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Separation of haloacetic acids in water by capillary zone electrophoresis with direct UV detection and contactless conductivity detection

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Abstract

The separation of haloacetic acids (HAAs) in water by capillary zone electrophoresis with direct UV and contactless conductivity detection was investigated using phosphate, citrate, and borate buffers, and the experimental data were compared to simulation data predicted by a computational program known as PeakMaster. Good agreement between the experimental data and simulation data predicted by PeakMaster was found. Using the phosphate buffer or the citrate buffer and electrokinetic injection it was possible to quantitate HAAs at 0.1 ppm levels in water.

Keywords: Contactless conductivity detection; Detection, electrophoresis; Water analysis; Haloacetic acids; Organic acids

1. Introduction

Haloacetic acids (HAAs), especially chloroacetic acids, are known to be formed from dissolved humic matter during the chlorine disinfection process of drinking water [1–3]. Among the disinfection by-products (DBPs), trihalomethanes [1,4] account for more than 85% of all DBPs measured, whereas HAAs account for about 14% [3]. Among HAAs (refer to Table 1 for the list of compounds and their abbreviations), the chloroacetic acids seem to be the principal fraction of the nonvolatile chlorinated organic compounds in drinking water [2], and several researchers have reported concentrations of trichloroacetic acid (TCAA) and dichloroacetic acid (DCAA) as high as 160 μg/L in drinking water [5–10]. Recently, Dojlido et al. [10] reported that the highest concentration of HAAs in the Warsaw Water Works occurred in May–June, when the water temperature was the highest, and that volatilization of HAAs during water boiling showed that removal of HAAs was rather small. The presence in water of monobromoacetic acid (MBAA), dibromoacetic acid (DBAA), and mixed bromochloroacetic acids has also been reported [6,11]. To support the development of regulations to control HAAs and DBPs in

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Table 1
Target compounds investigated in this study

Peak numbers in chromatograms	Analyte	Chemical Abstract Services Registry No.	Abbreviation
1	Monochloroacetic acid	79-11-8	MCAA
2	Monobromoacetic acid	79-08-3	MBAA
3	Dichloroacetic acid	79-43-6	DCAA
4	Bromochloroacetic acid	5589-96-3	BCAA
5	Dibromoacetic acid	631-64-1	DBAA
6	Trichloroacetic acid	76-03-9	TCAA
7	Bromodichloroacetic acid	71133-14-7	BDCAA
8	Dibromochloroacetic acid	7278-95-5	DBCAA
9	Tribromoacetic acid	75-96-7	TBAA

general, the US Environmental Protection Agency (EPA), the National Institute of Environmental Health Sciences, and the US Army are working together to develop a comprehensive DBP database, based on which they can make regulatory decisions [12]. For this purpose, sensitive analytical methods are needed in order to accurately measure these types of compounds at parts-per-billion levels [e.g., current regulations establish a maximum contaminant level of 60 ppb for the combined concentration of monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), TCAA, MBAA, and DBAA].

At the present time there are four standard methods (i.e., EPA Methods 552, 552.1, and 552.2 and Standard Method 6233B of the American Public Health Association) for the determination of HAAs in drinking water [13–15]. EPA Method 552 involves extraction of HAAs with methyl *tert*.-butyl ether (MTBE) and capillary gas chromatography with electron-capture detection (GC–ECD) of the derivatized acids.

EPA Method 552.1 replaces the liquid–liquid extraction procedure with a solid-phase extraction procedure using anion-exchange columns or disks. However, there have been reports questioning the recovery of HAAs, and thus the reliability of the method depends on the sample matrix. For example, the presence of anions, especially sulfate, was reported to have a negative effect on the recovery of some HAAs from drinking water [16]. In addition, the derivatization procedure in Method 552.1, which requires heating HAAs in a 10% solution of sulfuric acid in methanol under pressure for 1 h, does not give reproducible results for some HAAs and causes column degradation.

Shorney and Randtke [16] conducted a study to better understand the factors influencing HAA analysis by EPA Method 552.1 and Standard Method 6233B and they concluded that the microextraction procedure in Standard Method 6233B, which uses MTBE, appears to be significantly more rugged, precise, accurate, and less time-consuming than the solid-phase extraction procedure in Method 552.1.

Pawlecki-Vonderheide et al. [17] reported further improvements to EPA Method 552, in which the methylation procedure used in EPA Method 552.1 was modified to include a back-extraction with saturated sodium hydrogen carbonate solution to neutralize the acidic extract and prevent any damage to the GC column. This method is now known as Method 552.2.

An ion chromatography (IC) method for the determination of HAAs was reported by Lopez-Avila et al. [18]. The IC method uses the microextraction procedure from Standard Method 6233 B and gives comparable results to Standard Method 6233B and EPA Method 552.1. Results from drinking water samples spiked with HAAs at three concentrations indicate that the IC method recovery appears to be a function of analyte concentration, and MCAA and MBAA exhibited lower recoveries overall, probably due to their higher water solubility.

Capillary zone electrophoresis (CZE) appears to be a good alternative to the chromatographic methods described above because it does not require the derivatization step and the analysis time is approxi-

mately 10 min (including column rinsing and actual analysis).

Martinez and co-workers [19,20] have reported the determination of HAAs by CZE after extraction of HAAs from water samples using solid-phase extraction on a highly cross-linked styrene–divinylbenzene cartridge and elution of HAAs from the cartridge with methanol. Using 4 m*M* 2,6-naphthalenedicarboxylic acid dipotassium (NDC) and 0.5 m*M* hexadecyltrimethylammonium bromide (CTAB) at pH 7.5 and electrokinetic injection, Martinez et al. [19] reported the indirect UV detection of six HAAs [MCAA, MBAA, DCAA, TCAA, bromochloroacetic acid (BCAA)] in real samples at parts-per-billion levels.

Most of the applications reported in the literature that deal with the determination of HAAs by CZE use indirect UV detection. Typical buffers for indirect UV detection include phthalate at pH 6 [20], 4 m*M* 2,6-naphthalenedicarboxylic acid at pH 7.5 [20], 10 m*M* chromate at pH 8.7 [20] and 2.5 m*M* pyromellitic acid–0.75 m*M* hexamethonium hydroxide at pH 3.7 [21]. Electroosmotic flow (EOF) modifiers such as CTAB and tetradecyltrimethylammonium bromide (TTAB) have also been used [20]. Despite the fact that indirect UV methods seem to be adequate in terms of sensitivity and selectivity, many of the indirect UV buffers are expensive, and may be prone to matrix interferences (since their composition is not disclosed, it is hard to really troubleshoot), thus the need for the determination of HAAs by CZE with direct UV detection.

Contactless conductivity detection (CCD) is complementary to UV detection, especially for inorganic and organic ions that do not absorb UV–Vis radiation significantly. CCD was first used in electromigration separation techniques by Gaš et al. [22], then a coaxial modification of CCD was reported by Zemann et al. [23] and subsequently many research groups [24–32] demonstrated the benefits of this type of detection. In this study we investigated CCD as a complementary detection method to the direct UV detection of HAAs.

To select buffers for the separation of HAAs by CZE (UV in series with CCD) we referred to both literature publications and predictive methods. A literature review indicated that a borate buffer at pH 9.6 [20] and a phosphate buffer at pH 5.7 [33] with EOF modifiers such as CTAB and TTAOH (tetradecyltrimethylammonium hydroxide), respectively, would allow the baseline separation of five HAAs, but there was significant peak spreading for the trihalogenated compounds [33]. Large differences in electrophoretic mobilities between the sample ions and the buffer co-ion may cause significant peak broadening, which predominantly originates in electrodispersion [34]. Based on that, we chose both the phosphate and the borate buffers since they both match the electrophoretic mobilities of our target compounds, but have evaluated other EOF modifiers. We also evaluated a citrate buffer since citrate matches the EOF mobilities of HAAs.

To find a suitable electrolyte system for the CZE separation of a set of compounds similar to HAAs is a difficult and time-consuming task for any experimentalist. However, there are computational programs which can be used to investigate the separation and electromigration dispersion of analytes theoretically and thus, in a short time, find more appropriate conditions to start the experimental work. In this study, such computational software, called PeakMaster [35], was applied and the theoretical computational results were then critically compared with the CZE experimental results.

2. Experimental

2.1. Instrumentation

A ^{3D}CE system (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detection (DAD) system and a personal computer were used for all experiments reported here. The contactless conductivity detector used in this study consists of two cylindrical conducting surfaces (electrodes) made from two syringe cannulae each 6 mm in length. The electrodes were connected to an oscillator (frequency 625 kHz) through a resistor. The detection gap between the electrodes was 2 mm. Details of the construction of the conductivity cells and CCD can be found elsewhere [36]. The conductivity cell together with the input of the current-to-voltage (I/U) converter and the additional electronics were mounted in a cassette that fits the Agilent ^{3D}CE system. The separation capillary had

an outside diameter of 365 μm and first passed the CCD and then the UV detector. Data acquisition and processing were performed with the Agilent ^{3D}CE Chemstation. Fused-silica capillaries (Agilent Technologies) of various inner diameters (50–75 μm), an outer diameter of 365 μm and 80.5 cm (the effective length for UV detection is 72 cm and the effective length for CCD is 63.5 cm) extended path length (bubble cell) were used in the study. The applied voltage was −25 kV. Sample injection was carried out either by pressure injection (i.e., 50 mbar for 3–10 s) or electrokinetically (i.e., −5 kV for 3–10 s).

2.2. Reagents

All reagents used in this study were of analytical grade. High-purity analytical standards of the nine HAAs listed in Table 1 were obtained as neat materials from Supelco (Bellefonte, PA, USA). Individual stock solutions for the nine HAAs were prepared in ultrapure water; these stock solutions were used to prepare the composite solutions used in the development of the CZE procedure.

A composite solution of inorganic anions (chloride, bromide, nitrite, nitrate, sulfate, fluoride, and phosphate) at 10 ppm was obtained by dilution of a 1000 ppm anion stock solution available commercially from Agilent Technologies.

The various buffers were prepared in the laboratory using ultrapure water and EOF modifiers [e.g., diethylenetriamine (DETA) and hexadimethine bromide (HDMBr)] from Aldrich and a cationic surfactant from J&W Scientific (Folsom, CA, USA). Results of their evaluation are listed in Table 2.

2.3. Capillary conditioning

Each new capillary was rinsed with 0.1 *M* NaOH for 5 min followed by a reagent water rinse for an additional 10 min. At the beginning of each day and every time the buffer was changed, the capillary was again rinsed with 0.1 *M* NaOH for 5 min and reagent water for 10 min followed by a 5 min rinse with the running buffer from a separate vial than that used during analysis.

3. Results and discussion

3.1. Theoretical considerations and simulation of electrolyte system performance in CZE

The separation process in CZE is of inherently nonlinear nature, which makes it difficult to properly design the separation or even to understand the process. Electromigration dispersion is a consequence of the nonlinearity. This causes peak broadening and deformed peak shapes, which results in decreased separation efficiency. Attempts to calculate the composition of the sample zones in CZE have only been made recently and a few models for the calculation have been reported [37–39]. When dealing with electrolyte systems containing multiple ions co-migrating with the sample ion, the situation appears more complicated. The first consequence is the generation of new migrating system peaks [40] in addition to the ''normal water gap'' peak, which is caused by a jump in the Kolhrausch regulating function, and which moves in the column only due to the electroosmotic flow. Second, using multiple co-

Table 2
Buffer systems evaluated in this study

Buffer identification	Buffer composition	Buffer pH	Conductivity (S/m)
A1	12.5 m*M* Na_2HPO_4–12.5 m*M* NaH_2PO_4 and 5 ppm HDMBr	7.21	0.378
A2	6.25 m*M* Na_2HPO_4–6.25 NaH_2PO_4 and 200 ppm (5 m*M*) DETA	9.40	0.267
B1	50 m*M* citric acid–10 m*M* LiOH and 5 ppm HDMBr	2.63	0.154
B2	50 m*M* citric acid–25 m*M* LiOH and 5 ppm HDMBr	3.12	0.197
B3	50 m*M* citric acid–35 m*M* LiOH and 5 ppm HDMBr	3.43	0.254
B4	50 m*M* citric acid–50 m*M* LiOH and 5 ppm HDMBr	3.95	0.366
B5	50 m*M* citric acid–70 m*M* LiOH and 5 ppm HDMBr	4.61	0.572
C1	100 m*M* boric acid–80 m*M* Tris and 120 ppm (3 m*M*) DETA	8.62	0.134

ions can cause a severe broadening of the analyte peaks or even their loss [41–43]. Recently, it was pointed out by Bocek et al. [44] that even polyvalent electrolytes such as phosphate buffers, when used in the pH region where two ionic forms are present (e.g. at pH 7), behave as systems with multiple co-ions and cause unexpected system peaks and broadening of the analyte peaks.

Using the current version of the computational program PeakMaster [35], which can evaluate electrolytes with two co-ions and two counterions, we evaluated all the buffers investigated here using the Eigenpeaks and Electropherogram windows. In the Eigenpeak window, the electrophoretic mobility of the eigenpeaks of the tested electrolyte system, which are manifested by perpendicular lines, can be read. Moreover, the sensitivity of CCD for the analytes and their electrodispersion in the tested

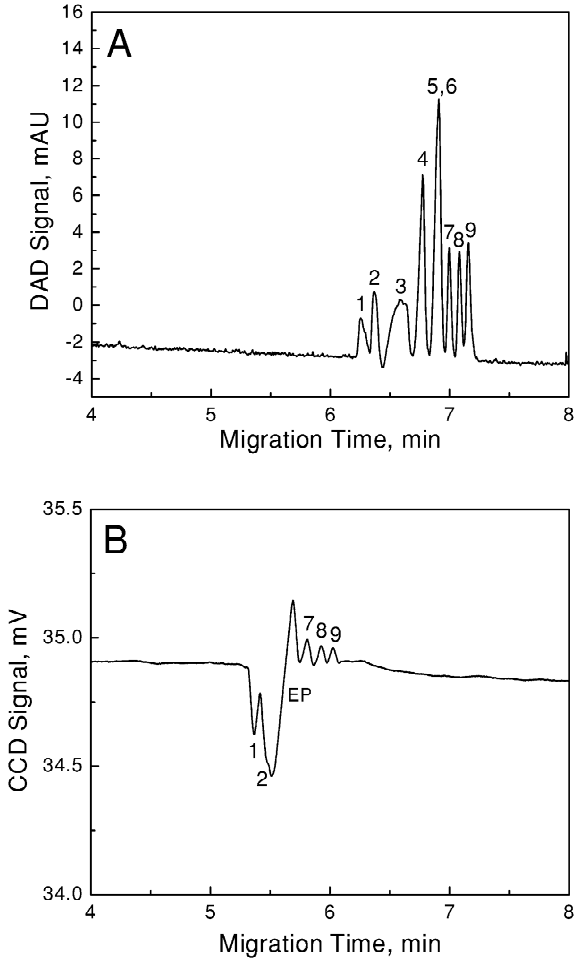

Fig. 1. Electrophoretic separation of nine HAAs in a buffer containing 12.5 m*M* NaH_2PO_4–12.5 m*M* Na_2HPO_4 and 5 ppm HDMBr using (A) direct UV detection at 200 nm (DAD, diode-array detection) and (B) CCD. For other conditions, see Experimental. Peaks: 1=MCAA, 2=MBAA, 3=DCAA, 4=BCAA, 5=DBAA, 6=TCAA, 7=BDCAA, 8=DBCAA, 9=TBAA, EP=eigenpeak.

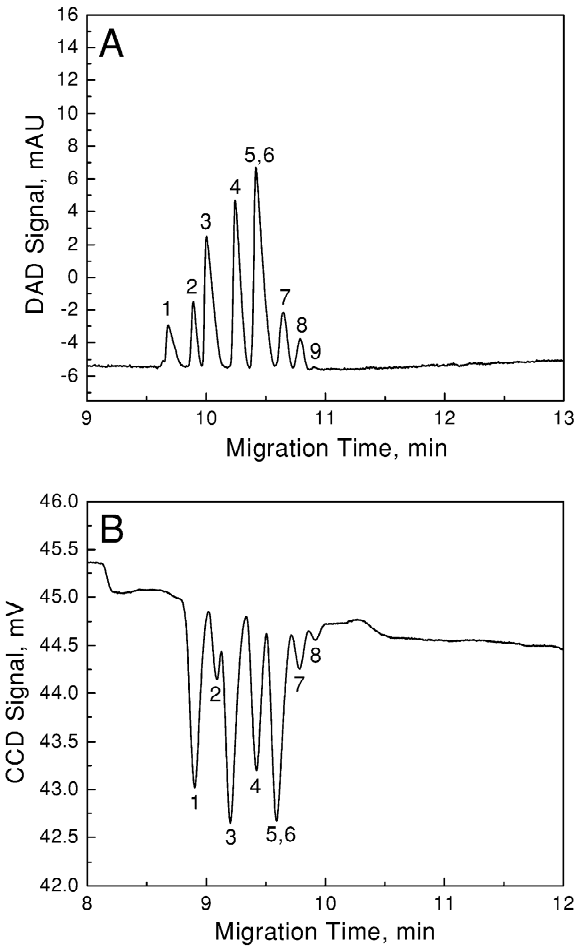

Fig. 2. Electrophoretic separation of nine HAAs in the buffer containing 6.25 m*M* NaH_2PO_4–6.25 m*M* Na_2HPO_4 and 5 m*M* DETA using (A) direct UV detection at 200 nm and (B) CCD. For other conditions, see Experimental. Peaks: 1=MCAA, 2=MBAA, 3=DCAA, 4=BCAA, 5=DBAA, 6=TCAA, 7=BDCAA, 8=DBCAA, 9=TBAA.

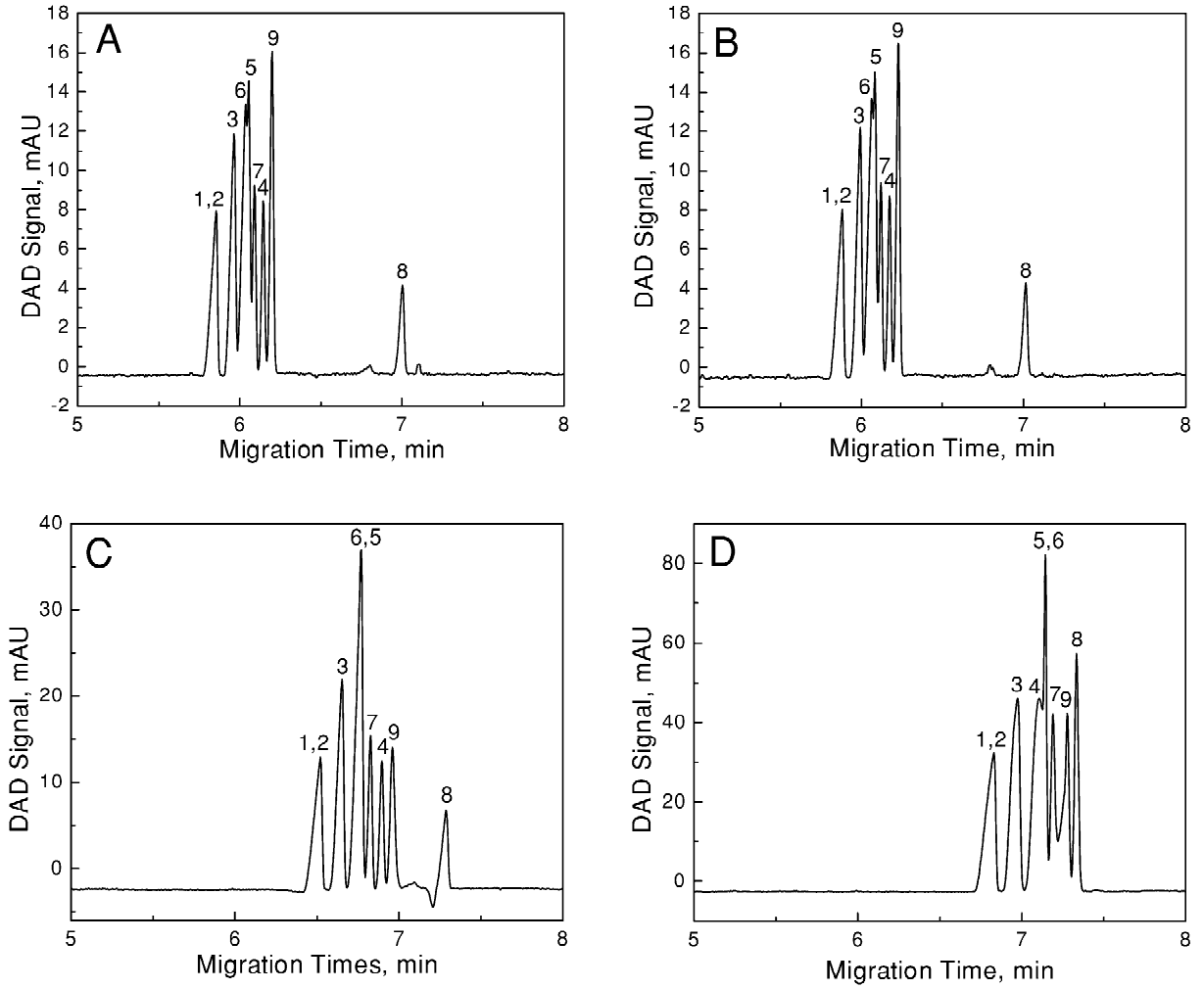

Fig. 3. Electrophoretic separations of nine HAAs in the buffer containing 50 m*M* citric acid, 5 ppm HDMBr and (A) 10 m*M*, (B) 25 m*M*, (C) 35 m*M* and (D) 50 m*M* LiOH using direct UV detection at 200 nm. For other conditions, see Experimental. Peaks: 1=MCAA, 2=MBAA, 3=DCAA, 4=BCAA, 5=DBAA, 6=TCAA, 7=BDCAA, 8=DBCAA, 9=TBAA.

electrolyte system can also be estimated in this window. The higher the absolute value of the molar conductivity detection response characterized by the CCD Signal curve, the better the sensitivity. Vice versa, the closer the absolute value of the velocity slope, represented by the Dispersion curve, to zero, the lower the electrodispersion of analytes in the tested electrolyte system. In the Electropherogram window, the position (i.e., migration time), shape (i.e., tailing or fronting) and orientation (i.e., positive or negative response) of peaks of individual analytes and positions of eigenpeaks and the electroosmotic flow marker are drawn. While the molar conductivity detection value should be as high as possible as it characterizes the sensitivity of CCD detection, the velocity slope is a measure of the tendency of the analyte to undergo electromigration dispersion, i.e. to attain triangular peak shapes. A suitable electrolyte system should have as low velocity slope value as possible.

3.2. Phosphate buffer

Fig. 1A shows an electropherogram for an HAA composite standard obtained by direct UV detection at 200 nm. The corresponding CCD electropherogram is shown in Fig. 1B. Only three of the common anions (Br^-, NO_2^-, and NO_3^-) can be detected by UV and none interfered with the determination of HAAs since they had much higher electrophoretic mobilities than the HAAs (e.g., the migration times

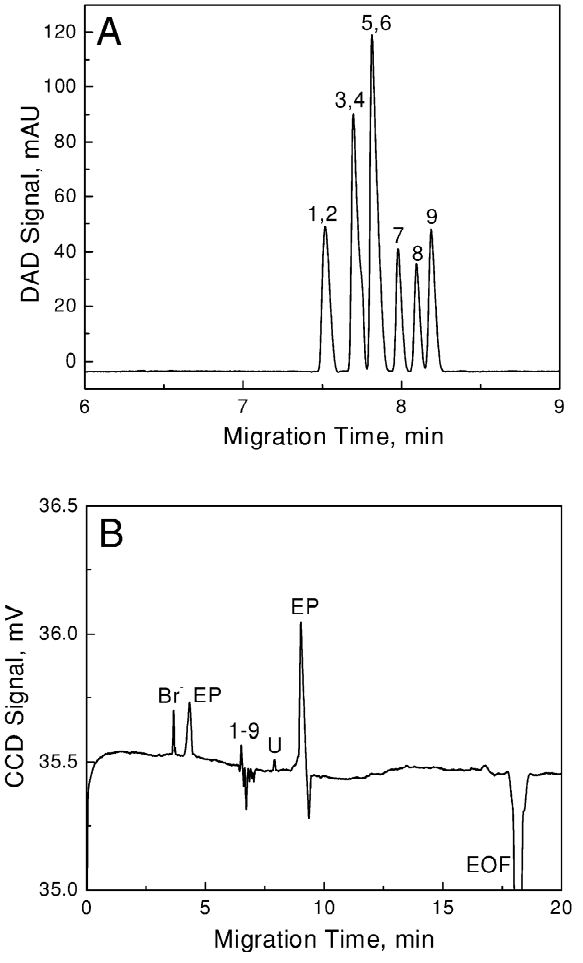

Fig. 4. Electrophoretic separation of nine HAAs in the buffer containing 50 m*M* citric acid–70 m*M* LiOH and 5 ppm HDMBr using (A) direct UV detection at 200 nm and (B) CCD. For other conditions, see Experimental. Peaks: 1=MCAA, 2=MBAA, 3=DCAA, 4=BCAA, 5=DBAA, 6=TCAA, 7=BDCAA, 8=DBCAA, 9=TBAA, Br^-=bromide, EP=eigenpeak, U=unknown impurity, EOF=water gap.

Table 3
Migration times (min) of HAAs using 12.5 m*M* NaH_2PO_4–12.5 m*M* Na_2HPO_4 buffer with 5 ppm HDMBr (pH 7.21)[a] or 50 m*M* citrate–70 m*M* LiOH buffer with 5 ppm HDMBr (pH 4.61)[b]

Compound	Standard 1 (nine HAAs)[a]	Standard 1 (nine HAAs)[b]	Standard 2 (seven HAAs)[b]
MCAA	6.255	7.521	7.571
MBAA	6.367	7.521	7.571
DCAA	6.585	7.702	7.800
BCAA	6.774	7.702	–
DBAA	6.909	7.820	–
TCAA	6.909	7.820	7.862
BDCAA	6.996	7.983	8.019
DBCAA	7.081	8.098	8.139
TBAA	7.155	8.191	8.237
Bromide	3.915	4.268	4.303

[a] Buffer A1. Electropherograms are shown in Fig. 1.
[b] Buffer B5. Electropherograms are shown in Fig. 4 and PeakMaster computational model in Fig. 5.

are 4.145 and 4.230 min for NO_2^- and NO_3^-, respectively). Using an 80.5 cm column (total length)×50 μm I.D., and 12.5 m*M* NaH_2PO_4–12.5 m*M* Na_2HPO_4 with 5 ppm HDMBr, the migration times of the HAAs are in the 6–7.5 min window. With the exception of DBAA and TCAA, which comigrate (see peaks 5, 6 in Fig. 1A), all the other compounds are baseline resolved. Peak shapes are quite symmetrical, with the exception of DCAA (peak 3 in Fig. 1A). HDMBr was added to reverse the EOF. The peak shape of DCAA was significantly improved by the addition of DETA, as shown in Fig. 2A, where a diluted phosphate buffer with DETA was applied. Whatever the mechanism may be, the data clearly indicate that DETA did indeed sharpen up the DCAA peak. However, DBAA and TCAA still comigrate when using the phosphate buffer with DETA. Experiments carried out at −15 kV indicate similar resolution, but the peaks were shifted by about 5 min at longer migration times.

The computational results for the phosphate buffer predict an eigenpeak in the CCD electropherogram (Fig. 1B) at the position of the migration of ions with an effective mobility of $34 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}. Moreover, any ions with effective mobilities greater than $34 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} will give negative peaks. Since the effective mobilities of two HAAs were higher than $34 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}, these peaks were negative and the remainder of the peaks were

positive in the CCD electropherogram. The predicted eigenpeak also appeared in the CCD electropherogram (Fig. 1B) and overwhelmed three HAA peaks.

When diluted phosphate buffer was used with DETA in place of HDMBr to reverse the EOF, all peaks were negative on the CCD as predicted by the computational model (Fig. 2B), since the model predicted that any peaks with effective mobilities less than $40{\cdot}10^{-5}$ cm^2 V^{-1} s^{-1} would be negative. The computational model also predicted that the HAA peaks would be broader in the case of the diluted phosphate buffer with DETA and no eigenpeaks should appear in the CCD electropherogram.

3.3. Citrate buffer

UV electropherograms obtained with the citrate buffer containing various concentrations of LiOH are shown in Figs. 3A–D and 4A and the migration times of the HAAs using 50 m*M* citrate–70 m*M* LiOH with 5 ppm HDMBr are given in Table 3. The corresponding CCD electropherogram recorded with the same buffer is shown in Fig. 4B. As the concentration of LiOH increases, the electrodispersion (band broadening) also increases, as predicted by the computational model and also visible in the peak shapes in Figs. 3 and 4. Additionally, there are more comigrating pairs with this buffer (e.g., MCAA/MBAA, DCAA/BCAA, and TCAA/DBAA). However, at 70 m*M* concentration for LiOH, the computational model predicted two eigenpeaks in the CCD electropherogram with effective mobilities of $20{\cdot}10^{-5}$ cm^2 V^{-1} s^{-1} (migration time 9.1 min) and $48{\cdot}10^{-5}$ cm^2 V^{-1} s^{-1} (migration time

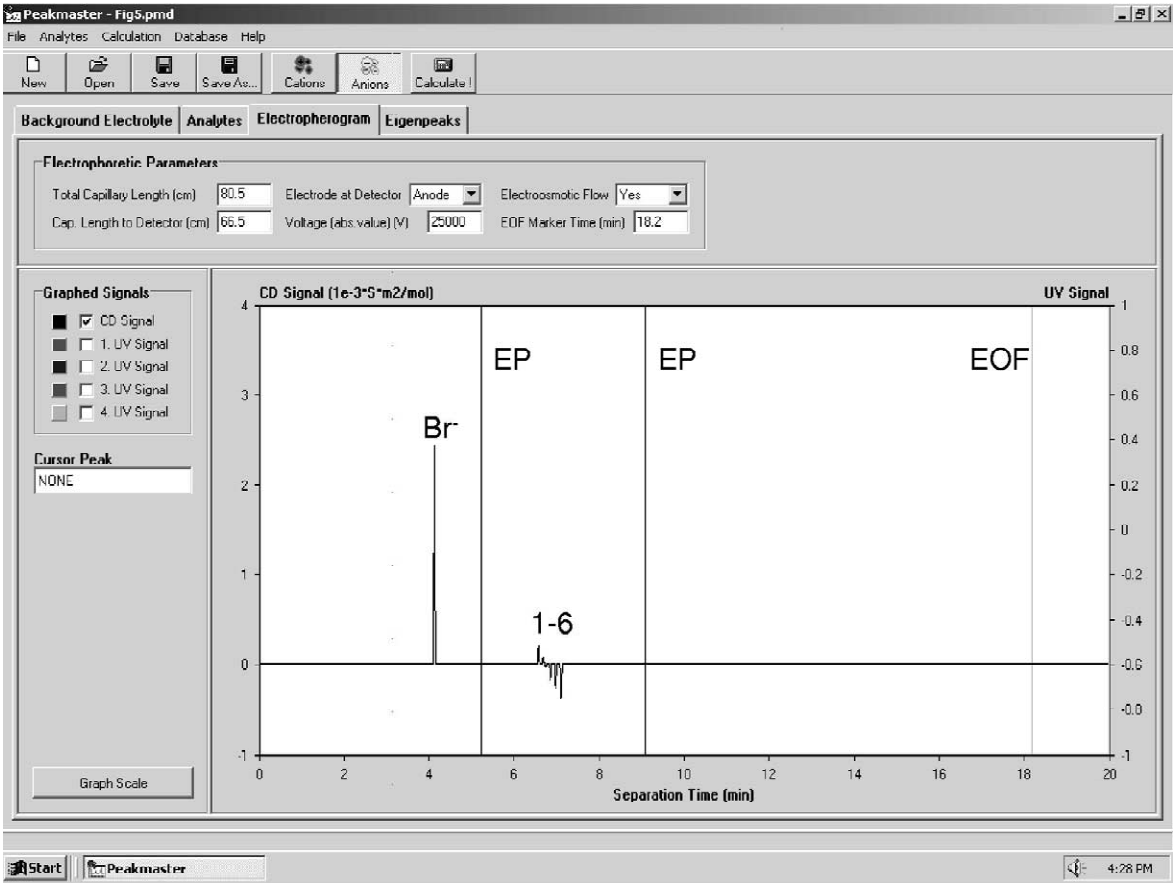

Fig. 5. PeakMaster's prediction of the electrophoretic separation of HAAs using CCD under the same experimental conditions as described in Fig. 4.

5.2 min) as demonstrated on the Electropherogram window output of PeakMaster in Fig. 5. Both eigenpeaks were found in the CCD electropherogram (Fig. 4B) at the predicted locations. Although the direct UV separation gave just positive peaks of HAAs at 70 m*M* LiOH, the CCD electropherogram exhibited HAA peaks which were both positive and negative, making the identification difficult. For all citrate buffers investigated here, there seems to be a good correlation between the experimental data and computational model prediction. All tested citrate buffers in this work contained HDMBr at either 5 or 10 ppm concentration. A cationic surfactant from J&W Scientific, identified as a fluoroalkyl quaternary ammonium iodide, was also evaluated at two concentrations (e.g., 250 and 25 ppm), but the separation seemed to be identical to the citrate buffer containing HDMBr.

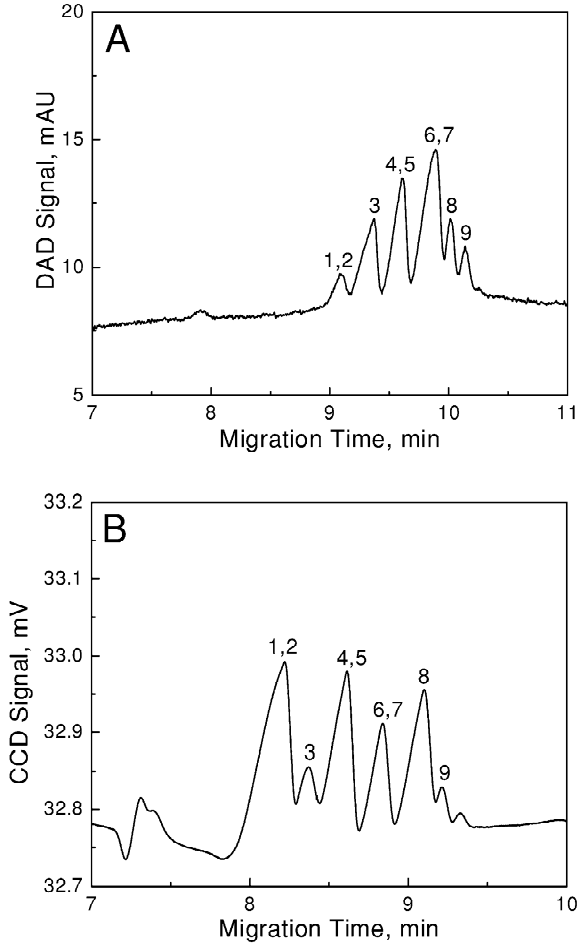

Fig. 6. Electrophoretic separation of nine HAAs in the buffer containing 100 m*M* H_3BO_3–80 m*M* Tris and 3 m*M* DETA using (A) direct UV detection at 200 nm and (B) CCD. For other conditions, see Experimental. Peaks: 1=MCAA, 2=MBAA, 3=DCAA, 4=BCAA, 5=DBAA, 6=TCAA, 7=BDCAA, 8=DBCAA, 9=TBAA.

3.4. Borate buffer

This buffer exhibited a considerably higher electrodispersion (Fig. 6A and B) than the two previously tested buffers and made separation of the nine HAAs difficult. The high electrodispersion observed with this buffer was in good agreement with predictions of the computational model of PeakMaster. However, this buffer seemed to perform quite well for the common anions (e.g., bromide, nitrite, and nitrate), which have higher mobilities than the HAAs and, according to the computation model, are not electrodispersed as much as HAAs. The migration times of the HAAs were in the 9–10.2 min window on the UV electropherogram and 8.2–9.5 on the CCD electropherogram and they were all positive, as correctly predicted by the model. These preliminary results did not justify additional work with this buffer system.

4. Conclusions

Analysis of HAAs by CZE is possible; however, none of the buffers investigated allowed complete separation of the nine compounds. Good agreement between the phenomena predicted by the computational program PeakMaster and the real observations in electropherograms was found. Using the phosphate buffer or the citrate buffer and electrokinetic injection it was possible to quantitate the HAAs at 0.1 ppm levels in water. To detect lower levels of HAAs, preconcentration procedures, such as those proposed by Martinez et al. [20], should be considered with real samples to clean up the matrix and preconcentrate the HAAs prior to CZE.

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