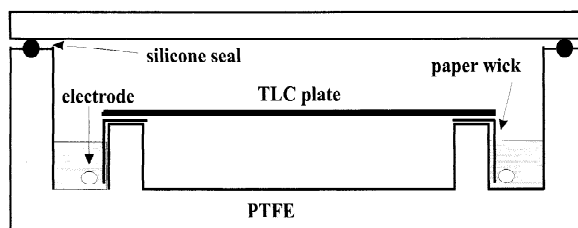


Fig. 2. Horizontal development chamber.

the laboratory. Reagent grade dichlorodimethylsilane was obtained from BDH, Poole, UK. Reagent grade pyrene (Fluka, Gillingham, UK) was used as the neutral flow marker, dissolved in ethanol at a concentration of 2 mg ml^{-1} .

2.1.4. TLC plates

Glass backed silica gel TLC plates (6 mm pore, $250 \mu\text{m}$ layer, $75 \times 25 \text{ mm}$ strips) were used throughout the experiments (Whatman International, Maidstone, UK). In an attempt to replicate the work carried out by Pretorius et al. [1] all plates were silanised by submerging them plate in a 5% solution of dichlorodimethylsilane in toluene for 15 min. The plates were then soaked in methanol for 15 min and, after the initial evaporation of the residual methanol in a fume cupboard, were then dried overnight at 80°C .

2.2. Methods

2.2.1. Vertical plate elution

The development chamber was primed with elution solvent to a level of ca. 10 mm above the base, sealed and allowed to come to equilibrium. Aliquots ($2 \mu\text{l}$) of sample were manually applied to the plate as a line of spots 25 mm from the edge of the plate. They were left to dry for 2 min and then the whole plate was dipped in the elution solvent. The plate was then connected in the development chamber, and a potential difference of 10 kV was applied between the top of the plate and the solvent in the base of the chamber (Fig. 1). After 4 min the power was switched off and the plate removed from the chamber and allowed to dry. The position of the spot was noted by viewing under a UV lamp (254 nm).

The experiments were performed with the top electrode being held either at a positive or negative potential with respect to the base electrode. Control experiments were also run with no applied potential.

2.2.2. Horizontal plate elution

The plates were spotted, dried and dipped in solvent as described previously, but with the sample spots being applied across the plate at its center. Each plate was then loaded into the horizontal development chamber with its silica surface facing downwards and making contact with the paper wicks at the solvent reservoirs (Fig. 2). The potential was then applied after closure of the chamber.

2.2.3. Thermally-induced solvent flow

Experiments were performed to investigate the effects of thermally-induced solvent flow. These were carried out in both the vertical and horizontal development chambers with no applied potential, but with a small 5 W heater placed at the top of the plate, to simulate the heating action of coronal discharge near the “top” of the plate (Fig. 3).

3. Results

Initial attempts to reproduce the work carried out by Pretorius et al. [1] were hampered by insufficient detail. This was particularly the case with regards to the materials and experimental apparatus that had been employed in the original thin layer experiments. The solvents investigated in this study were *n*-hexane and toluene, solvent choices that were based on information provided by one of the original authors of the Pretorius paper [14].

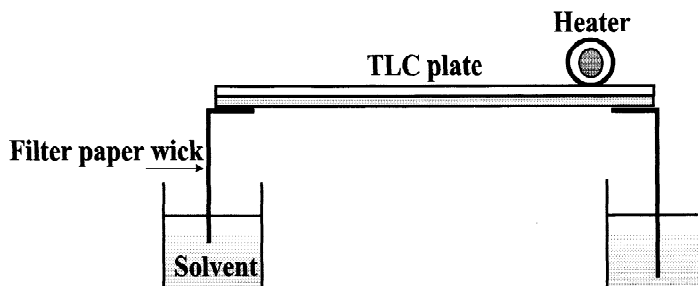


Fig. 3. Induction of thermal solvent migration.

In order to identify whether EOF could be induced in a TLC plate it was first necessary to identify a marker compound which could be employed to track the flow of solvent through the chromatographic media without being influenced by chromatographic retention effects or electrophoretic migration. The compound chosen for this work was pyrene. This compound has no electrophoretic migration characteristics and can be viewed under ultraviolet light. On the chromatographic media employed in this experiment, and when eluted with either *n*-hexane or toluene, pyrene exhibits an R_f of 1.00 ± 0.05 , indicating that it is not chromatographically retained. The pyrene acts as an indicator of the relative linear solvent migration velocity, and is not solely an EOF marker.

A number of experiments were initially carried out using the vertical development chamber, as used in the original work. Significant migration of the pyrene was observed without the application of an electric field. When a potential of 10 kV was applied to the plate the migration distance increased (Table 1). Whilst at first sight this might be interpreted as an electroosmotic flow being induced in the plate, reversal of the field was found to result in the same increased migration. The direction of increased migration was therefore independent of the direction of the applied field and could not therefore be due to an electroosmotic effect.

For the solvent migration to occur in the absence of an applied potential the migration had to result from a physical effect such as solvent evaporation at the top of the plate. This would create a solvent loading differential between the top and the bottom of the plate, causing solvent to flow up the plate by capillarity. When a potential of 10 kV was applied to the plate, migration increased and irrespective of the

polarity of the system, solvent was evaporating off the upper region of the plate as a result of Joule heating and coronal discharge. The current seen with both solvents, which is believed to be largely due to the presence of ionic impurities in the solvent, was around 20 μA , corresponding to a power of ca. 0.2 W at 10 kV. This current remained fairly constant through the run until the solvent evaporation finally resulted in circuit breakage. The power dissipated in the solvent layer would be sufficient to increase evaporation of the solvent near the top of the plate. More power would be transferred to a smaller volume of solvent when compared to the bottom of the plate.

Confirmation that thermally-induced solvent migration could account for the observed migration was obtained by using a small electrical heater at one end of a horizontally mounted plate to enhance the evaporation rate and to induce a solvent flow through the plate (Table 1).

Running the plates horizontally provided further confirmation of the hypothesis that the migration was being induced by the evaporation of solvent from the top of the vertically mounted plate. In the horizontal mode there is a solvent reservoir at each end of the plate thereby eliminating solvent depletion at the top of the plate due to gravitational effects. Under these conditions no migration is seen, irrespective of polarity (Table 2).

Placing the heater on the horizontal plates induced some migration but as there was less solvent depletion due to gravitational effects, the migration was less than with the vertical plates. No difference in spot shape between evaporation driven and standard TLC plates were observed on the lower half of the plate. As the spots reached the evaporation front, their shape tended to distort, becoming flatter at their front. This culminates in the entire spot becoming

Table 1
Migration of pyrene during vertical development ($n=3$, RSD=15%)

Potential (kV)	Migration (mm)	
	Hexane	Toluene
0	20	7
+10	28	13
-10	28	13
Heater	33	28

Table 2
Migration of pyrene during horizontal development ($n=3$, RSD=10%)

Potential (kV)	Migration (mm)	
	Hexane	Toluene
0	0	0
+10	0	0
Heater	19	11

concentrated into a narrow band at the evaporation front.

4. Discussion and conclusions

Current understanding of the origins of electroosmotic flow would suggest that pure non-polar aprotic solvents such as toluene and hexane should not be capable of supporting electroosmosis. The experiments reported in this paper have demonstrated that in vertical thin layer electrochromatography, using silanised silica plates and eluted with these solvents with non-polar aprotic solvents such as toluene and hexane, the observed solvent migration arises principally from the evaporation of solvent. This occurs from the upper portion of the thin layer chromatography plate and is enhanced by Joule heating and coronal discharges in this region. There is no evidence of EOF of solvent through the thin layer plate.

The observation of evaporation effects in thin layer electrophoresis and electrochromatography is not in itself new. The phenomena were first reported in the 1960s [15,16], when it was realised that evaporation driven migration can make up a significant proportion of the total solvent flow. The presence of such a strong flow induction mechanism complicates the measurement of electroosmotic migration velocities, necessitating the separate measurement of the evaporative flow component in order to determine the electroosmotic component.

The evaporative effects observed in a conventional vertical TLC system depend on a number of factors. In such systems a solvent vapour gradient normally forms between the bottom and top of the development chamber due to the density of the solvent vapour. With non-pretreated plates, as the solvent migrates up the plate it is influenced by both gravitational effects and the evaporation of solvent. This results in the distribution of solvent along the length of the plate differing significantly with the surface coverage of solvent at the top of the plate being significantly less than is the case at the bottom of the tank. With pre-wetted plates initially the layer is at its maximum solvent loading but this starts to decrease immediately after removal of the plate from the dipping solvent. When loaded into a vertical

development chamber some solvent drains to the base of the plate under gravity leading again to an uneven distribution of solvent along the length of the plate. Neither the uneven distribution of solvent vapour in the chamber nor the gravitational drainage of solvent should occur with a horizontal development chamber.

When a potential is applied across the layer, a current will flow, proportional to the potential applied and the resistance of the layer. Effective layer resistance is a function of the concentration of mobile ions present in the system. The electrical power input to the system is proportional to the square of the applied potential, and inversely proportional to the layer resistance. The power transferred to the layer will cause its temperature to rise, increasing solvent evaporation. The temperature will tend to stabilise when heat dissipation by evaporation is equal to heat input. Whether the system reaches equilibrium will depend on how readily fresh solvent is able to reach the area of the plate that has been subject to evaporative solvent loss.

The main conclusion to be drawn from this work is that in the vertical elution experiments of Pretorius et al. [1] the observed solvent migration can be wholly accounted for wholly by the evaporation of solvent from the upper portion of the TLC plate. No evidence has been found in this work to support the occurrence of EOF of solvent through the thin layer plate. Given the non-polar nature of the solvents that are believed to have been used in the original thin layer experiments, and their poor abilities to support the ionization necessary for the generation of EOF, this is a more plausible flow generation mechanism. Even if the toluene and hexane had not been employed in the original work, and the study had been carried out using the more polar potentially ionic methanol–benzene mixtures employed in their measurement of flow through microparticulate unmodified silica columns [1], this same evaporative mechanism would be expected to occur with their vertically-mounted thin layer plates.

The high migration rates observed on heated TLC plates suggests the intriguing possibility of using localised heating to induce solvent migration on pre-wetted plates. Such a technique would not however offer the advantage of a plug-flow profile resulting from EOF.

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