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Review

Stacking in capillary zone electrophoresis

Zak K. Shihabi*

Pathology Department, Wake Forest University, Baptist Medical Center, Winston–Salem, NC 27157, USA

Abstract

Due to the short light path of the capillaries, the CE detection limit based on concentration, is far less than that of HPLC and not sufficient for many practical applications. Several methods, based on different electrophoretic maneuvers, can concentrate the sample (stack) easily on the capillary before the separation step of capillary zone electrophoresis (CZE). These methods incorporate different types of discontinuous buffers as the means for invoking different velocities to the same analyte molecules to produce a sharpening of the band (stacking). In CZE, these buffers can be often very simple such as sample dilution or adding to the sample a high concentration of a fast mobility ion. However, in other applications these buffers can be as complicated as those required for isotachophoresis. Stacking can often yield a concentration factor of 5–30-fold, which can improve greatly in CZE the detection limits bringing them very close to those of HPLC. Different methods of stacking, the importance of discontinuous buffers and the different mechanism for concentration on the capillary are reviewed here. As there is a need for more practical applications, there will be more methods devised for stacking in CZE.

Keywords: Reviews; Stacking; Sample handling

Contents

*Tel.: +1-336-716-2639; fax: +1-336-716-9944.
E-mail address: zshihabi@wfubmc.edu (Z.K. Shihabi).

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1. Introduction

Capillary electrophoresis (CE) has a very good sensitivity based on mass detection. A feature, which is important when the sample size is very limited as in analyzing a single cell, thus a minute amount of sample is sufficient. However, CE has less than desirable sensitivity based on concentration especially when compared to high-performance liquid chromatography (HPLC). In the majority of the practical applications the latter feature is far more important especially for routine analysis of compounds present at low concentrations. More sensitive detection systems like laser-induced fluorescence (LIF) and bubble cells have been used. Because of the need for better sensitivity of detection in CE, sample concentration becomes crucial for the widespread use of this technique in the practical analysis. Sample concentration can be accomplished by physical means such liquid- and solid-phase extraction. It also can be accomplished much more easily and more conveniently by several electrokinetic maneuvers on the capillary such as by stacking, field amplified injection, isotachophoresis (ITP) or focusing. In this review we refer to all the electrokinetic methods of sample concentration on the capillary in broad terms as stacking. The majority of these technique have a common ground of changing the velocity (v) of the same analyte ions by some maneuver which involves changing the field strength, the charge, or the ionic shell to achieve sample concentration based on the two following formulas:

$$v = \mu H$$

$$v = q/6\pi\eta r$$

where μ = electrophoretic mobility; H = field strength; q = charge on the molecule; η = viscosity, and r = ionic radius.

In all the stacking techniques, discontinuous buffers of different kinds are the basic means for altering the charge and field strength to modify the ion velocity leading to sample concentration. Buffer discontinuity can be brought about in a very simple manner by altering the sample conductivity or pH slightly so as to be different from that of the separation buffer. Sometimes this can occur unintentionally; for example, the sample can be dissolved in the same separation buffer but at a lower ionic strength, or the sample, normally contains a high salt content. As the size of the sample is increased this effect of discontinuity becomes more substantial. On the other hand, these buffers can be more specialized or complicated as mandated by ITP or isoelectric focusing.

Stacking is one of the desirable features of CE which has not been fully explored or utilized. Stacking in CE is similar to sample enrichment in HPLC. However, from a practical point of view, it is easier to perform stacking in CE relative to sample enrichment in HPLC. There is no need for complicated steps or special equipment in the CE and it has better flexibility. It can be achieved using several different principles but it requires careful planning.

Sample stacking has been discussed or noted in very few reviews [1–4]. Here, the mechanism and the different methods to achieve stacking in capillary zone electrophoresis (CZE) for different types of compounds are reviewed with several examples. Some of these methods are easier to perform or more suited for certain applications than others.

2. Historical

One of the earliest methods for concentrating the sample electrokinetically on the capillary is ITP. The theoretical basis for ITP was laid by the early work of Kohlrausch using discontinuous buffers (leading and terminating ions). The leading ion dictates the velocity and the concentration of the zone behind. Kendall [5] has postulated that an electrochemical separation under the same velocity, i.e., ITP, can be used for the purification of isotopes and other compounds. Stacking or sample concentration occurs in ITP when the sample concentration is low and has an ionic mobility intermediate between the leading and terminating ions. In order for the sample ions to keep up with the leading ion's velocity, the diluted sample concentrates to regulate its velocity through its field strength, with the contiguous separated zones resembling a stack of coins [6].

Tiselius [77] described how a zone could be sharpened by continuous buffer dilution to bring along a conductivity gradient. The advantage of the discontinuous buffer on sample concentration was

also noted by Poulik [7]. He ascribed the band sharpening to the changes in field strength. He also described how the line between the two buffers was moving with the progress of the electrophoresis. The discontinuous buffers were cleverly utilized in the polyacrylamide gel electrophoresis by Ornstein [8] to concentrate diluted protein samples before the separation in the gel. For example, a small anion such as chloride in the sample buffer migrates rapidly towards the cathode leaving behind an area with a high field strength which can drag or speed up a very slow moving buffer ion such as glycine. As the boundary between the two ions moves in the sample zone the analytes such as protein in front of this boundary slow down while the ones behind feel the effect of the high field strength and concentrate. Thus the difference in the field strength at the boundary ''sweeps'' along the analytes in the sample concentrating them in stacks [9].

In conjunction with the discontinuous buffers, stacking was further enhanced in the disc polyacrylamide gel by loading the diluted samples in gel with large pores before the electrophoresis step on small pore gels. This enables the sample to concentrate at the start of the gel [9] before the separation step. Initially few discontinuous buffers were described for stacking in gel electrophoresis; however, more buffers were devised later on.

In CE, since the birth of this technique Mikkers et al. noticed that diluting the sample in water gave sharper peaks compared to those prepared in the same separation buffer [10].

3. Basic principles for stacking

In the majority of the methods a change in the ion velocity brought about by discontinuous buffers during the electrophoresis leads to stacking in CZE. Thus, stacking in general depends on the change in the ion velocity and on the presence of discontinuous buffers.

3.1. Discontinuous buffers

Continuous buffers where the buffer in the capillary, the sample, and at the electrodes is the same, are very good for separation in CZE provided the sample size is small ($<1\%$ of the capillary volume). Under these conditions all the ions of the same analyte move uniformly at the same speed leading to the migration of the sample zone almost at the same length as it was injected, provided the diffusion parameters are kept to the minimum. However, if the sample size is increased especially $>5\%$ of the capillary volume, very broad overlapping peaks, hardly recognizable, are observed (i.e., sample overload).

3.2. Ion velocity and stacking

In stacking, a large sample about 5–50% of the capillary volume usually is injected; however, the sample band is markedly sharpened. In order to obtain sample concentration or stacking, the same analyte ion at the different areas of the sample plug have to move at different velocities in such a way that the two edges of the sample migrate getting closer to each other, i.e., band sharpening. This can result from a difference in the field strength at different areas of the sample plug. Discontinuous buffers are the means for inducing this difference in the field strength. Several closely related maneuvers can be used to modify the field strength in the sample, e.g., changes in the ionic strength, differences in the conductivity, manipulation of the charge or addition of salts. Furthermore, to produce stacking, the process has to be accomplished before the sample enters the separation buffer. In order to visualize the process of stacking, one can look at that from different points of view; for example:

(A) Accelerating the analyte ions in the sample zone, due to a low ionic strength, until they reach the separation buffer with high ionic strength where they stack in a sharp zone. The positive ions stack in front of the negative ions.

(B) A sweeping action where as the boundary between a high and low field strength can speed up the molecules in the high region while slowing them down in the low region. As the boundary moves it produces a ''sweeping'' action of the molecules into sharp stacks. Sweeping can occur also from the movement of another type of molecule which can interact with the analyte and carry it along such as when the sodium dodecyl sulfate (SDS) micelle carries a neutral molecule [11].

(C) Band-narrowing can be accomplished by: speeding one edge through a high field strength in the sample; by slowing one edge of the zone through a high salt concentration in the sample; and by both, i.e., one edge slowing down while one edge is speeding up. In the latter case a better stacking can be achieved. This can happen when both salts and a low conductivity diluent (e.g., alcohol or acetonitrile) are present in the sample. Stacking by acetonitrile for biological samples, which contain sodium chloride, is a good example of this type [12–14] which can occur, in both hydrodynamic [12,13] and electromigration injection [14]. The salts, having fast mobility, migrate rapidly early on ahead of the analytes leaving behind an area of higher field strength. The analyte ions in the high field strength accelerate while those in front in the low field strength slow down leading to a sweeping or concentration of the sample ions as the boundary of the two field strengths move on as illustrated in Fig. 1. This difference in the field strength accelerates the stacking process. If the difference in the field strength is not large enough the stacking does not occur in the necessary time before the analytes enter the separation buffer. Thus, optimum conditions, e.g., enough salt concentration in the sample, must prevail for stacking. Exceeding these optimum conditions can also lead to the opposite effect. Thus the same factor, such as sodium chloride in the sample, can occasionally promote stacking while in other instances causes band broadening.

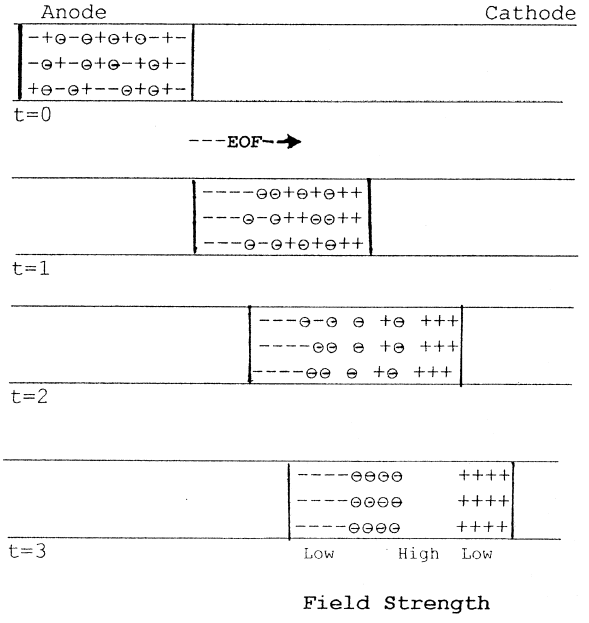

Fig. 1. Illustration of stacking of the analyte ions in the discontinuous buffers in presence of NaCl. At $t=0$, The sample analyte (θ) is injected in presence of salts (±). At $t=1$ as soon as the voltage is tuned on the salts ions move rapidly, especially if a low ionic strength or acetonitrile is present in the sample and slowing at the interface of the separation buffer. At $t=2$, because of the increased field strength in the area vacated by the small ions the analyte ion θ in that region begins to move relatively fast; while those in front or close to the inorganic cations slow down and remain behind. At $t=3$ this process proceeds further giving rise to stacking.

4. Methods for stacking

Different methods for stacking including discussion of the theoretical aspects have been described. Some of these methods are more suitable for certain types of compounds; while few are very specialized such as the ITP and isoelectric focusing and thus are mentioned briefly. A more detailed discussion of these latter methods can be found elsewhere.

4.1. Isotachophoresis and transient isotachophoresis

The method is based on the use of discontinuous buffers in respect to ions with different mobility; however, they have to migrate at the same velocity. A fast-mobility ion in high concentration is used as a leading ion and a slow-mobility ion is used as a terminating ion; while the sample ions have an intermediate mobility. At equilibrium the intermediate ions adjust their concentration so as they move at the same velocity of the leading ion. The electric field strength is inversely proportional to the ion mobility in that region.

ITP is often considered as both, a concentration and a purification method. The method can concentrate the sample 10–1000-fold. The concentrated segments can be coupled by several methods to CZE using a single or separate capillary for further separation and quantification. Chen and Lee [15]

have described an automated system for combined ITP–CZE. The sample is focused in the first capillary by ITP then injected repeatedly into a second capillary for CZE.

A simple form of ITP is transient ITP, which is easier to couple to CZE (in the same capillary) [16–19]. Under appropriate conditions, a concentration step due to a brief ITP is induced before the sample enters the separation buffer. In many instances the transient ITP step occurs accidentally in samples containing high concentrations of salts or it can also be induced by addition of an appropriate leading/terminating ion to samples with a complex matrix [20–23]. The method can concentrate both small and large molecules.

Karger and co-workers [24,25] have described two strategies for coupling ITP to CZE. The first method uses on-column transient ITP. After the sample is injected, a leading or terminating electrolyte is chosen based on the mobility of the analyte and the co-ion of the background electrolyte. This method gives about a 50-fold increase in sensitivity. In the second approach, a second CZE column is coupled to the ITP column. This involves a more complicated system but it gives up to a 1000-fold increase in sensitivity [25]. The group has also shown the advantages of coupling transient ITP with CZE for concentration of several model proteins such as cytochrome *c*, lactoglobulin, ribonuclease and lysozyme by both ultraviolet and mass spectra detection [25].

The concentration of a sample analyte ion with an intermediate ion mobility present at a low concentration is due to the need to change its concentration and in turn its field strength to keep up in pace with the velocity of the leading ion. At equilibrium, if few sample ions were encountered in the leading zone they would encounter lower field strength so they slow down. While if some sample ions slow down to migrate in the terminating ion region they are exposed to higher field strength and speed up to catch up with their own segment. Thus the difference in the field strength between terminating and leading zone dictates the stacking. Since the velocity of the ions are affected by factors such as the charge, pH, concentration and co-ions a successful ITP, unfortunately, requires careful attention to all these details.

4.2. High-field stacking (low ionic strength buffer in the sample)

As pointed out earlier, Mikkers et al. [10] noticed that diluting the sample in water gave sharper peaks in CE. Hjertén et al. [26] used lower conductivity buffer in the sample to sharpen the bands for polyacrylamide gel electrophoresis. The dilution and the lower conductivity cause the sample resistance and the field strength (V/cm) in the sample plug to increase. In turn, this causes the ions to migrate rapidly and stack as a sharp band at the boundary between the sample plug and the electrophoresis buffer with the positive ions lining up in front of the negative ones [27,28]. Once in the electrophoresis buffer, the components of the sample migrate in different zones according to their charge/mass characteristics. The sample can be injected hydrodynamically or by electromigration.

4.2.1. Hydrodynamic injection

The sample usually is dissolved in a lower ionic strength buffer (~10×) relative to the separation buffer. This is a very simple method to perform in practice and thus it is the most used type of stacking. One can look at the low ionic strength as another simple way of introducing discontinuity to the buffer in the sample zone. In this technique the amount of sample concentration is limited to about 10 times and often does not exceed about five-fold. Buffer removal is important when injecting very large sample volumes to avoid the mismatch of electroosmotic flow (EOF). After hydrodynamically injecting the sample, the polarity is reversed. The negatively charged ions are pumped out by the EOF while the negative sample ions will be stacked behind [29]. This can improve greatly the stacking.

4.2.2. Electromigration (field amplified sample injection)

Conventional electromigration can be used to inject on the capillary. However, sample stacking can occur if the samples are prepared also in very dilute buffer or water [30]. The analyte (cations) experience high field strength, move rapidly, and concentrate at the tip of the capillary at the boundary between the sample and the separation buffers. The concentration is related to the ratio of the two buffers. A plug of

water can be injected before the separation buffer, which can enhance this technique further [31]. Several hundred-fold enhancements in concentration have been shown to be possible by this technique for some drugs [31–35].

Three factors limit the stacking in this process. The heat generated by the current, the laminar flow generated by the mismatch of the local EOF and the presence of other ions in the sample all affect the outcomes. Biological samples have high slats or ion content which interfere in this technique [36]. Thus sample extraction to eliminate the interfering ions becomes very important. Electromigration favors the high-mobility anions in the sample. However, cations electromigration can be enhanced too by a reversed polarity injection [37].

4.3. pH (focusing)

Velocity-difference induced focusing of analytes using a dynamic pH junction allows injection of large sample volume with separation efficiencies close to a million theoretical plates [38]. This approach which does not depend on conductivity is useful for weakly acidic species and zwitterionic analytes that can possess different velocities in the sample and background electrolytes. Stacking based on adjusting the pH of the sample has been described also by Aebersold and Morrison [39]. They have concentrated peptides by dissolving the sample in buffer two units above the net isoelectric point (p*I*), so the peptides are negatively charged. As the potential is turned on, the peptides initially migrate towards the anode until they are stopped by the interface of the electrophoresis buffer, where they concentrate. After the short pH gradient of the sample dissipates in the electrophoresis buffer, the peptides become positively charged and migrate towards the cathode as a sharp zone. Using this method, a larger volume was introduced into the capillary obtaining a five-fold concentration. Catecholamine metabolites [38], and few drugs [13] have been shown to stack better by manipulating the sample pH.

4.4. Acetonitrile–salt mixtures (organic solvents)

Mixing acetonitrile with the sample (2:1, v/v) is used mainly to remove proteins. However, the presence of acetonitrile in the sample (not in the buffer), has several important additional advantages. For example, (1) it counteracts the deleterious effects of ions, (2) it yields better stacking for small molecules than that obtained in dilute buffers, and (3) it allows larger volumes of sample (in some cases half of the capillary volume) to be injected. The overall effect is an increased sensitivity of about 20-fold.

Because acetonitrile has low conductivity by itself it can bring some stacking due to the high field strength [13,14,36,40–42]. The stacking occurs in both the hydrodynamic and the electromigration injection. The latter gives much more concentration [14,36,40,41]. The sodium chloride present in serum at about 150 mmol/l together with the acetonitrile used in the deproteinization both bring about a further but unique type of sample stacking with even higher sample concentration than that obtained with acetonitrile only. This type of stacking occurs in many organic solvents and it is greatly enhanced by the presence of high concentrations of different inorganic ions in the sample [13,43]. It is more suited for practical work, especially for samples obtained from serum, food, or industrial sources and produces higher sample concentration compared to that of the low ionic strength buffers. Organic buffers for separation such as triethanolamine (TEA), Tris and 2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES) which generate low currents, also favor rapid migration of the ions and stacking similar to that when the sample is dissolved in acetonitrile and it also enhances the acetonitrile stacking [43].

Acetonitrile stacking is favored by a low voltage, a long capillary and a high ionic strength in the electrophoresis buffer. However, it is restricted to small molecules, which are soluble in acetonitrile and many small peptides. Basic compounds are more difficult to stack in acetonitrile because of the their interaction with the capillary wall. An organic buffer containing an amine or zwitterionic group performs better for the stacking of these compounds [44].

The mechanism behind stacking acetonitrile–salt is similar to that of transient ITP. The salts move rapidly in the acetonitrile, slowing at the separation buffer interface, creating two regions of field strength a low one (in the salts) and high one (in the

acetonitrile). As the boundary between the two regions moves in the sample, analyte ions behind the boundary, i.e., the region of higher strength move faster, while those ones in the front, i.e., in the low field strength slow down, Fig. 1. This produces a ''sweeping'' action for the analyte ion concentrating them into a very sharp band. This effect is very similar to ITP where the salts (in the sample) act as leading ions while the acetonitrile gives the high filed strength similar to the action of the terminating ions (relative to the leading ions) but without the rigid requirements of pH, concentration, or counter ions necessary in the ITP. Thus the acetonitrile–salt stacking mechanism can be considered as ''transient ITP-like'' [43]. After the bands stack they enter the separation buffer for separation into the distinct zones.

Compared to ITP, acetonitrile gives a good degree of stacking of about 10–30 times. However it is easier to perform since there is no strict requirements for matching the mobility of the leading/terminating ions and samples mobility or pH. It also eliminates the proteins from the sample. Furthermore, both anions and cations can be concentrated at the same time in the acetonitrile, Fig. 2.

4.5. Isoelectric focusing

Here the proteins are separated based on their isoelectric point in a pH gradient formed by carrier ampholytes when the electric field is applied. The ampholytes can also be considered as special discontinuous buffers in which they differ in their p*K*. After the voltage is turned on the ampholytes rearrange according to their pH while the sample analytes which occupy the whole capillary concentrate (focuses) into very narrow zones according to their p*K* where the net charge of the protein is zero. This technique gives very high resolution on its own without the need for a CZE step. It also gives high concentration since the sample occupies all the capillary volume. It is suitable for focusing zwitterionic compounds such as proteins. As performed on the CE equipment, the technique has a few problems; such as the absorption of light by the ampholytes at the high end of the UV range and the need for the focused zones to be transported to the detection window. The majority of the commercial CE instruments are not designed to accommodate this technique easily. Detailed discussion of this technique can be found in Ref. [45].

4.6. Mixed mode

A better stacking can be obtained if the combination of more than one variable is introduced in the sample such as a pH difference and a low ionic strength or an organic solvent. In many types of stacking an additional plug of water is introduced before the sample is injected to further increase the sample concentration on the capillary. A stacking has been described based on titration. Using a weak-acid buffer such as acetate as the background electrolyte, the electrokinetic injection of the sample is immediately followed by electrokinetic injection of an acid solution, which can result in titration of the background electrolyte producing a low-conductivity zone across which the sample cations are focused [46].

Quirino and Terabe [47] have devised a mixed mode for further stacking in which the sample is concentrated first by field amplified injection under non-micellar conditions. The buffers are changed and the polarity is reversed to induce sweeping of the analytes into a micellar (SDS) solution giving about a million-fold increase in sensitivity for some cations. On the other hand, Palmer et al. [76] concentrated neutral molecules in micellar electrokinetic chromatography by increasing the salt concentration in the sample while stacking the anionic micelle cholate at the interface of the buffer and the sample before entering the sample zone. The neutral molecules carried by electroosmotic flow enter the concentrated micelles and thus are stacked.

5. Applications

Many compounds have been stacked based on the different methods described. Table 1 lists examples for stacking many compounds by CZE. Ions can be stacked easily in water or weak buffers. The high mobility of the ions, which leads to fast analysis and a high degree of stacking makes ion analysis by CE very popular. Drugs and small molecules can be stacked also to some extent in acetonitrile. The

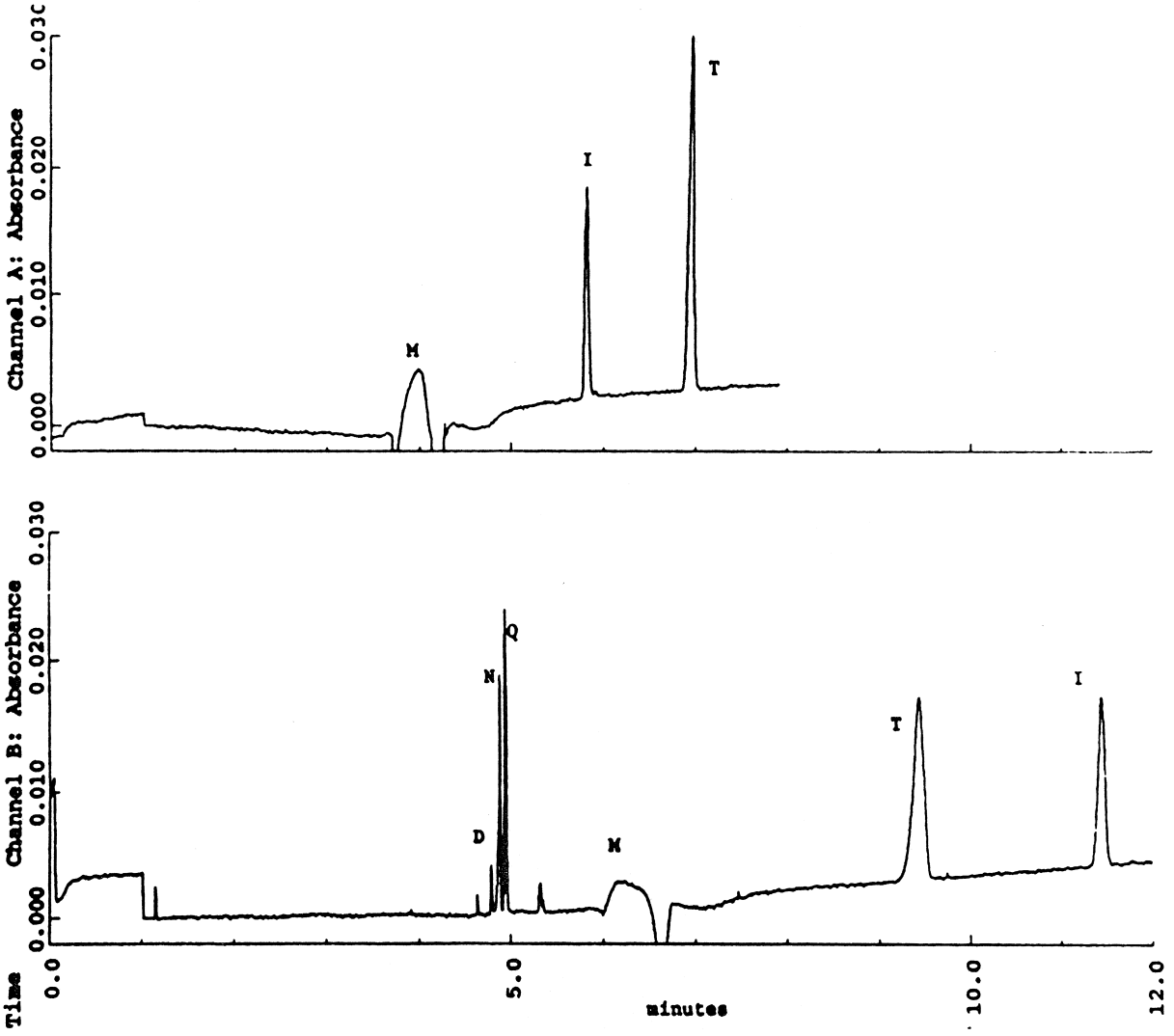

Fig. 2. Stacking of both cationic and anionic compounds. Effect of the separation buffer type on stacking at sample loading of 12% of the capillary volume: (Top) 210 m*M* borate buffer, pH 8.6; and (Bottom) 160 m*M* triethanolamine, 50 m*M* tricine, pH 8.6 containing 10% acetonitrile. Separation of a mixture of weakly cationic and anionic compounds in the same run: doxepin (D, 50 mg/l), *N*-acetylprocainamide (N, 50 mg/l), quinine (Q, 20 mg/l), theophylline (T 50 mg/l) and iothalamic acid (I, 20 mg/l) at 14 kV, 254 nm; (M = electroosmotic flow). From Ref. [44] with permission.

advantage here is that the acetonitrile can precipitate the excess of proteins present in the biological samples. ITP gives a high degree of stacking with the elimination of sample matrix but it is more difficult to perform. It is expected other combinations and new methods of stacking to be designed in the future.

6. Concluding remarks

As we see stacking depends on the use of discontinuous buffers. These buffers provide the means for giving a different velocity to the same analyte ions. Discontinuity in the buffer can be easily induced, e.g., by slight adjustment in the sample pH

Table 1
Examples for different compounds analyzed in CZE based on different kinds of stacking

Type	Compound	Basic principle	Ref.
Ions	Cations	pH mediated/field amplified	[46]
	Nitrate	Acetonitrile	[48]
	Hippurate	ITP	[49]
	Anions	pH mediated/field amplified	[50]
	Several	ITP	[18,19,51]
	Several	Electro-stacking	[52]
	Several	Electromigration	[53]
Drugs	Alkaloids	Field amplified	[41]
	Amiodarone	Field amplified	[34]
	Antibiotics	Field amplified	[40]
	Opiods	Field amplified	[35,36]
	Forensic	Polarity switching	[54]
	Phenobarbital	Acetonitrile	[3]
	Procainamide	Acetonitrile	[44]
	Iohexol	Acetonitrile	[55]
	Tricyclics (antidepressants)	Acetonitrile	[44]
	Quinidine	Acetonitrile	[44]
	Formoterol	Field amplified	[56]
	Drugs	Field amplified	[57]
Endogenous	Orotic acid	ITP	[57]
	Adenosine	ITP	[58]
	Catecholamines	pH	[38]
	Catecholamines	Acetonitrile	[44]
	Nucleotides	Acetonitrile	[59]
	AA	ITP	[60]
Peptides	Angiotension	Acetonitrile	[61]
	Angiotension	ITP	[15,62]
	Insulin	Acetonitrile, conductivity, ITP	[63]
	Several	pH	[38]
	Angiotensin, gonadorelin	ITP	[59,64]
	Different	ITP	[65]
Proteins	Several	Transient ITP	[24,25,66]
	General	Buffer removal	[67]
	Several	ITP	[68]
	Cathapsin D	Acetonitrile for peptides	[69]
DNA	PCR general	pH	[70]
	PCR general	pH	[71]
	DNA sequence	Low conductivity	[72]
	DNA sequence	Low ionic strength	[72]
	DNA sequence	Low conductivity	[41,73]
	DNA sequence	pH	[74]
	DNA adducts	Buffer removal	[75]

PCR = Polymerase chain reaction.

or by changes in the sample conductivity. On the other hand some of the buffers can be complicated and require careful planning such as those for ITP and isoelectric focusing. Stacking can give often a concentration factor from 5- to 50-fold depending on the type chosen which can bring the sensitivity of CE to that of HPLC or better [64]. The combination of stacking, sensitive cells and sensitive detectors will make this technique much more popular in practical applications. Examples for the use of different continuous buffers and their effect on stacking has been discussed recently [43].

References

[1] G. Hempel, Electrophoreis 21 (2000) 691.
[2] D.S. Burgi, R.-L. Chien, in: J.P. Landers (Ed.), Handbook of Electrophoresis, 2nd ed., CRC Press, Boca Raton, FL, 1997, p. 479.
[3] Z.K. Shihabi, in: S. Palfrey (Ed.), Clinical Applications of Capillary Electrophoresis, Humana Press, Totowa, NJ, 1999, p. 157.
[4] Z.K. Shihabi, in: J.P. Landers (Ed.), Handbook of Electrophoresis, 2nd ed., CRC Press, Boca Raton, FL, 1997, p. 457.
[5] J. Kendall, Nature 150 (1942) 136.
[6] A. Kolin, in: D. Stathakos (Ed.), Electrophoresis '82, Walter de Gruyter, Berlin, 1983, p. 2.
[7] M.D. Poulik, Nature 180 (1957) 1477.
[8] L. Ornstein, Ann. NY Acad. Sci. 121 (1965) 321.
[9] B.D. Hams, in: B.D. Hams, D. Rickwood (Eds.), Gel Electrophoresis of Proteins, IRL Press, Oxford, 1983, p. 1.
[10] F.E.P. Mikkers, F.M. Everaerts, Th.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 11.
[11] J.P. Quiarino, S. Terabe, Science (Washington, DC) 282 (1998) 465.
[12] Z.K. Shihabi, J. Cap. Electrophoresis 2 (1995) 267.
[13] M.A. Friedberg, M. Hinsdale, Z.K. Shihabi, J. Chromatogr. A 781 (1997) 35.
[14] Z.K. Shihabi, J. Chromatogr. A 853 (1999) 3.
[15] S. Chen, M. Lee, Anal. Chem. 72 (2000) 816.
[16] P. Gebauer, P. Bocek, Electrophoresis 18 (1997) 2154.
[17] P. Gebauer, P. Bocek, Electrophoresis 16 (1995) 1999.
[18] L. Krivankova, P. Bocek, J. Chromatogr. B 689 (1997) 13.
[19] L. Krivankova, P. Pantukova, P. Bocek, J. Chromatogr. A 838 (1999) 55.
[20] J.B. Bachmann, J. Chromatogr. A 734 (1996) 319.
[21] P. Gebauer, W. Thormann, P. Bocek, J. Chromatogr. 608 (1992) 47.
[22] P. Gebauer, W. Thormann, P. Bocek, Electrophoresis 16 (1995) 2039.
[23] C. Schwer, F. Lottspeich, E. Kenndler, Anal. Chem. 65 (1993) 2108.
[24] F. Foret, E. Szoko, B.L. Karger, J. Chromatogr. 608 (1992) 3.
[25] T.J. Thompson, F. Foret, P. Vouros, B.L. Karger, Anal. Chem. 65 (1993) 900.
[26] S. Hjertén, S. Jerstedt, A. Tiselius, Anal. Biochem. 11 (1965) 219.
[27] R.-L. Chien, J.C. Helmer, Anal. Chem. 63 (1991) 1354.
[28] D.S. Burgi, R.-L. Chien, Anal. Chem. 63 (1991) 2042.
[29] R.-L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 1046.
[30] L. Gross, E.S. Yeung, J. Chromatogr. 480 (1989) 169.
[31] R.-L. Chien, D.S. Burgi, J. Chromatogr. 559 (1991) 141.
[32] C.X. Zhang, Y. Aebi, W. Thormann, Clin. Chem. 42 (1996) 1805.
[33] C.X. Zhang, W. Thormann, Anal. Chem. 70 (1998) 540.
[34] C.X. Zhang, W. Thormann, Anal. Chem. 68 (1996) 2523.
[35] A.B. Wey, C.X. Zhang, W. Thormann, J. Chromatogr. A 853 (1999) 95.
[36] R. Taylor, S. Toasaksiri, R.G. Reid, Electrophoresis 19 (1998) 2791.
[37] R. Chien, D.S. Burgi, J. Chromatogr. 559 (1992) 153.
[38] R. Aebersold, H.D. Morrison, J. Chromatogr. 516 (1990) 79.
[39] P. Britz-Mckibbin, D. Chen, Anal. Chem. 72 (2000) 1242.
[40] A.K. Lalloo, S.C. Chattaraj, I. Kanfer, J. Chromatogr. B 704 (1997) 333.
[41] M. Unger, J. Stockigt, J. Chromatogr. A 791 (1997) 323.
[42] D. Figeys, H. Ahmadzedeh, E. Arriaga, N.J. Dovichi, J. Chromatogr. A 744 (1996) 325.
[43] Z.K. Shihabi, Electrophoresis 21 (2000) in press.
[44] Z.K. Shihabi, J. Chromatogr. A 817 (1998) 25.
[45] R. Rodriquez-Diaz, T. Wehr, M. Zhu, V. Levi, in: J.P. Landers (Ed.), Handbook of Electrophoresis, 2nd ed., CRC Press, Boca Raton, FL, 1997, p. 101.
[46] S. Park, C.E. Lunte, J. Microcol. Sep. 10 (1998) 511.
[47] J.P. Quirino, S. Terabe, Anal. Chem. 72 (2000) 1023.
[48] M.A. Friedberg, M.E. Hinsdale, Z.K. Shihabi, J. Chromatogr. A 781 (1997) 491.
[49] L. Krivankova, A. Vrana, P. Gebauer, P. Bocek, J. Chromatogr. A 772 (1997) 283.
[50] Y. Zhao, C.E. Lunte, Anal. Chem. 71 (1999) 3985.
[51] W.R. Jones, J. Soglia, M. McGlynn, C. Harber, J. Reineck, C. Krstanovic, Am. Lab. 28 (1996) 25.
[52] M.J. Wojtusik, M.P. Harold, J. Chromatogr. A 671 (1994) 411.
[53] P.E. Jackson, P.R. Haddad, J. Chromatogr. 640 (1993) 481.
[54] G. McGrath, S.W. Smyth, J. Chromatogr. B 681 (1996) 125.
[55] Z.K. Shihabi, M.S. Constantinescu, Clin. Chem. 38 (1992) 2117.
[56] J.Z. Song, J. Chen, S.J. Tian, Z.P. Sun, J. Pharm. Biomed. Anal. 21 (1999) 56.
[57] F. Tagliaro, G. Manetto, F. Crivellente, D. Scarcella, M. Marigo, Forensic Sci. Int. 92 (1998) 201.
[58] C.C. Wang, W.P. McCann, S.C. Beale, J. Chromatogr. B 676 (1996) 19.
[59] Z.K. Shihabi, M.E. Hinsdale, A.J. Bleyer, J. Chromatogr. B 669 (1995) 163.
[60] D.S. Stegehuis, H. Irth, U.R. Tjaden, J. van der Greef, J. Chromatogr. 538 (1991) 393.

[61] Z.K. Shihabi, J. Chromatogr. A 744 (1996) 231.
[62] J.C.M. Waterval, C.J.L. la Porte, R. van 't Hof, J. Teeuwsen, A. Bult, H. Lingeman, W.J.M. Underberg, Electrophoresis 19 (1998) 3171.
[63] Z.K. Shihabi, M. Friedberg, J. Chromatogr. A 807 (1998) 129.
[64] J.C.M. Waterval, H. Krabbe, J. Teeuwsen, A. Bult, H. Lingeman, W.J.M. Underberg, Electrophoresis 20 (1999) 2909.
[65] D.T. Witte, S. Nagard, M. Larsson, J. Chromatogr. A 687 (1994) 155.
[66] F. Foret, E. Szoko, B.L. Karger, Electrophoresis 14 (1993) 4.
[67] J. Li, C. Wang, J.F. Kelly, D.J. Harrison, P. Thibault, Electrophoresis 21 (2000) 198.
[68] J. Bergmann, U. Jaehde, M. Mazereeuw, U. Tjaden, W. Schunack, J. Chromatogr A. 734 (1996) 381.
[69] Z.K. Shihabi, T.E. Kute, J. Chromatogr. B 683 (1996) 125.
[70] Z.K. Shihabi, J. Chromatogr. A 853 (1999) 349.
[71] P.G. Righetti, C. Gelfi, J. Biochem. Biophys. Methods 41 (1999) 75.
[72] Y. Xiong, S.R. Park, H. Swerdlow, Anal. Chem. 70 (1998) 3605.
[73] P. Lindberg, M. Stjernstrom, J. Roeraade, Electrophoresis 18 (1997) 1973.
[74] H. Tan, E.S. Yeung, Anal. Chem. 70 (1998) 4044.
[75] S.M. Wolf, P. Vouros, Anal. Chem. 67 (1995) 891.
[76] J. Palmer, N.J. Munro, J.P. Landers, Anal. Chem. 171 (1999) 1679.
[77] H. Haglund, A. Tiselius, Acta. Chem. Scand. 4 (1950) 957.