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Nonaqueous capillary electrophoresis equipped with amperometric detection for analysis of chlorinated phenolic compounds

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Abstract

Nonaqueous capillary electrophoresis (NACE) equipped with amperometric detection has been developed for separation and detection of an 11-member model mixture of chlorinated phenolic compounds. With triacetyl-β-cyclodextrin (TACD) as a novel selectivity selector, acetonitrile proved to be an excellent solvent for this water-insoluble cyclodextrin derivative. Resolution of the analytes was achieved by using an optimized acetonitrile medium consisting of 500 m*M* acetic acid, 10 m*M* sodium acetate, 12 m*M* TACD and 50 m*M* tetrabutylammonium perchlorate. Separation of analytes was attributed to differential electrostatic and/or inductive interactions of the analytes with the TACD/TBA^+ complex and charged tetrabutylammonium phases. A simple end-column amperometric detector (Pt vs. Ag/AgCl, poised at +1.6 V) in conjunction with NACE was used to analyze chlorophenols. Amperometric detection of such target compounds in acetonitrile-based media offers high sensitivity and alleviates electrode fouling compared to aqueous buffers. The detection limits obtained, ranging from 30 n*M* to 500 n*M*, are 3–8-fold lower than those obtained with aqueous buffers.

Keywords: Nonaqueous capillary electrophoresis; Electrochemical detection; Instrumentation; Buffer composition; Chlorophenols; Cyclodextrins

1. Introduction

Capillary electrophoresis (CE) using aqueous buffers as background electrolytes and organic additives has been effective and useful for analysis of charged components in a mixture [1]. Other modified electrophoretic techniques such as micellar electrokinetic chromatography (MEKC) [2] and cyclodextrin-mediated CE [3] have also been developed to resolve uncharged and water-insoluble molecules. Their effectiveness, however, is significantly reduced when used to separate highly hydrophobic and neutral compounds such as aromatic hydrocarbons and polycyclic aromatic hydrocarbons (PAHs). Electrophoretic separation in nonaqueous and mixed media may be an attractive alternative to MEKC for the separation of hydrophobic solutes having a low solubility in aqueous media. Both selectivity and efficiency of the non-aqueous electrophoretic separation are greatly influenced by the physicochemical properties of the solvent used such as viscosity, dielectric constant, dipole moment and the autoprotolysis [4]. Since the important characteristics of separation can be manipulated on a wider scale than with aqueous CE, nonaqueous CE (NACE) may be

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applicable for the analysis of small molecules with equal or nearly equal charge-to-mass ratio in aqueous CE. Nonaqueous media are also useful in preventing solute aggregation and solute–wall interaction and therefore could improve resolution and separation efficiency.

Tetraalkylammonium (TAA^+) perchlorate added to acetonitrile (MeCN) or mixtures of MeCN and water was demonstrated to effect the separation of five neutral PAH solutes [5]. The mobility of the solutes increased with increasing hydrophobicity and the separation mechanism was attributed to the solvophobic association of the PAHs with tetraalkylammonium ions and thereby forming a positively charged species that migrates faster than the neutral formamide in an electrical field. The ammonium ions induce a dipole on the PAH, allowing an electrostatic interaction to take place, i.e., the π-electrons of the aromatic analytes may be attracted to the TAA^+ ion [6]. The separation of PAHs in non-aqueous systems based on MeCN was improved if planar organic cations such as tropylium tetrafluoroborate and/or 2,4,6-triphenylpyrylium were used instead of tetraalkylammonium perchlorate in the separation medium [7]. Charge-transfer interactions, electrostatic as well as dispersive forces were attributed to the separation of the PAHs and such interactions are more pronounced in most nonaqueous solvents than in water. This hypothesis can be supported by citing the work of Ahuja and Foley [8] in which sodium dodecyl sulfate, a negatively charged ion, was used in the separation medium. In this case, all of the solutes (naphthalene, anthracene and phenanthrene) comigrated with the electroosmotic flow. To date, NACE has been developed for separation and analysis of organic compounds [9], inorganic ions [10], pharmaceutical compounds [11], surfactants [12] and polyethers [13]. Several papers have been published to describe the physical properties of nonaqueous solvents and their applicabilities for improving separations in NACE [14–16]. However, little attention has been paid to finding more appropriate additives for nonaqueous systems since the organic additives such as surfactants and cyclodextrins are often not as effective in nonaqueous solvents as they are in water [6].

The development of NACE equipped with amperometric detection is of importance for analysis of phenol and its hydrophobic chlorinated derivatives. This family of phenolic compounds raises a significant pollution concern since they are major components of pulp and paper effluents. Such chlorinated pollutants are also formed by processes such as degradation of pesticides, chlorination of drinking water, wood preservation and herbicide synthesis [17]. In general, CE (aqueous or nonaqueous) is usually equipped with UV detection which is not sufficiently sensitive for detection of chlorophenols. In addition, organic solvents used in NACE possess higher background UV absorbance than water. On the basis of the electroactivity of chlorinated phenols, a simple end-column amperometric detection may be used for sensitive analysis of such pollutants. A recent study revealed that the interference between the high separation voltage and the electrochemical detection circuit is greatly reduced by using organic media [18].

In this article, NACE equipped with amperometric detection has been developed for analysis of chlorophenols. MeCN, acetic acid and sodium acetate were used to formulate the electrophoretic medium, aided by tetrabutylammonium perchlorate (TBAP) and a water-insoluble cyclodextrin derivative, triacetyl-β-cyclodextrin (TACD). To the best of our knowledge, TACD has not been used in electrophoretic separation since it is water insoluble and its solubility in MeCN is greatly reduced in the presence of water. However, this cyclodextrin is highly soluble in MeCN to form an ideal separation medium for NACE. Amperometric detection was accomplished with a detector equipped with a platinum electrode, similar to the arrangement that has been applied to aqueous CE [19]. Cyclic voltammetry (CV) was applied to characterize electrochemical oxidation and reduction of chlorophenols in organic media to provide a basis for CE with amperometric detection of the chlorinated phenols.

2. Experimental

2.1. Materials

Platinum wires (0.127 mm, detecting electrode and 0.5 mm diameter, counter electrode), silver wire (0.5 mm diameter, reference electrode), chloro-

phenols and all other chemicals were purchased from Aldrich (Milwaukee, WI, USA).

2.2. Sample preparation

Stock solutions of chlorophenols were prepared in MeCN (HPLC grade, >99.93%, Aldrich), with a predetermined volume of the resulting solution added to MeCN to obtain the desired concentrations. All samples were protected from light and kept in a ventilated hood.

2.3. Cyclic voltammetry

CV experiments used a potentiostat/galvanostat (Model 263A, EG&G, Princeton Applied Research, Princeton, NJ, USA) to operate a three-electrode system consisting of a saturated Ag/AgCl reference electrode [RE-1, Bioanalytical Systems (BAS), West Lafayette, IN, USA], a platinum wire counter electrode (0.5 mm) and a working platinum disk electrode (3 mm diameter active surface, MF 2013, BAS). Analog signals from the voltammograph were digitized by an A/D board (DP 500-AD) supplied with an interface box (Labtronics, Guelph, Canada) that was installed on a PC 486 computer. The data were stored in ASCII files and converted to PRN files for treatment by a graphic program.

2.4. Preparation of detecting electrodes

The amperometric detection cell required a working electrode prepared with platinum wire. A 3 cm platinum wire (0.127 mm diameter) was inserted into a glass capillary (1.2 mm I.D., commonly used for melting point determination, 1 cm long), with the wire recessing about 2 mm from one capillary end. This capillary end was heated with a Bunsen burner to seal the glass around the wire. The resulting capillary end was then polished on fine silicon carbide abrasive paper aided by an alumina slurry (CF-1050, BAS). Microscopic inspection was performed in order to retain only those having a circular cross section well sealed with the glass capillary. A piece of 0.5 mm silver wire was attached to the other end of the small wire so that the 0.5 mm wire almost touched the end of the glass capillary. Epoxy glue was used to secure a rigid connection between the 0.5 mm wire and the glass capillary (Fig. 1).

2.5. Capillary conditioning

For reproducible results all new capillaries were conditioned before use. They were washed sequentially for 10 min with MeCN, 10 min with water, 10 min with 1 *M* NaOH followed by a sequence of 10 min of water and 10 min of the separation medium. The daily pretreatment was 10 min with MeCN, 15 min with the separation medium followed by a preconditioning of 20 min under +20 kV to obtain

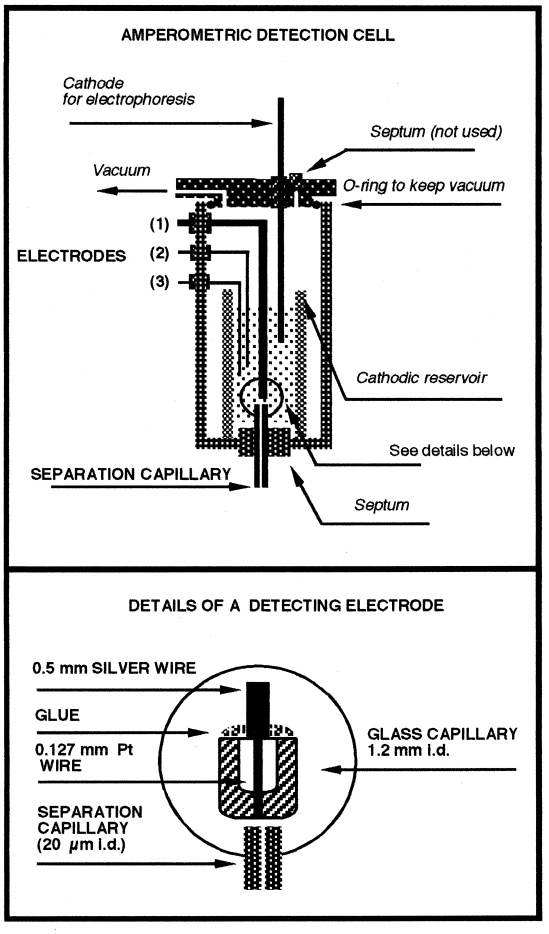

Fig. 1. Amperometric detection cell with details of the detecting electrode. Components are not drawn to scale. Electrodes: (1) working: 0.127 mm platinum wire (2) counter: 0.5 mm platinum wire (3) reference: 0.5 mm Ag/AgCl.

good equilibrium and a stable baseline. After use the capillaries were rinsed with MeCN and filled with a solution of 1 m*M* of NaOH. Well-closed vials must be used because of the volatility of the solvent.

2.6. Amperometric detection and electrophoretic capillary arrangement

Polyimide-coated fused-silica capillaries of 20 μm I.D. were purchased from Polymicro Technologies (Phoenix, AZ, USA). Vacuum injection (10 s at 14.5 p.s.i. or about 100 kPa, corresponding to an injection volume of 5.6 nl), and unless otherwise indicated, 20 kV separation potential, and a standard 70 cm capillary length were used in all cases. Electropherograms were obtained with the Applied Biosystems CE instrument (ABI-270A, Perkin-Elmer, Foster City, CA, USA). For amperometric detection, one capillary end was top mounted into the anodic reservoir. However, the other capillary end was inserted into the cathodic reservoir through a septum opening, created at the bottom (Fig. 1). The cathodic reservoir supplied with the CE instrument was made of acrylic polymer that is susceptible to acetonitrile. To circumvent this problem, a glass insert, cut from a regular Pasteur pipette, was slipped over the septum and positioned inside the acrylic reservoir to serve as an outlet container. All three electrodes for amperometric detection as well as the cathode for electrophoresis were immersed in the separation medium that was contained in the glass container as shown in Fig. 1. Three other septa were installed on the side of the acrylic reservoir to accommodate insertion of the three electrodes. The reference electrode was a silver wire with AgCl formed at the tip by electroformation, while a platinum wire (0.5 mm diameter) served as the counter electrode. The platinum electrode, as described above, was used as the detecting electrode and positioned directly at the capillary outlet. The separation capillary was positioned so that its outlet was as close to the tubing center as possible. Due to the large diameter of the detecting electrode (0.127 mm) compared to the internal diameter of the capillary (20 μm), good electrochemical efficiency was achieved without precise microalignment. However, to ensure the reproducibility of the results obtained, the distance between the detecting electrode and the capillary outlet was controlled by monitoring the detector's background current before the sample was introduced to the separation capillary. Notice that a close distance should influence the background noise whereas too large a distance reduces electrochemical efficiency. In this set-up, a background current with a fluctuation of about ±10 nA was considered as a good compromise. With this procedure, each time the detection chamber was reassembled, the peak height could change up to 5% (compared with a previous assembling) but the migration times were not affected as anticipated, During electrophoresis, a CV-1B voltammograph (BAS) was used to apply +1.6 V to the working electrode for detecting the target analytes that migrated to the detector with different mobilities. The time response data were digitized and treated by the same procedure used for cyclic voltammetry.

2.7. Separation medium

MeCN behaves as a very weak base or acid and possesses low viscosity. Although its aprotic characteristics makes it a less suitable solvent for the electrolytes, the addition of acetic acid will improve the solubility of electrolytes considerably. In this study, the separation medium consists of MeCN, 500 m*M* acetic acid, and 10 m*M* sodium acetate. TBAP and TACD were added to the separation medium as discussed in Results and Discussion. The separation medium was filtered through a 0.45-μm Gelman nylon aerodisk filter before use. All experiments were performed at room temperature (21–23°C).

2.8. Peak identification

The electropherogram peaks were identified by spiking with individual components. The augmentation in peak height from one run to another after spiking with those of standards was used for peak identification.

2.9. Safety

Pentachlorophenol (PCP) and several other chlorinated phenolic compounds such as 2,3,4,6-tetrachlorophenol (tetraCP) and 2,4,6-trichlorophenol (TCP) are carcinogenic and their vapor is very

dangerous. Therefore, proper eye and skin protection is recommended. Stock solutions of the chlorophenols (a few ppm or less) must be prepared and handled in a ventilated hood. Disposable latex gloves and safety glasses must be worn to avoid any contact or exposure while working with chlorophenols because they may be absorbed through the skin. Special care must be taken to dispose of waste solutions because of their toxicity and carcinogenicity,

3. Results and discussion

3.1. Electrochemical oxidation of chlorinated phenols in organic medium

Electrochemical oxidation was considered as a promising method for destroying phenols in aqueous solutions, with potential applications in wastewater treatment, and has been studied extensively [20,21]. The oxidation process has been shown to be quite complex involving polymer formation resulting in electrode poisoning and significant decrease in efficiency, particularly when pure metal electrodes were used [22]. Highly dispersed electrodes, prepared from oxides such as SnO_2, SbO_5 and PbO_2 were demonstrated to produce mostly CO_2 instead of polymer forming species and only very slight electrode poisoning was observed [23]. The electrochemical properties of chlorinated phenols were less well understood but some studies have also shown that chlorobenzoquinones were some common intermediates that also polymerized with subsequent electrode fouling [24]. Thus, the use of pure metal electrodes in electrochemical detectors always presented some difficulty in aqueous media. However, a platinum electrode modified with electrodeposited tin mostly eliminated electrode fouling and such electrodes were used to detect phenol and many of its chlorinated derivatives in a capillary electrophoretic separation [19].

The study began with cyclic voltammetry performed in MeCN since this organic solvent has been used in many nonaqueous electrophoretic separations [25]. Sodium acetate was necessary as the supporting electrolyte for amperometric detection although it might not be required for electrophoretic separation. As a hydrogen bond donor, acetic acid was added to facilitate the solubilization of sodium acetate in MeCN and this electrolyte system was preferred for NACE with electrochemical detection [25]. Fig. 2A presents cyclic voltammograms obtained with 20 m*M* phenol in an aqueous buffer (solid line) and in MeCN (dotted line). In the aqueous buffer, the

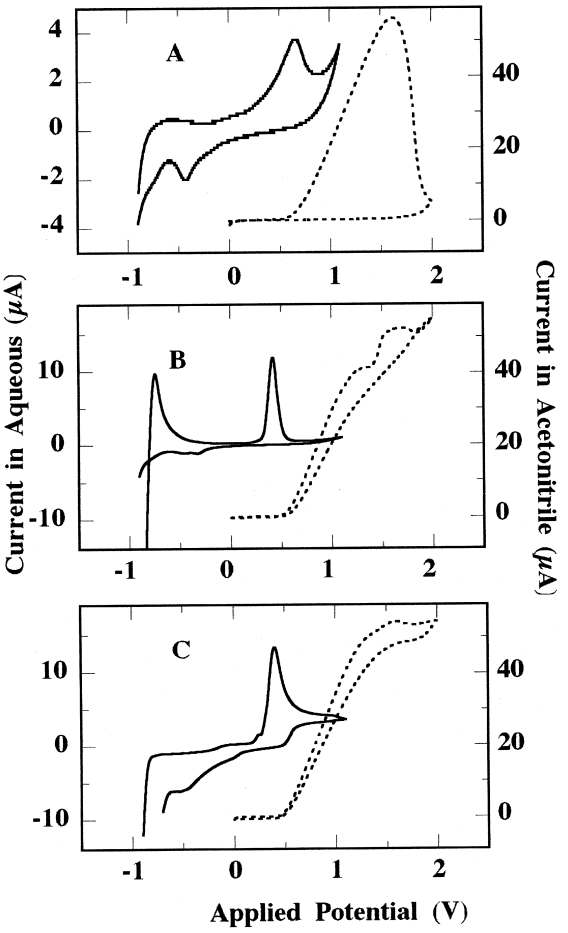

Fig. 2. Cyclic voltammograms of the working platinum electrode (3 mm active surface) recorded at 50 mV s^{-1} (vs. Ag/AgCl) and 25°C in presence of: (A) 20 m*M* phenol in 25 m*M* phosphate and 25 m*M* borate, pH 8.1 (solid line) and in acetonitrile containing 500 m*M* acetic acid and 10 m*M* sodium acetate (dotted line); (B) 20 m*M* 2,4-dichlorophenol in 25 m*M* phosphate and 25 m*M* borate, pH 8.1 (solid line) and in acetonitrile containing 500 m*M* acetic acid and 10 m*M* sodium acetate (dotted line); (C) 20 m*M* 2,4,6-trichlorophenol in 25 m*M* phosphate and 25 m*M* borate, pH 8.1 (solid line) and in acetonitrile containing 500 m*M* acetic acid and 10 m*M* sodium acetate (dotted line).

oxidation peak appeared around +0.66 V during the increasing potential sweep whereas a noticeably smaller reduction peak was registered at −0.45 V. The resulting irreversible voltammogram was due to the fact that the phenoxy radicals formed during the electrooxidation are prone to polymerization that results in the formation of a passive film. If the polymer formed deposits on the electrode surface, electrode fouling occurs. The severity of electrode fouling was confirmed since the oxidation peaks obtained at +0.66 V diminished with each sweep cycle, resulting in continuous depletion in electroactivity (figure not shown). The oxidation peak of phenol in MeCN appeared at +1.6 V and the peak current was several-fold higher, indicating a higher level of electroactivity of phenol in MeCN. However, there was no reduction peak in the reverse sweep, an indication of totally irreversible oxidation. The absence of electroactivity during the decreasing voltage sweep was not totally unexpected due to the polymerization of the phenoxy radicals that could eventually foul the electrode as discussed earlier. For comparison, cyclic voltammetry was also performed with solutions of 2,4-dichlorophenol (2,4-DCP, Fig. 2B) and 2,4,6-TCP (Fig. 2C). For both compounds, the resulting voltammograms in MeCN were virtually peakless even though the electroactivity was comparable with that of phenol (dotted lines), confirming that amperometric detection should be feasible. Another important feature that was revealed in the voltammograms was the wide voltage window for electrochemical activity in MeCN solutions. In aqueous buffer, if +0.45 V was selected as the detection voltage, in order to maximize the response to 2,4-DCP and 2,4,6-TCP, the response to phenol would be minimal. In MeCN solution, any potential between +1.5 to +1.7 V would be suitable for detection of all three compounds.

In view of the irreversible voltammograms obtained, the extent of the electrode fouling was further investigated by monitoring the current versus time response at a constant applied potential (Fig. 3). A 20 m*M* solution of phenol in MeCN and in water, respectively yielded a response current of ca. 67 μA (+1.6 V) and 4 μA (+0.66 V). Unlike the weak response in aqueous buffer that drastically diminished with time (solid line with a final current of 0.05 μA), the response in MeCN yielded a strong response current, about 90% of the initial value after 5 min (dotted line). Only after 15 min, this response current diminished to 0.1 μA. Apparently, the polyphenol formed during electrooxidation tended to deposit much more quickly on the electrode surface in the aqueous medium than in MeCN. The constant

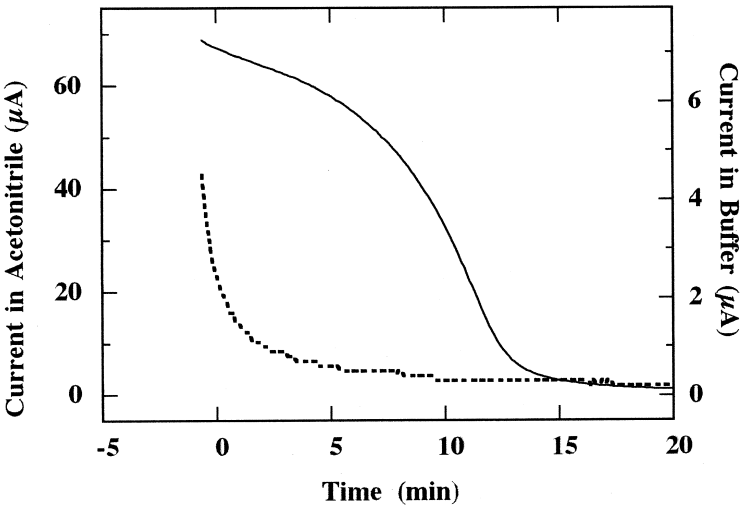

Fig. 3. Variation in currents of the working platinum electrode (3 mm active surface) during the electrolysis of 20 m*M* of phenol at 25°C. Solid line: in 25 m*M* phosphate and 25 m*M* borate, pH 8.1 at +0.66 V vs. Ag/AgCl. Dotted line: in acetonitrile containing 500 m*M* acetic acid and 10 m*M* sodium acetate at +1.6 V vs. Ag/AgCl.

voltage time-variation of 2,4-DCP and 2,4,6-TCP were somewhat similar to that of phenol. Therefore, detection of phenol and its derivatives in MeCN could offer a significant improvement in sensitivity as well as alleviate the severity of electrode fouling.

A series of experiments was then conducted to examine the electrode response (poised for only 1 min) after several repeated analyses. The data obtained confirmed that 10 successive injections of phenol in MeCN only resulted in a 4% loss of current intensity whereas the chlorinated phenols underwent losses of less than 1%. In CE separation, an analyte normally takes one min or less to pass through the electrode, therefore it was reasonable to expect that a platinum electrode would be able to monitor a separation accomplished in MeCN.

Before proceeding with further experimentation, a 5.6 nl sample (10 μ*M*, vacuum injection at 70 p.s.i. for 10 s) of each analyte of interest was repeatedly injected into the CE capillary to verify that a current response was obtained at +1.6 V applied potential. The peak height was observed to diminish about 2% after each run, a clear indication of electrode fouling, although the severity was less problematical with amperometric detection in an aqueous buffer (ca. 4%, Ref. [19]). This problem could be effectively circumvented by cycling the applied potential between 0 to +2 V at 600 mV/s, followed by a constant applied potential at +2 V for 30 s. With this conditioning procedure, the peak height between runs was never exceeded by more than ±1%. It should be noted that for operation in aqueous buffers, the cleaning step required several cycles of cyclic voltammetry [19]. A repetition could be performed with 0.02 min variation of migration time for each analyte, if between runs, the separation capillary was washed for 3 min with MeCN and then 3 min with the separation medium. The treatment in organic solution allowed the electrode to regain a stable baseline very quickly (30 s) while cleaning in aqueous media required 3 to 5 min for the electrode to restabilize.

3.2. Separation of chlorinated phenolic analytes

The pK_a values of chlorinated phenols in aqueous solutions are dependent on the position and number of chlorines in the aromatic ring, 4.74 for PCP and 9.37 for 4-chlorophenol (CP) [26]. Since pH may not be compared directly in aqueous and nonaqueous solutions, the term apparent pH (pH*) will be used. For chlorophenolic compounds, the relationship between the pK_a values in MeCN (pK_{MeCN}) and in aqueous medium (pK_{H_2O}) can be taken as pK_{MeCN} = pK_{H_2O} +3 [27]. According to this expression, the pK_{MeCN} value for PCP should be 7.74, thus in a separation medium of pH*=5, phenolic compounds would be mainly in the neutral form.

An initial attempt with MeCN containing 500 m*M* or 1 *M* acetic acid and 10 m*M* sodium acetate revealed that only a large peak around 8 min was noted with a resultant separation current of 0.6 μA at +20 kV separation potential (data not shown). With sodium acetate as the electrolyte (it breaks down at 350 mV, a potential higher than that of ammonium acetate), acetic acid was required to facilitate the solubilization of the salt [25]. However, in the presence of 50 m*M* TBAP, four discernible peaks were observed with PCP emerging as the last peak, indicating PCP interacted most weakly with the TBA^+ ion (figure not shown). The addition of perchloric acid, up to 100 m*M*, to the separation medium instead of TBAP failed to provide any degree of separation. In such separations, the resultant separation current was always less than 2 μA. It appears that the positively charged center of tetrabutylammonium (TBA^+) cation was primarily responsible for the partial separation of eleven chlorophenolic compounds. Improved separation was not attained by any further increase or decrease in the TBAP concentration. Notice that the solvophobic interactions commonly dominated in aqueous CE will only play a limited role in NACE [28]. It was somewhat difficult to determine the electroosmotic flow since small and neutral markers such as formamide, thiourea and mesityl oxide (detected with an on-line UV detector at 229 nm) emerged after only 6.5 min of separation, slightly ahead of the phenol peak (experiments conducted with a 35 cm length capillary with UV detection).

3.3. NACE in the presence of TACD

To our knowledge, TACD never has been used in aqueous CE because this cyclodextrin is completely

water insoluble. However, its solubility in organic solvents such as acetonitrile is extremely high. In nonaqueous media, the positively charged amine (TBA^+) has been reported to interact with β-cyclodextrin [28]. Therefore, TACD could be assumed to interact with TBA^+ ion and migrate in the electrical field. The effectiveness of TACD in NACE using tetrabutylammonium reagent for separations of the chlorophenolic compounds is illustrated in Fig. 4. The detecting electrode exhibited a satisfactory stable baseline before and after the sample plug had arrived at the electrode's surface (±10 nA fluctuation or better). The migration times of all compounds increased noticeably when TACD was added to the MeCN medium (containing 10 m*M* sodium acetate, 50 m*M* TBAP and 500 m*M* acetic acid) indicating a considerable change in the electroosmotic flow (Fig. 4A). At +20 kV separation potential, the presence of cyclodextrin had to be above 6 m*M* to improve separation (Fig. 4B) and at 12 m*M* TACD baseline separation was achieved except for the phenol/2,4,6-TCP pair (peaks 2/3 Fig. 4C). Increasing TACD from 12 m*M* to 15 m*M* or higher did not resolve these two analytes. Improved resolution was also attained by decreasing the separation potential from +20 kV to +15 kV albeit at the expense of analysis time (Fig. 4D). Notice also that the PCP peak was very small and only emerged after 62 min of separation (data not shown). Similarly, a modest improvement in resolution was attained by increasing sodium acetate in the separation medium from 10 m*M* to 13.5 m*M* or higher (data not shown). However, the phenol/2,4,6-TCP pair was still not separated and analysis time was significantly longer. For instance, 2,3,4,6-tetraCP (peak 10) only emerged after 36 min of separation instead of 26 min as shown in Fig. 4C. The low level of TACD required in this study (12 m*M*) was somewhat surprising since much higher concentrations of β-cyclodextrin were necessary in nonaqueous systems. As an example, 100 m*M* β-cyclodextrin was necessary to achieve similar chiral separations for trimipramine in NACE in comparison to only 0.2 m*M* β-cyclodextrin in aqueous CE [28]. Several cyclodextrins including α-, β- and γ-cyclodextrins are completely insoluble in the separation medium used in this study.

Although it might be tempting to suggest, based on Fig. 4, that TACD demonstrates noticeable selectivity for the individual chlorophenolic compounds,

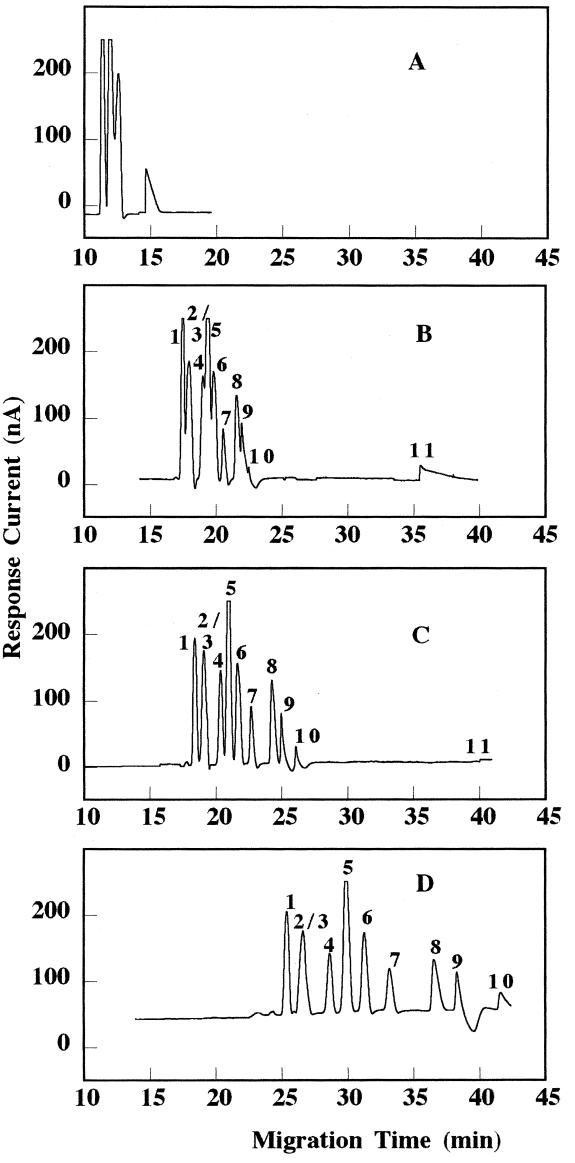

Fig. 4. Electropherograms obtained with the platinum detecting electrode (+1.6 V vs. Ag/AgCl). Separation was performed at +20 kV using a 70 cm×20 μm I.D. separation capillary and 10 s sample vacuum injection at 14.5 p.s.i. (100 kPa). Peak identification is given in Table 1, and analyte concentrations ranged from 0.5 to 10 ppm. The separation medium consisted of 500 m*M* acetic acid, 10 m*M* sodium acetate and 50 m*M* tetrabutylammonium perchlorate in acetonitrile containing different concentrations of triacetyl-β-cyclodextrin (A) 2 m*M*, (B) 6 m*M*, and (C) 12 m*M*. In (D), the separation potential was +15 kV using an acetonitrile medium containing 12 m*M* triacetyl-β-cyclodextrin, 500 m*M* acetic acid, 10 m*M* sodium acetate and 50 m*M* tetrabutylammonium perchlorate.

the improved separation could be due to several factors such as changes in viscosity, zeta potential, ionic radii of the analytes, electroosmotic flow, etc., instead of the analyte–cyclodextrin/TBA^+ interaction. However, baseline separation obtained at a relatively low TACD concentration (12 m*M*) implied that the interaction between the analyte and the TACD/TBA^+ complex indeed played an important role in the separation mechanism. In addition to the ion–dipole interaction between the analyte with the TBA^+ ion as discussed previously, electrostatic interactions (dipole–dipole) between the analytes and the unsubstituted hydroxyl groups around the rim of TACD could be the rationale behind the electrostatic interactions of the analyte with the TACD/TBA^+ complex. The cyclodextrin derivative used in this study has an average molecular mass of 2017 and a degree of substitution of 6.2, i.e., there are still 14–15 unsubstituted hydroxyl groups (primary and secondary) on the rim of the cyclodextrin molecule to interact with the analytes and the TBA^+ ion.

The experimental data further confirmed that the presence of both TBAP and TACD in the separation medium was essential for baseline separation of the 11 chlorophenolic compounds tested in this study. Without TBAP, only four discernible peaks were observed in an attempt to use an acetonitrile medium containing only 10 m*M* sodium acetate, 500 m*M* acetic acid and 12 m*M* TACD (figure not shown). Therefore increasing concentrations of TBAP were added to the separation medium to reveal the influence of this additive and a complete resolution at 50 m*M* TBAP was attained as previously shown in Fig. 4C. In view of this, the resolution of the analytes was accomplished by differential association–interaction of the chlorophenolic analytes between the tetrabutylammonium and tetrabutylammonium/cyclodextrin complex phases. Some parallels can be drawn between this technique and aqueous CE using two different phases such as sodium dodecyl sulfate and a negatively charged β-cyclodextrin derivative [29]. In this mixed-mode separation TACD/TBA^+ complex and TBA^+ ion displayed different selectivities and mobilities in an electrical field. Hence, the optimal resolution of the mixed-mode system could be manipulated by adjusting the concentration ratio of the two phases. With the exception of 2,6-DCP and 2,4,6-TCP, the migration order of the analytes was related to the position as well as the number of chlorines in the aromatic ring. Apparently, the number and position of the chlorine adversely affected the ion–dipole interaction between the analytes and the TBA^+ ion and/or increased the electrostatic interaction between the analytes and the TACD/TBA^+ complex. This could be the reason why 2,4,5-TCP, 2,3,5-TCP and 2,3,5,6-tetraCP emerged very late in the electropherogram.

3.4. Detection limits

The MeCN system enabled more sensitive detection compared with the reported results obtained in aqueous buffer [19]. To establish the detection limit for each chlorinated phenol, a standard sample was analyzed by NACE with 10 s injection time. A calibration plot of each peak area versus concentration was constructed for each analyte by running electropherograms of the standard mixture diluted in MeCN at several ratios. The detection limit was estimated as the concentration at which the peak area was equal to three-times the standard deviation of the peak area versus concentration plot. In all cases, excellent linearity (ranging from 0.03 to 1 μ*M*) was obtained (r^2>0.99). The detection limit is defined as peak area>3× standard deviation of area. The detection limit obtained for each analyte was then compared with the reported value obtained in aqueous buffer [19]. In general, the detection limits obtained for other chlorinated phenols were about 3–8-fold lower than those obtained with aqueous buffers (Table 1).

Notice that a noble detecting electrode such as gold can be integrated on the capillary by depositing a gold film onto the end of the separation capillary to improve reproducibility as well as to facilitate the experimental procedures [30,31]. Alternatively, on-capillary electrodes can be fabricated by using a metal wire epoxy-glued across the outlet of a separation capillary [32]. A laser beam could also be used to drill a hole on the separation capillary and a gold metal wire was then inserted into the hole and served as the detecting electrode [33].

4. Conclusions

The applicability of electrochemical detection with NACE was performed without any electrical-field

Table 1
Migration order and detection limits of the chlorophenols

Compound[a]	Migration order	Detection limit (n*M*)	
		Acetonitrile medium (at 95% confidence interval, $n=3$)	Aqueous buffer [19][b]
2,6-DCP	1	45±1	350
Phenol[c]	2	30±2	100
2,4,6-TCP[c]	3	80±3	360
4-CP	4	30±2	180
2,4-DCP	5	45±2	355
2,5-DCP	6	45±1	–
3,5-DCP	7	45±3	–
2,4,5-TCP	8	85±3	380
2,3,5-TCP	9	90±3	385
2,3,5,6-TetraCP	10	130±4	–
PCP	11	>500	450

[a] CP: Chlorophenol, DCP: dichlorophenol, TCP: trichlorophenol and PCP: pentachlorophenol.
[b] Ref. [19]: the separation buffer consists of 25 m*M* phosphate and 25 m*M* borate, pH 8. The platinum (0.127 mm diameter) was poised at +0.9 V vs. Ag/AgCl. The capillary length (70 cm), diameter (20 æm) and the injection volume (5.6 nl) were identical to the operating conditions used in this study.
[c] Peaks 2 and 3 comigrated, therefore only 10 peaks were observed in the electropherograms.

decoupler and the alignment was simple due to the large diameter of the detecting electrode in comparison with that of the separating capillary. Separation of chlorophenolic compounds in acetonitrile-based media offers high sensitivity and less electrode fouling compared to aqueous buffers. The detection limits obtained are 3–8-fold lower than those obtained with aqueous buffers. The resolution of 11 chlorophenolic compounds was achieved by a combination of neutral triacetyl-β-cyclodextrin and a charged additive, tetrabutylammonium perchlorate. The separation of the analytes was attributed to differential interaction between the charged tetrabutylammonium/cyclodextrin complex and the charged tetrabutylammonium phases. The addition of these selectors in the separation medium exhibited a great change in the selectivity and the migration time without a significant decrease in the peak height except for PCP.

NACE is useful in preventing or minimizing aggregation and solute–wall interactions to improve resolution and separation efficiency [12,34]. In this respect, NACE is a viable alternative to MEKC or cyclodextrin modified CE to resolve large PAHs and fullerenes [35]. Although many questions remain to be answered including the separation mechanism, controlling electrophoretic behavior and selectivity, specific applications, etc., NACE using TACD and TBAP does appear to offer some promise as an alternative to the more popular MEKC or cyclodextrin modified CE for analysis of hydrophobic and neutral compounds.

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