# 8
# Modern Injection Modes (Stacking) for CE

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## 8.1
## Introduction

Capillary electrophoresis (CE) is an attractive analytical liquid-phase separation technique due to its high separation efficiency, small sample requirement, and low running costs. CE is also flexible due to the availability of different CE modes that allow separation of almost any analyte. For example, capillary zone electrophoresis (CZE) [1], electrokinetic chromatography (EKC) [2], and capillary gel electrophoresis (CGE) [3] are the CE modes used to separate analytes based on charge, interaction with a "pseudo" stationary phase, and size, respectively. Different analyte detection schemes such as photometric, conductivity, mass spectrometric (MS), and laser-induced fluorescence are employed. Standard commercial CE instrumentations are conveniently equipped with an online photometric detector and thus UV detection is most commonly used. The light path length is short due to the dimensions of the capillary used (i.e., 25–75 μm inner diameter); therefore according to the Beers Law concentration sensitivity in CE using UV detection is quite poor. In addition, to preserve the high efficiency separation in CE, the injected sample amount or plug length must be small or narrow, respectively. Significant efforts from the CE community to overcome this issue have been made over the years. Online sample concentration or stacking and online solid-phase extraction (SPE) and liquid–liquid extraction (LLE) have been shown to lower the limit of detection (LOD) and quantification (LOQ) of analytes. This has made CE relevant for the analysis of real samples and at par with the more commonly used high-performance liquid chromatography (HPLC).

## 8.2
## Online Sample Concentration or Stacking

Stacking is an elegant, simple, and effective way to improve detection sensitivity and thus is a highly active research area in CE [4–6]. Stacking comprises a family

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**Table 8.1** Online concentration or stacking techniques in capillary electrophoresis.

| Technique | Mechanism |
|---|---|
| 1. Field amplification or enhancement | Reduction in the electrophoretic velocity of charged samples at the concentration boundary |
| 2. Transient isotachophoresis (tr-ITP) | Focusing of charged sample zones between leading and terminating ionic zones |
| 3. Dynamic pH junction | Change in electrophoretic mobility of basic, acidic, or amphoteric samples due to the pH |
| 4. Sweeping | Chromatographic partitioning of neutral or charged samples into a pseudophase (e.g., micelle) |
| 5. Analyte focusing by micelle collapse (AFMC) | Transport, release, and accumulation of neutral or charged samples facilitated by surfactant micelles |
| 6. Micelle-to-solvent stacking (MSS) | Change in charged samples' effective electrophoretic mobility due to the pseudophase and presence of an organic solvent |

of techniques that allow the injection of larger amounts or longer plugs of sample solution into the capillary. The injected sample is concentrated by squeezing the analytes in the long sample plug into a narrow band in the so-called stacking boundary or zone. In essence, the use of stacking also integrates sample preparation and CE separation in one run. Interestingly, the promise of high-performance CE and the concept of stacking in CE were published at the same time by Mikkers, Everaerts, and Verheggen in 1979 [7,8]. They diluted the analytes in two sample diluents, the separation background electrolyte/solution (BGS) and water. When the samples were injected for longer than usual, the analytes prepared in the lower conductivity diluent (i.e., water) were detected as narrower and taller peaks. This indicated a sample concentration effect (field amplification) before detection of the analytes.

The stacking techniques in CE are summarized in Table 8.1. They rely on electrophoretic (e.g., field amplification [7,8], transient isotachophoresis (tr-ITP) [9], and dynamic pH junction [10]) and/or chromatographic phenomena (i.e., sweeping [11], analyte focusing by micelle collapse (AFMC) [12], and micelle-to-solvent stacking (MSS) [13]). Enrichment is induced by changing the chemical properties (e.g., conductivity or pH) of the sample or/and BGS. In some cases, modification of the sample prior to injection into the CE instrument may involve only simple filtration.

### 8.2.1 Field Amplification/Enhancement

A stacking boundary in between the separation electrolyte and the sample solution (inside the capillary) is formed when the sample is prepared in a low-conductivity diluent. The stacking mechanism was explained on the basis of field amplification (also called field enhancement as suggested by Prof. S. Terabe).

The electric field is enhanced and reduced in the sample and separation solution zone, respectively. The electrophoretic velocity of the charged sample then becomes faster in the sample than in the separation solution zone, thus the analytes accumulate at the stacking boundary. Field enhancement can be implemented using hydrodynamic (i.e., field-amplified/enhanced sample stacking or FASS/FESS) and electrokinetic (i.e., field-amplified/enhanced sample injection or FASI/FESI) injection modes. Significant advancements in this area were made in the early 1990s by Chien and Burgi [14].

In FASS, the length of the low-conductivity sample that remains in the capillary during separation should be controlled in order to obtain high-sensitivity enhancement factors (SEF) and preserve the separation efficiency. In most stacking studies, the SEF values are typically calculated by dividing the peak signal with stacking by the signal without stacking or the LOD with stacking by the LOD without stacking. An SEF value of ~10 is typically obtained and both anionic and cationic analytes can be enriched [15]. However, higher SEF values can be obtained if the sample matrix is removed prior to separation. Removal of the sample matrix allows larger sample loads and reasonably sharp peaks [16–19]. This strategy can be implemented using polarity switching (different polarity during stacking and separation), by the application of pressure, and more conveniently by the clever use of the electroosmotic flow (EOF). Only the analytes of the same charge can be effectively stacked at a time since the EOF or applied pressure and analyte electrophoretic velocity must move in opposite directions. More importantly, there is only one stacking boundary where either anionic or cationic species are enriched since the other end of the sample zone is submerged in the separation electrolyte during matrix removal. The stacking boundary must be maintained inside the capillary prior to separation; otherwise, the concentrated samples will be lost into the inlet vial. In addition, the ions of charge opposite to that of the target ions are concentrated at the edge of the capillary. These ions will eventually diffuse into the inlet vial. In the literature, this is commonly called as large-volume sample stacking (LVSS) or reversed electrode polarity switching mode (REPSM). However, since the stacking mechanism is field amplification, this technique will be referred to as FASS with sample matrix removal by polarity switching, pressure application, or EOF modulation.

FASS with sample matrix removal by pressure application was recently demonstrated by Tuma and coworkers for the *in vivo* monitoring of neurotransmitters and drugs in biosamples [20,21]. Low detection limits were obtained using capacitively coupled contactless conductivity detection (CE/$C^4D$). In another report, Dziomba *et al.* performed FASS with counter pressure [22]. By performing the stacking injection 4× (repetitive injection), detection limits in the ng/ml range were reached for antipsychotic drugs in human urine samples after liquid–liquid extraction. FASS with sample matrix removal by polarity switching was applied by Muller and coworkers for monitoring of nucleotide pyrophosphatase/phosphodiesterase and purine nucleoside phosphorylase and adenosine deaminase reactions [23,24]. In FASS with matrix removal, EOF modulation is the most convenient way to achieve high sensitivity, since after sample loading, no

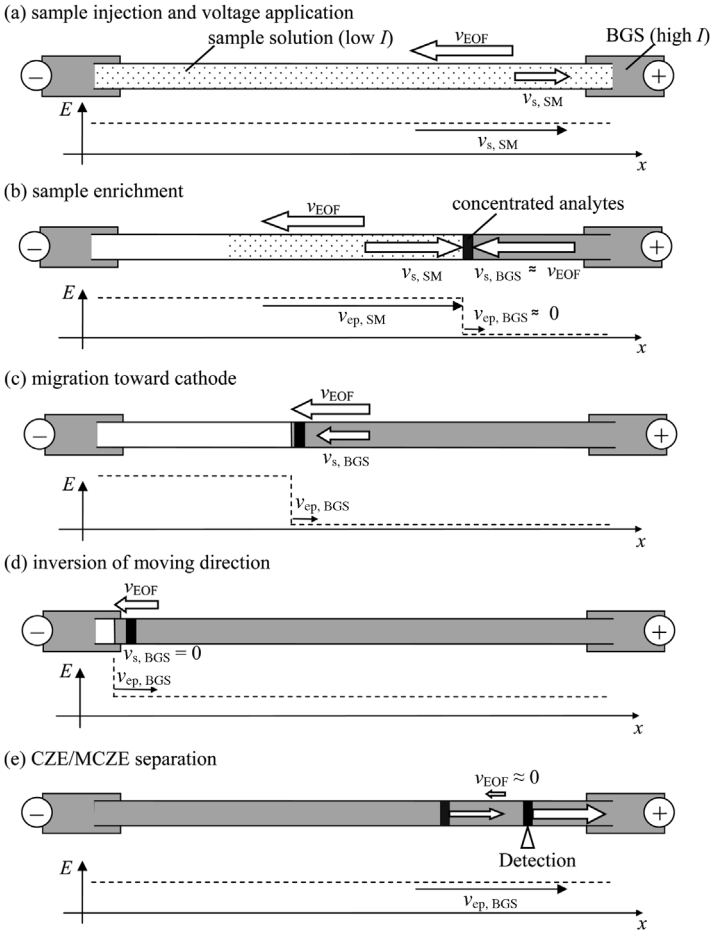


**Figure 8.1** Whole capillary sample injection using FASS with matrix removal by EOF modulation for the stacking of anionic analytes [25].

additional steps are required during the stacking step. In general, the EOF is suppressed such that the stacking is completed before the entire sample matrix is removed. Otsuka and coworkers developed several innovative assays for high-sensitivity analysis of oligosaccharides, chiral molecules, and cations using FASS with matrix removal by EOF modulation [25–28]. Figure 8.1 shows the scheme used by them for the stacking of anionic analytes [25]. The entire column was filled with the sample and a voltage was applied with the separation electrolyte at both ends of the column (a). The separation electrolyte at the anodic end enters the column and this forms the stacking boundary. The analytes migrate to the stacking boundary where they are concentrated, while the EOF pumps the sample matrix out of the column (b–c). The sample matrix is completely out of the column and stacked analytes separated by CZE (d).

In FASI or FESI, sample ions of similar charge are selectively introduced into the capillary. The injected sample ions can exceed the total amount of sample ions injected by filling the capillary with the sample solution; thus, the sample load is extremely large. Keeping this in mind, FESI is one of the most powerful and popular stacking techniques. FESI can also be combined with the other stacking techniques shown in Table 8.1 and these combinations will be tackled later. For complex matrices, an appropriate sample preparation approach is required to fulfill the field-amplification condition (the sample should be in a low-conductivity electrolyte or water). Notable applications are possible for drugs in the environment and biological fluids [29–33], natural products [34–36], human and animal foods [37–39], pesticides and pollutants [40–42], and large molecules and cells [43,44].

The key to the success of FESI is that the stacking boundary should be maintained inside the capillary and preferably close to the injection point. For example, Sihvonen and coworkers [45] used pressure while Wuethrich, Haddad, and Quirino [46] used a hydrogel at the outlet end of the capillary in order to keep the stacking boundary at the capillary inlet. For the simultaneous analysis of cationic and anionic species, Hou *et al.* [47] cleverly performed FESI at the inlet and outlet ends, where the anions and cations were selectively injected into the cathodic and anodic ends of the capillary.

### 8.2.2
### Transient Isotachophoresis or tr-ITP

In isotachophoresis or ITP, the sample ions are squeezed in between the leading and the terminating ions of the same charge. The electrophoretic mobility of the sample ions is between the fast-leading and slow-terminating ions. The concentration of the sample ions is adjusted to the concentration (Kohlrausch law) of the leading electrolyte; thus, a dilute sample can be concentrated during an ITP analysis. As a stacking strategy, ITP can be induced during the early stages of a CE analysis and can thus concentrate the sample ions before the analytical separation. This process is now called as tr-ITP, which was first described by Foret, Szoko, and Karger to stack acidic and basic proteins and then separate them using CZE with coated capillaries [9]. The typical strategy in tr-ITP is to add a leading ion into the background electrolyte or sample solution and separation is done by CZE that is performed under no-EOF or co-EOF conditions. After injection of the sample, voltage is applied using a terminating ion at the inlet of the capillary to facilitate the tr-ITP stacking.

This stacking method is popularly used for the analysis of charged drugs, biomolecules, and natural products [48–57]. As a method for preselection of binding ligands, Riley *et al.* used tr-ITP and CZE to analyze protein-aptamers and free aptamers [58]. There is also an interesting work conducted by Timerbaev and coworkers, who studied the tr-ITP of charged micelles to facilitate the concentration of neutral analytes that are not affected by ITP [59,60].

### 8.2.3 Dynamic pH Junction

The use of a sample solution with a different pH for the separation electrolyte for stacking was first proposed by Aebersold and Morrison [61]. If the analytes concentrate at a pH boundary due to a change in the analytes' electrophoretic mobility or velocity, the stacking technique was coined as dynamic pH junction [10]. This technique is reminiscent of the effect of capillary isoelectric focusing on amphoteric analytes or analytes with a pI. In a pH gradient, the analytes migrate along the capillary until they reach a pH zone that is equal to its pI. The analytes accumulate at this pH zone because they are neutral and therefore do not have electrophoretic mobility.

For the focusing of acidic compounds by a dynamic pH junction, let us take the example of a sample prepared in an acidic sample diluent where the analytes are neutral. The separation electrolyte is basic and normally with a pH that is above the p$K_a$ of the analytes. The initial pH junction is formed at the cathodic side of the sample zone and the anodic side of the separation electrolyte. In the presence of an electric field, hydroxide ions from the separation electrolyte are neutralized by the hydronium ions from the sample solution. The flux of the hydroxide ions must be greater than that of the hydronium ions from the sample. This will cause the pH junction to sweep through the entire sample zone. The acidic analytes in the sample zone are ionized by the hydroxide ions and thus migrate electrophoretically in the same direction as the pH junction. The change in electrophoretic mobility from 0 to a certain value at the pH junction caused the stacking of the analytes. Similar scenarios can be imagined for weakly basic and amphoteric analytes. However, tr-ITP also plays a role in the stacking with a pH junction with the coion in the sample solution and the hydroxide or hydronium ions as the leading and terminating ions, respectively [49,52].

The dynamic pH junction made effective the sensitive CE analysis of sugars [62], amines [63], amino acids [64], peptides [65–68], proteins [68], and other biologically active compounds [69]. It has also been recently applied to food analysis [70,71]. The use of a dynamic pH junction in CZE with electrospray ionization (ESI) MS detection was a particularly interesting application in bottom-up proteomics analysis [68]. When compared to regular CE injection (Figure 8.2a and b), a larger injection focusing on the dynamic pH junction (Figure 8.2c and d) allowed the identification of additional peptides and proteins. For example, 19 and 20 more peptides and protein, respectively, could be identified from an *Escherichia coli* digest.

### 8.2.4 Sweeping

The fundamental condition for sweeping is a sample solution that is void of the pseudostationary phase (e.g., micelle) or any additive (e.g., borate or ethylene diaminetetraacetic acid) that will interact with the analyte [11,72,73]. The

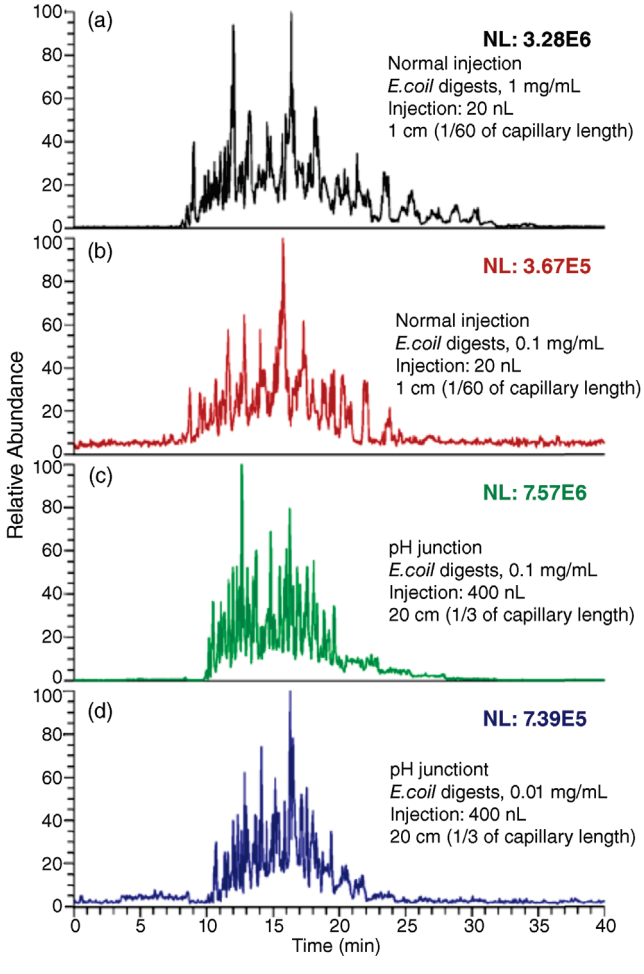


**Figure 8.2** Base peak elecropherograms of *E. coli* digests analyzed by conventional (a, b) and dynamic pH junction-based (c, d) CZE–ESI–MS/MS using an LTQ mass spectrometer. Conventional CZE–MS/MS analysis of 1 mg/ml (a) and 0.1 mg/ml (b) *E. coli* digests in 0.1% (v/v) FA with an injection volume of 20 nl. Dynamic pH junction-based CZE–MS/MS analysis of 0.1 mg/ml (c) and 0.01 mg/ml (d) *E. coli* digests in 10 mM ammonium acetate (ph ~6.5) with injection volumes of 400 nl. NL, normalization level [68].

additive penetrates the sample zone and the additive–analyte interaction causes the concentration of the analytes at the additive front. The concentration effect for charged and neutral analytes is thus dependent on the magnitude of the interaction. Analytes that have very high affinities for the additive can yield extremely high SEFs. In MEKC, the affinity is directly proportional to the retention factor $k$. The SEF value for sweeping is approximately equal to $(1 + k)$ implying that the injected sample plug is narrowed by a factor $1/(1 + k)$. If the $k$ value is very high, the entire capillary can be filled with the sample solution for

sweeping. Full-capillary sample injection in sweeping MEKC was recently applied by Wang *et al.* for the analysis of anabolic androgenic steroids that are misused in sport doping [74].

It is also well known that the strength of analyte–additive interactions can be controlled by changing the concentration of the additive. The concentration of the additive entering the sample solution for sweeping the analytes is affected by the conductivity of the sample solution. There is a higher and lower concentration of the additive if the sample is prepared in high- and low-conductivity sample diluents, respectively. If a high-conductivity sample solution is used, the additives are stacked at the concentration boundary and then the stacked additives sweep through the sample. At the other side of the sample zone, the stacked additives will dilute or destack leading to the slight broadening of the stacked analytes [75]. Stacking and destacking of the additive due to conductivity differences between the sample and the separation electrolyte was revisited by Pyell and coworkers [76,77]. Their results were similar to those initially reported by Quirino, Terabe, and Bocek [75]. In practice, however, the conductivity of the sample matrix relative to the separation electrolyte with the additive should be optimized in order to achieve the acceptable SEF values.

The sweeping boundary is normally at the front of the additive that penetrates the sample. A special case was observed for the sweeping of cationic alprenolol enantiomers using anionic sulfated beta-cyclodextrin (β-CD) and an organic solvent in the separation and sample solution, respectively [78]. The enantiomers' effective electrophoretic mobility was the same as the electrophoretic mobility due to the low interaction between alprenolol and β-CD. When the amount of the organic solvent in the sample was increased (i.e., 60%), the interaction between the analytes and additive became negligible. This caused the sweeping boundary to shift from the electrophoretically moving β-CD front to the zone between the sample and organic solvent-rich separation solution.

Sweeping nowadays is popularly used in the sensitive analysis of drugs and bioactive compounds in complex matrices that were normally subjected to a suitable sample cleanup step [79–87]. Another notable application of sweeping is in food analysis for the determination of vitamins, herbicides, and contaminants in cereal, vegetables, milk, and alcoholic drinks [88–91]. There are also applications of sweeping in environmental analysis [92–94] for the determination of polar and nonpolar pollutants and in consumer product analysis [95–97] for the determination of preservatives and active ingredients.

### 8.2.5
### Analyte Focusing by Micelle Collapse and Micelle-to-Solvent Stacking

AFMC involves the transport, release, and accumulation of analytes into a stacking boundary where micelles are made to collapse [12,98]. The analytes are prepared in a micellar solution of a surfactant such as sodium dodecyl sulfate (SDS). The collapse is due to the dilution of the surfactant micelles at the boundary between the sample and the electrolyte to below the critical

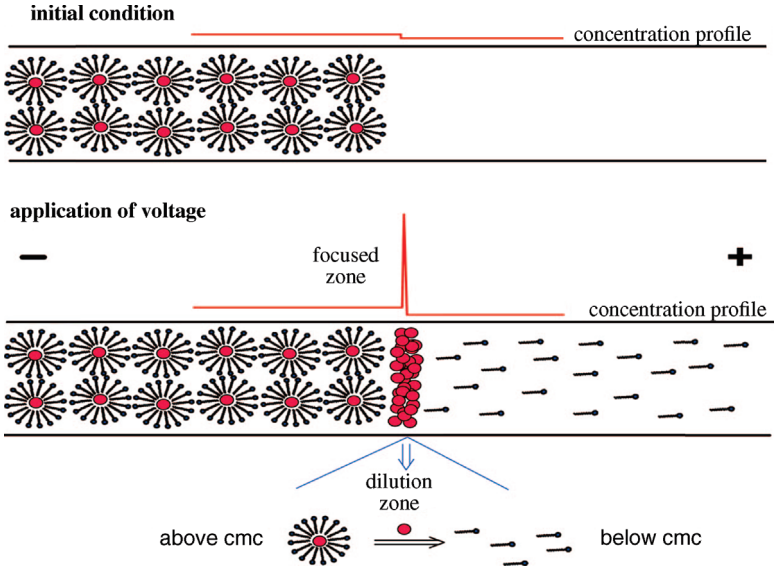


**Figure 8.3** Transport, release, and accumulation of analytes by micelle collapse in a dilution zone [12].

micelle concentration (cmc) as shown in Figure 8.3. The collapse of the micelles can also be facilitated by the addition of an organic solvent into the electrolyte [99,100]. The cmc of the surfactant increases in the presence of the organic solvent. Therefore, if the surfactant concentration in the micellar solution is lower than the cmc of the surfactant in the electrolyte with a high percentage of the organic solvent, the micelles will collapse at the boundary during electrophoresis. For example, the cmc of SDS in 30% acetonitrile in the electrolyte is >10 mM. Thus, the micelles from a 10-mM SDS solution will collapse at the boundary. AFMC is used for both neutral and charged analytes.

MSS is similar to AFMC in a number of ways; MSS also relies on the transport and release of the analytes by the micelles into a stacking boundary [13,99]. The stacking boundary is found between a micellar solution and an organic solvent-rich zone. However, the unique stacking mechanism of MSS is the reversal in the effective electrophoretic mobility of the analytes at the boundary, thus MSS is relevant only for charged analytes. The effective electrophoretic mobility in the micellar zone is dictated by the micelles, while the mobility in the solvent-rich zone is controlled by the electrophoretic mobility of the analytes. The micelles must have a charge opposite to that of the analytes. Therefore, the effective electrophoretic mobilities in the micellar and organic solvent-rich zone are in the opposite direction or on the opposite sign. The reversal in direction may not necessarily be accompanied by micelle collapse at the boundary. The first configuration of MSS is that the sample is prepared in a micellar solution. The organic solvent-rich zone is produced by adding an organic solvent to the separation electrolyte, by injection of a solvent-rich plug, or by using nonaqueous CE for separation.

AFMC has been applied to neutral and charged pollutants, drugs, and natural products employing separation using MEKC or CZE [96,101–107]. MSS has been applied to mainly cationic molecules such as drugs (e.g., tricyclic antidepressants and antihistamines) and alkaloids using SDS micelles in the sample and separation using CZE, nonaqueous CE, and MEKC [100,107–115]. A commonly used SDS microemulsion was also used by Kukusamude, Srijaranai, and Quirino for effective electrophoretic mobility reversal, and the MSS variant was called microemulsion to solvent stacking [116]. MSS of anionic drugs was accomplished using cationic cetyltrimethyl ammonium bromide or 1-dodecyl-3-methylimidazolium tetrafluoroborate micelles with separation using CZE [99,117].

The other configuration of MSS is where the analytes are prepared in the organic solvent-rich solution [118]. Figure 8.4 shows the MSS of cationic analytes using SDS micelles in co-EOF CZE. A micellar solution is injected prior to the sample solution (Figure 8.4a). When a voltage is applied, the analytes stack at the MSS boundary (MSSB), which is between the micellar and the sample solution, due to the reversal in the effective electrophoretic mobility ($\mu_{ep}{}^{*}(a)$) of the analytes (Figure 8.4b). The analytes in the sample solution migrate to the micellar solution with a velocity dictated by its electrophoretic mobility ($\mu_{ep}(a)$) that is directed to the cathode. They are captured by the micelles that transport them with a velocity dictated by their $\mu_{ep}{}^{*}(a)$ that is directed to the anode. The affinity of the analytes for the micelles is significantly reduced by the organic solvent in the MSSB; thus, the direction of the $\mu_{ep}{}^{*}(a)$ at the MSSB ($\mu_{ep}(a)$ MSSB) is reversed to the cathode. The micelles in this configuration can be

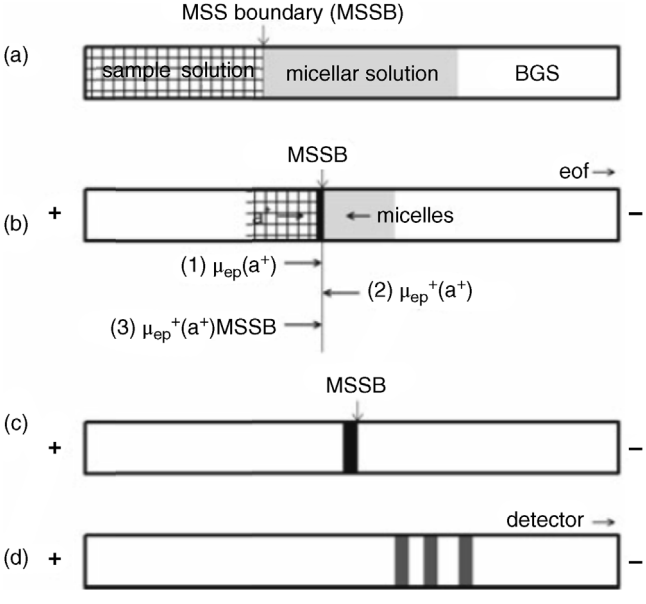


**Figure 8.4** Evolution of zones in MSS co-EOF CZE using a sample prepared with organic solvent-rich solution [118].

imagined as online transient micellar-phase concentrators because the concentration ends (Figure 8.4c) and CZE separation (Figure 8.4d) begins when all the micelles migrate through the MSSB. A similar strategy was developed for anionic analytes using cationic surfactants as transient concentrators [119].

## 8.3 Combination of Stacking Techniques

### 8.3.1 Stepwise Use of Stacking Mechanisms

The stepwise combination of stacking techniques to further boost concentration sensitivity or as an academic exercise has attracted much attention since FESI was combined with sweeping [120]. The main idea is to overdo the first stacking step in order to load more analytes into the capillary. This is followed by another stacking mechanism to concentrate the broad zones produced in the first stacking step. Theoretically, the overall SEF is the product of the SEF values obtained in the first and second stacking steps. The combination of two powerful stacking mechanisms such as FESI, sweeping, and tr-ITP should easily provide 10 000-times improvement in the concentration sensitivity. To date, only two-step stacking approaches have been realized, which are FESI–sweeping [120,121], FESI–tr-ITP [122,123], sweeping–AFMC [105–107], and sweeping–MSS [124,125]. In addition, stepwise stacking approaches work only for the enrichment of analytes of the same charge. The stepwise use of more than two stacking mechanisms or the simultaneous stepwise stacking of both anions and cations is yet to be realized.

FESI or field-enhanced sample injection with electrokinetic injection allows the largest amount of sample that can be loaded into the capillary. FESI with sweeping or tr-ITP provide the largest improvements in concentration detection sensitivity in CE to date; almost a million-fold with FESI and sweeping (also known as cation- or anion-selective exhaustive injection and sweeping). FESI with sweeping and tr-ITP is followed by separation using EKC and CZE, respectively. The strategy for electrokinetic supercharging (EKS) (developed by Hirokawa, Okamoto, and Gaš), which involves FESI with tr-ITP of cationic analytes, is described in Figure 8.5 [122]. The leading electrolyte is injected after conditioning the capillary with the supporting electrolyte (1). The sample in a low-conductivity diluent is injected electrokinetically or FESI of the sample is performed (2). Then, the terminating electrolyte is injected to induce tr-ITP (CZE) and stack the analytes introduced by FESI (3). There is localized analyte depletion in the area between the electrode and capillary tip in the sample solution at the inlet vial due to long FESI. This causes issues with repeatability and reduces the amount of analytes injected with time. Hirokawa and his team optimized the FESI parameters such as the development of new electrode configurations and have demonstrated a 100 000- fold improvement in sensitivity via EKS [126–128].

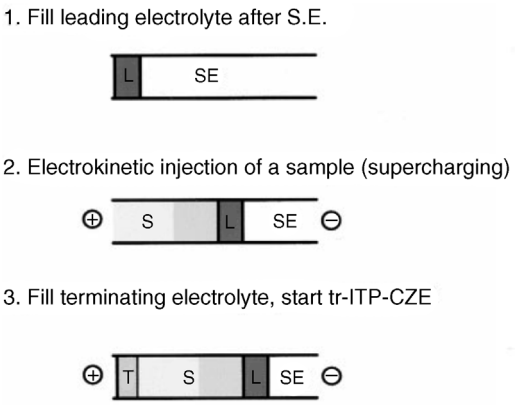


**Figure 8.5** Operation of EKS for cationic analytes. SE is the supporting electrolyte for CZE; L is the leading electrolyte, S is the sample plug; and T is the terminating electrolyte. Both reservoirs are not shown in this scheme. After these three steps, tr-ITP-CZE is started [122].

EKS is popularly used for the trace analysis of inorganic ions, drugs, bioactive small molecules, natural products, and DNA found in biological, herbal, and environmental samples [129–140]. The most popular application of FESI and sweeping is for cationic analytes (cation-selective exhaustive injection-sweeping). FESI and sweeping were combined for the trace analysis of organic pollutants such as nitroimidazone, malachite green, and anti-inflammatory drugs in water samples [141–143]. In food analysis, melamine and malachite green were determined in milk samples [144,145] and ractopamine was determined in porcine meat [146]. There were also several applications for the determination of metabolites and drugs in biofluids such as urine and serum [147–152]. In addition, chemometrics was used to optimize the experimental steps involved in the FESI and sweeping combination [146,153,154]. The sweeping and AFMC combination was applied to the analysis of steroids and natural products in two-dimensional CE separations [105–107]. The new two-step stacking approach of sweeping and MSS was applied to the analysis of phenolic compounds, nitroimidazoles, and alkaloids in real or spiked real samples [155–158].

### 8.3.2
### Stacking Mechanisms Working in Synergy

Two or even more stacking mechanisms can occur when the sample is prepared in a diluent that is different from that of the separation electrolyte. For example, when the sample is prepared in a diluent of a pH different from that of the separation electrolyte and with no additive, the stacking mechanisms of dynamic pH junction and sweeping are induced [159,160]. Compared to the stepwise use of stacking mechanisms in Section 8.3.1, the stacking mechanisms described in this section occur at the same time.

The use of FESI and MSS synergy was recently introduced [161] and the mechanism can also be explained by Figure 8.4. However, in Figure 8.4a the sample is prepared in a low-conductivity electrolyte for FESI. To induce MSS, a low-conductivity organic solvent-rich solution is injected before electrokinetic injection of the sample or the sample itself is prepared in a low-conductivity organic solvent-rich diluent. The FESI increases the sample load while the analytes introduced into the capillary were focused by MSS. After FESI, the analysis occurred as per Figure 2.4b–d.

When stacking mechanisms are combined in this fashion, longer injections of the sample are possible compared to when only one stacking mechanism is utilized. Thus, better sensitivity enhancements are possible with such combinations. Dynamic pH junction and sweeping synergy was recently reported for high-sensitivity analysis of dipeptides with laser-induced fluorescence detection and determination of benzoic and sorbic acids in food products [162,163]. FESI-MSS was used for the sensitive analysis of racemic drugs with ESI-MS, sulphonamides in water samples, and herbicides in milk [164–166].

## 8.4 Method Development for Stacking in CE

The stacking mechanisms must be chosen based on the nature of the compound (charged or neutral) and the CE mode used for separation (CZE or EKC) (see Table 8.2). All the stacking techniques presented are applicable to charge analytes, while sweeping and AFMC are the most versatile ones. The stacking techniques can be implemented with separation by CZE and EKC except for tr-ITP. The stacking technique of tr-ITP is difficult to implement in EKC because of the pseudo-stationary phase added in the separation electrolyte. This phase could interact with the ITP-formed zones and thus could disturb the stacking process for charged analytes.

**Table 8.2** Summary of stacking techniques and applications based on the nature of the analyte (charged or neutral) and the separation mode (CZE or EKC).

| Technique (see Table 2.1) | Charged | Neutral | CZE | EKC |
|---|---|---|---|---|
| 1. | Yes | Na | Yes | Yes |
| 2. | Yes | Na | Yes | No |
| 3. | Yes | Na | Yes | Yes |
| 4. | Yes | Yes | Yes | Yes |
| 5. | Yes | Yes | Yes | Yes |
| 6. | Yes | Na | Yes | Yes |

*Note:* na is not applicable.

## 8.5 Conclusions

Stacking techniques allowed the use of CE for the trace analysis of various analytes. The detection sensitivity of CE with stacking and UV detection is comparable or even better than HPLC. Stacking can easily be performed by simply changing the chemistry of the sample solution and thus stacking is one of the popular ways to improve detection sensitivity in CE. In practice, an appropriate sample preparation is chosen such that the sample is converted to a form that is amenable for stacking. The development of microextraction or microsample preparation schemes compatible with stacking CE should be an exciting area of research because of the small sample requirement of CE.

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