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Evaluation of different electrolyte systems and on-line preconcentrations for the analysis of haloacetic acids by capillary zone electrophoresis

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Abstract

This study compares the sodium chromate, potassium hydrogenphthalate, 2,6-naphthalenedicarboxylic acid dipotassium and sodium tetraborate electrolyte systems, for determining haloacetic acids using capillary zone electrophoresis with and without modified electroosmotic flow. In order to detect low concentrations of these compounds, an on-line preconcentration step was carried out. Results were good when standard solutions were analysed, but when the method was applied to real samples, the sample had to be pretreated to decrease the interference of the matrix. For this reason, a solid-phase extraction process with LiChrolut EN cartridges was applied to clean up the sample before the injection.

Keywords: Buffer composition; Water analysis; Chloroacetic acids; Bromoacetic acids

1. Introduction

The US Environmental Protection Agency (EPA) has promulgated regulations to control haloacetic acids (HAAs) [1] because they are toxic organohalogen compounds formed by the process of disinfecting water [2–4]. The haloacetic acids selected for control are monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), dibromoacetic acid (DBAA) and trichloroacetic acid (TCAA). Organobromide compounds are formed when the water being disinfected contains large amounts of bromide [5]. Toxicological studies indicate that these compounds may have adverse effects on health and DCAA and TCAA are animal carcinogens [6–8].

A maximum contaminant level (MCL) of 60 μg l^{-1} for the sum of these five haloacetic compounds in drinking water has been proposed by the EPA. Gas chromatographic methods are usually used to determine these compounds in water samples, but this involves a derivatization step because of their low volatility and high polarity [9,10].

Capillary electrophoresis (CE) is useful in many different areas of separation science and it is becoming more important all the time [11–13]. In the environmental field, CE has become increasingly popular because it gives high resolution separation for certain pollutants [14,15]. It can be a good alternative to chromatographic methods for determin-

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ing haloacetic acids because it does not require the derivatization step and it can reduce analysis times.

In CE, the carrier electrolyte ions should be as mobile as the analyte ions so that the shape of the migrating zone can be maintained as an approximately Gaussian distribution and to prevent any deterioration of the resolution. So, the electrolyte system is important because it affects the resolution efficiency and the analysis times.

One of the major problems in the analysis of haloacetic acids using CE is that the limits of detection are poor. In order to solve this problem, an off-line liquid–liquid extraction can be used as a preconcentration step [16]. However, this type of extraction has some problems: generally, a toxic and inflammable organic solvent must be used and the concentration step is time consuming.

One way of increasing the sensitivity in CE is to optimize the sample introduction. In many studies, electrokinetic injection has been used to preconcentrate the sample [17–19]. When this type of injection is used, the sample end of the capillary is placed in the vial and the analytes are transported by applying an electric field. The amount of sample injected will depend on the electric field.

The aim of our work was to study different electrolyte systems and the electrophoretic behaviour of the five regulated haloacetic acids, establishing an analytical method using capillary zone electrophoresis (CZE). Electrokinetic injection was used to introduce the sample and a solid-phase extraction (SPE) with a highly crosslinked styrene–divinylbenzene sorbent (LiChrolut EN) was applied to determine haloacetic compounds in real samples.

2. Experimental

2.1. Instrumentation

Measurements were made on a Hewlett-Packard (HP, Waldbronn, Germany) Model ^{3D}CE instrument equipped with a UV detector. Data were collected with the HP Chemstation version A.05.01 chromatographic data system. All experiments were performed using uncoated fused-silica capillary tubing (64.5 cm×75 μm I.D.) supplied by Supelco (Bellefonte, PA, USA). The detection window was prepared by burning off a small part of the polyimide coating 56 cm away from the capillary inlet.

2.2. Chemicals

The haloacetic acids studied were: (1) MCAA, (2) MBAA, (3) DCAA, (4) DBAA and (5) TCAA. Standards were obtained from Merck (Darmstadt, Germany). An individual standard solution of 2000 mg l^{-1} of each compound was prepared with water which had been purified by a Milli-Q system (Millipore, Bedford, MA, USA). Standard working solutions were prepared weekly or daily, depending on their concentration. All solutions were stored at 4°C in the refrigerator.

Potassium hydrogenphthalate (phthalate) (Aldrich, Milwaukee, WI, USA), 2,6-naphthalenedicarboxylic acid dipotassium (NDC) (Aldrich), sodium chromate tetrahydrate (Aldrich) and sodium tetraborate (Fluka, Buchs, Switzerland) were studied as electrolytes. Hexadecyltrimethylammium bromide (CTAB) (Sigma, St. Louis, MO, USA) was used as electroosmotic flow (EOF) modifier, sodium hydroxide (Aldrich) to adjust the electrolyte pH, and formamide (Fluka) as a neutral EOF marker. In all cases, when the electrolyte solutions contained CTAB as EOF modifier, they were prepared daily and passed through a 0.45-μm filter in order to avoid precipitation problems.

2.3. Electrophoretic conditions and system operation

The separation of haloacetic compounds was studied using four different electrolyte systems: a solution of 12 m*M* phthalate (pH=6), a solution of 4 m*M* NDC (pH=7.5), a solution of 20 m*M* borate (pH=9.6) and a solution of 10 m*M* chromate (pH=8.7). In order to reverse the EOF, the effect of adding 0.5 m*M* of CTAB in all electrolyte systems was also studied.

All separations were carried out by rinsing the capillary for 3 min with a background electrolyte immediately before the injection. At the beginning of each experimental day, the capillary was washed with 0.1 *M* NaOH for 15 min and then rinsed with deionized water (10 min) and electrolyte (10 min). The capillary temperature was kept constant at 25°C.

The detector was set at different wavelengths as a function of the working electrolyte: 254 nm, 235 nm, 195 nm and 254 nm for phthalate, NDC, borate and chromate electrolytes, respectively. In all cases, the detection was indirect, except for borate electrolyte.

During the study of the different electrolyte systems, the injection was made hydrodynamically at a pressure of 40 mbar for 20 s. However, when electrokinetic injection was used, a −5 kV voltage was applied for 20 s.

2.4. Sample preparation

Some matrix interferences were observed when electrokinetic injection was used in the real samples studied. In order to prevent this, an SPE step was used as a sample pretreatment and highly crosslinked styrene–divinylbenzene cartridges (LiChrolut EN, 200 mg, Merck) were used as sorbents.

The extraction process was carried out using the Bond Elut/Vac Elut system (Varian, Harbor City, CA, USA). Before extracting, the cartridges were rinsed using 10 ml of methanol followed by 5 ml of deionized water adjusted to pH 0.5 using sulphuric acid. The samples were acidified with sulphuric acid to pH 0.5, approximately, in order to prevent the analytes from taking their ionic form, and were passed through the cartridges at a flow-rate of approximately 2 ml min^{-1}. A clean up step was carried out using 0.5 ml of deionized water. Finally, 1 ml of methanol was used to elute the retained compounds. However, the eluted solution had to be diluted 1:3 with deionized water before the electrokinetic injection since it was observed that the haloacetic acids could not be injected directly from the methanolic solution.

3. Results and discussion

Many applications of CE have been reported for the determination of anions [20–22] most of which use a modifier to reverse the direction of the EOF. However, flow reversal is unnecessary for the separation of many organic anions [23,24]. Using small-diameter columns and high pH, the EOF is faster than the electrophoretic mobility of many anions which reach the detector in a reasonable time even though their electrophoretic migration is away from the detector. Nevertheless, some very fast anions cannot be determined without flow reversal because their electrophoretic velocities go against the EOF and are also much faster.

In order to obtain the best separation of the haloacetic acids, both the separation modes, with and without flow reversal, were studied using different electrolyte systems.

3.1. Separation of haloacetic acids without flow reversal

The choice of electrolyte is extremely important for the success of any CE analysis. The mobility of the electrolyte should be as similar as possible to the mobility of the analytes of interest. For this reason, four different electrolyte systems were studied to separate these haloacetic acids. Chromate has often been used as the electrolyte system to analyse inorganic anions since it has a high mobility [22,25]. Phthalate, NDC and borate electrolytes have been used in the separation of organic acids [24,26].

First, the concentration and pH of each electrolyte was studied so that the efficiency of the separation would be the best, i.e., short analysis time and good resolution. The concentration and pH ranges selected were: 5–14 m*M* and pH 4–10 for phthalate, 2–8 m*M* and pH 7–10 for NDC, 10–25 m*M* and pH 8.5–10 for borate and 3–10 m*M* and pH 7.5–9 for chromate. Results were best for: 12 m*M* (pH=6) of phthalate, 4 m*M* (pH=7.5) of NDC, 20 m*M* (pH=9.6) of borate and 10 m*M* (pH=8.7) of chromate.

Table 1 shows the absolute value of the mobility of these compounds in the best conditions for each electrolyte system. In all cases, 30 kV was applied in the separation step. As can be seen, the mobility was

Table 1
Mobilities of the haloacetic acids studied

Compound	Mobility ($\mu_{eo}\cdot 10^{-4}$, $cm^2\ V^{-1}\ s^{-1}$)			
	Chromate	Borate	Phthalate	NDC
MCAA	0.75	0.73	0.68	0.74
MBAA	0.73	0.70	0.66	0.72
DCAA	0.70	0.68	0.65	0.70
DBAA	0.66	0.63	0.61	0.64
TCAA	0.66	0.63	0.61	0.64

For explanation, see Section 3.1.

similar for all these compounds. However, the peak shape was different in each case. When chromate, phthalate, and NDC were used the peaks had a fronting shape but they did not when borate was used. This can be seen in Fig. 1 which shows the optimum separation for the haloacetic acids studied using each electrolyte system. In all cases, TCAA and DBAA comigrated and only four peaks were obtained. Another aspect that should be mentioned is the increase in the signal when NDC (Fig. 1d) is used; this can be explained by the high molar absorptivity of this electrolyte in comparison to the other electrolyte systems. The instability of the baseline when phthalate is used as electrolyte (Fig. 1c) should also be pointed out.

3.2. Separation of haloacetic acids with flow reversal

There are various ways of modifying the EOF for the determination of low-molecular-mass anions. These include pH control [27], the addition of organic solvents [28], the use of modified capillaries with bonded phases [29] and the addition of cationic surfactants [22,30]. The last method is the most

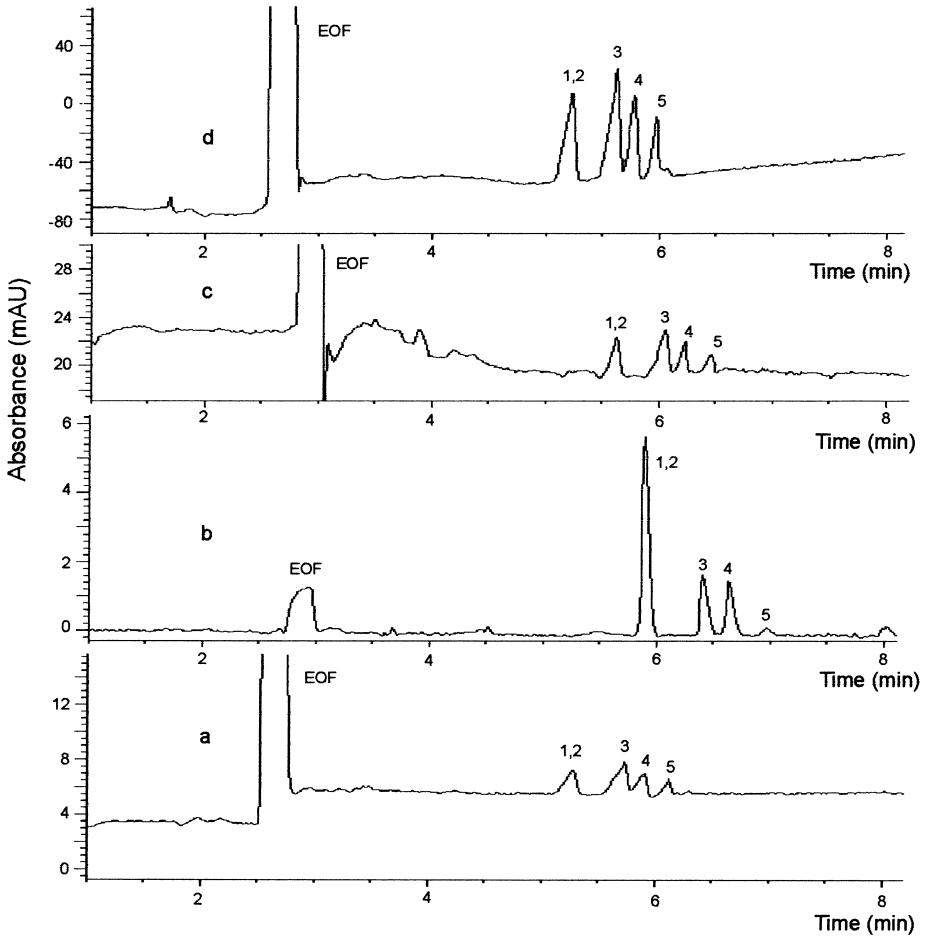

Fig. 1. Electropherograms of a standard solution of the haloacetic acids studied under optimum conditions without reversal flow using (a) chromate, (b) borate, (c) phthalate, (d) NDC electrolytes. Peak designation: 1=TCAA (10 mg l^{-1}); 2=DBAA (10 mg l^{-1}); 3=DCAA (12 mg l^{-1}); 4=MBAA (10 mg l^{-1}); 5=MCAA (8 mg l^{-1}).

common. The surfactant is adsorbed on the capillary wall and produces a net positive charge on the surface. Under these conditions and applying a negative voltage, anions can be detected at the anode.

CTAB is one of the cationic surfactants that has been used to modify the EOF [22,25,31]. In order to study the separation of the haloacetic acids using flow reversal, this surfactant was added to the four different electrolyte systems. The surfactant was studied at concentration between 0.2 and 0.7 m*M*. The final value selected was 0.5 m*M* because in all the electrolytes studied the EOF was stable and reversed. The voltage applied in the separation was −20 kV for chromate, phthalate and borate, but for NDC the separation was initially made at this voltage for 4.5 min and then a linear gradient to −15 kV in 0.5 min was applied.

Under these conditions, all the compounds studied were separated when this surfactant was used, except in the case of borate where the separation was not possible and only distorted bands were obtained. Fig. 2 shows the electropherograms obtained using chromate, phthalate and NDC electrolytes with flow reversal. When chromate was used (Fig. 2a) the baseline was distorted at 2.5 min and sensitivity was poor. In the case of phthalate (Fig. 2b), the peak corresponding to TCAA gave a poor sharp shape because of the drop on baseline. Moreover, sensitivity was low and baseline noise high with this electrolyte. When the NDC electrolyte system was used (Fig. 2c), resolution between peaks was good and sensitivity higher.

By comparing the results obtained in the separation of haloacetic acids with and without flow reversal, it can be seen that in the second mode TCAA and DBAA peaks comigrate and the migration order of haloacetic acids is opposite to the order obtained with flow reversal, because without CTAB, anions move in the opposite direction to the EOF. For this reason, the smaller the anions (MCAA, MBAA), the faster their electrophoretic speed and the longer it takes them to reach to the detector. However, in the presence of CTAB, anions move in the same direction as the EOF, i.e., to the detector, and migration time is shorter for smaller anions that have higher net speed. Moreover, in this case, large anions are expected to interact with positively charged CTAB and this can be responsible for the improved resolution between TCAA and DBAA.

Finally, it can be concluded that results were best when a solution of 4 m*M* NDC and 0.5 m*M* CTAB was used as electrolyte system. Under these conditions, correlation results, within the concentration range studied (2–30 mg l^{-1}), were good (r^2>0.99) and the limits of detection were at low mg l^{-1} levels, between 0.4 mg l^{-1} for MCAA and 0.9 mg l^{-1} for DBAA.

3.3. Study of an on-line preconcentration step

In order to detect haloacetic acids with CE at the μg l^{-1} levels that are found in real samples, they must be preconcentrated.

Field amplified injection techniques were used to enhance detectability in CE. These techniques are based on the fact that the electrophoretic velocity of an ion depends linearly on the field strength, i.e., the voltage applied divided by the length of the capillary, and the ions are preconcentrated and focused in the capillary. The main advantage of this method is that without modifying the instrument, enrichment factors are high. For example, the field amplified sample stacking with sample matrix removal gave enrichment factors of more than 500 [32]. However, when this preconcentration system was applied to determine haloacetic acids, using an electrolyte composed of NDC and CTAB, no enrichment factor was obtained. This may be due to the step where the voltage with reverse polarity is applied [33], and the haloacetic acids are pushed out of the capillary before the sample plug. Consequently, these anions cannot be preconcentrated using this technique.

Another system that can be used as a preconcentration technique for diluted samples in CE is electrokinetic injection. To initiate this injection, the sampling end of the capillary is placed in the sample vial and the sample ions are transported by applying an electric field. If the electrokinetic injection is performed with a polarity that allows the ions of interest to migrate from the sample toward and across the sample/electrolyte boundary, and if the conductivity of sample segment is lower than that of the carrier electrolyte, the ionic components are electrostacked and trace enriched [20].

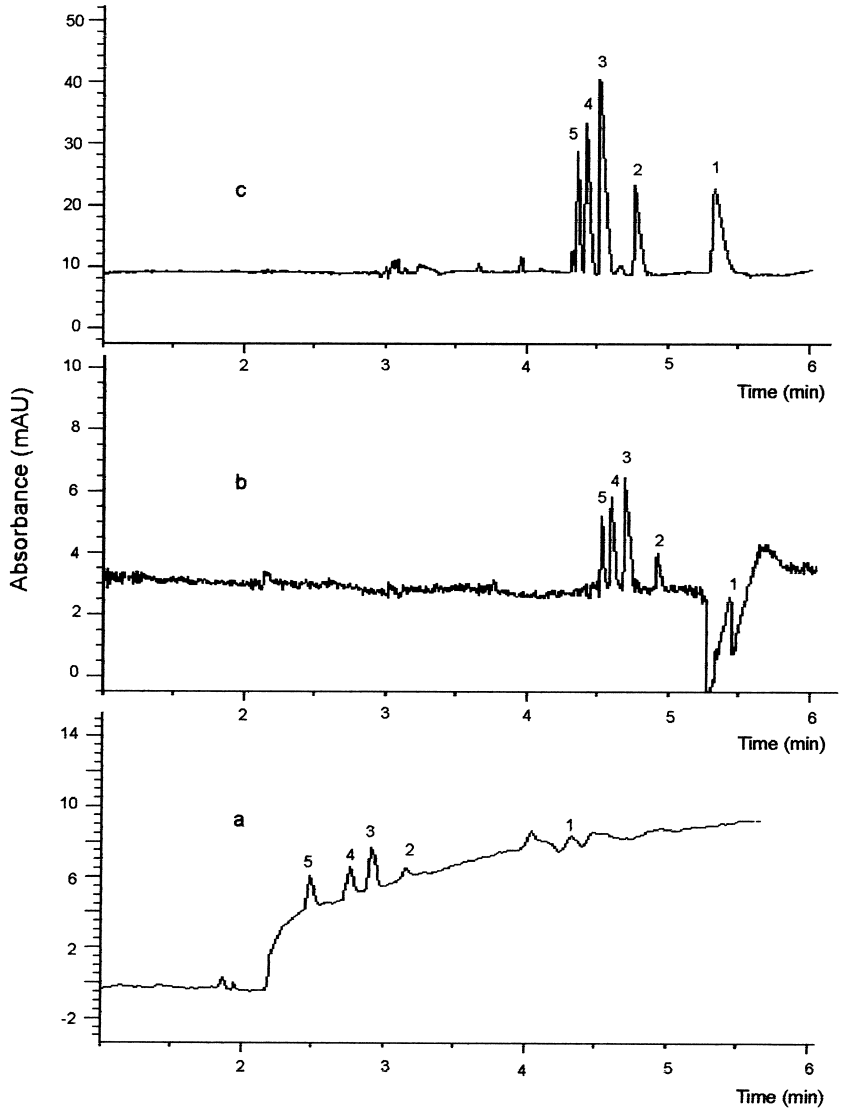

Fig. 2. Electropherograms of a standard solution of the haloacetic acids studied under optimum conditions with reversal flow using (a) chromate, (b) phthalate, (c) NDC electrolytes. In all cases a concentration of 0.5 m*M* CTAB was used. For peak designation and concentrations, see Fig. 1.

When this injection technique was applied to determine haloacetic acids, detection improved. This can be seen in Fig. 3. The results were recorded using −5 kV for 20 s of a standard solution of 0.5 mg l^{-1} of all haloacetic acids, except for MCAA and DCAA (0.4 mg l^{-1} and 0.6 mg l^{-1}, respectively).

3.4. Application to real samples

CE using electrokinetic injection for the determination of haloacetic acids was applied to analyse real sample of swimming pool water. The chlorination of this type of water generates many chlorinated com-

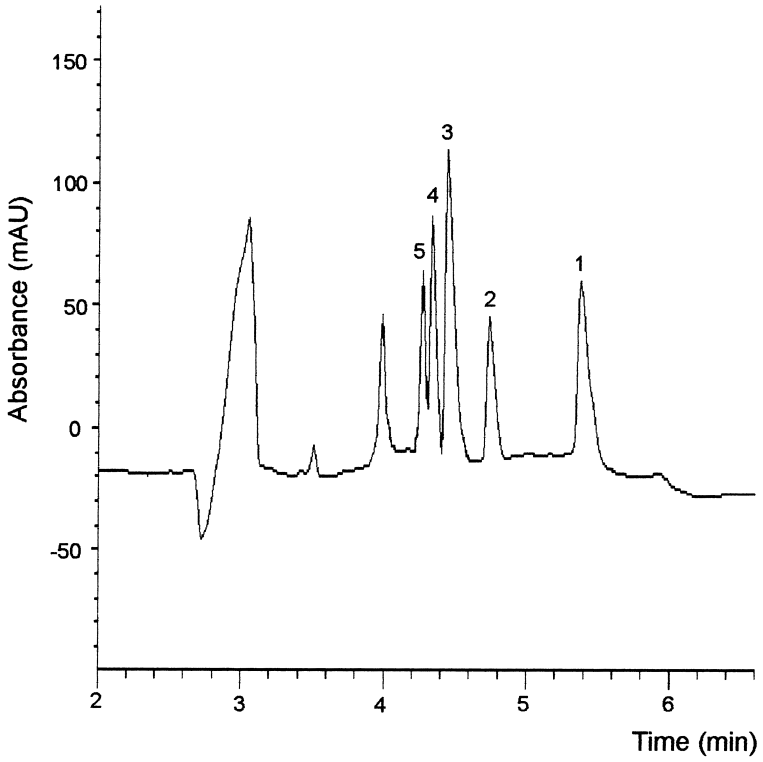

Fig. 3. Electropherogram of a standard solution of 0.5 mg l^{-1} of all haloacetic acids, except for MCAA and DCAA (0.4 mg l^{-1} and 0.6 mg l^{-1}, respectively) using electrokinetic injection: −5 kV for 20 s. Electrolyte system: 4 m*M* NDC, 0.5 m*M* CTAB, pH=7.5. For peak designation, see Fig. 1.

pounds and haloacetic acids are one type of these compounds [34].

However, when this type of water sample spiked with 0.5 mg l^{-1} of each haloacetic acid was analysed, no peaks corresponding to these compounds appeared in the electropherogram. This may be due to matrix interference since this sample has many ions that can negatively affect the electrokinetic injection of haloacetic compounds. In order to overcome this problem, a SPE with highly cross-linked styrene–divinylbenzene (LiChrolut EN) cartridges was applied to clean up the sample before the injection. In this study, 25 ml of swimming pool water was adjusted to pH 0.5 using concentrated sulphuric acid. Then, after the sample had passed through the cartridge, a clean up step was carried out using 0.5 ml of deionized water. Finally, to elute the compounds 1 ml of methanol was used.

Nevertheless, the eluted solution had to be diluted 1:3 with deionized water to be injected electrokinetically. The reason for this was that no peaks were obtained for the haloacetic acids when these compounds were directly injected from the methanolic solution. After studying different ratio of dilution, results were good with a dilution ratio of 1:3.

Fig. 4a shows the electropherogram of the sample of deionized water and Fig. 4b the electropherogram correspond to the sample of swimming pool water. It can be seen that different peaks at similar migration times to haloacetic acids appear in the second figure. To confirm the peaks, a swimming pool water sample spiked with a standard solution of 0.12 mg l^{-1} of the studied compounds was analysed using the same procedure (Fig. 4c). The signal peak of the haloacetic acids increased in the last electropherog-

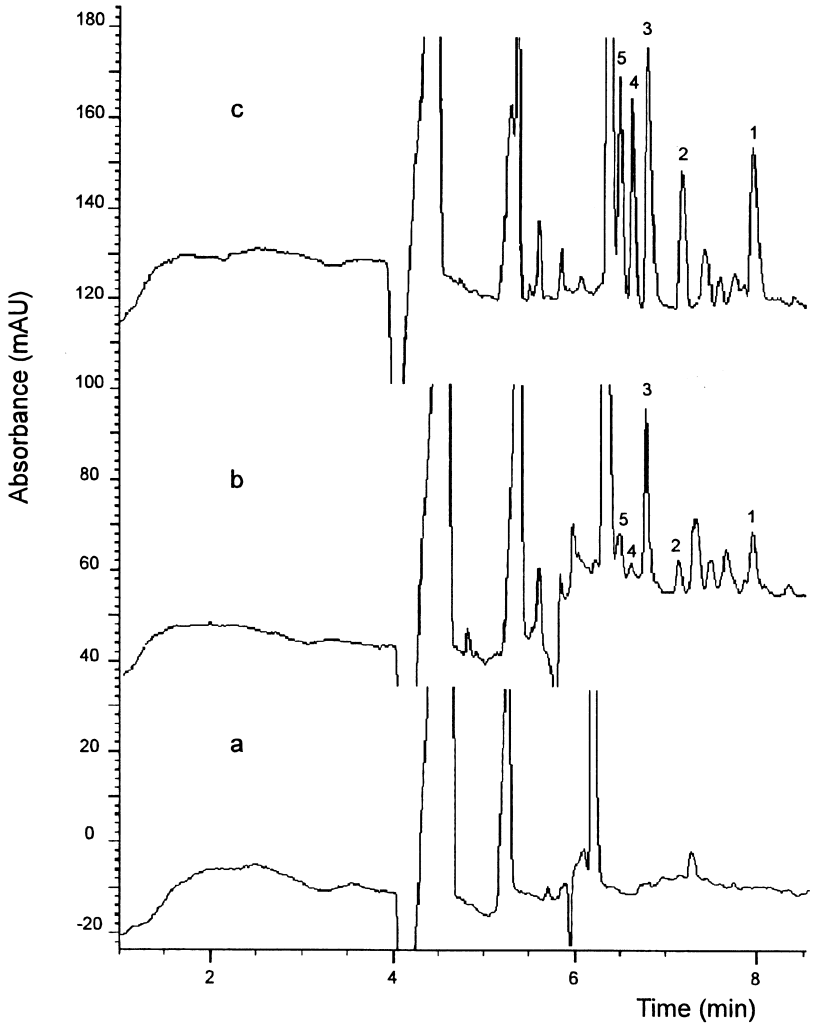

Fig. 4. Electropherograms obtained from the analysis of 25 ml of deionized water and swimming pool water samples using the proposed method. (a) Deionized water sample, (b) swimming pool water sample and (c) swimming pool water spiked with a standard solution of 0.12 mg l^{-1}. For peak designation, see Fig. 1.

ram. These results agree with those in the literature [34,35] where the presence of these compounds in this type of sample has been demonstrated.

The recovery of each compound, and the linearity and correlation (r^2) for the total analytical system, including the SPE treatment, were studied with this type of water. The analytical data are shown in Table 2. Within the concentration range studied for each compound (40–160 μg l^{-1}), there was a good correlation between peak area and concentration, with values between 0.991 for TCAA and 0.996 for MCAA. The relative standard deviation (R.S.D.) of five analyses of swimming pool water spiked with 80 μg l^{-1} of each haloacetic acid was between 14.5% for DBAA and 6.6% for DCAA.

The results obtained suggest that the proposed method is suitable for analysing haloacetic acids in these samples. The concentrations found for these compounds in the swimming pool water sample were: 24.7 μg l^{-1}, 7.1 μg l^{-1}, 68.8 μg l^{-1}, 15.2 μg

Table 2
Calibration data, relative standard deviation and recovery for the five haloacetic acids studied using the proposed method

Compound	Linear range ($\mu g\ l^{-1}$)	Slope	Intercept	r^2	R.S.D. (%)[a]	Recovery (%)
MCAA	40–160	1.22	30.1	0.996	7.3	54
MBAA	40–160	1.20	8.5	0.992	8.3	83
DCAA	40–160	1.18	81.2	0.993	6.6	60
DBAA	40–160	0.92	14.0	0.994	14.5	58
TCAA	40–160	1.19	50.1	0.991	10.2	58

[a] Obtained for 80 $\mu g\ l^{-1}$ of the five haloacetic acids ($n=5$).

l^{-1} and 42.1 $\mu g\ l^{-1}$ for MCAA, MBAA, DCAA, DBAA and TCAA, respectively. These results were obtained using the standard addition method.

4. Conclusions

Our research has shown that a surfactant must be added to the electrolyte system to completely separate the five haloacetic acids studied using CE. The best electrolyte system studied was a solution of 4 m*M* NDC with 0.5 m*M* CTAB. Using this electrolyte and electrokinetic injection, it has also been demonstrated that these haloacetic acids can be determined at $\mu g\ l^{-1}$ levels.

This CE method enables the HAAs studied to be detected in only 8 min, whereas in GC methods a derivatization step and longer analysis times are required. However, when the proposed method was applied to determine these compounds in swimming pool water samples, the samples had to be pretreated to prevent the matrix from having a negative effect on the electrokinetic injection. For this reason, a SPE process with LiChrolut EN cartridges was applied to clean up the sample before the injection.

Acknowledgements

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