ELSEVIER

Journal of Chromatography A, 887 (2000) 115–124

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Review

Gradient elution techniques for capillary electrochromatography

Catherine A. Rimmer, Stephanie M. Piraino, John G. Dorsey*

Department of Chemistry, Florida State University, Tallahassee, FL 32306-4390, USA

Abstract

Capillary electrochromatography (CEC) is a rapidly maturing technique, but still in need of further instrumental development and in need of unique applications that are not possible by traditional pressure-driven LC. We review the development of gradient elution schemes for CEC, beginning with pH gradients initially developed for capillary electrophoresis. Step gradients are the most easily instrumentally implemented, but provide less flexibility in separation than continuous gradients. Pressure-assisted CEC is easily adapted to gradient elution schemes, but does not offer the advantages of very high column efficiency provided by totally electro-driven mobile phases. The development of flow-injection interfaces allows a true solvent gradient to be generated by μ-LC pumps, with the mobile phase drawn into the separation capillary by pure electroosmotic flow. While requiring both a CEC instrument and a traditional pump or pumps capable of generating the gradient, this method offers advantages of greatly reduced column handling, prolonging column lifetimes, and allows simple autosampling. We also discuss voltage gradients, which provide a mobile phase velocity gradient.

Keywords: Gradient elution; Electrochromatography; Reviews

Contents

*Corresponding author. Tel.: +1-850-644-4496; fax: +1-850-645-5644.
E-mail address: dorsey@chem.fsu.edu (J.G. Dorsey)

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00534-3

1. Introduction

Capillary electrochromatography (CEC) has exploded in interest in the past few years. But is this new technique a revolution in the practice of liquid chromatography (LC), or is it merely a niche technique that will eventually have limited application in modern analytical laboratories? Traditional, pressure-driven LC is not broken and it can still more easily provide answers for most routine analytical problems.

The development of capillary electrophoresis (CE), micellar electrokinetic chromatography (MEKC), and the continued interest in (pressure driven) open tubular LC are all driven by the limited peak capacity of traditional LC. In spite of the popularity and utility of CE and MEKC, there are difficulties in using either of these techniques for routine analyses, and they have not become the commercial success that was predicted during their early development. Electroosmotic flow (EOF) is an important component of the driving force for both techniques, and is generated only at the solution–capillary interface. As there is a very small surface-to-volume ratio, this means that the capillary wall chemistry must be *exactly* reproduced between separations for the EOF to remain constant. A second limitation of these, and all capillary separations methods, is that the small column diameter also dramatically limits sample injection volume, and adversely affects concentration limits of detection (LOD). While extremely impressive absolute LODs are reported by researchers in these areas, injection volumes are typically in the pl or nl range, making concentration LODs difficult to lower beyond about the ppm range.

CEC may ultimately address both of these problems. Simply put, electrochromatography is a liquid chromatographic experiment run in a CE instrument. That is, EOF drives the mobile phase through the column, rather than a pressure drop. Since the capillary is now packed with chromatographic stationary phase, the surface-to-volume ratio is increased by orders of magnitude. This will ultimately allow much larger injection volumes, because of the increased volume phase ratio, lowering the concentration LOD, and should also give *much* improved EOF reproducibility. The ultimate driving force for the interest in CEC, however, is not these two minor advantages, but rather the solution to the limited peak capacity of traditional LC.

What is still needed for CEC to become an important tool in the arsenal of practicing analytical chemists? Certainly one very important need is the development of simple, reliable, reproducible gradient elution schemes. Likely the greatest use of CEC will ultimately be for the analysis of extremely complex samples, where very high peak capacities are necessary, and traditional LC fails due to pressure limitations. These complex samples *must* be run in a gradient mode, and a useful solvent gradient will approximately double the peak capacity of an isocratic separation, allowing CEC to approach the peak capacities currently offered only by open-tubular gas chromatography (GC)!

2. Step gradients

Step gradients have been used quite commonly in CEC. A step gradient is one which has a large, near instantaneous increase in solvent strength at a defined time, as opposed to the gradual continuous increase of a linear gradient. Step gradients for CEC have their roots in capillary zone electrophoresis (CZE) as most CEC has been done with CE instrumentation. Since it is possible to produce step gradients without changing the basic instrumentation these were the first gradients generated in electroosmotically-driven systems.

2.1. Mechanical step gradients

The first and most straightforward method for producing step gradients was performed by Euerby et al. [1]. The gradient was generated by starting the separation using weak mobile phase in the mobile phase reservoirs. Then at predetermined time intervals the voltage was turned off and the weak mobile phase was replaced with a stronger mobile phase, and the voltage reapplied. When the separation was complete the capillary was placed in the original mobile phase vials and the system was allowed to reequilibrate.

Euerby et al. used this system to separate a test

mixture of six diuretics. When the diuretics were separated using isocratic conditions so that the critical pair had a resolution of approximately 1.0 the separation took about 35 min. However when one mobile phase step was used the separation took approximately 16 min for all of the solutes to be adequately resolved. Three consecutive gradient runs showed retention time relative standard deviation (RSD) values ranging from 0.48 to 1.0%, indicating that the gradient step was reproducible.

This method of generating gradients has the same problem that many do. The inlet frit is often disturbed as the mobile phase composition is changed. This occurs in both homebuilt and commercial systems as the capillary must either be moved to a new mobile phase vial or a new, capped mobile phase vial must be moved to it. Ultimately, this shortens the useful lifetime of the packed capillary. Therefore, this type of step gradient is not necessarily useful for large numbers of samples. In spite of this disadvantage there are several redeeming qualities to this technique. The first and most important is that it is very easy to generate a gradient which utilizes purely EOF. There are no instrumental difficulties with sample injection and the procedure can be easily automated using commercial CE instrumentation. Finally the gradient can be carefully adjusted by varying the number of steps, the height of the steps, and the length of time each step is applied. A multistep gradient would approach a true linear gradient, but would require multiple movements of the separation column, and multiple voltage off/on steps.

2.2. Electrically gated step gradients

Another method for generating step gradients for use in CE allowed for a step change in the ionic matrix [2] or pH [3] of the mobile phase solution. This was performed using multiple buffer reservoirs. However, instead of physically switching the buffer vials, the system was electrically gated. Thus the applied potential could be switched from the primary reservoir to a secondary reservoir without disrupting the capillary.

In the case of changing the ionic matrix the primary buffer was a solution containing a mixture of HCl and KOH at pH 3.5. The secondary buffer was set to pH 6.5. The capillary was equilibrated and the injection was made using the primary buffer. At a predetermined time the electric gate was switched and the potential was applied to the secondary buffer vial, generating a gradient from pH 3.5 to pH 6.5.

Finally, the system was tested using five solutes ranging in pK_a from 0.7 to 4.8. A comparison of the separation at pH 3.5, 4.0, 6.5, and a gradient from 3.5 to 6.5, shows that there is a dramatic improvement when the gradient is used. With the gradient all five of the solutes were well resolved and they appeared as relatively narrow bands, with the last peak eluting in under 9 min.

Although this technique was developed solely for use in CE separations it represents an important step in the development of gradients in electroosmotically-driven systems. It allowed for the generation of a purely electroosmotic gradient without disturbing the capillary. The technique is similar to the use of dual power supplies in CEC [4]. However, in this case the electric gating system was designed to allow only one step. Thus it is more difficult to control the separation than it is in a system which allows for many varying steps or that allows for a linear gradient using two power supplies.

2.3. Step gradients using mixing

Balchunas and Sepaniak investigated gradient elution MEKC to extend the elution range for the separation of an 11-component test mixture [5]. A pseudo-step gradient was achieved by the addition of 0.5-ml aliquots of 2-propanol to 2.5 ml of the starting mobile phase. A magnetic stirring bar was used to facilitate the mixing, and the power supply was shut down each time solvent was added.

As more 2-propanol was added, both the EOF rate and the net micellar velocity decreased, increasing the effective retention time of the micelles. To decrease the analysis time and maintain a constant current, the voltage was increased throughout the gradient run from 15 to 27 kV. Triton X-100 (a nonionic surfactant) was also added to the 2-propanol to increase the net velocity of the micelles for a faster separation. The gradient conditions broadened the elution range to adequately resolve

later-eluting peaks and revealed two previously unresolved impurities with the eighth compound.

Zhang et al. used a similar instrumental setup to perform a gradient separation of nine DNPH derivatized ketones and aldehydes for a comparison of the retention behaviors and column efficiencies of CEC, pressurized electrochromatography (PEC), and micro-high-performance liquid chromatography (μ-HPLC) [6]. One column and one system were used to perform all three chromatographic techniques. The mobile phase was introduced via a pump or pipette and was stirred with a magnetic stirrer.

The nine components were successfully separated using a mobile phase of acetonitrile–buffer (60:40, v/v) from 0 to 12.83 min and then titrating to 80:20 (v/v). A mixture of 15 aromatic components was also separated using both isocratic and gradient conditions on a reversed-phase CEC column. The mixture was separated using isocratic and gradient conditions; however, the gradient elution effectively resolved the later-eluting peaks and decreased separation time.

The instrumentation setups described in both papers have several limitations. Pipetting the mobile phase into the system and stopping the voltage during a separation adds complexity and raises concern for reproducibility. The homogeneity of the gradient once additional mobile phase is added is also a consideration since the mobile phase is stirred with a magnetic stirrer. Whether or not sampling starts before or after the solution is thoroughly mixed will potentially yield different retention times. These systems also limit the gradient conditions that may be used because 100% organic mobile phase can never be reached unless the initial mobile phase is removed.

On the other hand, these papers also illustrate the usefulness of gradient elution CEC to separate complex mixtures. Although Balchunas and Sepaniak [5] report the use of gradient conditions for MEKC, their findings are easily applied to CEC. The experiment can be performed with relative ease even though the instrumentation is not fully automated. Zhang et al. [6] describe a more automated system. Using the same column and system for different modes of chromatography provides for a more accurate comparison of the three techniques.

3. Linear mixing gradients

3.1. Linear mixing in buffer vials

Linear mixing gradients for electro-driven separations were first generated by Tsuda [7] and Sepaniak et al. [8]. This method bridges the gap between the previously discussed mixing step gradient and the gradients generated using a flow injection interface. The systems work by constantly pumping a fresh source of mobile phase into the reservoirs. Tsuda accomplished this using a split injector system supplying both the inlet and the outlet reservoirs with mobile phase [7]. Sepaniak et al.'s [8] system varied in that two pumps were used, one to deliver the new mobile phase to the inlet reservoir and the other to remove excess solvent from the reservoir.

Tsuda's system was tested using a pH gradient to separate a series of solutes in CZE. Not surprisingly, he found an increase in resolution and a decrease in retention time when the pH gradient was used. Additionally, current versus time was plotted and shows the reproducibility of the gradient as well as allowing for the estimation of the pH of the solution at any point in time during the separation. Sepaniak et al. generated both pH gradients and solvent gradients for use in MEKC. The pH gradients were tested by adding bromcresol green to the mixture and measuring the change in absorbance with pH using a photodiode array detection (PDA) system. Solvent gradients were studied by adding a fluorophore to the solution and tracing the increase in response with time, allowing them to study the reproducibility and the shape of the gradients. Linear, concave, and convex gradients were generated and a series of *n*-alkylamines was separated. The studies showed that a linear gradient using acetonitrile was ideal for the optimization of the separation in MEKC.

It is important to note that these techniques were used in CZE and MEKC rather than CEC. However, they could have easily been adapted to generate gradients in CEC. Linear mixing gradients require the use of additional equipment and solvent overflow can be cumbersome to manage. Additionally the capillary must be moved out of the buffer vial in order to make injections. In spite of these minor

disadvantages these techniques laid the necessary groundwork for the use of flow injection interfaces to generate gradients and perform on-line injections.

3.2. Flow injection interface

Huber et al. [9] and Lister et al. [10] have recently developed similar schemes for generating gradients in CEC, using a flow injection interface to introduce solvents into the separation capillary. This was based on work by Kuban et al. [11] who used a flow injection interface for on-line injections in CE. The system works simply, Fig. 1. Solvents are pumped from a set of μ-LC gradient pumps through an injector, through the interface, past the capillary and out to waste. Simultaneously, a potential is applied across the interface, electroosmotically introducing a portion of the solvent gradient and sample into the capillary. Although the principles of Huber et al.'s and Lister et al.'s systems are similar, the instrument specifics vary. Huber et al. used an Applied Biosystem CE system with an Upchurch microcross as the flow injection interface whereas Lister et al. used a laboratory-built CE system with an interface machined to the specifications in Kuban et al.'s paper.

The gradient profile was measured by both groups using 5% acetone in acetonitrile to trace the increase in absorbance with increasing organic solvent. The gradient tested by Lister et al. went from acetonitrile (ACN)–water (50:50) to 100% ACN in 20 min. The gradient was first tested by attaching an open capillary directly to the injector outlet. Then the column was placed in the interface and the same tests were run. The results of the increase in absorbance with time were plotted. The shape in both cases was the same indicating that a linear gradient was being produced using the interface. There is a slight time delay using the interface, simply to the increase in dwell volume.

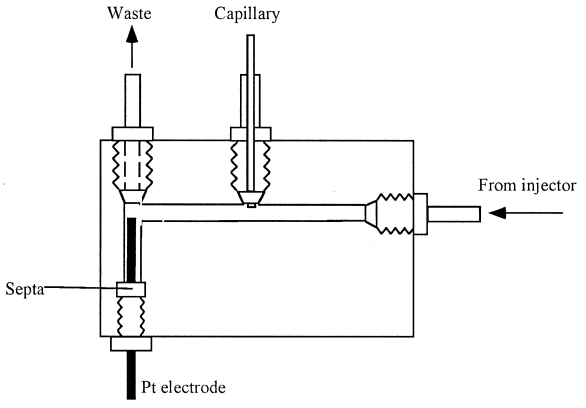

Fig. 1. Schematic representation of the flow injection interface in the configuration used by Lister et al. [10].

Since there is a pressure-driven component in the flow injection interface scheme an important test was to determine if there was a significant hydrodynamic contribution to flow. This was tested by equilibrating an open capillary with ACN–water (50:50) at 15 kV. As with the gradient profile experiments the ACN contained 5% acetone. The voltage was then turned off and the solvent was switched to 100% ACN. No increase in absorbance was seen, indicating that there was no significant forward hydrodynamic flow through the capillary. Next, ACN with 5% acetone was placed in the outlet vial and the column was equilibrated using ACN–water (50:50). When the voltage was turned off there was an increase in absorbance indicating that solvent was being pulled from the outlet vial. These tests were then performed using a packed capillary. When a packed capillary was used there was no significant hydrodynamic flow seen in either direction, even when the measured for 24 h.

Finally Lister et al.'s system was tested for the separation of polycyclic aromatic hydrocarbons (PAHs). The solutes were first separated using isocratic conditions. At ACN–water (90:10) all of the solutes eluted quickly; however, the resolution suffered. At ACN–water (60:40) the first two solutes were just baseline resolved, however, the last eluting solute appeared as a wide band just after 70 min. Using a 5-min gradient from the weak composition to the stronger composition the first solutes were adequately resolved and the later eluting peaks eluted within 20 min, indicating that there is a good gradient generated. The results from Huber et al. were similar as they found an RSD of less than 3% for five injections of PTH amino acids.

More recently Ericson and Hjerten examined the use of similar equipment using monolithic columns for the separation [12]. They found they could

separate charged proteins using both the conventional gradient elution method and using counterflow gradients. In the counter flow mode the gradient is generated as usual, however, the sample is injected at the capillary outlet. In order for this technique to work the electrophoretic mobility of the solutes must be greater than the electroosmotic mobility of the mobile phase. They found that when the system was optimized the reverse flow gradient allowed for the separation of solutes using shorter columns without sacrificing resolution.

There are minor disadvantages to the flow injection interface technique. Under isocratic conditions peaks from solutes injected using the interface were much more broad and asymmetrical than those injected directly into the capillary. This is possibly due to the fact that although the flow through the capillary is electroosmotic, the flow past the capillary is hydrodynamic. It is possible to solve this problem by rearranging the interface so that the flow from the pumps aims directly at the capillary inlet.

A second disadvantage is that the amount of solute injected is difficult to determine. This is because the amount injected is dependent on several factors such as the applied voltage, charge of the solutes, the flow-rate past the capillary, and the volume of the injection loop. A minor disadvantage of this system is that a relatively large amount of solvent is used when compared with traditional CE and CEC experiments.

In spite of these disadvantages there are several good reasons to use a flow injection interface for the generation of gradients in CEC. It is similar to pressurized CEC in that it is easy to couple with information rich detection systems. Tan et al. have shown the use of a flow injection interface to make on-line injections and to generate gradients for CEC–nuclear magnetic resonance (NMR). Using the flow injection interface allowed them to avoid reshimming the magnet after each injection [13]. Additionally Choudhary et al. have recently coupled this system with a mass spectrometer allowing for separation, detection and identification of amino acids [14].

One of the biggest advantages to using the flow injection interface is the ease of automation, as shown by Huber et al. who used a Perkin-Elmer Applied Biosystems CE system. This system could be used for CE, CEC, pressurized CEC, and microbore LC. Since the same column can be used for all of the separations it is a convenient setup for the comparison of microcolumn techniques. Additionally the use of automated pumps and injectors leads to reproducible gradients and injections. A very important advantage of this method is the greatly reduced handling of the separation capillary, which leads to increased lifetimes.

4. Pressurized flow electrochromatography

Pressurized electrochromatography, or pressure-assisted electrochromatography was especially popular during the early development of electrochromatography as it avoids some of the problems seen in purely electro-driven CEC. The instrumentation is a hybrid of microbore LC and CE in which mobile phase is pumped through the capillary and a potential is applied across it [15–17]. Typically there is also a restrictor capillary which is said to prevent voltage and current leakage, protecting the instrumentation. Although this is a technique which has been utilized by several groups, the instrumentation schemes are similar. This technique naturally lead to gradient elution pressurized electrochromatography as the only instrumentation change necessary is the use of gradient microbore LC pumps rather than an isocratic pump. Behnke and Bayer were the first to use gradient pressurized CEC, their instrument diagram is shown in Fig. 2 [18].

Although the generation of solvent gradients in pressure-driven systems is a well established phenomenon, few experiments have been reported showing the gradient profile when a voltage is applied in addition to the pressure-driven flow. Instead, the studies have focused on the difference between pure microbore LC separations and those enhanced with an applied voltage.

Pressurized CEC does avoid some of the problems associated with CEC, however, many of these problems have been minimized as electrochromatography has become better understood. One of the early cited advantages was that the increased pressure minimized bubble formation. When CEC was first developing bubble formation was a significant problem which frequently lead to the breakdown of flow through the capillary. However, it is not as much of a

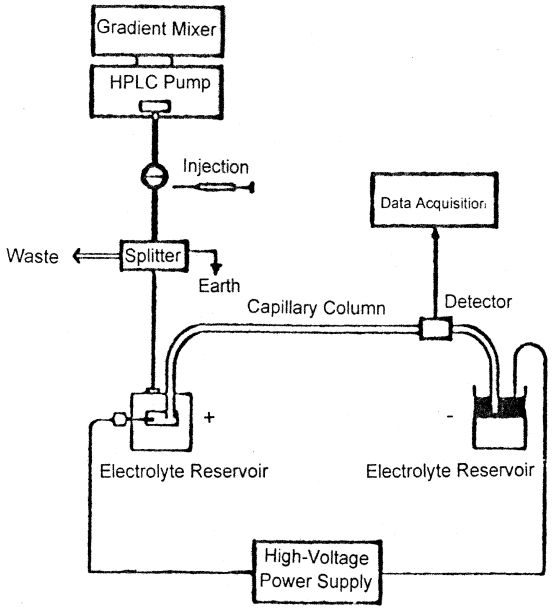

Fig. 2. Schematic representation of a pressurized flow electrochromatography system [18].

problem now with the careful selection and low concentration of organic buffers, the use of non-aqueous mobile phases and mobile phases without supporting electrolyte, and a better understanding of the preparation of frits [19–22].

Sample introduction is also greatly simplified when pressurized CEC is used. A traditional LC autoinjector can be used for injection, which eliminates the uncertainty in timed injections on a laboratory-built system as well as the sample biasing that occurs when charged solutes are injected electrokinetically.

The most controversial advantage to the use of pressurized flow electrochromatography is the claim that the flow profile becomes more ''plug like'' in nature with the applied electric field [17]. Although there is an increase in efficiency when a potential is applied across the capillary this may be due to focusing effects of the solutes rather than a change in flow profile. Additionally there will be solvent focusing effects when a gradient elution is used making the peaks appear even more narrow.

All of these advantages make pressurized CEC attractive and easier to use than the purely electroosmotically-driven version of CEC. The ease of use and similarity to LC has encouraged groups to use it for practical separations. It has been used for measuring drug components in horse urine [23] and for the mapping of tryptic digests of peptides [24]. In addition the low solvent volume makes it easier to couple with information rich detectors such as mass spectrometers [25] and nuclear magnetic resonance [26] than traditional bore LC.

Although these are often cited advantages of pressurized electrochromatography, the technique also suffers from the disadvantages of *both* electro-driven separations and pressure-driven LC. That is, as pressure is the principle driving force for the mobile phase, column lengths cannot exceed the pressure limits of the pump, and the use of very small diameter stationary phases is also impossible. The advantage of selectivity tuning appears to be minor, and it is unlikely this technique will ever be more than a research curiosity.

5. Dual power supply gradient

One of the most elegant methods for generating a gradient in CEC was developed by Yan et al. [4]. In contrast to traditional CEC instrumentation their method uses two inlet mobile phase vials, each of which is controlled by an independent power supply. Short, open capillaries connect the two inlet mobile phase vials to a tee where the solvents are mixed. The third arm of the tee is then connected to the inlet of the separation capillary which is grounded at the outlet as in traditional CEC, as shown in Fig. 3. The amount of each solvent which reaches the mixing tee is controlled by the voltage applied to each vial.

The gradient generated for the test was from 55:35 to 80:20 acetonitrile–4 m*M* sodium tetraborate buffer and this was accomplished simply by setting one of the mobile phase reservoirs at the starting conditions and the other at the end conditions. Approximately 10^{-7} *M* fluoranthene was added to the stronger mobile phase so that an increase in fluorescence could be traced with an increase in organic solvent. The tests to show the shape of the gradient were performed in an open capillary as it is difficult to find a fluorescent marker which is totally unretained. A plot comparing measured and calculated changes in fluorescence showed good agreement

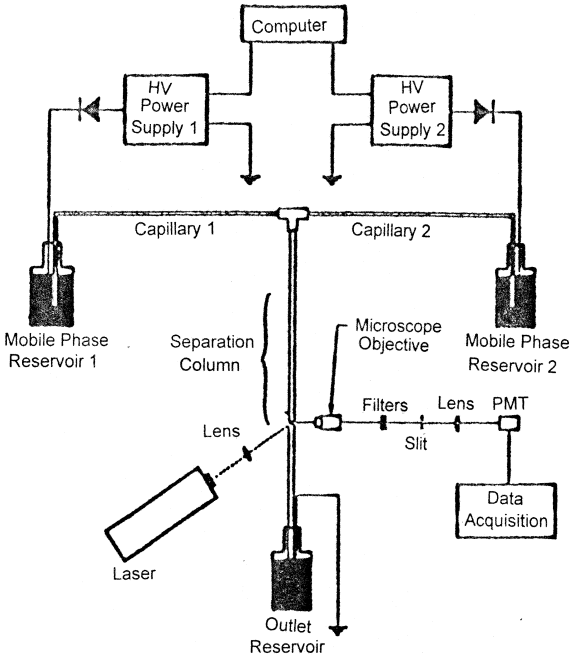

Fig. 3. Schematic representation of the dual power supply solvent gradient CEC apparatus [4].

indicating that a linear gradient is generated. The RSD of the changes in intensity of fluorescence from three consecutive runs was less than 3% indicating that the gradient generated is reproducible.

A packed capillary was used to separate 16 PAHs. Four consecutive runs from 55:35 to 80:20 acetonitrile–4 m*M* sodium tetraborate buffer gave retention time RSD ranging from 2.8 to 8.1% for the different solutes.

In many ways this is the ideal method for generating gradients in CEC. However, there are still some serious drawbacks to this technique. The first and most notable is that sample injection is not a trivial task. In order to sample the separation capillary must be disconnected from the tee, then reconnected for the mobile phase to flow through the capillary. As well as being tedious this runs the risk of disrupting the packed bed, thus reducing the lifetime of the capillary. The difficulty sampling indicates that it would be difficult to automate the separation without making significant changes to the instrument.

Another challenge associated with this system is that the flow is dependent on the dielectric constant (ϵ) and viscosity (η) of the solution, thus, EOF changes with solvent composition [22]. Because of these changes in mobility control of gradient shape is more difficult than simply linearly changing applied voltage with time. Mobility increases sharply as the mobile phase composition approaches 100% acetonitrile, so solvent and flow-rate gradients are occurring simultaneously. Also, temperature becomes an important issue as it affects not only chromatographic retention, but also electroosmotic velocity of the mobile phase. Finally, due to the differences in the surface of the fused-silica capillaries the electroosmotic mobility must be measured every time a new capillary is used in order to calculate appropriate voltage gradient programs. Although these factors make the control of gradients more difficult it is important to note that it is possible to generate well controlled reproducible gradients of any desired shape.

In spite of these drawbacks Yan et al. [4] offered the first linear gradient which uses solely EOF to drive the mobile phase. This is very important as previous linear gradients utilized pressurized flow, thus removing the real advantages CEC has to offer.

6. Chemistry on a chip

Chemistry on a chip has become an exciting area of study and microchips are useful for electroosmotically-driven isocratic and gradient separations. The microchip in Fig. 4 has two solvent reservoirs each of which is controlled by an individual power supply [27]. The two solvent reservoirs feed into a mixing arm and then into a separation channel. Two additional arms at the sample/solvent introduction end of the capillary contain the electrically gated analyte and analyte waste. The chip also has two sites for detection, one is in the arm of the mixing tee and the other is at the end of the separation channel. The reservoirs and channels in the chip are manufactured using either photolithography or wet chemical etching and there are glass reservoirs for the mobile phase components. In order to complete the channels the chip is covered with a glass plate and the buffer reservoirs are capped in order to minimize evaporation.

The gradient shape was tested using the fluorescent tag rhodamine B in the stronger mobile phase reservoir and detected in the mixing tee. By carefully considering Ohm's and Kirchhoff's laws, linear,

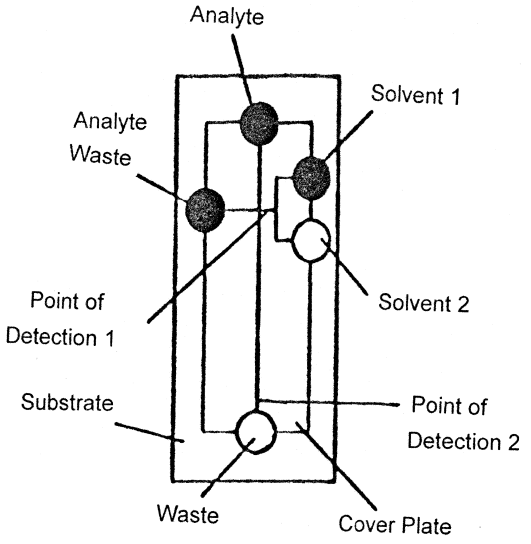

Fig. 4. Schematic representation of gradient elution system on a chip [27].

concave, convex and sinusoidal gradients were reproducibly generated. A charge couple device (CCD) was also used to show that when voltage is applied only to one reservoir there is no flow from the other. The CCD experiments also indicated that the mixing process produces turbulence where the solvents first meet, but that the mixing is complete before the solvent mixture enters the separation channel.

The system was tested for the separation of five coumarin dyes using linear, concave and convex gradients as well as isocratic separations for comparison. The separations using methanol and acetonitrile gradients resulted in increases in apparent efficiency as well as decreases in the retention time of the last solute. These increases in apparent efficiency are most likely due to solvent focusing effects similar to those which are found in gradient LC. In addition the linear gradients provided adequate separation of all solutes in a minimum period of time when compared with the isocratic separations.

Although these tests were performed using MEKC, it should be possible to utilize the same instrument configuration for packed or open channel CEC. The instrument design is similar to the Yan et al. [4] system in that the flow is purely electroosmotic. However, the microchip has the benefit of easy injection. There are several advantages to using microchips for separations. Since the channels are short it is easy to produce high field strengths, thus producing high velocities which take advantage of the flat portion of the Van Deemter curve. This makes it possible to perform very fast separations which use little solvent without sacrificing efficiency. Additionally there are examples of microchips coupled with other analytical instrumentation such as mass spectrometry. Although the authors indicate that the gradients need to be optimized and that other types of gradients need to be attempted this technique presents the ideal electroosmotic gradient if detection is not an issue.

7. Voltage gradients

Voltage gradients have been explored throughout the history of CEC. When using voltage gradients it is not necessary to make any instrument changes. The only necessary piece of equipment is a power supply which can be interfaced with a computer. Most groups that perform voltage gradients use the same basic equipment to generate the gradient. The differences lie in the programming of the voltage ramps and the reason for changing the applied voltage.

An interesting approach to voltage gradients was used in MEKC. In this case the capillary was coated with a conductive metal in order to create radial gradients [28,29]. These gradients were used to change the electroosmotic mobility, thus allowing control of the retention window. A series of separations using amino acids was shown to be quite reproducible (RSD less than 3%) using the radial voltage gradient.

Xin and Lee demonstrated that strongly retained solutes can be eluted in a more timely manner by increasing the voltage with time in CEC [30]. Increasing the field strength results in an increase in flow-rate, analogous to an increase in pressure in traditional LC. They also demonstrated that there are few changes in plate height and resolution with increasing voltage.

There may be hazards to increasing voltage too quickly. Euerby et al. made ‘‘short end injections’’ to produce very fast separations [31]. Additionally a voltage gradient was used to force the more strongly retained solutes to elute quickly. It was shown that

the very steep voltage gradients distort early eluting peaks, thus they must be carefully controlled to avoid distortion.

The Van Deemter plot for CEC appears to be relatively flat at increasing flow-rates. Since it is possible to run at high velocities without sacrificing much in terms of plate height, efficiency, and resolution, it appears that there is little advantage to running at anything but the highest possible mobile phase velocity. It is claimed that an advantage to using voltage gradients over solvent gradients is that there is no change in selectivity [30]. Although this is true it is not necessarily an advantage. Changes in retention time should be proportional to changes in flow-rate, thus doubling the flow should decrease the retention time by half. The selectivity changes seen in solvent gradients may allow for a greater decrease in retention time, thus making them more practical for the optimization of the separation of complex mixtures.

Voltage gradients do have valuable places in gradient CEC. They were especially useful during the early days of CEC as strongly retained solutes could be forced to elute more quickly without necessitating changes in instrumentation. However, it appears that their most significant role will be assisting with solvent gradients. This can be accomplished several ways. For example Yan et al. [4] and Kutter et al.'s [27] gradient elution schemes depend on voltage gradients to deliver specific volumes of solvent to a mixing tee. Horváth and co-workers [9,14] and Lister et al.'s [10] gradient elution schemes could benefit from voltage gradients in order to compensate for the changing mobility at different mobile phase compositions.

8. Conclusions

The development of CEC today can be compared to reversed-phase LC in the middle 1970s. That is, the possibilities of the technique are very clear, the solution to the limited peak capacity of pressure-driven LC is a very powerful driving force. However, developments in stationary phase chemistry, and in instrumentation are both necessary before this technique can really join the arsenal of the practicing separations chemist.

References

[1] M.R. Euerby, D. Gilligan, C.M. Johnson, K.D. Bartle, Analyst 122 (1997) 1087.
[2] J. Sudor, Z. Stransky, J. Pospichal, M. Deml, P. Bocek, Electrophoresis 10 (1989) 802.
[3] P. Bocek, M. Deml, J. Pospichal, J. Sudor, J. Chromatogr. 470 (1989) 309.
[4] C. Yan, R. Dadoo, R.N. Zare, D.S. Anex, Anal. Chem. 68 (1996) 2726.
[5] A.T. Balchunas, M.J. Sepaniak, Anal. Chem. 60 (1988) 617.
[6] Y. Zhang, W. Shi, L. Zhang, H. Zou, J. Chromatogr. A 802 (1998) 59.
[7] T. Tsuda, Anal. Chem. 64 (1992) 386.
[8] M.J. Sepaniak, D.F. Swaile, A.C. Powell, J. Chromatogr. 480 (1989) 185.
[9] C.G. Huber, G. Choudhary, Cs. Horváth, Anal. Chem. 69 (1997) 4429.
[10] A.S. Lister, C.A. Rimmer, J.G. Dorsey, J. Chromatogr. A 828 (1998) 105.
[11] P. Kuban, A. Engstrom, J.C. Olsson, G. Thorsen, R. Tryzell, B. Karlberg, Anal. Chem. Acta 337 (1997) 117.
[12] C. Ericson, S. Hjerten, Anal. Chem. 71 (1999) 1621.
[13] X.J. Tan, M.E. Lacey, W.P. Kelley, A.G. Web, J.V. Sweedler, The Pittsburgh Conference 1999, paper 388.
[14] G. Choudhary, Cs. Horváth, J.F. Banks, J. Chromatogr. A 828 (1998) 469.
[15] T. Tsuda, Anal. Chem. 59 (1987) 521.
[16] T. Tsuda, Anal. Chem. 60 (1988) 1677.
[17] T. Eimer, K.K. Unger, T. Tsuda, Fresenius J. Anal. Chem. 352 (1995) 649.
[18] B. Behnke, E. Bayer, J. Chromatogr. A 680 (1994) 93.
[19] R.A. Carney, M.M. Robson, K.D. Bartle, P. Myers, J. High Resolut. Chromatogr. 22 (1999) 29.
[20] B. Behnke, E. Grom, E. Bayer, J. Chromatogr. A 716 (1995) 207.
[21] R.J. Boughtflower, T. Underwood, C.J. Paterson, Chromatographia 40 (1995) 329.
[22] P.B. Wright, A.S. Lister, J.G. Dorsey, Anal. Chem. 69 (1997) 3251.
[23] M.R. Taylor, P. Teale, S.A. Westwood, D. Perrett, Anal. Chem. 69 (1997) 2554.
[24] B. Behnke, J.W. Metzger, Electrophoresis 20 (1999) 80.
[25] M.R. Taylor, P. Teale, J. Chromatogr. A 768 (1997) 89.
[26] P. Gfrorer, J. Schewitz, K. Pusecker, L. Tseng, K. Albert, E. Bayer, Electrophoresis 20 (1999) 3.
[27] J.P. Kutter, S.C. Jacobson, J.M. Ramsey, Anal. Chem. 69 (1997) 5165.
[28] P. Tsai, B. Patel, C.S. Lee, Anal. Chem. 65 (1993) 1439.
[29] P. Tsai, B. Patel, C.S. Lee, Electrophoresis 15 (1994) 1229.
[30] B.M. Xin, M.L. Lee, J. Microcol. Sep. 4 (1999) 271.
[31] M.R. Euerby, C.M. Johnson, M. Cikalo, K.D. Bartle, Chromatographia 47 (1998) 135.