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Review

Sweeping: concentration mechanism and applications to high-sensitivity analysis in capillary electrophoresis

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Abstract

Sweeping in capillary electrophoresis (CE) involves the interaction of a pseudostationary phase (PS) in the separation solution and a sample in the matrix that is free of the PS used. The PS includes not only the PSs employed in electrokinetic chromatography, but also complexation reagents such as borate. The sample matrix could have a lower, similar, or higher conductance than the separation solution. Thus, the basic condition for sweeping is a sample matrix free of the additive. The accumulation of analyte molecules during the interaction makes this interesting phenomenon very useful as an on-line preconcentration method for CE. Preconcentration occurs due to chromatographic partitioning, complexation, or any interaction between analytes and PS. Contact between analyte and PS is facilitated by the action of electrophoresis and is independent of electroosmosis. The analyte, PS, or both should have electrophoretic velocities when an electric field is applied. The extent of preconcentration is dictated by the strength of the interaction involved. From tens to several thousand-fold improvements in detector response for many neutral and charged analytes have been achieved with this technique, suggesting sweeping as a general approach to on-line preconcentration in CE. The mechanism and applications of the sweeping phenomenon under different experimental conditions are discussed in this review, with particular emphasis on a better understanding of the sweeping mechanism under reduced electric field (high conductivity) in the sample zone.

Keywords: Reviews; Sweeping; Preconcentration

Contents

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1. Introduction

Capillary electrophoresis (CE) may be regarded as the most important milestone in the separation sciences during the latter part of the 20th century. CE has been applied to simple problems such as the assay of pharmaceutical products and to more complex problems such as the mapping of the human genome and proteome. Separations involve electrophoresis, electroosmosis, and chromatography. Although CE can easily separate very complex mixtures, low concentration sensitivity prevents the detection of trace levels of analytes. Low sensitivity emanates from two sources, namely small sample volumes (e.g., 2–10 nL) and short optical pathlengths (e.g., 25–100 μm).

In response to the sensitivity problem, several on-line or on-capillary focusing methods have been developed to preconcentrate analytes inside the capillary before separation and detection. The use of adsorbents placed at the inlet tip of the capillary to trap analytes in the eluent that are consequently released with the use of another solvent afforded a many-fold improvement in detection sensitivity. The principle of preconcentration is based on chromatographic partitioning, binding, or sorption effects. Both charged and neutral analytes can be preconcentrated [1–3].

The use of electrophoretic effects, such as sample stacking, transient isotachophoresis, and a dynamic pH junction, may provide up to a thousand-fold improvement in detection sensitivity. Sample stacking [4] in capillary zone electrophoresis (CZE) results from the movement of sample molecules along a boundary that separates regions of different electric field strengths. Molecules move faster in one region (high electric field sample region) and slow down in the other (low electric field separation region). The change in electrophoretic velocity causes the focusing of analytes [4,5]. Isotachophoretic preconcentration results from the adjustment of the sample ion concentration to the concentration of the leading electrolyte used based on Kohlrausch rules [6]. Dynamic pH junction preconcentration results from the change in electrophoretic mobilities when the analytes experience changes in pH [7,8]. For amphoteric analytes, preconcentration may be similar to that in isoelectric focusing. A transient pH gradient is produced by two buffers of different pH used as sample and separation solutions. It should be noted that only charged analytes can be preconcentrated using electrophoretic effects. Sample stacking of neutral analytes in the electrokinetic chromatography (EKC) mode is an exemption [5,9–11].

In this review, on-line sample preconcentration by sweeping is discussed. Emphasis is given to the focusing mechanism under different experimental conditions. Sweeping relies on the interaction between analyte and PS and electrophoresis to induce interaction. Both charged and neutral analytes can be preconcentrated, which makes this enrichment technique versatile. The applications and combination with sample stacking are also considered.

2. Sweeping

2.1. Partitioning with pseudostationary phases in electrokinetic chromatography

EKC is a mode of CE where an additive called the pseudostationary phase (PS) is added to the separation buffer. PSs include micelles, polymers, dendrimers, etc. Partitioning of the analytes between PS and the surrounding phase promotes the separation of a mixture of analytes. Neutral analytes are separated due to partitioning alone, while charged analytes are separated based on partitioning and electrophoresis [12,13]. Two types of PS can be used in EKC, namely charged and neutral PSs. Only charged analytes can be separated using a neutral PS.

2.1.1. Charged PS

Sweeping was first described in electrokinetic chromatography (EKC) using a charged PS (i.e., micelle). EKC with micelles is commonly referred to as MEKC. The fundamental condition for sweeping is a sample matrix free of the PS used. Sweeping in EKC is defined as the picking and accumulating of analytes by the charged PS that fills or penetrates the sample zone during application of a voltage. It is like carefully ''sweeping'' grains of rice scattered on the floor using a broom, where the broom and the grains of rice are analogous to the PS and analytes, respectively [14]. Picking and accumulating of analytes occurs due to partitioning or interaction of analytes with the PS, therefore sweeping preconcentrates due to a partitioning mechanism. It should be emphasized that partitioning is not possible without electrophoresis, which induces the movement of the charged PS into the sample zone. Sulfated cyclodextrins and microemulsions have also proved to be useful charged PSs, aside from micelles, for the sweeping and separation of diverse samples [15].

2.1.1.1. In a homogenous electric field

The evolution of an analyte zone in EKC under sweeping and homogenous electric field conditions is depicted in Fig. 1. Maintaining a constant resistance along the capillary by preparing the sample in a matrix having a similar conductance to the background solution (BGS) produces a homogenous electric field. The BGS contains the PS. A negatively charged PS is used and, for simplicity, the electroosmotic flow (EOF) is zero. This form of EKC where the electrophoretic velocity of the PS is greater than the EOF and analytes are brought to the detector with the aid of the PS is termed reversed migration EKC (RM-EKC) [13]. Fig. 1A shows a long injection of the sample solution (S) into a capillary previously filled with the BGS. The gray area and dotted area depict the analyte molecules in the S zone and anionic PS in the BGS zone, respectively. It should be noted that the analytes are electrically neutral and will not migrate by themselves without being incorporated by the PS.

Fig. 1B shows the application of a voltage across the capillary with two reservoirs filled with BGS at both ends. Anionic PSs from the cathodic end enter the capillary and the S zone. Although the conductance is kept constant throughout the capillary, the concentration of PS entering the PS zone may be

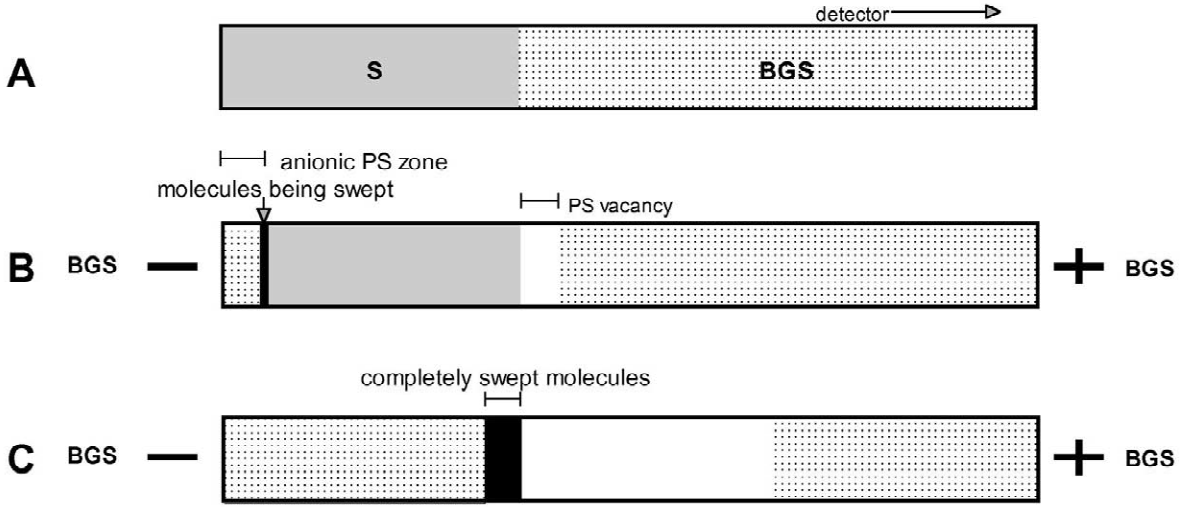

Fig. 1. Sweeping in a homogenous electric field. Progress of an analyte zone in EKC using a negatively charged PS and a zero EOF environment. (A) Starting situation, a longer than a typical injection of sample solution (S) prepared in a matrix having a conductivity similar to the micellar background solution (BGS). (B) Application of a voltage with the cathode at the inlet end and the anode at the outlet end; the capillary is dipped into two reservoirs filled with the BGS, PS enters the S zone and sweeps (concentrates) the analyte molecules. (C) The final swept zone is formed when the PS completely fills the S zone. For further explanation, see the text.

different from that in the BGS zone. This is caused by the difference in electrophoretic mobilities of PS and buffer components. However, in this figure the concentration of PS throughout the column is assumed equal. The concentration of PS in the S plug is initially zero and rises to a certain value from the front boundary. As the PS passes through the S zone, analyte molecules are picked up and accumulated. The accumulated zone, shown as a darker area in the figure, has a concentration greater than that found in the original. This dark area also contains the PS which continually penetrates the S zone in the presence of an electric field. The velocity of the PS is always greater than the velocity of the analyte molecules incorporated in the PS. A PS vacancy (white area) or a zone without the PS develops at the interface between the S and BGS zones. Here, the PS moves to the anode and is replaced by the anionic components of the S zone in order to preserve electrical balance. After a certain period of time, all analyte molecules are picked and accumulated by the PS (Fig. 1C). The accumulated analytes are then separated by EKC. The migration velocities of the analytes are slower than that of the PS and, therefore, the analytes will never enter the vacancy zone.

For two-component samples, Fig. 2 depicts the resulting analyte zones after sweeping. The swept zone of the higher retention factor (k) analyte is narrower than the lower one, which will be explained later. k can be expressed as:

$$k = K\phi \tag{1}$$

where K is the partition or distribution coefficient (concentration of the solute in the PS/concentration of the solute in the surrounding liquid phase) and ϕ is the phase ratio (volume of the PS/volume of the surrounding liquid phase). In general, the greater the affinity of the solute for the PS, which leads to high K, and the greater the volume of PS, which produces high ϕ, the higher the value of k. Quantitatively, the resulting length of the swept zones (l_{sweep}) can be approximated by [14]:

$$l_{\text{sweep}} = l_{\text{inj}} \cdot \frac{1}{1+k} \tag{2}$$

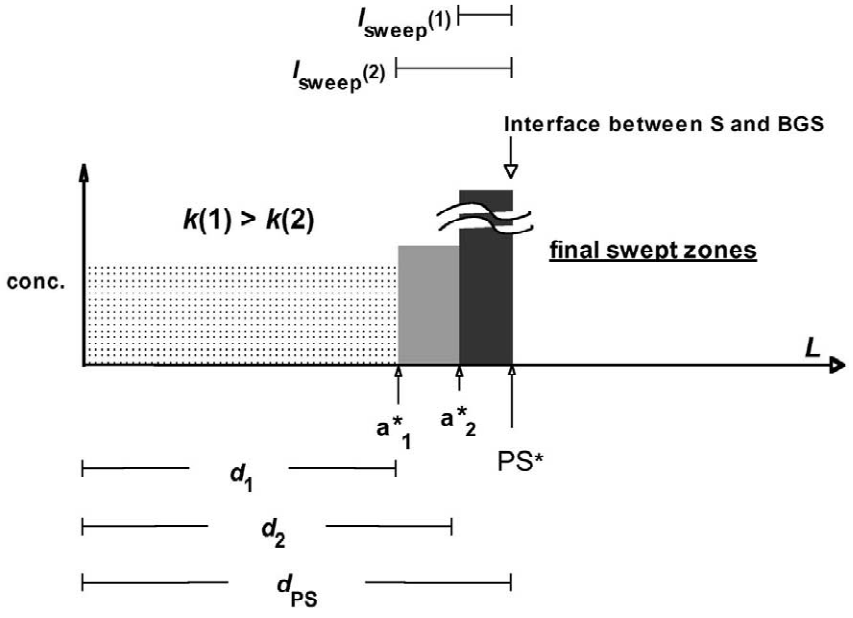

Fig. 2. Effect of retention factor on the final swept zone lengths. Legend: first batch of anionic pseudostationary phase (PS) that enters the S zone (PS*); first batch of analyte molecules (a_1^* or a_2^*) that is picked by the PS; distance traveled by PS* (d_{PS}); distance traveled by a_1^* or a_2^* that is picked by the PS (d_1 or d_2, respectively); retention factor of analyte 1 or 2 ($k(1)$ or $k(2)$, respectively); l_{sweep} of analyte 1 or 2 ($l_{sweep}(1)$ or $l_{sweep}(2)$, respectively). For details, please consult the text.

where l_{inj} is the length of the injected S zone. Since the concentration of the PS entering the S zone is assumed equal, the *k* values in the S zone when filled with anionic PS are equal to the *k* values in the BGS. With Eq. (2) in mind, sweeping is basically dependent on the retention factor and the length of the initial zone, which suggests narrower zones for high *k* analytes. This equation also predicts an almost limitless improvement in detection sensitivity for analytes having very high *k*. On the other hand, the focusing effect on lower *k* analytes should not be overlooked and may be sufficient for some real applications.

Eq. (2) was derived with the hypothesis that the analyte zones are completely swept when the first batch of PS that enters the S zone (PS*) reaches the initial interface between the S and BGS zones. The difference in the distance traveled by PS* (d_{PS}) and the distance traveled by the first batch of analyte molecules (a_1^* or a_2^*) that are picked up by the PS (d_1 or d_2) is l_{sweep} (see Fig. 2). The distance traveled is the product of the (effective) electrophoretic velocity and the time elapsed after PS* reaches the S and BGS interface. In accordance with Eq. (2), l_{sweep} of the higher *k* analyte ($l_{sweep}(1)$) is narrower than that of the lower *k* analyte ($l_{sweep}(2)$) (see Fig. 2).

A theoretical study of sweeping under a homogenous electric field of neutral and charged solutes in the absence and presence of EOF also gave rise to the same equation as Eq. (2). It should be noted that the calculation of *k* for neutral or charged solutes is different. In summary, for neutral or charged solutes in a homogenous electric field system and a fixed l_{inj}, sweeping is independent of EOF and is only affected by *k* [16]. It should be noted that although Eq. (2) is derived irrespective of EOF, a strong EOF usually causes low concentration efficiency in comparison to a low or suppressed EOF, as reported in Ref. [16]. This is probably due to a slight difference in EOF velocity between the sample zone and BGS zone, which may generate mixing at the boundary, resulting in increased band dispersion. The EOF may also have an additional band broadening effect that is not yet well characterized. In any case, compared with most other preconcentration methods, sweeping appears to be the most versatile since it preconcentrates both neutral and charged solutes.

As seen in many examples of sweeping-MEKC, peaks are usually narrower than those observed in conventional MEKC. One explanation is due to the shorter capillary length utilized for the separation, because a significant portion of the capillary effective length is utilized to fill the sample solution. Another probable explanation is the high focusing efficiency of sweeping to give very narrow concentrated zones of analytes in comparison with the usual injection plug length. The high concentration efficiency of sweeping was clearly shown using sweeping microchip electrophoresis [17]. Since the detection position is movable in microchip electrophoresis, one can directly observe the sweeping process by successively changing the detection position along the separation channel. Based on experiments, the width of the analyte swept zone was found to be extremely narrow and was equal to the size of the focused laser spot (ca. 25 μm) in our equipment. The focused zone started to broaden immediately after the end of sweeping due to thermal diffusion. The dependence of zone broadening was accurately described by molecular diffusion. In other words, sweeping provides an ideal sample injection technique in terms of minimizing extra column band broadening effects.

2.1.1.2. Reduced electric field in the sample region

The use of high salt concentrations in sample solutions or high-conductivity sample matrices in sample preconcentration MEKC was proposed by Palmer et al. in 1999 [18]. The sample is prepared in a matrix two to three times the conductivity of the separation solution and run under high EOF conditions using micelles as PS. An alternative focusing mechanism was suggested based on their observations of enhanced analyte focusing in a high-salt sample matrix. Based on the proposed mechanism, analytes experience a reduction in velocity upon encountering a stacked micelle/sample zone interface. The same scheme was also described for electrokinetic injection with a high-conductivity sample matrix [20,21]. Since their conditions correspond to sweeping under a reduced electric field in the S zone (sample is void of the PS used), our group has attempted to explain their concentration process in terms of sweeping [19]. However, further investigation is still required to clarify the relationship between sweeping and high-salt concentration sam-

ple stacking [20,21]. Here, we discuss the focusing mechanism observed with high-salt sample matrices in further detail in terms of sweeping based on additional experimental data.

The development of analyte zones in EKC under a reduced electric field in the sample zone is illustrated in Fig. 3. Similar to Fig. 1, a negatively charged PS is used and EOF is zero. Fig. 3A is similar to Fig. 1A but the sample zone contains a high concentration of salts. The high-salt concentration sample matrix produces a reduced electric field in the sample zone and an enhanced electric field in the BGS region. The field enhancement can be described quantitatively by the enhancement factor, γ', which is equal to the ratio of the conductivity of S and BGS [4]. The value of γ' is always greater than 1.

When voltage is applied (Fig. 3B), anionic PS from the cathodic end enters the capillary and stacks into the high conductivity S zone [18]. In the figure, zones with more dots than the BGS zone depict stacked PS. The concentration of PS entering the S zone (C_{PS}(S)) is higher than that in the BGS zone (C_{PS}(BGS)) as predicted by [4,19]:

$$C_{PS}(S) = C_{PS}(BGS)\gamma' \quad (3)$$

The higher concentration of PS entering the S zone can also be explained by the principle of the self-sharpening effect of isotachophoretic boundaries and of adjustment of concentration in conformity with the Kohlrausch regulating function [19,22,23].

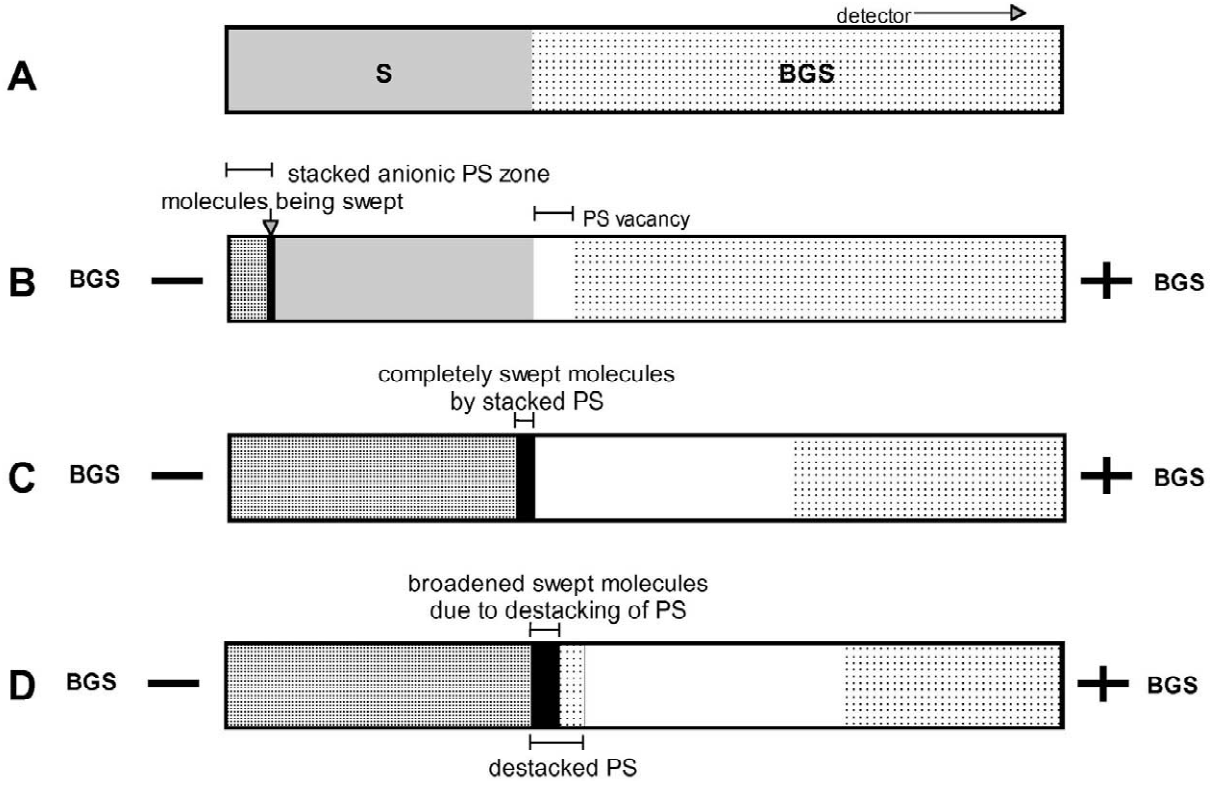

Fig. 3. Sweeping in a reduced electric field. Progress of an analyte zone in EKC using a negatively charged PS and a zero EOF environment. (A) Starting situation, a longer than typical injection of sample solution (S) prepared in a matrix having a conductivity higher than the micellar background solution (BGS). (B) Application of a voltage with the cathode at the inlet end and the anode at the outlet end; the capillary is dipped in two reservoirs filled with the BGS, PS enters and stacks at the S zone, stacked PS sweeps (concentrates) the analyte molecules. (C) The final swept zone is formed when the stacked PS completely fills the S zone. (D) Stacked PS destacks at the initial boundary between the S and BGS zones; destacking of PS causes broadening of the swept analyte zone. For further information, see text.

The analyte molecules are picked up and accumulated by the stacked PS penetrating the S zone (Fig. 3B and C). Examination of Eqs. (1)–(3) suggests that the swept zone depicted in Fig. 3C should be narrower compared to the final swept zone in a homogenous electric field system of the same analyte, BGS, and l_{inj}. This is explained by the high k in the S zone due to the increased concentration of PS caused by stacking (C_{PS}(S)).

In Fig. 3D, the stacked PS destacks at the concentration boundary or interface between the S and BGS zones. The concentration boundary is stationary since this boundary moves with the velocity of the EOF, which is zero. The concentration of PS in the destacked zone (C_{PS}(destacked)) can be approximated by:

$$C_{PS}(\text{destacked}) = \frac{C_{PS}(S)}{\gamma'} \quad (4)$$

which, in principle, is similar to Eq. (3). Analysis of Eqs. (3) and (4) indicates that C_{PS}(destacked) is equal to C_{PS}(BGS). The decrease in concentration leads to a decrease in k for each analyte. The decrease in k then leads to a broadening effect and an increase in focused zone length. The extent by which k increased in the stacking process is equal to when k decreased in the destacking of PS, therefore the final swept zones in a homogenous and reduced electric field system should give similar lengths. In effect, the resulting analyte zone length in a reduced electric field system can also be approximated by Eq. (2) [19].

In a previous paper, it was suggested that the broadening of swept zones caused by the destacking of the PS is reminiscent of the broadening of separated zones in partial filling MEKC [19]. The broadening of zones in partial filling MEKC is essentially caused by the loss of analyte k when the sample moves from the micellar to the non-micellar region. Here, broadening is caused by the decrease in analyte k when the sample moves from a stacked PS zone to a destacked PS zone. The loss of retention also causes a decrease in the effective electrophoretic mobility [product of $k/(1+k)$ and the electrophoretic mobility of PS].

To verify the destacking process shown in Fig. 3D, several experiments were designed. The effect of the concentration of PS in the sample matrix on band broadening was first examined with three phenol derivatives dissolved in four different SDS concentration solutions in the same buffer (40 m*M* phosphate buffer, pH 2.5) used to prepare BGS. The results are shown in Fig. 4, where the dependence of the peak widths at the half peak height on sample plug lengths is shown. In these experiments, we attempted to compare MEKC separations starting from the end of sweeping described in Figs. 1C and 3D. When the sample matrix contains a higher concentration (240 m*M*) of SDS than that (80 m*M*) in BGS, it mimics stacking (sweeping) with a high salt concentration [18] mentioned above. When the sample matrix does not contain PS or SDS (plot 1), peak widths are the minimum irrespective of sample plug lengths between 1.5 and 3.7 mm, which is explained by the sweeping effect. It should be noted that plot 1 does not mimic the conditions given in Fig. 1C and 3D, but shows a simple sweeping condition. While the sample matrix contains a three times higher SDS concentration (240 m*M*) than that of BGS (80 m*M*) (plot 4), the peak width increased with an increase in the sample plug length. The peak width at half-height of 2,3,5-trimethylphenol dissolved in 240 m*M* SDS was 5.3 mm, while that in 80 m*M* SDS was 4.2 mm for 2.2 mm sample injection. The measured conductivities of 240 and 80 m*M* SDS solutions in 40 m*M* phosphate buffer (pH 2.5) were 12.8 and 6.3 mS/cm, respectively. These results do not seem consistent with the prediction from Eq. (4). However, it should be mentioned that the peak width observed with the 80 m*M* SDS matrix was not the sample plug length but the result of band broadening ascribed to MEKC in addition to the injected zone width (2.2 mm). If we assume that the sample plug length was expanded about twice ($=12.8/6.3$) by destacking with the 240 m*M* SDS matrix, the observed peak width (5.3 mm) could be the sum of the sample plug length (2.2×2 mm) and band broadening due to the MEKC process. Therefore the result seems reasonable. When the sample matrix is equal to the BGS (plot 3), which corresponds to conventional MEKC after sweeping (Fig. 1C), peak widths increase with an increase in the plug length, but the broadening effect is not very significant because the contribution of band broadening due to MEKC separation to the total peak widths is relatively high. With a lower concentration of SDS (40

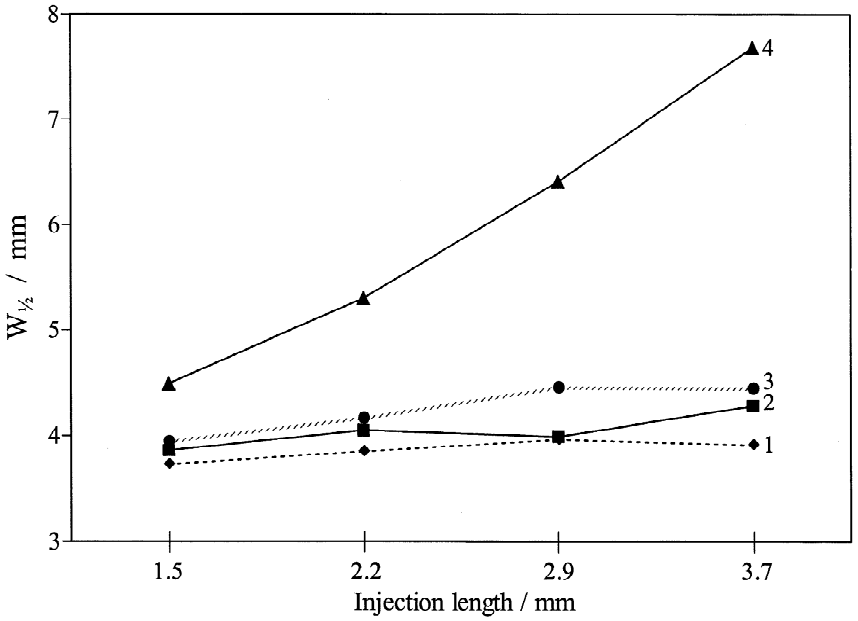

Fig. 4. Dependence of peak widths at half heights on the sample plug lengths and sample matrices. Analytes, 2,3,5-trimethylphenol; BGS or separation solution, 80 m*M* SDS in 40 m*M* phosphate buffer (pH 2.5); sample matrix, 1=phosphate buffer (pH 2.5) having the same conductivity as that of BGS; 2=40 m*M* SDS in 40 m*M* phosphate buffer (pH 2.5); 3=80 m*M* SDS in 40 m*M* phosphate buffer (pH 2.5); 4=240 m*M* SDS in 40 m*M* phosphate buffer (pH 2.5); sample injection, pressure injection at 50 mbar; capillary, 50 μm I.D.×60 cm (51.5 cm to the detector); applied voltage, −20 kV; temperature, 25 °C; detection, UV absorbance at 210 nm; CE instrument, Hewlett-Packard ^{3D}CE.

m*M*) in the sample matrix (plot 2), sweeping was still observed.

To mimic more precisely the destacking process that occurs in the high-salt concentration sample matrix [18] (Fig. 3D), an injection of 2.2 mm sample solution in 240 m*M* SDS in BGS buffer was followed by an injection of 15 mm of the same 240 m*M* SDS without the sample. The 240 m*M* SDS zone without the analyte corresponds to the stacked SDS zone shown to the left of the swept analyte zone in Fig. 3D. The peak width of 2,3,5-trimethylphenol was compared with that when BGS (80 m*M* SDS) was used for both the 2.2 mm sample solution and the following 15 mm SDS solution. The results are shown in Fig. 5. Plot 1 is the same as part of the data given in Fig. 4. Although two points at 80 m*M* SDS in Fig. 5 should show the same peak widths, the width in plot 2 was slightly larger, probably due to zone broadening of the injected analyte zone during

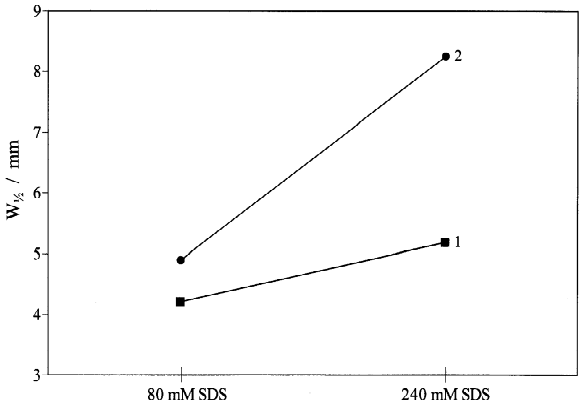

Fig. 5. Effect of sample matrices on peak widths at half heights. Sample plug, 1=2.2 mm sample solution in 80 m*M* SDS or 240 m*M* SDS in 40 m*M* phosphate buffer (pH 2.5); 2=2.2 mm sample solution in 80 or 240 m*M* SDS in 40 m*M* phosphate buffer (pH 2.5)+15 mm 80 or 240 m*M* SDS in 40 m*M* phosphate buffer (pH 2.5). Other conditions are the same as in Fig. 4.

the following injection of the SDS solution. The injected sample plug lengths are equal between plots 1 and 2, but the following injection of 240 m*M* SDS caused significant differences in peak width, as shown in Fig. 5. It is apparent from these results that the concentrated SDS zone formed by stacking of the SDS micelle due to the high-salt concentration sample matrix adversely affects peak widths.

There remains the question of why sweeping seems not to work well with low-salt concentration sample matrices [18,20]. Fig. 6 shows electropherograms obtained with different salt concentrations using various injection plug lengths under the same conditions given in Ref. [18], except for the analytes. If the sample plug length was reduced to 14 mm from the 36 mm employed in Fig. 2 of Ref. [18], sweeping of even low retention factor analytes (peaks 1 and 2) is apparent even at 25 m*M* NaCl. When a 50 m*M* NaCl sample matrix was employed, a sample plug of 21 mm did not deteriorate the concentration efficiency. The results obtained with a 35 mm sample plug and 150 m*M* NaCl were similar to those reported previously [18]. These results clearly show that the sample plug lengths are too long to produce narrow focused peaks in low salt sample matrices. The reason why high-salt concentration sample matrices can produce a high concentration efficiency is explained by the fact that the retention factors are significantly increased in high-salt concentration matrices; e.g., the retention factor of progesterone increased from ca. 24 at 25 m*M* NaCl to ca. 40 at 150 m*M* NaCl with 80 m*M* sodium cholate in 10 m*M* tetraborate (pH 9.2). This significant change in the retention factor explains why the progesterone peak (3) in Fig. 6C is narrow, which can be expected from Eq. (2). It should be noted that, although 10% ethanol was added in Fig. 6 and in Ref. [18], the retention factors given above are values without ethanol because ethanol is not contained in the sample matrix where sweeping occurs. Thus, sweeping works under any salt concentrations of sample matrices, but the injection sample plug

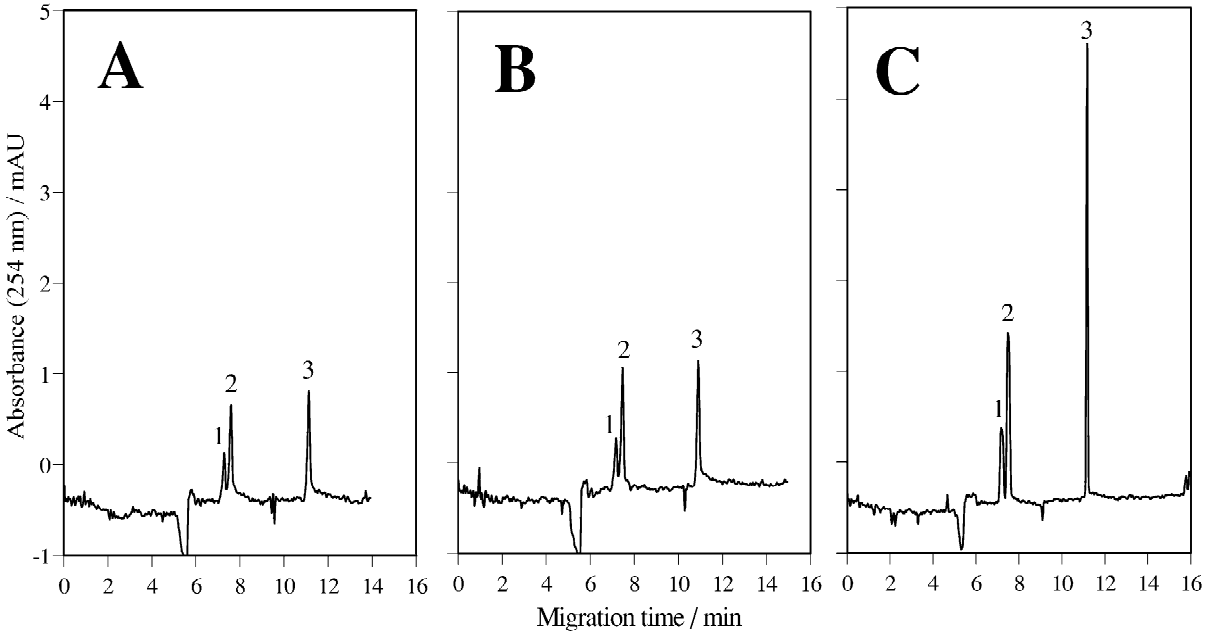

Fig. 6. Effects of salt concentration in sample matrices on maximum sample injection lengths to obtain highest concentration efficiency in sweeping. Peak identification, 1 = cortisone (3.3 ppm); 2 = hydrocortisone (4.0 ppm); 3 = progesterone (5.0 ppm). BGS or separation solution, 80 m*M* sodium cholate in 10 m*M* sodium tetraborate containing 10% ethanol; sample matrix, 25 m*M* NaCl (A), 50 m*M* NaCl (B), 150 m*M* NaCl (C); injected sample plug length, 14 mm (pressure injection for 20 s at 50 mbar) (A), 21 mm (30 s) (B), 35 mm (50 s) (C); applied voltage, 30 kV; detection, UV absorbance at 254 nm. Other conditions are as in Fig. 4.

length must be optimized depending of the magnitude of the retention factor of the analyte. Therefore, the high-salt concentration stacking observed by Palmer et al. [18] is in fact a specific mode of sweeping when applied to some analytes with high retention factor values.

In a recent paper [21], Palmer et al. describe electrokinetic injection for the concentration of neutral analytes with high-salt concentration sample matrices to give a shorter injection time and lower detection limits in comparison to pressure injection. The lowest limit of detection (LOD) given in Ref. [18] is 50 ppb at $S/N = 5$ for some steroids, which is comparable to the value obtained by sweeping without using a high-salt concentration sample matrix under strong EOF (70 ppb at $S/N > 5$) for the same compounds [16]. Under suppressed EOF, sweeping in an homogenous electric field generated a much higher concentration efficiency and the LOD was <7 ppb [16]. Another independent group, Harino et al., reported a notable thousand-fold preconcentration of steroidal compounds with excellent plate numbers by sweeping with an homogenous electric field in RM-MEKC [24].

2.1.1.3. Enhanced electric field in the sample region

The progress of the analyte zones in EKC under sweeping and an enhanced electric field in the

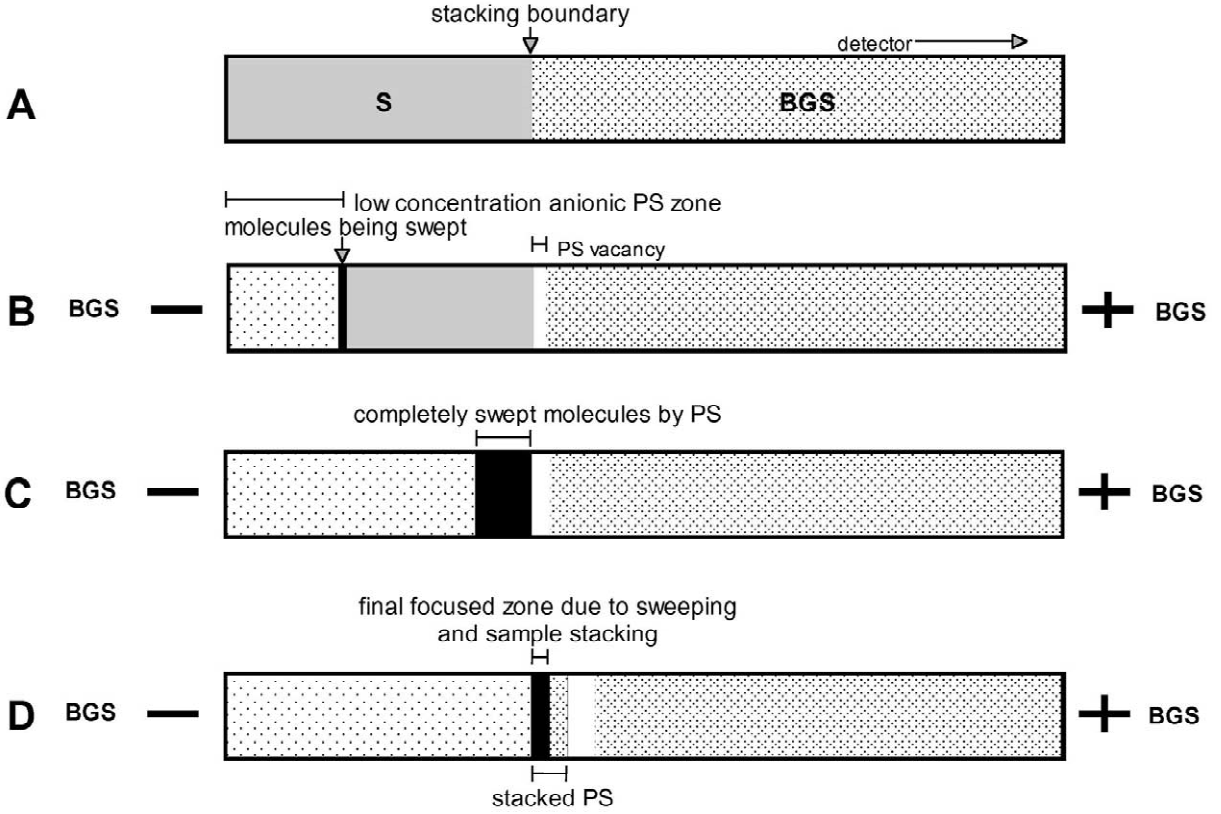

Fig. 7. Sweeping in an enhanced electric field. Progress of an analyte zone in EKC using a negatively charged PS and a zero EOF environment. (A) Starting situation, a longer than typical injection of sample solution (S) prepared in a matrix having a conductivity lower than the micellar background solution (BGS). (B) Application of voltage with the cathode at the inlet end and the anode at the outlet end; the capillary is dipped into two reservoirs filled with the BGS; PS enters the S zone at a lower concentration compared to the BGS; PS sweeps (concentrates) the analyte molecules. (C) The final swept zone is formed when the PS completely fills the S zone. (D) PS stacks or focuses at the initial boundary between the S and BGS zones; stacking of PS causes an additional focusing of the swept analyte zone. For further information, see text.

sample zone is depicted in Fig. 7. Preparing the sample in a matrix having a conductance lower than the BGS yields an enhanced electric field in the sample zone and a reduced electric field in the BGS region. Here, the enhancement factor (γ) is equal to the ratio of the conductivity of BGS and S. Similar to Figs. 1 and 3, a negatively charged PS is used and EOF is zero. Fig. 7A is the same as Figs. 1A and 3A. When voltage is applied, anionic PSs from the cathodic end enter the capillary [25–27]. A PS vacancy (white area) or a zone without the PS develops from the concentration or stacking boundary (SB), which is located at the interface between the S and BGS zones. The SB is stationary because the EOF is zero. It is also interesting to note that the influx of ions to and from the SB is constant [4]. The PS vacancy has been observed experimentally in vacancy MEKC [28]. The concentration of PS entering the S zone (C_{PS}(S)) is lower than that in the BGS zone (C_{PS}(BGS)) as predicted [25]:

$$C_{PS}(S)\frac{C_{PS}(BGS)}{\gamma} \quad (5)$$

The analyte molecules are picked up and accumulated by the PSs filling the S zone (Fig. 7B and C). Analysis of Eqs. (1), (2) and (5) implies that the swept zone depicted in Fig. 7C should be broader than the swept zone in an homogenous electric field system of the same analyte, BGS, and l_{inj}. This is explained by the low k in the S zone due to a lower concentration of PS (C_{PS}(S)). The PS, upon reaching the SB, then stack and form a more concentrated zone of PS. The concentration of PS in the stacked zone (C_{PS}(stacked)) can be approximated by:

$$C_{PS}(\text{stacked}) = C_{PS}(S)\gamma \quad (6)$$

which, in theory, is similar to Eqs. (3)–(5). Evaluation of Eqs. (5) and (6) indicates that C_{PS}(stacked) is equal to C_{PS}(BGS). The increase in concentration leads to an increase in k for each analyte. The increase in k then leads to a focusing effect and a decrease in analyte zone length.

Furthermore, the change in the effective electrophoretic velocity in the SB is reminiscent of that in sample stacking in CZE causes focusing [4,27]. The increase in k as the PS passes the SB and the change in effective electrophoretic velocity appear to be two similar mechanisms, since the change in the effective electrophoretic velocity is dictated by the increase in k. On the other hand, the difference in electric fields also causes an additional factor, which is a change in the electrophoretic velocity of the PS. The electrophoretic velocity of the PSs in the S zone is greater than when the PS approaches the SB. At the SB, the increase in k and the change in electrophoretic velocity of the PS are then the factors that affect the narrowing of neutral analyte zones. These two processes are cumulatively termed here as EKC sample stacking.

We started the study of on-line sample preconcentration for neutral analytes using low conductivity sample matrices to obtain efficient focusing under an enhanced electric field in the sample zone [25,26]. The concentration mechanism with low conductivity sample matrices was explained without using the term ‘‘sweeping’’, but it can be described more clearly in terms of two focusing mechanisms: sweeping as the PS fills the S zone and EKC sample stacking at the SB. In previous studies, it was found that low and high k samples are more effectively enriched when prepared in a low and high (or the same conductivity as the BGS) conductivity matrix, respectively. However, preconcentration of high k samples in a high conductivity matrix (pure sweeping, 1000-fold) is 10 times better than preconcentration of low k samples in a low conductivity matrix (sweeping in an enhanced electric field and EKC sample stacking, 100-fold) [27,29]. It was suggested previously that the lower sensitivity enhancement for low k in a low conductivity matrix can be explained by the disturbance caused by the mismatch of electroosmotic velocities, lower retention factors in the sample zone, and differences in composition between the S and BGS zones [27]. From the practical point of view, sweeping under an enhanced electric field is recommended for analytes having retention factors less than one in the running PS solution. It is recommended to prepare the sample matrix in the lowest conductivity to facilitate EKC sample stacking. In addition, on-line preconcentration of ionic polar analytes that possess low retention factors may be realized using alternative focusing formats, such as sample stacking and dynamic pH junction.

2.1.2. Neutral PS

EKC with charged PSs is the most common format, but EKC with uncharged PSs can also provide useful separations of many important charged molecules [30–32]. The interaction between an uncharged PS and charged molecules can then be utilized to perform sweeping. Unlike sweeping mechanisms with charged PS, where the PS penetrates the S zone, the sample penetrates the neutral PS zone to incur preconcentration. A theoretical study generated the same equation for sweeping (Eq. (2)). The separation and preconcentration of phenol derivatives using nonionic surfactants of alkyl polyoxyethylene ether type (Brij 35 and Brij 58) yielded peak height enhancements of up to 100-fold in 20 m*M* borate buffer (pH 11.25) [33]. The fair enhancements in detection response can be attributed to the moderate interaction of solute with the neutral PS.

2.2. Other interactions

2.2.1. Complexation in CZE

Separation of solutes containing vicinal diol groups in CZE can be achieved by the addition of borate to the separation buffer [34,35]. Borate anions interact or form complexes with the analytes to form zwitterionic or anionic species, thus altering the electrophoretic mobility which causes separation selectivity. On-line preconcentration by sweeping is then applied by merely preparing the sample in a matrix that is free of borate [36]. Borate enters the S zone and forms complexes in situ with the analytes, which eventually leads to narrowing of the analyte zones. Previous studies have used a dynamic pH junction and borate complexation to focus weakly acidic analytes with vicinal diols [7,8]. Sample focusing with a dynamic pH junction depends on the change in electrophoretic mobility due to borate complexation as well changes in pH, which makes it applicable to weakly acidic diol solutes [7,8]. The concentration mechanism with a dynamic pH junction is partly the same as that of sweeping for vicinal diols with borate complexation. It is not essential to use a low pH buffer in sample matrices in the concentration of diols with borate complexation. It should be emphasized that the dynamic pH junction can also be applied to the preconcentration of weak acids and bases. In this review the concentration mechanism of uncharged diols with borate complexation is described in terms of sweeping.

The evolution of neutral analyte zones under sweeping via complexation with borate would be similar to that above or with the use of charged PS. The length of the analyte zone after sweeping (l_{sweep}(complex)), however, is given by [36]:

$$l_{sweep}(\text{complex}) = l_{inj}\left(\frac{\mu_{ep}(\text{b}) - \mu^{*}_{ep}(\text{a})}{\mu_{ep}(\text{b})}\right) \quad (7)$$

where μ_{ep}(b) and μ^{*}_{ep}(a) are the electrophoretic mobility of borate ion and the effective electrophoretic mobility of the neutral solute (a) after complexation, respectively. μ^{*}_{ep}(a) is given by:

$$\mu^{*}_{ep}(\text{a}) = \frac{K[\text{b}]}{1 + K[\text{b}]}\mu_{ep}(\text{ab}) \quad (8)$$

where K is the formation constant, [b] is the concentration of borate, and μ_{ep}(ab) is the electrophoretic mobility of the analyte–borate complex. In summary, the important factors are the formation constant, the borate concentration and the mobility of the analyte–borate complex. Moreover, the pH, which can affect borate complexation, is also important [36]. The low formation constants between borate and the analytes produced up to 40-fold sensitivity improvement.

2.2.2. Other possibilities

Several possibilities for sweeping in CE will be discussed briefly below. First, sweeping can be extended to CZE systems involving other complexing agents [36]. For example, organic compounds capable of complexation with metal ions [37]. Second, in hydrophobic interaction EKC, where separation of neutral solutes is facilitated, for example by the addition of a tetraalkylammonium salt to a non-aqueous separation solution [38–40]. Tetraalkylammonium cations would sweep neutral solutes prepared in a non-aqueous matrix free of the ammonium additive. Lastly, in chemical derivatization. This is amenable to chemical reaction with rapid kinetics, such as naphthalene-2,3-dicarboxaldehyde for the determination of amines, including amino acids. An important factor for sweeping using derivatization is the reaction time. In all of the above-mentioned possibilities, the major factor that will affect the

extent of preconcentration is given by the strength of the interaction involved and the resultant change in analyte velocity (electrophoretic mobility).

3. Selective exhaustive injection–sweeping

Selective exhaustive injection–sweeping (SEI–sweep) is a combination of two on-line preconcentration techniques that could provide a more than 100 000-fold increase in detection sensitivity, which is the highest ever reported in CE [41,42]. SEI is field-enhanced sample injection (FESI) or sample stacking with electrokinetic injection performed for a longer period of time than typical, for example 60 and 400 s for typical FESI and SEI, respectively. Either organic cations or anions are selectively injected. Cations were first reported with this method, but anions have also been reported recently [43]. A limitation with SEI–sweep comes from the SEI step, which, in principle, is sample stacking in CZE. In order to perform sample stacking with electrokinetic injection effectively, the sample has to be prepared in a low conductivity matrix, which is different from that found in real-world samples.

Fig. 8 shows the steps for selective exhaustive injection followed by sweeping of cations (cation selective exhaustive injection–sweeping, CSEI–sweep). The water plug during the long FESI or CSEI step helps maintain field enhancement at the tip of the capillary, especially when the sample matrix contains salt, and may also improve reproducibility. The presence of a high conductivity buffer free of organic solvent (HCB) improves the total focusing effect. The conductivity of the HCB is greater than the buffer. The HCB increases the amount of sample molecules injected and creates a narrower stacked zone after the CSEI step, but does not affect the focusing effect of the sweeping step [41].

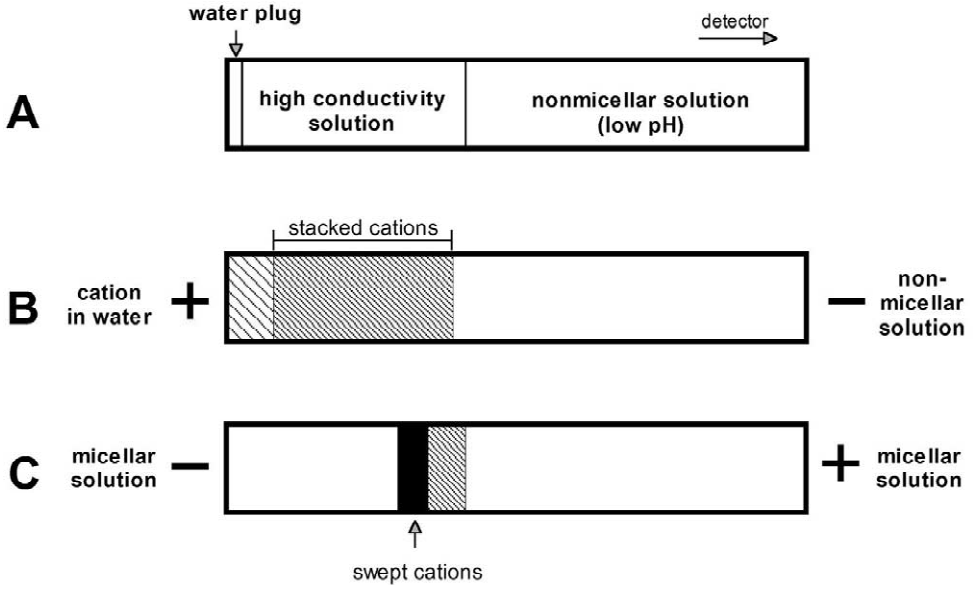

Fig. 8. Cation selective exhaustive injection–sweeping model. (A) Starting situation. The capillary is first conditioned with a nonmicellar background solution, then injection of a high conductivity solution devoid of organic solvent, followed by injection of a short water plug. (B) Electrokinetic injection for a longer period than usual (e.g., 400 s) at a positive polarity of cationic analytes prepared in a low conductivity matrix or water, wherein the cationic analytes focus or stack at the interface between the water zone and the zone of high conductivity solution devoid of organic solvent. (C) Injection is stopped and micellar solutions are placed at both ends of the capillary followed by application of a voltage at negative polarity; this will permit entry of micelles from the inlet vial into the capillary and sweep the stacked and introduced analytes to narrower bands. Reprinted, with permission, from Ref. [42].

4. Applications

Sweeping is a relatively new on-line sample preconcentration method, thus only a few papers have been published reporting its applicability to real-world analysis. However, an increasing number of reports demonstrates that sweeping is easily transferable to other laboratories because of its simplicity [24,44–47]. In addition to anionic PS commonly used in EKC, cationic PS in the form of micelles has proved useful for the sweeping of neutral and negatively charged solutes [44,48,49]. In the area of environmental analysis, a ppb level of a chiral herbicide spiked in lake water was separated and detected by sweeping MEKC using SDS as the PS and γ-cyclodextrin as a chiral selector [14]. Harino et al. showed the applicability of sweeping MEKC for the analysis of estrogens in water [24]. Takagai and Igarashi reported the combination of sweeping MEKC with liquid–liquid extraction for the analysis of benzo[*a*]pyrene and pyrene [46]. Taylor et al. is the first group reporting the utility of sweeping MEKC to urine and plasma extract analysis [47]. Fig. 9 shows sweeping MEKC analysis of basic drugs spiked in urine and plasma. This demonstrates that sweeping is a robust technique that can be applied to any sample matrix for FESI or sample stacking. This suggests a promising role for this method in the analysis of trace amounts of biological samples where a significant concentration of salt is present.

Microchip electrophoresis is rapidly gaining attention as a unique format to perform chemical separations. Detection in microchip electrophoresis is required to be highly sensitive since the amount of analyte is minute and the detection volume is very small (on the order of pL). Therefore, laser-induced fluorescence has been the most popular detection format. Nevertheless, on-line sample concentration is useful to obtain higher concentration sensitivity. So far, two examples of sweeping in microchip electrophoresis have been reported [17,21]. Palmer et al. employed electrokinetic injection with a high-salt concentration sample matrix [21] and obtained a 20-fold peak height improvement for BODIPY. Our group performed sweeping in conventional microchip electrophoresis to directly observe the sweeping process, and demonstrated a more than 100-fold increase in concentration sensitivity for some rhodamine derivatives [17]. In sweeping, the sample zone is extremely narrow and a high separation efficiency can be expected even within the short channel of a microchip.

5. Conclusion

To date, sweeping is the most versatile (applicable to both neutral and charged analytes) and one of the most effective ($>$1000-fold increase in sensitivity) on-line preconcentration methods in CE. Preconcentration results from the interaction of analytes and the PS (including complexation reagents such as borate or tetraalkylammonium ion) which is driven by electrophoresis. Theoretical and experimental studies indicate that the extent to which the injected analyte zones are narrowed is dictated by the strength of the interaction involved. Interactions reported thus far are partitioning and complexation. Up to several thousand-fold improvements in detector response have been demonstrated, thereby lowering concentration detection limits more than three orders of magnitude. When there is sufficient resolution of the sample and matrix components, sweeping will be very useful in real-world analysis since it appears to be less affected by the sample matrix than other preconcentration methods. Furthermore, a combination of sample stacking with electrokinetic injection and sweeping demonstrated a more than 100 000-fold increase in detection sensitivity. Hence sweeping appears to have wide applications in chemical separations by CE.

For charged PS and sample matrices with slightly lower, equal, or greater conductivity than the separation solution, the optimum matrix conductivity for focusing may sometimes be slightly lower or higher than the separation solution in order to obtain better peak shapes [18,44]. This can be explained by the possibility that the concentration of charged PS entering the sample zone is different with various buffer or sample matrix components with a fixed conductance. For example, although the conductivity is the same, the concentration of charged PS entering the sample zone may be lower or higher than the

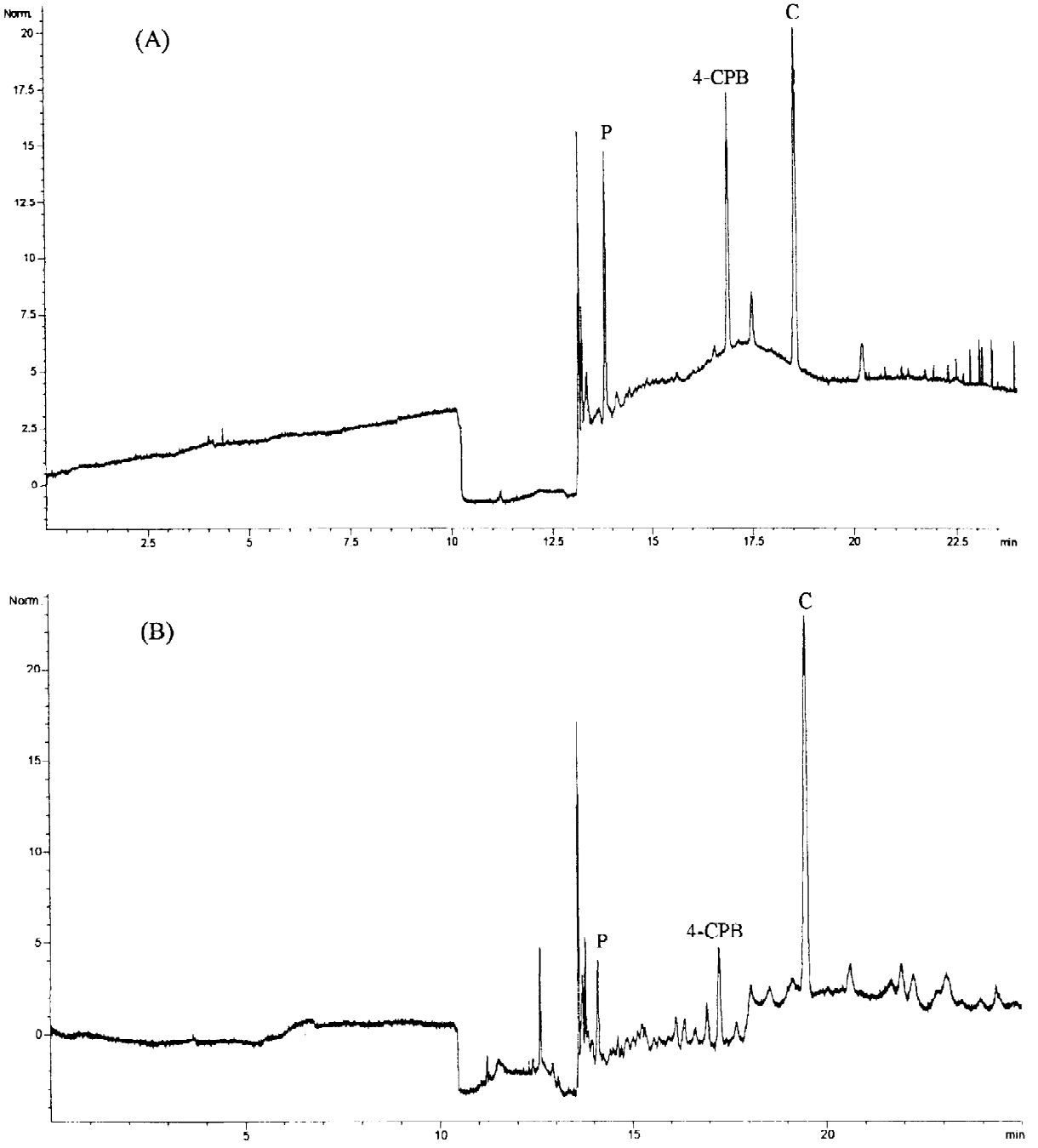

Fig. 9. Representative electropherograms showing the separation of proguanil (P), 4-chlorophenylbiguanide (4-CPB) and cycloguanil (C) in buffer solution following extraction from (A) spiked plasma containing 0.10 μg/ml P, 0.20 μg/ml 4-CPB and 0.13 μg/ml C, and (B) spiked urine containing 1.7 μg/ml P, 2.5 μg/ml 4-CPB and 2.1 μg/ml C. Conditions: capillary total length 760 mm (effective length 680 mm)×50 μm I.D.; phosphate buffer (50 m*M*, pH 2.0) containing 80 m*M* SDS and 40% methanol. Detection at 200 nm. Injection time 500 s at 50 mbar. (From Ref. [47].)

concentration of PS in the separation solution. This is consistent with the Kohlrausch rule, which implies that the charged PS should adjust to the Kohlrausch value of the sample zone. The Kohlrausch value is equal to the sum of C/μ, where C is the concentration and μ is the electrophoretic mobility. In other words, solutions of the same conductivity may have different Kohlrausch values, in this case the sample and separation solution. Therefore, for a given experimental system there exists a lower limit of matrix conductivity where the concentration of charged PS that enters the sample zone is optimum for sweeping. This lower limit of conductivity may then be slightly lower or higher than the separation solution. For example, Lin et al. observed, with cationic surfactants and phosphate buffers, that a sample matrix conductivity of 1.2 to 1.4 times the conductivity, and not more, of the separation solution gives the optimum focusing effect of sweeping [44]. In our group, with SDS and phosphoric acid buffers, slightly lower to equal conductivity between sample and separation solutions provides an optimum enrichment effect. With this in mind, the focusing effect of sweeping using charged PS is also affected by the nature of the PS and buffer components used. A thorough experimental study should be performed in the future on this aspect.

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