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ARTICLE *in* ANALYTICAL CHEMISTRY · FEBRUARY 2014

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Stacking and Separation of Neutral and Cationic Analytes in Interface-Free Two-Dimensional Heart-Cutting Capillary Electrophoresis

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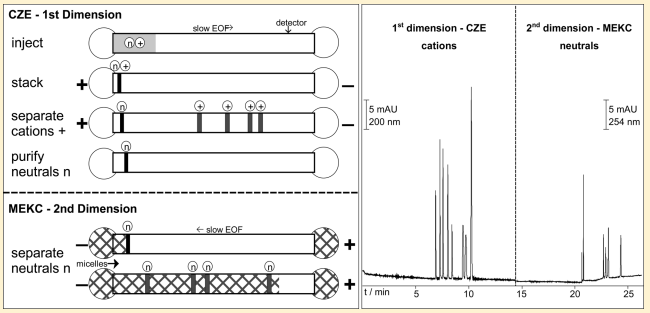
Supporting Information

ABSTRACT: Interface-free two-dimensional heart-cutting capillary electrophoresis is typically used to purify/concentrate and separate a single target fraction. Here, we demonstrate the simultaneous stacking and orthogonal separation of target fractions that belong to two different classes of compounds. The cationic species were first analyzed by capillary zone electrophoresis using an electrolyte of low pH in a fused-silica capillary. The neutrals that remained inside the capillary after the first dimension analysis were then separated by micellar electrokinetic chromatography using sodium dodecyl sulfate micelles that were electrophoretically introduced into the capillary. The suppression of electro-osmotic flow using low pH was essential in shifting the separation selectivity from electrophoresis to electrokinetic chromatography. Two approaches to stacking based on the solubility of the species present were developed. Stacking was achieved during the first dimension analysis via sweeping and/or analyte focusing by micelle collapse. Two artificial mixtures (eight cationic drugs/five neutral steroids, and three quaternary ammonium/three neutral organophosphate pesticides) were successfully analyzed with sensitivity enhancement factors from 15 to 100. Some analytical parameters and application to a spiked real water sample were also studied.



Multidimensional separation, a flourishing research area, increases the peak capacity and thus allows for the analysis of very complex mixtures.[1−5] Orthogonal techniques are combined to separate compounds that belong to different classes or structural characteristics.[6−8] Examples of orthogonal separation systems include hydrophilic interaction and ion-pair reversed-phase liquid chromatography,[9,10] reversed-phase and size exclusion liquid chromatography,[11,12] ion-exchange and reversed-phase capillary electrochromatography,[13] nonpolar and polar column gas chromatography,[14] and nonpolar and ionic liquid column gas chromatography,[15] among others.

In two-dimensional heart-cutting capillary electrophoresis (2-D heart-cutting CE), the focus is on one or several parts of the entire electrophoretic or electrochromatographic profile. The developments were on (1) interfaces that transfer fractions from the first to the second dimension and (2) interface-free strategies in order to perform two CE modes in a single capillary. Majority of the developments were on interfaces which were also used in comprehensive analysis.[16−24] Interfaces were designed and constructed mainly by Dovichi's group to provide a leak-free connection between the two dimensions.[16−22] The success of such an approach often relied on the skill of the chemists who make such meticulous devices. Others have used an etched porous interface in a single capillary where pressure was used to transfer the fractions across the interface.[23,24]

Interface-free 2-D heart-cutting CE was first described by Cottet et al.[25] This multidimensional approach is attractive because it can be performed in commercial CE instrumentation with minimal or no modification. Currently, reports were limited to purification and separation of a target fraction in the first and second dimension, respectively. In the initial paper, the first dimension was capillary zone electrophoresis (CZE) to purify the fraction that contained anionic synthetic polymers. The unwanted neutral components in the sample mixture were made to migrate out of the capillary (charge density-based separation). The target fraction was then subjected to CE using a separation solution with entangled polymer (size-based separation). The analysis in the second dimension was via counter electro-osmotic flow (EOF) mode, where the separation media entered the capillary via EOF and the anionic analytes in the target fraction electrophoretically migrated opposite the direction of EOF.[25,26] Variations of the approach

Received: January 5, 2014
Accepted: February 13, 2014
Published: February 13, 2014

   dx.doi.org/10.1021/ac500090n | *Anal. Chem.* 2014, 86, 3159−3166

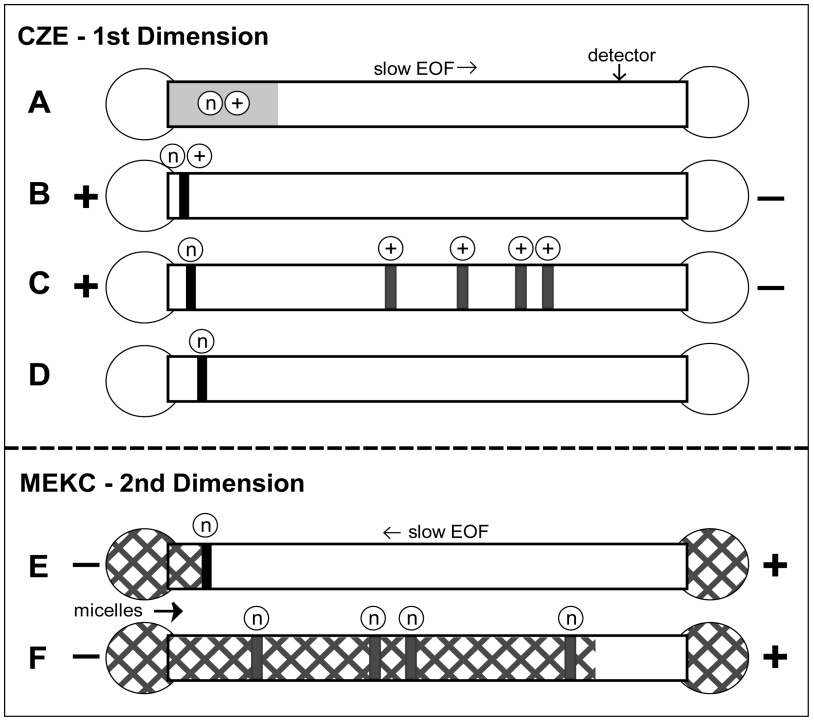


Figure 1. Stacking and separation of two different classes of compounds [cationic (+) and neutral (n) analytes] in interface-free 2-D heart-cutting CE (CZE × MEKC). (A) The fused-silica capillary was filled with a low-pH CZE electrolyte. The sample was injected as a long plug. The CZE electrolye was placed at both ends of the capillary. (B) A voltage was applied at positive polarity (cathode at the detector end). The analytes were focused by stacking. (C) Continued application of voltage caused the migration of the concentrated cationic analytes to the detector. (D) The cationic analytes migrated out of the capillary, and the concentrated neutral analytes were purified and remained inside the capillary. The first dimension analysis ended. (E) The start of the second dimension analysis was the replacement of the CZE electrolye at both ends of the capillary by the low-pH MEKC electrolyte with SDS micelles. The presence of micelles is depicted by the square patterned zones. Application of voltage at negative polarity (anode at detector end) caused the electrophoretic migration of the SDS micelles into the capillary. The micelles eventually penetrated or swept the neutral analyte fraction. (F) Continued application of voltage caused the micelle-bound analytes to separate and migrate to the detector. The second dimension and analysis ends when all the analytes were detected.

in order to extended applicability to other systems have been demonstrated,[27−29] for example, sample cleanup by CZE in the first dimension and separation of the target anionic derivatized amino acids by micellar electrokinetic chromatography (MEKC) in the second dimension.[27]

Stacking or online sample concentration techniques[30,31] have also been implemented in interface-free 2-D heart-cutting CE in order to improve detection sensitivity.[32−35] Aside from purification, the target fraction was also concentrated in the first dimension via isotachophoresis,[36] dynamic pH junction,[37] or sweeping.[38,39] In addition, analyte focusing by micelle collapse (AFMC)[40−43] was used to refocus the swept or concentrated bands that were broadened during pressure mobilization. Moving of the swept band to the inlet end was required for the second dimension separation.[34,35] To date, an interface-free 2-D heart-cutting CE approach that could concentrate and separate two or more different classes of compounds is yet to be realized.

In this study, the stacking and purification of two target fractions that contained cationic and neutral species was investigated for the first time in interface-free 2-D heart-cutting CE. Included was the separation of the constituents of each fraction using CZE and MEKC in the first and second dimension, respectively. Two test mixtures were studied. First was a variety of eight cationic drugs and five neutral steroids, where all compounds were soluble in water. The other was three quaternary ammonium herbicides and three poorly water-soluble neutral organophosphate pesticides. All the above compounds were chosen because they are (emerging) environmental pollutants. Stacking of both species was with one or a combination of two online concentration techniques. For the water-soluble mixture, two-step stacking by sweeping and AFMC was used. For the second mixture, AFMC was employed where the presence of micelles in the sample aided in the dissolution of the organophosphates. In addition, applicability was tested using a fortified sample of river water.

■ EXPERIMENTAL SECTION

Reagents and Materials. Sodium dodecyl sulfate (SDS) and phosphoric acid were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Methanol (MeOH) and acetonitrile (ACN) were all HPLC grade obtained from Merck. Stock solutions at appropriate concentration were prepared in distilled deionized water. Phosphate buffer was prepared by adjusting the pH of phosphoric acid with 5 M sodium hydroxide. All stock solutions were sonicated and filtered by a 0.45 μm filter prior to use.

Standards of cationic drugs (neostigmine bromide, doxepin hydrochloride, dibucaine hydrochloride, ticlopidine hydrochloride, verapamil hydrochloride, diphenhydramine, propranolol, labetalol), neutral steroids (progesterone, hydrocortisone, cortisone, prednisone, triamcinolone), quaternary ammonium herbicide diquat, and organophosphate pesticides (parathion, fenitrothion, and azinphos-methyl) were all purchased from Sigma-Aldrich (Germany). The other two

dx.doi.org/10.1021/ac500090n | Anal. Chem. 2014, 86, 3159−3166

quaternary ammonium herbicides (paraquat and difenzoquat) were obtained from Chemservice (West Chester, PA, U.S.A.). Care should be taken when handling the above chemicals as they may be harmful to humans. Stock solutions of the standard were prepared in ACN or MeOH to a concentration of 1 mg/mL each. The solutions were stored at 2−8 °C when not in use. The sample diluent and separation (CZE and MEKC) electrolytes were freshly prepared each day. The working sample solutions were prepared by appropriate dilution of stock standard in either separation electrolyte or the sample diluent.

Capillary Electrophoresis. All experiments were performed on a Hewlett-Packard 3D capillary electrophoresis system (Waldbronn, Germany). Polyimide-coated untreated fused-silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ). Fused-silica capillaries of 50.0 cm (effective length 41.5 cm) × 50 μm i.d. were thermostatted at 20 °C. New capillaries were conditioned with 1 M NaOH (20 min), water (10 min), and separation electrolyte (15 min). After each run, the capillary was conditioned by flushing with the CZE electrolyte (4 min). Detection wavelength was performed at 200 (cationic drugs and herbicides/pesticides) and 254 (steroids) nm where analytes showed the best signals. Other conditions are found in the texts and figures.

Typical Injection and Stacking in Interface-Free 2-D Heart-Cutting CE (CZE × MEKC). The typical injection was conducted by hydrodynamic injection of sample at 25 mbar for 6 s. Two stacking approaches were demonstrated: (1) two-step stacking with sweeping and AFMC for the sample mixture with water-soluble analytes (drugs and steroids) and (2) AFMC for the mixture containing soluble and poorly water-soluble analytes (cationic herbicides and neutral pesticides). Separation electrolyte for typical injection and stacking was 100 mM phosphate (pH 2.5) containing 20% ACN in the first dimension (CZE) and 100 mM SDS in 10 mM phosphate (pH 2.5) and 20% ACN in the second dimension (MEKC). After injection, the first dimension was performed at positive polarity (+20 kV) and switched to second dimension by replacing the CZE electrolyte to MEKC electrolyte at negative polarity (−20 kV).

Two-Step Stacking with Sweeping and AFMC. Cationic drugs and neutral steroids were prepared in 80 mM phosphate (pH 2.5). The micellar solution [5 mM SDS in 80 mM phosphate (pH 2.5)] was injected first at 50 mbar for 65 s followed by sample solution at 50 mbar for 60 s and organic solvent rich solution [30% ACN in 100 mM phosphate (pH 2.5)] at 50 mbar for 10 s.

AFMC. The herbicides and pesticides were prepared in a micellar solution of 5 mM SDS in 80 mM phosphate (pH 2.5). The sample solution was introduced into the capillary at flush mode (∼950 mbar) for 0.19 min, and then the organic solvent rich solution [30% ACN in 100 mM phosphate (pH 2.5)] was injected at 50 mbar for 10 s.

Spiked River Water Sample Preparation. The river water sample was collected from a river in Tasmania, Australia. An amount of 1 mL of the sample spiked with 0.01 μg/mL of herbicides and pesticides was evaporated in vacuum oven at 40 °C to dryness. Then, the samples were reconstituted to 0.1 mL with the micellar solution [5 mM SDS in 80 mM phosphate (pH 2.5)]. The resulting concentration of the analytes was 0.1 μg/mL. This was performed in triplicate. The samples and a standard sample with 0.1 μg/mL of each analyte were analyzed by AFMC in 2-D CE. The recoveries were calculated by dividing the corrected peak area obtained in the reconstituted sample by the standard sample (× 100%).

RESULTS AND DISCUSSION

Stacking and Separation in Interface-Free 2-D Heart-Cutting CE (CZE × MEKC): Scheme. The scheme for the separation of cationic and neutral species by interface-free 2-D heart-cutting CE is shown in Figure 1. In the first dimension or CZE, online sample concentration of both species is also shown. CZE was performed using a low-pH electrolyte in order to ionize the cationic species as well as to suppress the EOF. In Figure 1A or starting situation, a long plug of sample solution (gray zone that contained cationic and neutral analytes) was injected into a capillary that was previously filled with the CZE electrolyte. The CZE electrolyte was also at both ends of the capillary. In Figure 1B, a voltage was applied at positive polarity (cathode at the detector end), and this caused all analytes to concentrate. A detailed discussion of the two stacking approaches will be given later.

In Figure 1C, after stacking, the cationic species separated via CZE and migrated to the UV detector (see Figure 1C). The concentrated neutral analytes moved a bit toward the outlet or cathode due to the slow EOF. When the last cationic analyte migrated out of the capillary, the voltage was stopped as shown in Figure 1D. This is the end of the first dimension analysis. The neutrals remained inside the capillary.

The second dimension analysis started by replacing the CZE electrolyte at both ends of the capillary by the MEKC electrolyte with SDS micelles. In Figure 1E, a voltage is applied at negative polarity (anode at detector end), and this caused the micelles to electrophoretically migrate into the capillary. The square patterned zone depicts the presence of micelles. The micelles eventually reached the concentrated neutral analytes. A sweeping effect on the purified neutral fraction was expected because this fraction was free of the pseudostationary phase. In Figure 1F, continued application of voltage caused the separation via MEKC principles in reversed migration mode.[44,45] The SDS micelles carried all the analytes to the detector, in the order of decreasing hydrophobicity. The more hydrophobic analytes have more affinity to the micelles.

The suppression of EOF was vital in the proposed interface-free 2-D heart-cutting CE because it allowed (1) the purification of the neutral analytes while the cationic analytes were being analyzed by CZE, (2) the retention of the purified neutral analyte fraction inside the capillary after the cationic analytes migrated out of the capillary in the first dimension, and (3) the reversed migration MEKC in the second dimension of neutral analytes by allowing the negatively charged pseudostationary phase (i.e., micelles) to enter the capillary via electrophoresis.

2-D Heart-Cutting CE (CZE × MEKC) Separation of Cationic and Neutral Analytes: Proof of Concept. Two CE experiments of the model mixture of eight cationic drugs and five neutral steroids are shown in Figure 2. Parts A and B (A × B) of Figure 2 are from the proposed 2-D heart-cutting CE (CZE × MEKC) method. Figure 2A is the first dimension analysis where the cationic drugs were separated by CZE using 100 mM phosphate buffer (pH 2.5) and 20% ACN. Figure 2B is the second dimension analysis where the neutral steroids that remained inside the capillary were separated by MEKC using 100 mM SDS with 10 mM sodium phosphate (pH 2.5) and 20% ACN. For simplicity, the conductivities of the CZE and MEKC electrolytes were similar in order to avoid stacking or

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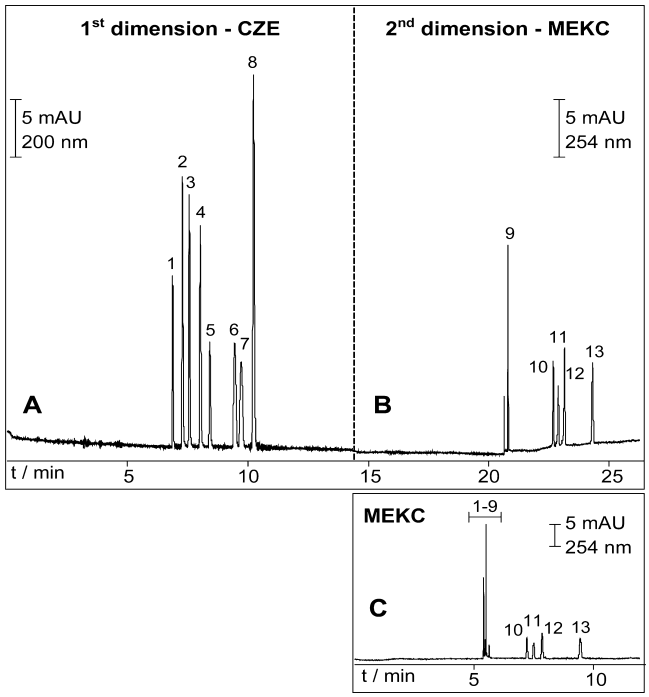

Figure 2. Interface-free 2-D heart-cutting CE (CZE × MEKC) (A × B) vs MEKC only (C) of cationic drugs and neutral steroids. (A) First dimension or CZE with electrolye of 100 mM sodium phosphate (pH 2.5) and 20% ACN. (B) Second dimension or MEKC with electrolyte of 100 mM SDS, 10 mM sodium phosphate (pH 2.5), and 20% ACN. Sample of 5−10 μg/mL of drugs and steroids in CZE electrolyte was injected at 25 mbar for 6 s. Separation voltage was 20 kV in CZE (A) and −20 kV in MEKC (B and C). Detection was at 200 nm in panel A and 254 nm in panels B and C. Peak identifications: cationic drugs were neostigmine (1), ticlopidine (2), diphenhydramine (3), doxepin (4), propranolol (5), labetalol (6), dibucaine (7), and verapamil (8); steroids were progesterone (9), hydrocortisone (10), cortisone (11), prednisone (12), and triamcinolone (13). The switching from CZE (A) to MEKC (B) is explained in the text. Other CE conditions are found in the Experimental Section.

destacking effects due to electric field differences. Figure 2C shows the one-dimension or 1-D MEKC analysis of the sample mixture using the MEKC electrolyte. It is emphasized that the separation conditions used in MEKC were the same in the 2-D and 1-D experiments. Detection in CZE and MEKC where the drugs and steroids have better signals was 200 and 254 nm, respectively. The two experiments were also typical injections of the same sample prepared in the CZE electrolyte.

The cationic drugs were not separated, and they also migrated closely with progesterone in the 1-D MEKC experiment (see Figure 2C). The cationic drugs and progesterone had the most affinity to the SDS micelles, and thus they migrated fastest to the detector. The analytes were all separated in the 2-D CE method (see Figure 2, parts A and B), and this shows the potential of the 2-D approach in separating complex mixtures of cationic and neutral analytes.

The switch from the first to the second dimension was conducted after the last peak (i.e., peak 8) migrated out of the capillary during the CZE analysis. The calculated apparent velocity of peak 8 in B was 4.1 cm/min, and the distance from detector to end of capillary was 8.5 cm. Thus, this peak after detection should migrate out of the capillary at 12.3 or 10.2 min (migration time of peak 8) + 2.1 min. To ensure complete removal, an additional 2 min was added to the above time. The additional time conveniently allowed us to automate the 2-D procedure with the Hewlett-Packard ChemStation software. When the switch was done too early, the last peak was detected twice during the 2-D analysis. Peak 8 migrated past the detector from the anodic end via electrophoresis. It was captured by the micelles from the inlet vial and was subsequently detected again at the front of the micelles.

The analytical figures of merit for the 2-D heart-cutting CE (CZE × MEKC) conditions in Figure 2, parts A and B, are summarized in Table S1 of the Supporting Information. The coefficient of determination (r^2) values were better than 0.999. The intraday repeatability (% RSD, n = 4) for corrected peak area and migration time was 1.0−3.2% and 0.4%, respectively. For interday repeatability (% RSD, n = 4), it was 1.9−4.4% and 0.4−0.5%, correspondingly. Corrected peak area was calculated by dividing the peak area with the migration time. The limits of detection (LODs) (S/N = 3) for the cationic drugs and steroids were 0.2−0.4 and 0.1−0.6 μg/mL, respectively.

Online Sample Concentration or Stacking in Interface-Free 2-D Heart-Cutting CE. The stacking of all species was performed in the first dimension via approach 1, two-step stacking with sweeping and AFMC[46,47] for the test mixture with water-soluble analytes and approach 2, AFMC for the mixture that contained soluble and poorly water-soluble analytes. In both approaches the AFMC or micelle collapse was facilitated by the injection of an organic solvent rich solution.[48]

Stacking Approach 1: Two-Step Stacking with Sweeping and AFMC. The steps involved in stacking approach 1 are depicted in Figure S1 of the Supporting Information. In a capillary that was previously conditioned with the CZE electrolyte, the micellar solution was injected hydrodynamically, followed by the sample solution, and finally the solvent-rich solution (see Supporting Information Figure S1A). Sweeping was performed using a micellar solution of 5 mM SDS with 80 mM sodium phosphate (pH 2.5). The test analytes were prepared in a sample diluent [i.e., 80 mM sodium phosphate (pH 2.5)] that was void of the micelles, which is the fundamental requirement of sweeping. The organic solvent rich solution was 30% ACN in 100 mM sodium phosphate. The conductivities of the sample solution, solvent-rich solution, micellar solution, CZE electrolyte, and MEKC electrolyte were considered similar (within 90%). It is noted that the critical micelle concentration (cmc) of SDS in the presence of electrolytes is <3 mM,[40] and in 30% ACN with electrolytes it is 11 mM.[49]

When a voltage is applied with the CZE electrolyte at both ends of the capillary, the micelles entered and swept the analytes in the sample zone (see Supporting Information Figure S1B). The sweeping of the cationic analytes was faster because these analytes migrated in the opposite direction of the micelles. The concentration of SDS entering the sample zone was approximately 5 mM since the conductivities of the micellar and sample solutions were similar. The micelles eventually traversed through the sample zone (see Supporting Information Figure S1C). The analytes were all accumulated at the front of the micelles.

The second focusing mechanism of AFMC started when the micelle front reached the boundary between the sample solution and the organic solvent rich zone (see Supporting Information Figure S1D). The cmc of SDS was 11 mM in the organic solvent solution, and thus the concentration of the SDS was not enough to sustain micelles in this boundary. This caused the collapse of the electrophoretically migrating micelles

dx.doi.org/10.1021/ac500090n | Anal. Chem. 2014, 86, 3159−3166

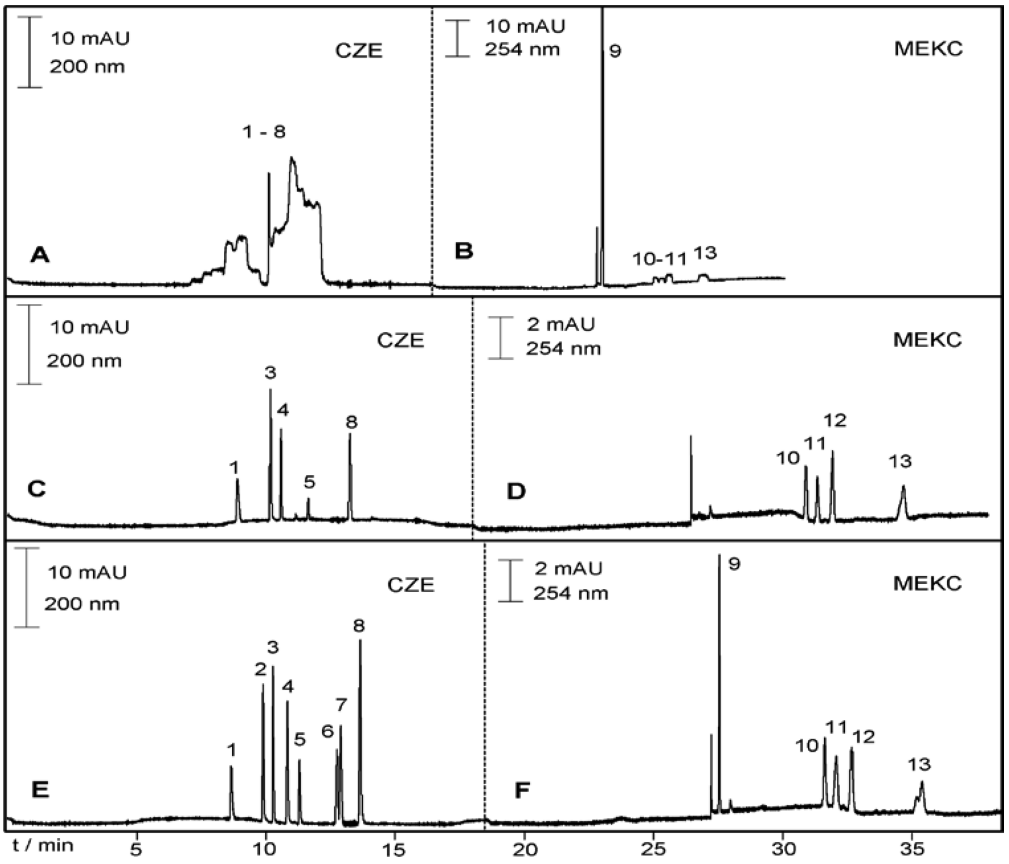

Figure 3. Electropherograms obtained from interface-free 2-D heart-cutting CE (CZE × MEKC) [(A × B), (C × D), and (E × F)] of cationic drugs and neutral steroids using different sample diluents and sample injection schemes. CZE and MEKC electrolytes were the same as in Figure 2. Sample solution was 0.25−0.5 μg/mL of analytes in CZE electrolyte (A × B) and in 80 mM sodium phosphate (pH 2.5) [(C × D) and (E × F)]. Micellar solution was 5 mM SDS with 80 mM sodium phosphate (pH 2.5). Organic solvent rich solution was 30% ACN in 100 mM sodium phosphate (pH 2.5). Sample injection scheme: (A × B) was injection of sample at 50 mbar for 60 s; (C × D) was injection of micellar solution at 50 mbar for 60 s then sample at 50 mbar for 60 s; (E × F) was same as in (C × D) but with injection of organic solvent rich solution at 50 mbar for 10 s after the sample injection. Other conditions and identification of peaks were the same as Figure 2.

and the subsequent release of the bound analytes in the boundary. Both neutral and charged analytes were concentrated. In the case of the cationic analytes, the focusing mechanism is also known as micelle to solvent stacking (MSS).[41,43] MSS is the reversal in the analytes' effective electrophoretic mobility, where in this case was accompanied by micelle collapse. The two-step stacking procedure ends when all the micelles from the injected micellar solution migrated through the boundary (see Supporting Information Figure S1E). The concentrated cationic analytes separate and electrophoretically migrate to the detector (Figure 1C). The concentrated neutral analytes remained in the boundary and moved with the EOF.

Experimental verification of two-step stacking in interface-free 2-D heart-cutting CE is elaborated in Figure 3. The effect of different kinds of sample and injection conditions is shown. The CZE and MEKC conditions were the same as in Figure 2, parts A and B, respectively. There are three 2-D (CZE × MEKC) experiments: (A × B), (C × D), and (E × F). The sample solution was injected at 50 mbar for 60 s in all experiments. In (A × B), the sample solution was prepared in the CZE electrolyte. In (C × D) and (E × F), the sample was prepared in 80 mM phosphate (pH 2.5). The micellar solution was injected for 60 s at 50 mbar before the sample solution. In (E × F), the organic solvent rich solution was injected for 10 s at 50 mbar after the sample.

In (A × B), all the analytes were detected as broad peaks in the first dimension or CZE, as expected since the sample diluent and CZE electrolyte were the same. However, in the second dimension analysis or MEKC, the most hydrophobic neutral analyte (i.e., progesterone or peak 9) was detected as a sharp peak. The less hydrophobic steroids (i.e., hydrocortisone, cortisone, prednisone, and triamcinolone, or peaks 10−13, respectively) were detected as broad peaks. This was because of the effect of sweeping on this neutral sample fraction without micelles. Note that the focusing effect of sweeping depends on the strength of the interaction between micelles and analyte. Progesterone had the most affinity to the micelles.

In (C × D), in the absence of a solvent-rich plug, the peaks that corresponded to neostigmine, diphenhydramine, doxepin, propranolol, and verapamil were sharp, while the rest of the cationic drugs (i.e., ticlopidine, labetalol, and dibucaine) were not detected. No peak was observed for progesterone and sharp peaks for the other steroids. In (E × F) or the proposed stacking procedure which involved the injection of the solvent-rich plug, all the peaks were acceptably sharp except for the last peak or least hydrophobic steroid triamcinolone. The peak shape for triamcinolone in (E × F) is characteristic of an incompletely stacked peak due to the incomplete transport of

dx.doi.org/10.1021/ac500090n | Anal. Chem. 2014, 86, 3159−3166

the injected analyte zone. Triamcinolone will be completely stacked by adjusting or increasing the micellar solution injection.[42]

The sharp peaks for 1, 3, 4, 5, and 8 in (C × D) was due to the MSS mechanism. MSS occurred at the boundary between the CZE electrolyte with 20% ACN and the micelle front that first swept through the sample zone. A concentration of 20% ACN was sufficient to induce effective electrophoretic mobility reversal for these analytes. On one hand, the other cations, ticlopidine, labetalol, and dibucaine, required a higher concentration of ACN to induce analyte focusing by MSS and most effectively by MSS accompanied by collapse of carrier micelles. Progesterone was not detected in (C × D) because it was highly bound to the micelles and thus it migrated with the micelles to the inlet and then out of the capillary during CZE. The less hydrophobic steroids which were loosely bound to the micelles remained inside the capillary.

There was analyte focusing of the less hydrophobic steroids (i.e., last four peaks) in (C × D) where the peak heights were similar to that in (E × F). The focusing mechanism in (C × D) was, however, different from (E × F). The mechanism for (C × D) involved three steps. First was the sweeping of the steroids as described in Supporting Information Figure S1, part B to C. Upon migration of the swept analytes to the CZE electrolyte, 20% ACN in the CZE electrolyte reduced the affinity of these steroids to the micelles. This prevented the migration of these analytes to the inlet, but it also broadened the swept zones. Finally, the switch to the second dimension caused the sweeping of the broadened zones with a higher concentration of SDS coming from the MEKC electrolyte.

The two-step stacking (E × F) was further optimized by (1) varying the organic solvent rich solution injection, (2) increasing the injection time of the sample solution, and finally (3) adjusting the micellar solution injection. In part 1, the injection time at 50 mbar for the 30% ACN with 100 mM sodium phosphate (pH 2.5) was varied from 2, 5, 10, 20 to 50 s. There were no significant differences in the results, and the 10 s injection was chosen. In part 2, the injection time of the sample solution was increased in order to obtain the best gain in sensitivity without significant compromise to resolution. For convenience, this was performed at a fixed sample and micellar solution injection ratio of 1:1. The injection of the sample solution at 50 mbar was increased from 60, 90, 120, 150, 180 to 210 s. For the cationic species, the peak heights increased and the resolution was acceptable when the injection time was increased from 60 to 180 s. However, the peaks obtained for the steroids were broad for sample injections >60 s. In part 3, the maximum sample injection time to nicely stack both species was 60 s at 50 mbar (at a fixed sample and micellar solution ratio of 1:1). The ratio was modified where the injection of the micellar solution was increased from 60 to 65, 70 to 75 s at 50 mbar. The last peak was nicely stacked using the 65 s injection, and thus longer injections were unnecessary. The final injection regimen for stacking was 65 s of micellar solution, followed by 60 s of sample solution, and finally 10 s of organic solvent rich solution (all at 50 mbar).

The analytical figures of merit for two-step stacking in 2-D heart-cutting CE are summarized in Supporting Information Table S1. The coefficient of determination (r^2) values for all analytes were greater than 0.999. The intraday repeatability (% RSD, n = 4) for corrected peak area and migration time was 0.5−2.0% and 0.2−1.3%, respectively. For interday repeatability (% RSD, n = 4), it was 1.4−3.0% and 0.6−1.9%, correspondingly. There were no significant differences in repeatability between typical and stacking injections. Repeatability and linearity in both injections were considered acceptable. The LODs (S/N = 3) for the cationic drugs and steroids were 0.003−0.01 and 0.003−0.03 μg/mL, respectively. The sensitivity enhancement factor (SEF) was calculated by dividing the LOD in typical injection by the LOD in stacking injection. The SEFs for the cationic drugs and steroids were 30−100 and 15−33, respectively. The LOD values obtained with stacking injection were 1−2 orders of magnitude better compared to typical injection in the proposed 2D-CE methodology.

Stacking Approach 2: AFMC. The steps involved in stacking approach 2 are depicted in Figure S2 of the Supporting Information. The sample prepared in a micellar solution was injected hydrodynamically into a capillary that was previously conditioned with CZE electrolyte (see Supporting Information Figure S2A). The micelles aided in the dissolution of poorly water-soluble analytes (i.e., organophosphate herbicides). This was followed by injection of the organic solvent rich solution. The conductivities of the sample solution, solvent-rich solution, CZE electrolyte, and MEKC electrolyte were similar. The presence of micelles in the sample zone and the solvent-rich zone facilitated the stacking by AFMC (or MSS for cationic species). For simplicity, the CZE electrolyte, MEKC electrolyte, organic solvent rich solution, and micellar solution used here were the same as in the previous section.

When a voltage is applied with the CZE electrolyte at both ends of the capillary, the micelles in the sample zone transported the analytes to the organic solvent rich zone (see Supporting Information Figure S2B). At the boundary between the sample solution and the organic solvent rich zone, the electrophoretically migrating SDS micelles collapsed, and this led to the release of the bound analytes in the boundary. Both species were concentrated. The stacking procedure ends when all the micelles (with the analytes) migrated through the boundary (see Supporting Information Figure S2C). The next step was the same as in Figure 1C.

Experimental verification of stacking approach 2 is demonstrated in Figure 4. The CZE and MEKC conditions were the same as in Figure 2, parts A and B, respectively. (A × B) is a 2-D (CZE × MEKC) experiment from a typical injection of sample solution prepared in the CZE electrolyte. (C × D) is the current optimized stacking 2-D experiment where the sample was prepared in the micellar solution. Typical injection and stacking injection of sample was 6 s at 25 mbar and 0.19 min at flush mode, respectively. In stacking, the solvent-rich solution was injected for 10 s at 50 mbar after the sample. The analytes in the typical were 40× more concentrated than those in the stacking injection. The improvements in peak height were from around 20× to 70× better compared to typical injection.

This stacking approach was optimized by (1) varying the organic solvent rich solution injection, (2) increasing the injection time of the sample solution, and finally (3) employing an injection of micellar solution after the sample. The chosen starting injection condition (all at 50 mbar) was 0, 60, and 10 s of micellar, sample, and organic solvent rich solution, respectively. In part 1, the injection time at 50 mbar for the 30% ACN with 100 mM sodium phosphate (pH 2.5) was varied from 2, 5, 10, 20 to 50 s. There were no significant differences in the results, and the 10 s injection was chosen. This injection was the same as in the two-step stacking. In part

dx.doi.org/10.1021/ac500090n | Anal. Chem. 2014, 86, 3159−3166

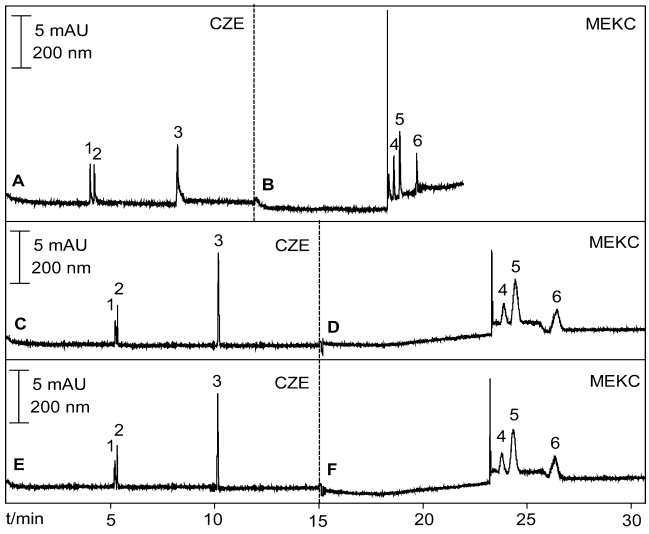

Figure 4. Electropherograms obtained from interface-free 2-D heart-cutting CE (CZE × MEKC) of cationic herbicides and neutral organophosphate pesticides using different sample diluents and sample injection schemes. Sample solution was 4 μg/mL each (1–6) in CZE electrolyte (A × B), 0.1 μg/mL each in 80 mM sodium phosphate (pH 2.5) containing 5 mM SDS (C × D), and spiked river sample at 0.01 μg/mL which was concentrated 10× by evaporation (E × F). Sample injection scheme: (A × B) was injection of sample at 25 mbar for 6 s; (C × D) and (E × F) were injection of sample by flushing for 0.19 min and finally organic solvent rich solution at 50 mbar for 10 s. Peak identifications: (1) paraquat, (2) diquat, (3) difenzoquat, (4) parathion, (5) fenitrothion, and (6) azinphos-methyl.

2, the injection time of the sample solution at 50 mbar was increased from 60 to 270 s at 30 s increments. The maximum peak heights were observed at 210 s.

To decrease the total analysis time, the flush mode was implemented for injection. The velocities of the 50 mbar and flush mode injections were determined. The 210 s at 50 mbar injection was approximately equal to 0.18 min at flush mode. Flush mode injections at 0.16, 0.17, 0.18, 0.19, and 0.20 min were then studied, and the 0.19 min gave the best results as shown in Figure 4B. In part 3, the rationale behind the injection of micellar solution after the sample prepared in micelles was to improve the transport of the analytes to the stacking boundary.[42,43] However, for the herbicide/pesticide mix, injection of the micellar solution did not provide any improvements. Under the current conditions, the affinity of the analytes to the micelles was sufficient to provide efficient transport for stacking. For other analytes, the micellar solution injection will improve the stacking. For example, when we tested the water-soluble mixture of drugs and steroids with this AFMC approach, a micellar injection was needed (data not shown).

The analytical figures of merit for the typical (A × B) and stacking (C × D) 2-D CE conditions in Figure 4 are summarized in Table S2 of the Supporting Information. The coefficient of determination (r^2) values for all analytes were greater than 0.999. For typical injection, the intraday repeatability (% RSD, n = 4) for corrected peak area and migration time was 1.5–3.9% and 0.01–0.5%, respectively. For interday repeatability (% RSD, n = 4), it was 1.4–4.3% and 0.04–1.7%, correspondingly. For stacking injection, the intraday for area and migration time was 0.9–2.0% and 0.4–0.9%, respectively. For interday, it was 1.2–2.1% and 0.5–1.0%, correspondingly. Repeatability and linearity in both injections were considered acceptable. The LODs (S/N = 3) for typical and stacking were 0.2–0.4 and 0.004–0.02 μg/mL, respectively. The SEFs for the quaternary ammonium herbicides and organophosphate pesticides were 40–75 and 20–30, respectively.

The stacking 2-D CE conditions in Figure 4 (C × D) were tested to the analysis of a spiked real water sample. The water sample was spiked with the herbicides at a concentration of 0.01 μg/mL. An amount of 1 mL of the spiked sample was evaporated to dryness and then was reconstituted with 0.1 mL of micellar solution. A representative stacking 2-D CE result is shown in Figure 4 (E × F). The concentration of the injected samples was 0.1 μg/mL due to the evaporation/reconstitution procedure with a 10-fold concentration factor. After comparison with the standard injection of the same concentration [Figure 4 (C × D)], the recoveries were very good at 95–98% (see Supporting Information Table S2).

CONCLUSION

A simple scheme to the analysis of two target fractions (cationic and neutral small molecules) in interface-free 2-D heart-cutting CE was shown. CZE and MEKC were in the first and second dimension, respectively. Two stacking approaches were shown, one for a mixture of water-soluble and the other for a mixture of water and poorly water-soluble analytes. It is noted that the stacking mechanisms of sweeping and AFMC employed the presence of electrolytes in the sample diluents; thus, the 2-D CE methods should be amenable to samples with salt. Stacking provided 1–2 orders of magnitude improvement in concentration sensitivity. The increase in peak capacity by the multidimensional separation approach and improved concentration sensitivity by stacking may further expand the applicability of CE for the analysis of complex and dilute samples. As with most separation assays, a sample preparation step prior to injection is necessary. Interface-free 2-D heart-cutting CE of mixtures of anionic and neutral analytes and other mixtures will be studied in capillaries having no EOF. The possibility of mass spectrometric detection and use of other stacking techniques such as transient isotachophoresis for high-salt matrixes in the first dimension analysis will also be explored.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

C.K. and S.S. express thanks for the financial supports from The Thailand Research Fund (TRF) through the Royal Golden Jubilee (RGJ) Ph.D. Program (Grant No. PHD/0334/2550) and Center of Excellence for Innovation in Chemistry (PERCH–CIC), Office of the Higher Education Commission, Ministry of Education. This work was supported under the

Australian Research Council Future Fellowship to J.P.Q. (FT100100213).

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