ELSEVIER

Journal of Chromatography A, 761 (1997) 259–268

JOURNAL OF CHROMATOGRAPHY A

Capillary electrophoretic separation of chlorophenols using amperometric detection

Abdelkader Hilmi*, John H.T. Luong, An-Lac Nguyen

Biotechnology Research Institute, National Research Council Canada, Montreal, Quebec H4P 2R2, Canada

Received 18 June 1996; revised 16 September 1996; accepted 2 October 1996

Abstract

A simple end-column amperometric detector, without a porous junction, was designed and attached to a capillary electrophoresis instrument to analyze eight mono-, di- and tri-chlorophenols. The platinum detecting electrode was modified by electrodeposition of tin, to enhance sensitivity as well as reduce electrode fouling owing to phenol oxidation. The modified electrode (0.127 mm in diameter) in combination with a 20 μm I.D. separation capillary yielded sharp peaks and enabled us to detect phenol at levels as low as 0.10 μ*M*. Satisfactory separation of the eight chlorophenols was achieved by using a mixed buffer of phosphate–borate.

Keywords: Detection, electrophoresis; Amperometric detection; Chlorophenols

1. Introduction

Chlorophenols are major components of pulp bleach plant effluents and constitute a significant category of pollutants. Pentachlorophenol and 2,4,6-trichlorophenol have been used widely as wood preservatives and pesticides whereas 2,4-dichlorophenol and 2,4,5-trichlorophenol are used as precursors for the synthesis of herbicides [1,2]. The large-scale use of chlorophenols has led to the contamination of terrestrial and aquatic ecosystems, resulting in the classification of chlorophenols as priority pollutants. Therefore, analytical studies for the monitoring of such aromatic compounds are developing rapidly along with the understanding of their high toxicity, environmental persistence and accumulation in the food chain.

Capillary electrophoresis (CE) is well established as one of the most efficient methods for separation of charged components in mixtures [3]. Modified CE methods which can separate uncharged molecules have also emerged, most notably micellar electrokinetic capillary chromatography [4] and cyclodextrin modified capillary electrophoresis [5,6]. CE systems are usually equipped with an UV or a laser induced fluorescence detector which are not sufficiently sensitive for detection of chlorophenols. Electrochemical detectors, which promise high sensitivity, simplicity and low cost, have been coupled with CE to detect carbohydrates, amino acids and neurotransmitters such as dopamine and catecholamine. However, there is a challenge in isolating the detecting electrode from the high voltage applied across the separating capillary since the electrophoretic current produced in the capillary upon application of separation potential (10–30 kV) can be five to six orders of magnitude (up to 200 μA) greater than the electrochemical currents measured at

*Corresponding author.

PII S0021-9673(96)00834-5

the amperometric detector (<1 nA). The pioneering study of Wallingford and Ewing [7] used a porous glass junction (also known as an electrical decoupler) and was followed by several significant improvements to the same concept [8,9]. In general, the glass junction was complex and would pose difficulties in adapting to commercial CE systems [10]. Junctions of Nafion (a negatively charged polymer) were devised later to improve durability but they induced the loss of some analytes due to the cation-exchange nature of Nafion [11,12]. Other studies reported the feasibility of amperometric detection without the porous junction [13,14] but the sensitivity was compromised. All of the set-ups for amperometric detection, with or without the porous junction, as described in the above-mentioned references, are rather complicated and are not readily adaptable to commercial CE instruments. More recent publications [15,16] described simpler configurations but the compatibility with commercial instruments is still not evident.

The objective of this work is to develop a simple end-column amperometric detector that is compatible with a commercial instrument for routine capillary electrophoretic separation of chlorophenols in contaminated soil and water. To minimize electrode fouling due to phenol oxidation as well as to improve detection sensitivity, metallic tin is electrochemically deposited on the platinum surface. To assess the performance of the detector, capillaries of 50 μm I.D. will be used with detection electrodes of 0.5 mm diameter. A negatively charged β-cyclodextrin, sulfobutylether-β-cyclodextrin, is added to the running buffer to improve the electrophoretic separation efficiency and a smaller electrode (0.127 mm) is used with a narrower capillary (20 μm I.D.) to attain a lower detection limit.

2. Experimental

2.1. Materials

A platinum disk electrode (1.5 mm diameter platinum surface, MF2013) was obtained from Bioanalytical System (BAS, West Lafayette, IN, USA). Platinum wires (0.127 mm and 0.5 mm diameter), silver wire (0.25 mm diameter), stanous chloride, and all chemicals were products of Aldrich (Milwaukee, WI, USA). Polyimide coated fused-silica capillaries of 50 μm I.D. and 20 μm I.D. were purchased from Chromatographic Specialties (Brockville, ON, Canada) and Polymicro, Phoenix, AZ, USA), respectively. Sulfobutylether-β-cyclodextrin was obtained from Applied Biosystems, Perkin-Elmer (Foster City, CA, USA).

2.2. Modification of platinum electrode

Stanous chloride solution (0.1 m*M*) in 50 m*M* phosphate, pH 7 was used to deposit metallic tin on platinum according to a well described process [17]. The platinum electrode (BAS-MF2013) was maintained at −0.6 V against an Ag/AgCl reference electrode by a voltammograph (BAS-CV-1B). After 10 min the electrode was rinsed several times in distilled water and was immediately installed for voltammetric study. During voltammetric studies the solution contained 20 m*M* phenol in 50 m*M* phosphate, pH 7. A platinum wire (0.25 mm diameter) served as the counter electrode in a three-electrode arrangement. The applied potential was cycled between −0.6 V and +0.9 V. Time response was obtained with the same experimental set-up but the applied potential was maintained at +0.9 V.

2.3. Preparation of detecting electrodes

The amperometric detection cell required a detecting electrode prepared with platinum wire. Two sizes of detecting electrodes were used and prepared differently. The larger electrode was prepared by inserting 7 cm of platinum wire (0.5 mm diameter) in a 1 cm segment of polyvinyl chloride tubing (1.2 mm O.D., 0.19 mm I.D., Cole-Palmer, Chicago, IL, USA) and was polished to produce a smooth surface, flush with the tubing end. After deposition of tin (conducted in the same manner as was done with the BAS electrode) the modified electrode was installed immediately in the detection cell.

Preparation of the smaller platinum electrode was somewhat more complicated because tubing of suitable diameter was not available. A 3 cm platinum wire (0.127 mm diameter) was inserted into a 1 cm segment of silica capillary (0.180 mm I.D., 0.40 mm O.D., Chromatographic Specialties) and epoxy glue

was aspirated to fill the capillary segment. After setting of the glue, one end of the wire was cut and polished to be flush with the capillary end. A piece of 0.5 mm platinum wire was attached to the other end of the small wire. The silica capillary was in turn inserted into a 1 cm glass tube (0.365 mm nominal I.D., 1.2 mm O.D., Zero dead volume union, part number 3-2402S, MicroQuartz Sciences, Phoenix, AZ, USA) and the end having the protruding 0.5 mm wire was fixed with epoxy glue. The piece of 0.5 mm Pt wire and the glass tube provided the rigidity for aligning the detecting electrode with the separation capillary (see details in Fig. 1). Deposition of tin was conducted in the same manner as with the larger electrode.

2.4. Amperometric detection cell and capillary electrophoresis

Electropherograms were obtained with the Applied Biosystems CE instrument 270A (Perkin Elmer). The capillary was installed in the normal fashion at the anodic end but was inserted to the cathodic reservoir through a septumed opening, created at the bottom, instead of the one provided on top (Fig. 1). Three other septa were installed on the side of the reservoir to allow insertion of the electrodes. The reference electrode was a silver wire with AgCl formed at the tip by electroformation and a platinum wire served as a counter electrode. The modified platinum electrode, as described above, was used as the working or detecting electrode and positioned directly at the outlet of the capillary. The separation capillary was positioned so that its outlet was as near the center of the tubing as possible. Due to the large diameter of the detecting electrode (0.5 mm) compared to the I.D. (50 μm) of the capillary, good oxidation efficiency was achieved without precise microalignment. During electrophoresis the BAS voltammograph was used to apply +0.9 V to the detecting

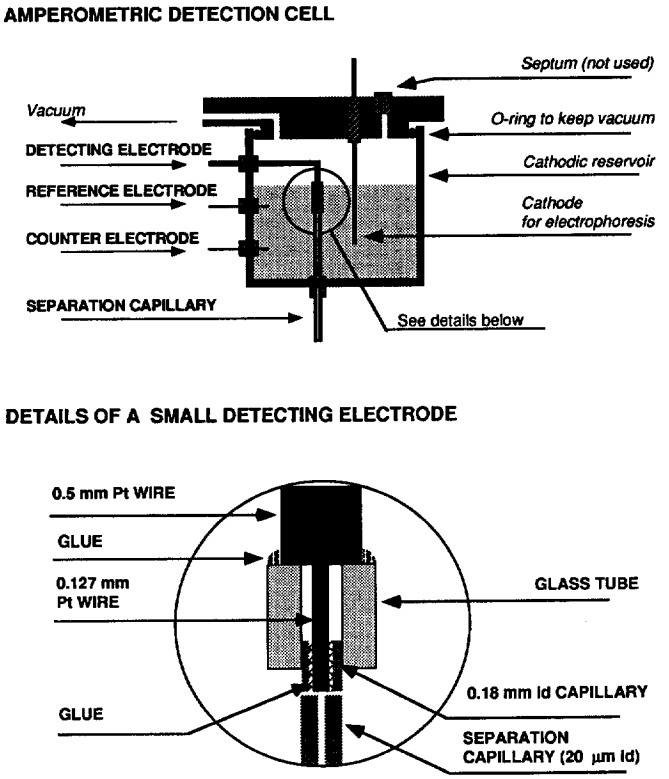

Fig. 1. (Top) Amperometric detection cell, showing original elements (italic lettering) and the added elements (bold lettering). (Bottom) Details of a small detecting electrode. Details not drawn to scale.

electrode. The response current was displayed on the monitor of a PC486 equipped with an A/D board (DP500-AD supplied with an interface box, Labtronics, Guelph, ON, USA). The time response data were stored in ASCII files and translated to PRN files for treatment by a graphic program. The number of theoretical plates (N) for a peak was calculated as $5.54\ (t/w_{1/2})^2$ where t is the peak migration time and $w_{1/2}$ is the peak width at half-maximum [18].

3. Results and discussion

3.1. Characteristics of the modified platinum disk electrode

The electrochemical oxidation of phenol is a feasible route for synthesizing hydroquinone or benzoquinone and it has been exploited in waste water treatment [19]. However, as commonly observed in electrochemistry of phenolic compounds, the oxidation peaks are irreversible on the reversed sweeps and electrode fouling progresses with each sweep cycle, resulting in continuous depletion in electroactivity. At a platinum electrode phenol and some of its derivatives are oxidized to generate hydroxyl radicals [20]. If a radical forms in the electro-oxidation of phenolic compounds after the loss of the first electron, the radicals will easily polymerize and the resulting polymer, i.e., polyquinone, is deposited on the electrode surface. Therefore electrodes prepared with metal oxides such as SnO_2, SbO_5 and PbO_2 were studied in view of overcoming this poisoning effect [21,22]. These studies reported a five-fold efficiency increase and attributed the improvement to a modification of the electrode surface structure [23].

This study confirmed that phenol was oxidized when the unmodified platinum disk electrode was poised at +0.7 V vs. Ag/AgCl and a yellowish film was formed on the electrode after prolonged exposure to cyclic voltammetry (CV) in the phenol solution. The modified platinum disk electrode (with deposited Sn) produced a noticeably higher current response, particularly during the initial CV cycles, but the response was only twice that of the unmodified platinum disk electrode after each was subjected to a few dozen voltammetric cycles (Fig. 2). It should be noted that the modified platinum disk electrode used in this study was prepared by electrodeposition of Sn on the platinum surface and the preparation procedure may allow the formation of SnO_2 as well as PtO_2. Therefore, the performance characteristics of the Pt/Sn disk electrode described here could not be compared directly with the electrode prepared by forming metal oxides on titanium base. Nevertheless, the CV results indicated a higher sensitivity of Pt/Sn compared with unmodified platinum. A two-fold improvement was further confirmed with each type of electrode exposed to a phenol solution for more than one minute and the electrode was maintained at +0.9 V vs. Ag/AgCl (Fig. 3). However, the initial behavior (Fig. 3 inset) was more essential; after 0.05 min (3 s) of potential application, the Pt/Sn disk electrode produced a current as high as 202 μA compared with 34 μA by the unmodified platinum electrode. The response ratio was even larger at shorter time from the initial application of voltage.

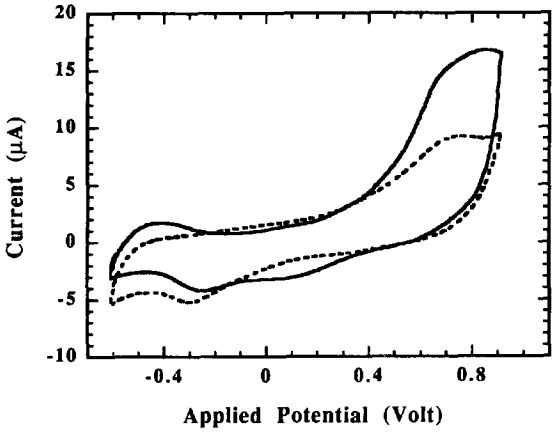

Fig. 2. Voltammograms obtained with a BAS-Pt electrode; unmodified (- - -) and deposited with Sn (———). Solution: 20 mM phenol, 50 mM phosphate, pH 7.

3.2. Characteristics of the detection cell assembly

The capillary inlet and the anode are normally immersed in a buffer solution that is maintained at a high potential (10–30 kV) relative to the cathode that is in the same solution with the capillary outlet. An end-column amperometric detector consists of a reference electrode, a counter electrode and a work-

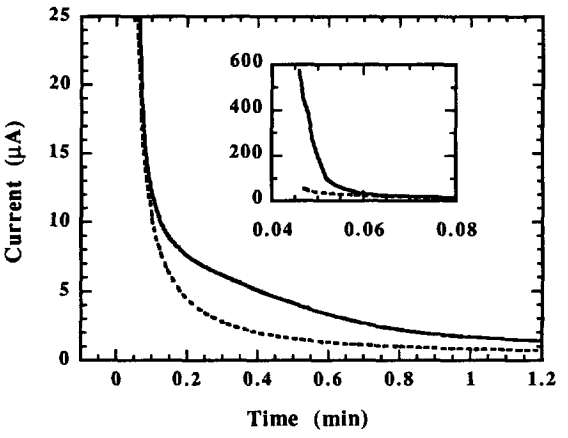

Fig. 3. Current–time response of an unmodified BAS electrode (- - -) and one deposited with Sn (———). Applied voltage: +0.9 V vs. Ag/AgCl. Solution: 20 m*M* phenol, 50 m*M* phosphate, pH 7. Inset shows response shortly after voltage application.

ing electrode that is maintained at +0.8–1.0 V vs. Ag/AgCl. When an amperometric detection cell is installed in a capillary electrophoresis (CE) system each analyte will arrive at the detecting electrode at a different migration time (assuming baseline separation), the concentration is very small and the detecting electrode will be exposed to each analyte for a very short time. Therefore, the higher response exhibited by the Pt/Sn electrode, compared with that of the unmodified platinum electrode, was an indication that the Pt/Sn would be more effective in detecting the CE signals.

A CE apparatus is always grounded for shock prevention. When a potentiostat such as the EG and G Model 400 (Princeton Applied Research, Princeton, NJ, USA) which is also grounded to protect the internal circuit in case of overload, was used to poise the detecting electrode, proper potentials cannot be maintained for electrophoresis as well as for amperometric detection when the two grounds were connected. In one set of experimental conditions (90 cm×50 μm I.D., 50 m*M* phosphate buffer, pH 7) the ABI system showed an electrophoretic current of 40 μA when a potential of 20 kV was applied. As soon as the EG and G instrument was connected to the detection cell, the electrophoretic potential and the current dropped very quickly and within a few seconds the circuit was broken. At the same time the current output from the electrochemical detector displayed erratic response. Conversely, a simple voltammograph such as the BAS model CV-1B is not grounded and when this apparatus was used to poise the working electrode of the detection assembly, no disturbance in either electrophoretic potential or current was observed. The detecting electrode exhibited a steady baseline current (2±0.05 nA) when the running buffer was allowed to flow under electroosmosis (20 kV, 40 μA).

The ABI electrophoresis system is normally set up with the capillary entering from the top of the outlet buffer vial. In this study, the amperometric detection assembly was arranged with the capillary inserted from the bottom of the buffer vial. This reverse arrangement allowed the analytes exiting the capillary to strike the working electrode (that was positioned directly above) quickly, to cause a sharp current response, before dispersing to the periphery. When the working electrode was positioned below the capillary outlet, a more broadened and less reproducible response resulted. The detecting electrode could be inserted from the top but it would be difficult to align it with the capillary. In this study, the amperometric detection cell (Fig. 1) required very little modification of the cathodic reservoir supplied with the ABI-270A. Only four septa (supplied by ABI) were required for inserting the electrodes while still maintaining vacuum in the reservoir.

3.3. Separation of phenol and chlorophenols

The beneficial effect of the Pt/Sn electrode was evident as the corresponding peaks were about twenty-fold higher than those obtained with the unmodified Pt electrode (Fig. 4). Despite the peak broadening effect as manifested by the peak asymmetry, base line resolution was obtained and the number of theoretical plates for all components are more than 70 000. This separation efficiency compares very favorably with values previously reported for dopamine and other neurotransmitters (60 000 to 160 000) using more elaborate end-column detectors [14].

CE with amperometric detection has mostly been applied to samples of dopamine and other neurotransmitters. There was only one short note describing the separation of chlorophenols using a 25 μm-

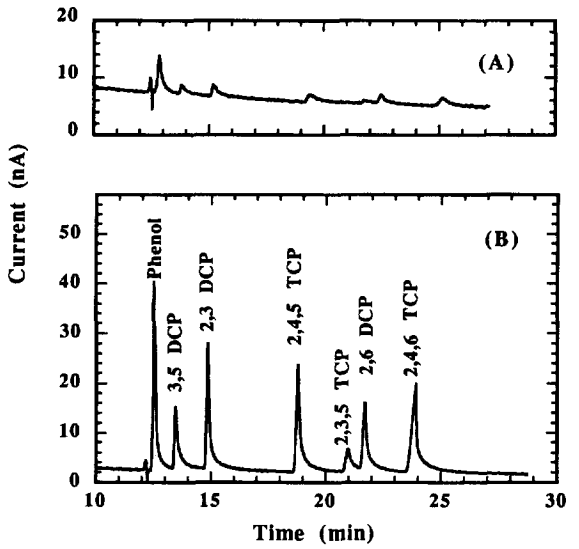

Fig. 4. Electropherograms with detection by 0.5 mm Pt electrode (A) and Pt/Sn (B); applied potential +0.9 V vs. Ag/AgCl. Injection: 2 s; capillary: 70 cm×50 μm I.D.; potential: 20 kV; current: 45 μA. Analyte concentrations: 0.62–2.35 m*M*. Buffer: 50 m*M* phosphate, pH 7. DCP: dichlorophenol; TCP: trichlorophenol.

bore capillary with detection by a 10 μm carbon fiber [24]. However, it was not clear whether a porous glass joint was used and a current response of 50–200 pA was reported for a standard mixture containing analytes at 5–8 ng/L. It should be noted that the concentrations mentioned were unusually small and the specific current response was far out-of-line from those reported by other studies. For example, a recent study dealing with samples of catecholamines [25], using 50 μm capillary, 33 μm carbon fiber, reported response current about 25 pA for analytes at 100 n*M* (about 10 μg/L) and this was in agreement with the results previously reported in the literature. Although the cited note dealt with a different class of analytes; the large response current reported for such small concentrations raised some doubt, particularly when other discrepancies can also be spotted in the note.

In this study, a 50 μm I.D. column was used with a large detecting electrode (500 μm) to obtain response current from 5 to 40 nA for analyte concentrations around 100 mg/L. In theory, if each individual analyte concentration is 100 μg/L, a corresponding response peak of 5–40 pA would be expected. As mentioned earlier, catecholamines in the range of 10 μg/L were reported to produce peak currents of about 25 pA. Thus the chlorophenols were less effectively oxidized by the electrodes in the configuration of this study. Therefore, samples in the range of 10–30 μ*M* were analyzed to obtain current peaks around 0.5–1 nA and established the limits of detection from 5 μ*M* (for phenol, most sensitive) to 20 μ*M* (for 2,5 dichlorophenol, least sensitive).

Although the detection assembly was simple and precise positioning of the capillary (relative to the detecting electrode) was not required, reproducibility was considered satisfactory. Once the detecting electrode (installed on the outlet buffer container) and the capillary were in place, a repetition could be performed with 0.02 min variation of migration time, and less than 4% reduction of peak height, for each analyte. If a third run was effected each peak height would again reduce further but always less than 4%. This was a clear indication of electrode poisoning therefore a treatment procedure was devised and applied. With the detection assembly in place the applied potential was quickly swept for 30 s between −0.6 V and 1.2 V at 600 mV/s. Such a treatment after each run insured less than 2% peak height variation between repetitions. Each time the buffer solution was changed and the buffer container was reassembled the peak height could change up to 5% (compared with a previous assembling) but the migration times were not affected.

3.4. Separation improvement using negatively charged cyclodextrin

Charge and molecular geometry are the two principal factors that influence migration during capillary electrophoresis. The compounds cited in Fig. 4 are resolved principally because of their different molecular geometry, since they are all uncharged. When 2-chlorophenol and 3-chlorophenol were added to the mixture they appeared to co-migrate with phenol (Fig. 5A) although three species could be observed at the top of the large peak at 12–14 min. Previous studies have demonstrated that uncharged polynuclear aromatic hydrocarbons and benzene derivatives could be separated by introducing a negatively charged inclusion agent such as

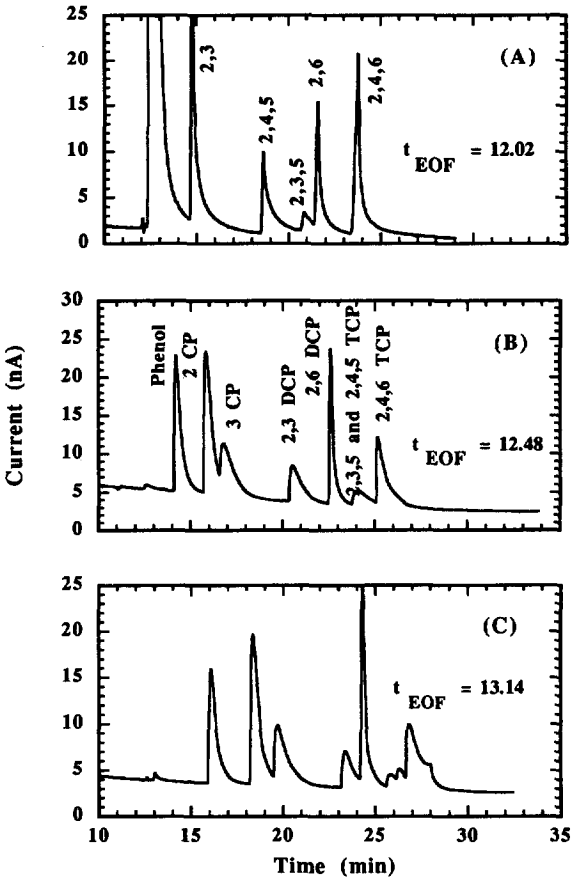

Fig. 5. Electropherograms detected by Pt/Sn electrode, same conditions as Fig. 4. Buffer: (A) 50 m*M* phosphate, pH 7; (B) with 2 m*M* sulfobutylether-β-cyclodextrin (SBCD); (C) with 4 m*M* SBCD. All analytes in samples are indicated in (B). CP: chlorophenol; DCP: dichlorophenol; TCP: trichlorophenol.

sulfobutylether-β-cyclodextrin (SBCD) in the running buffer [5,6]. Fig. 5B and C clearly show that SBCD exhibited a great influence on the migration pattern. Although the electroosmotic flow (EOF) was not significantly altered the migration time of every species was increased with increasing SBCD concentration. Apparently, the degree of chloro-substitution was an important factor in determining the complexation equilibrium since the mono-substituted derivatives emerged first followed by the di- and tri-substituted chlorophenols. As discussed in the cited article, separation by CE with cyclodextrin containing buffer is governed by the equilibrium constant of the complexation process [6]. With the addition of SBCD, a negatively charged compound with the propensity to move in the opposite direction of the EOF, analytes manifesting more tendency to form complexes with SBCD (i.e., showing high equilibrium constant for complexation) would have longer migration time. This study also illustrated that the mono-substituted derivatives, which are more water soluble than the di-and tri-substituted phenols, displayed the shortest migration times. There was good resolution of components within each subgroup and 2 m*M* SBCD was sufficient to resolve the mono-substituted derivatives. However, 4 m*M* SBCD was necessary to resolve 2,3,5-trichlorophenol from 2,4,5-trichlorophenol.

As the peak responses due to trichlorophenols were significantly reduced with increasing SBCD concentration, one may suspect the poisoning effect of 2,6-dichlorophenol, or some other components that reach the detector before the trichlorophenols, but this possibility was ruled out by the experimental observation that a sample of trichlorophenols without any other component showed the same response. The complexation with SBCD was observed to reduce the current responses of all studied phenol derivatives except for 2,6-dichlorophenol. In this particular case the complex actually produced an increased current response but this out-of-line behavior was not totally unexpected. Cyclodextrins and their derivatives are known to have a catalytic action resulting from the particular conformation that certain molecules must assume upon entering the cyclodextrin cavity [26]. Some conformations render such guest molecule more vulnerable to chemical modification. The results obtained here suggested that 2,6-dichlorophenol was more susceptible to electrochemical oxidation when complexed with SBCD.

3.5. Improvement of detection limit

Although the modified platinum electrode permitted a better sensitivity compared with the UV or fluorescence detectors, the detection limit was still far above the regulatory concentration. The use of SBCD enabled the separation of closely resembling components but reduced the responses to most of them. Therefore, experiments were conducted using a mixed buffer of phosphate and borate which was reported to be effective [24]. To compare with the

literature data [24], phenol, 2-chlorophenol, 2,6-dichlorophenol and 2,4,6-trichlorophenol exhibited the same order of migration although the buffer composition was somewhat different (Fig. 6).

CE separation normally requires a very small injection volume (5–20 nL). Therefore, for samples in micromolar concentration only femtomol quantity of an analyte will be sensed by the detecting electrode. Consequently, one may expect that the response current is not limited by the electrode area. Meanwhile the background noise is directly proportional to the sensing surface area. Therefore, it was anticipated that small electrodes would permit lower detection limit and this was confirmed by using the 0.127 mm diameter electrode (Fig. 6). Considering 2,4,6-trichlorophenol as an example, the concentration was 0.42 m*M* and the response current was 2.2 nA, over a background current of 0.6 nA, the specific current response was therefore (2.2−0.6)/0.42=3.55 nA/m*M*. When the larger electrode (0.5 mm diameter) was used (Fig. 4A) the same compound at 1.54 m*M* only gave a response of 6.6 nA, over a background current of 5.4 nA, the specific current response was 1.2/1.54=0.78 nA/m*M*. Since the small electrode greatly reduced the background current and the noise, 0.5 μ*M* of phenol, as well as the chlorinated derivatives, could be detected with a signal-to-noise ratio of 3:1.

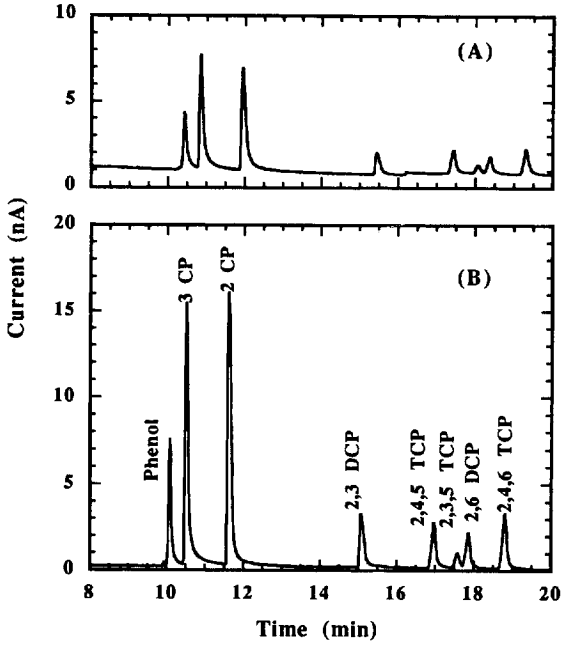

Fig. 6. Electropherograms with detection by 0.127 mm Pt electrode (A) and Pt/Sn (B); applied potential +0.9 V vs. Ag/AgCl. Injection: 10 s; capillary: 70 cm×20 μm I.D.; potential: 20 kV; current: 3 μA. Analyte concentrations: 0.62–2.35 m*M*. Buffer: 25 m*M* phosphate, 25 m*M* borate pH 8. All analytes identified by individual spiking. CP: chlorophenol; DCP: dichlorophenol; TCP: trichlorophenol.

Further improvement in detection limit was obtained with the modified small electrode. Fig. 6B shows a two-fold increase in the response current for a sample of the same analyte concentrations and the detection limit was experimentally established to be 100 n*M*. One may also note the resolving power of the mix buffer. In fact, the peaks were better resolved than when SBCD-containing buffer was used and they also appear to be symmetrical with the number of theoretical plates varying from 88 000 to 145 000. The five-fold increase in the specific current response displayed by the smaller electrodes affirmed that the exposed sensing surface area was the key sensitivity determinant, in agreement with statements in the literature [15]. In addition, the electrodes displaying elliptical platinum surfaces (because the platinum wire was at an angle) invariably displayed larger base currents and noises. Therefore it is evident that better control of the electrode preparation would improve the sensitivity. However, the electrode diameter must always be greater than the capillary bore to minimize the chance of the analyte not being sensed by the electrode.

Fig. 6 reveals a two-fold increase in response when Sn was deposited on a 0.127 mm Pt electrode but the larger electrode (0.5 mm) accomplished a 20-fold improvement for the same modification (Fig. 4). The remarkable improvement obtained with the 0.5 mm electrode (50 μm I.D. capillary, 2 s injection) was due to the ability of the deposited Sn to reduce fouling of the Pt surface. In accordance with the well-known Hagen-Poiseuille equation [3], the injected volume is proportional to the injection time and to the fourth power of the capillary diameter. Therefore when the 0.127 mm electrode was used with the 20 μm capillary (10 s injection) the injected volume was $(2/10)\times(50/20)^4=7.81$-fold smaller. With so many less analytes the Pt electrode would be poisoned to a lesser degree and one could expect the poisoning reduction benefit conferred by the de-

posited Sn to be less significant. The different degrees of improvement obtainable (by the Sn deposition) with 0.5 mm and 0.127 mm Pt electrodes were verified repeatedly and even smaller electrodes (0.06 mm and less) were prepared and used with 2–10 μm capillaries. Smaller capillaries (with smaller sample quantity) could be hypothesized to reduce the poisoning effect so much that the deposition of Sn may be no longer necessary. However, the material at hand did not permit reproducible assembling of small electrodes, with circular sensing surface.

No fouling was observed when the small electrodes were used, an amperometric detection cell could be assembled and used in a full day working session with less than 4% variation in peak height, for each component. The electrode treatment that was essential for the large electrodes was never required.

3.6. Requirements for amperometric detection without an electrical decoupler

This study used end-column detection without a porous junction. The original porous glass joint allowed impressive sensitivity (60 and 8.5 n*M* for catechol [9] and serotonin [27], respectively) but was recognized as being fragile, difficult to produce reliably and inexpensively [13]. The first studies on end-column detector (without a porous junction) reported a rather high limit of detection (0.5 μ*M* for catechol [13] and 1 μ*M* for dopamine [14]) therefore the more durable Nafion was devised that permitted greater sensitivity (31 and 0.5 n*M* for cysteine [11] and hydroquinone [12], respectively) but posed some problems with reproducibility due to adsorption of analytes. A later study [13] reported a significant sensitivity improvement (66 n*M* for catechol) of the end-column detector. The use of a narrow bore capillary (2–5 μm I.D.) was commonly considered a prerequisite for elimination of the porous junction [14,28] but very recent results [16,29] clearly showed that a larger capillary (50 μm I.D.) posed no problem. Nevertheless, the literature does not provide a clear understanding of the requirements for an amperometric detection assembly.

Observations in this study confirmed that a porous junction was not required if 2–5 μm capillaries were used for separation (data not shown). Interference-free operation can be explained by realizing that the internal resistance of the capillary was 10^{12}–10^{13} Ω compared with 10^{6}–10^{8} Ω between the capillary and the detecting electrode [14] Small detecting electrodes (about 10 μm, carbon fiber type) must be inserted into the capillary to assure that the analytes reach the sensing surface. This setup requires some adaptation at the capillary outlet [13,14]. If the sensing electrode is positioned outside the capillary it must be a disk electrode [15] to be placed as close to the capillary exit as possible. Since it is desirable to avoid the need for accurate alignment, electrode diameters equal to 10 times the capillary I.D. are used to assure total recognition of the analyte. Higher electrode/capillary diameter ratios were observed to increase the base line and the noise thus reducing the sensitivity; but ratios less than five incurred the risk of analytes escaping without being recognized leading to irreproducibility (data not shown). A ratio of five appears to be the best compromise and any commercial potentiostat can be used with 2–5 μm capillaries. Without an electrical decoupler an un-grounded potentiostat must be used with larger-bore capillaries (>10 μm I.D.). The data acquisition circuit (for computer linkage) may also be a source of interference and must be chosen accordingly.

4. Conclusions

This paper demonstrated the feasibility of performing end-column amperometric detection in capillary electrophoresis using relatively large bore capillary without an electrical decoupler. Platinum electrode modified with metallic tin minimized electrode fouling and provided significant improvement in detecting phenol and chlorinated phenolic compounds. Assembling the detection system was easy and there was no need for precise alignment or insertion of the microelectrode in the capillary. While negatively charged cyclodextrin improved the resolving power but reduced the detecting ability; the mixed buffer of phosphate–borate could separate all components without detrimental effects. The smaller electrode yielded sharper peaks and offered further improvement to enable the detection of nanomolar

concentration. Apparently, if small capillaries are used, in conjunction with proportionally small detecting electrodes, the poisoning effect would be reduced and electrode modification may not be required. The acquired information ascertains that simple amperometric detectors can be assembled for use with many existing CE systems to permit sensitive detection of electroactive compounds. Some further effort in selecting the electrodes suitable for different classes of electroactive analytes would significantly broaden the applicability of CE with amperometric detection. Work is in progress to analyze all 19 chlorinated phenols including 2,4-dichlorophenol and pentachlorophenol in contaminated soil and water and in other samples.

References

[1] U.J. Ahlborg and T.M. Thunberg, CRC Crit. Rev. Toxicol., 7 (1980) 1.
[2] K. Lindstrom and J. Nordin, J. Chromatogr., 128 (1976) 13.
[3] P.D. Grossman and J.C. Colburn (Editors), Capillary Electrophoresis, Theory and Practices, Academic Press, San Diego, CA, 1992.
[4] S. Nie, R. Dadoo and R.N. Zare, Anal. Chem., 65 (1993) 3571.
[5] R.J. Tait, D.O. Thompson, V.J. Stella and J.F. Stobaugh, Anal. Chem., 66 (1994) 4013.
[6] O.H. Szolar, R.S. Brown and J.H.T. Luong, Anal. Chem., 67 (1995) 3004.
[7] R.A. Wallingford and A.G. Ewing, Anal. Chem., 59 (1987) 1762.
[8] R.A. Wallingford and A.G. Ewing, Anal. Chem., 61 (1989) 98.
[9] T.M. Olefirowicz and A.G. Ewing, Anal. Chem., 62 (1990) 1872.
[10] Y.F. Yik and S.F.Y. Li, Trends Anal. Chem., 11 (1992) 325.
[11] T.J. O'Shea, R.D. Greenhagen, S.M. Lunte, C.E. Lunte, M.C. Smyth, D.M. Radzik and N. Watanabe, J. Chromatogr., 593 (1992) 305.
[12] T.J. O'Shea and S.M. Lunte, Anal. Chem., 66 (1994) 307.
[13] X. Huang, R.N. Zare, S. Sloss and A.G. Ewing, Anal. Chem., 63 (1991) 189.
[14] S. Sloss and A.G. Ewing, Anal. Chem., 65 (1993) 557.
[15] M-C. Chen and H-J. Huang, Anal. Chem., 67 (1995) 4010.
[16] A.M. Fermier, M.L. Gostkowski and L.A. Colón, Anal. Chem., 68 (1996) 1661.
[17] G. Kokidinis, J. Electroanal. Chem., 217 (1988) 201.
[18] J.W. Jorgenson and K.D. Lukacs, Anal. Chem., 53 (1981) 1298.
[19] H. Scharifian and D.W. Kirk, J. Electrochem. Soc., 133 (1986) 921.
[20] Ch. Comniellis and C. Pulgarin, J. Appl. Electrochem., 21 (1991) 703.
[21] D. Kotz, S. Stucki and B. Carcer, J. Appl. Electrochem., 21 (1991) 14.
[22] S. Stucki, B. Kotz, B. Carcer and W. Suter, J. Appl. Electrochem., 21 (1991) 99.
[23] Ch. Comniellis and C. Pulgarin, J. Appl. Electrochem., 23 (1993) 108.
[24] C.D. Gaitonde and P.V. Pathak, J. Chromatogr., 514 (1990) 389.
[25] S. Park, S.M. Lunte and C.E. Lunte, Anal. Chem., 67 (1995) 911.
[26] E.T. Chen and H.L. Pardue, Anal. Chem., 65 (1993) 2563.
[27] R.A. Wallingford and A.G. Ewing, Anal. Chem., 60 (1988) 1972.
[28] C. Haber, I. Silvestri, S. Roosli and W. Simon, Chimia, 45 (1991) 117.
[29] Y. Guo, L.A. Colón, R. Dadoo and R.N. Zare, Electrophoresis, 16 (1995) 493.