Pulsed amperometric detection with poly(dimethylsiloxane)-fabricated capillary electrophoresis microchips for the determination of EPA priority pollutants

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A miniaturized analytical system for separation and detection of three EPA priority phenolic pollutants, based on a poly(dimethylsiloxane)-fabricated capillary electrophoresis microchip and pulsed amperometric detection is described. The approach offers a rapid (less than 2 min), simultaneous measurement of three phenolic pollutants: phenol, 4,6-dinitro-*o*-cresol and pentachlorophenol. The highly stable response (RSD = 6.1%) observed for repetitive injections ($n > 100$) reflects the effectiveness of Au working electrode cleaned by pulsed amperometric detection. The effect of solution conditions, separation potential and detection waveform were optimized for both the separation and detection of phenols. Under the optimum conditions (5.0 mM phosphate buffer pH = 12.4, detection potential: 0.7 V, separation potential: 1200 V, injection time: 10 s) the baseline separation of the three selected compounds was achieved. Limits of detection of 2.2 μM (2.8 fmol), 0.9 μM (1.1 fmol), and 1.3 μM (1.6 fmol) were achieved for phenol, 4,6-dinitro-*o*-cresol and pentachlorophenol, respectively. A local city water sample and two over-the-counter sore-throat medicines were analyzed in order to demonstrate the capabilities of the proposed technique to face real applications.

Introduction

In recent years, there has been a growing concern regarding the consequences of exposure of wildlife and humans to xenobiotics. A considerable number of organic pollutants have a phenolic-based structure. Phenol and substituted phenols are also widely known as components in both industrial and natural waste.[1] Their presence in water is partially responsible for its toxicity and affects aquatic organisms including bioaccumulation in fish tissues. Phenols are introduced into the environment in a variety of ways. For example, 2-methylphenol can be released in wood pulping, diesel exhausts, and petroleum refining. Nitrophenols are released from mines, metal, and petroleum plants. Chlorophenols can be a by-product of fossil fuel burning or used as bactericides and wood preservatives.[2,3] In general, phenols can produce alteration in the cell membrane structures, mutation of the genetic material and change the speed of photosynthesis. Clinically, these compounds can produce a wide range of symptoms and pathologies.[4,5] Phenol poisoning can occur by skin absorption, vapor inhalation, or ingestion and, regardless of route of exposure, can result in detrimental health effects.[5–7] Furthermore, even at low concentrations in drinking water, they give off strong tastes and odors.[8] Most phenols and their derivatives can be oxidized by oxygen, allowing the use of some phenolic compounds as antioxidants.[9–11]

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Various methods have been reported for the determination of phenolic compounds, such as GC[12] or HPLC.[7,13] However, these methods require a sample pretreatment step,[12,14–16] expensive and bulky instrumentation and/or highly skilled personnel. Among others, capillary electrophoresis (CE)[17] with UV,[16–18] chemiluminescence,[19] MS,[20] ICP-MS,[21] or electrochemical[22] detection has been recently reported. CE can be also coupled with flow-injection analysis,[23] allowing on-line sample pretreatment. Additionally, CE can be miniaturized, offering high performance, versatility, reagent economy, speed, and automation capabilities. These miniaturized chemical analysis systems are particularly attractive for on-site environmental analysis.[24]

A wide variety of polymeric materials have been recently used to reduce fabrication complexity and cost for electrophoresis chips.[25] Poly(dimethylsiloxane) (PDMS) is an elastomeric material that has been used extensively because it is robust, optically transparent, non-polar, impermeable to aqueous solutions and allows the easy, fast, and inexpensive fabrication of devices using micromolding techniques.[26] Many detection modes have been coupled with capillary electrophoresis microchips.[27] Electrochemical detectors (EC) also offer great possibilities for those microenvironments with very good sensitivity, selectivity and on-chip integration capabilities.[28–31] Working electrodes have been fabricated from a number of materials,[29] including carbon,[32,33] gold, platinum,[34] palladium,[35] and copper.[36] Likewise, different electrochemical techniques have been applied to the detection of phenols.[37–39] However, the majority of applications using CE[22,29,40–43] or microchip-CE[28,39,44–47] utilized amperometric detection. Nevertheless, one drawback to the use of

amperometry is the fact that electrodes become fouled when they interact with analytes such as phenol or carbohydrates.[48–50] Different approaches have been proposed to increase signal stability. However, given the growth of microchip CE coupled with electrochemical detection, the ability to solve surface-related challenges is profoundly important. To overcome this problem, a potential waveform can be used to clean metal electrodes. This technique, known as pulsed amperometric detection (PAD), was introduced by Johnson[51,52] and LaCourse.[53] In PAD, the working electrode is first stepped up to a large positive potential. During this phase, an oxide layer is formed on the surface, and any adsorbed organic material is simultaneously stripped off from the surface. Following formation of the oxide and removal of adsorbate, the oxide layer is dissolved by applying a reducing potential to the electrode. This redox cycle serves to regenerate the clean, oxide-free noble metal surface. Finally, the potential is stepped to the detection potential for analytes. PAD has been used in combination with HPLC, CE and microchip-CE for the quantification of a wide number of analytes in various samples.[54]

In this report, an unmodified gold electrode was used to accomplish sensitive and stable detection of phenol, pentachlorophenol and 4,6-dinitro-*o*-cresol on a PDMS microchip. This is the first report on the detection of phenolic compounds using a PDMS microchip with PAD. The use of PAD allows an effective surface cleaning and results in the highest signal stability for phenols pollutants using microchip CE-EC to date.

Experimental

SU-8 2035 photoresist was purchased from MicroChem Co., Sylgard 184 silicone elastomer and curing agent were obtained from Dow Corning. Aqueous solutions were prepared using analytical grade reagents and 18 MΩ cm^{-1} resistance water (NANOpure Diamond, Barnstead). Electrolytes were prepared by weighing the desired amount of phosphate NaH_2PO_4 (Fisher) and adjusting the pH with 2 M NaOH (Fisher). Phenol (Ph), pentachlorophenol (PCP) and 4,6-dinitro-*o*-cresol (DNOC) were purchased form Sigma. (*Phenolic compounds are toxic/irritant/dangerous for both the environment and living organisms, and should be handled in a fumehood. Skin and eye contact, and accidental inhalation or ingestion should be avoided*). Sample stock solutions of phenols were prepared daily by dissolving the desired amount in the corresponding electrolyte solution.

The sore-throat medicines, Menthol Spray (CVS) and Cepastat (Insight Pharmaceuticals), were purchased from a local pharmacy. A dilution (1/2000) was performed to the liquid spray before the injection. An aliquot of 0.2952 g of lozenges was dissolved in 2 mL of buffer, diluted 400 times and injected.

The pH measurements were performed with a combined glass electrode and a digital pH meter (Orion 420A+, Thermo). A home-made high voltage power supply (HVPS) was used for all the experiments.[55] The HVPS consists of two positive and one negative DC-DC converters, a microprocessor controlled timer and a power supply. This arrangement allows the control of the potentials applied in the 0 to ±4000 V range with noise values in the nA range. All experiments were performed at room temperature (22 ± 2 °C).

2.1 Fabrication of the PDMS microchip

A 100 mm silicon wafer (Silicon Valley Microelectronics Inc.) was cleaned and oxidized with piranha solution (2 : 1 H_2SO_4 : H_2O_2) (*Caution! Piranha solution is a powerful oxidizing agent that reacts violently with organic compounds; it should be handled with extreme care*) and rinsed with deionized water, then dried at 200 °C for 15 min. The wafer was then coated with SU-8 2035 negative photoresist using a spin coater (Laurell Technologies) by dispensing approximately 3 ml of photoresist onto the wafer. A spread cycle of 500 rpm for 10 s followed by 3000 rpm for 30 s was performed followed by two pre-exposure baking steps at 65 and 95 °C for 5 and 10 min, respectively. A digitally produced mask containing the channel pattern was placed on the coated wafer, exposed to light *via* a near-UV flood source (365 nm, 13 mW cm^{-2}, Optical Associates Inc.) for 30 s and then baked at 95 °C for 10 min. The positive relief was developed by placing the wafer in propylene glycol methyl ether acetate for 15 min, rinsing with methanol, and drying under a N_2 stream. The height of the positive patterns on the molding masters, which is equal to the channel depth created in the PDMS layer, was 50 μm as measured with a profilometer (Dektak IIA, Sloan Technology). Two PDMS layers were fabricated by pouring a degassed mixture of Sylgard 184 silicone elastomer and curing agent (10 : 1) onto either a molding master or a blank wafer, followed by curing for at least 2 h at 65 °C. The cured PDMS was separated from the mold and reservoirs were made at the end of each channel using a 6 mm circular punch. As it was previously reported, a 25 μm-diameter, 99.99% gold wire (Goodfellow, England), was then aligned at the end of the separation channel in a perpendicular channel designed for working electrode.[56–60] Next, the two PDMS layers were placed in an air plasma cleaner (Harrick Plasma Cleaner/Sterilizer PDC-32G), oxidized for 20 s and immediately brought into conformal contact to form an irreversible seal. The extremities of the electrode channel were sealed with two drops of super glue. Finally, an electrical connection to the working electrode was made using silver paint (SPI Supplies) and a copper wire. A double-T injector with a 500 μm gap between side channels was used for all experiments defining a 1.2 nL sample plug. The separation channel was 52 mm long. A schematic drawing of the microchip is included in Fig. 1. The standard amount of buffer dispensed in each reservoir (B, C and D) was 50 μl, except for the sample reservoir (A) where only 45 μl were dispensed in order to avoid leakage into the separation channel. Electrical connections were made to the microfluidic devices with platinum electrodes placed into the reservoirs at the ends of each channel. The potentials used during injection and separation are summarized in Table 1.

2.2 Electrochemical detection

Pulsed amperometric detection was performed using a CHI812 electrochemical detector (CH Instruments) and according to the potential scheme described below and shown in Table 2. A gold wire was used as the working electrode according to the

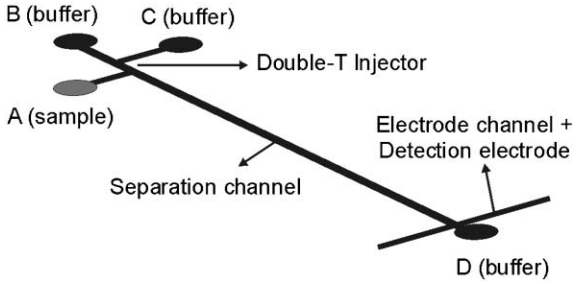

Fig. 1 Schematic drawing of the microchip-CE with PAD. Channels: 50 μm wide, 50 μm deep. Injector (double-T) volume: 1.2 nL, separation channel: 52 mm. Double-T arms: 10 mm long. Solution reservoirs: 6 mm diameter. Chip thickness: 2 mm.

Table 1 Reservoir solutions, injection and separation potentials used

Reservoir	Containing	Injection/V	Separation/V
A	Sample	+500	+500
B	Buffer	+500	+1200
C	Buffer	−50	+500
D	Waste	Ground	Ground

Table 2 Pulsed amperometric detection parameters for the detection of phenols

	Potential/V	Time/s
Clean	+1.6	0.05
Reactivate	−0.5	0.025
Detect	+0.7	0.15

previously presented arrangement.[56–60] The detection area was estimated to be 0.039 mm^2 (25 μm electrode diameter, 50 μm channel width). Ag wire and Pt electrodes were used as pseudo-reference[61] and auxiliary electrodes, respectively, and placed in the waste reservoir. A separate ground electrode was used for the separation system to minimize noise and also positioned in the waste reservoir.

Results and discussion

3.1 Pulsed amperometric detection waveform

Phenols can be oxidized under a wide range of conditions.[8,40,62–65] However, if amperometry is used, a decrease in the anodic current is frequently observed. As stated before, PAD was selected in order to minimize the electrode fouling and increase the signal stability. On each PAD cycle, the electrode surface was cleaned by the use of both a highly positive potential to remove the adsorbed material and a negative potential to reconstruct the surface. After that, a potential should be chosen in order to maximize the electrode response. The effect of the detection potential on the signal was analyzed between 0.0 V and +1.1 V for the three selected compounds, as no such data was available. Fig. 2 shows the hydrodynamic voltammograms corresponding to pentachlorophenol, 4,6-dinitro-*o*-cresol, and phenol. The curves were developed point wise by making 100 mV increments in the potential to the detection step. As can be seen, the peak current (I_p) increases as the detection potential increases until a

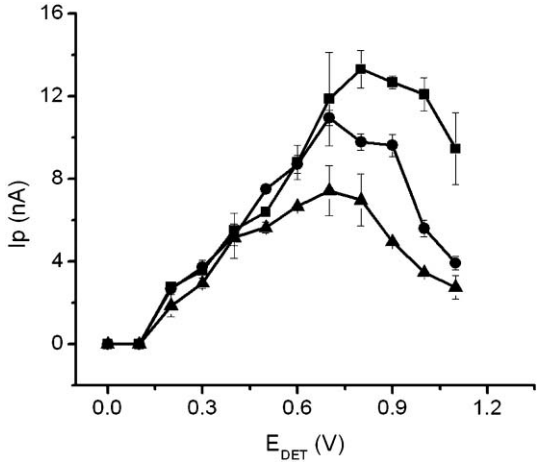

Fig. 2 Hydrodynamic voltammograms corresponding to 66 μM phenol (-■-), 28 μM pentachlorophenol (-●-), 20 μM 4,6-dinitro-*o*-cresol (-▲-). Other conditions: 5 mM phosphate buffer pH = 12.4, injection time: 10 s, separation potential: +1200 V.

maximum is obtained at +0.7 V. The current decrease observed at potentials higher than +0.7 V can be explained as the result of the formation of oxide on the working electrode surface during the measurement step. The formation of this oxide inhibited the pre-adsorption of the phenols on the electrode surface, reducing the signal intensity. Similar profiles were found for all three compounds. Therefore, +0.7 V was chosen as the optimum detection potential. These results are comparable to other molecules analyzed by the same technique.[56–59,66]

The effect of other parameters, such as oxidation and reduction potentials and the applied time for each step, were also studied and optimized considering not only the signal/noise ratio but also signal stability (data not shown). Typically, the higher the cleaning potential and applied time was, the larger the noise current produced. The optimum PAD parameters are summarized in Table 1.

3.2 Effect of electrolyte concentration

It is well known that the electrolyte concentration affects both the electro-osmotic flow (EOF) and the separation. Therefore, the effects of different electrolytes at different concentrations on both the resolution and signal/noise ratio were evaluated. Stock solutions of 50 mM (phosphate, borate, and phosphate + borate) were prepared by dissolving the corresponding amount of salt and adjusting to 12.4 with 2 M NaOH (the volume change was less than 1% in all the cases). Working solutions were prepared by dilution from the stock at final concentrations of 0, 1.0, 2.5, 5.0, 10.0, and 20.0 mM. According to the results (data not shown) there is a compromise relation between separation efficiency and baseline noise. Lower buffer concentrations produced lower baseline noise but the separation was compromised. Higher buffer concentrations produced not only better separations but also higher baseline noise values. The use of phosphate, borate or mixtures of them produced also very similar electropherograms with baseline separation of phenol, PCP and DNOC. However, higher baseline noise values (peak to peak average current) were obtained when borate was used

(borate = 1.6 ± 0.5 nA, phosphate = 0.7 ± 0.2 nA). As can be expected, the higher the buffer concentration (phosphate, borate or mixtures of them) the higher the noise. According to these experiments, 5.0 mM phosphate (pH = 12.4) was selected as the optimum electrolyte for the separation and detection of the selected compounds.

3.3 Effect of solution pH

Phenols can be separated by CE using uncoated fused silica capillaries.[23] Under alkaline conditions the majority of the phenolic compounds become anions and migrate according to their charge to mass ratio.[67] Although phenols can be oxidized at a wide range of pH values,[24,62,68] lower limits of detection are typically obtained under alkaline conditions.[11,48,49,60,69] For that reason, the effect of solution pH on the separation of phenols was studied between 10 and 12.6 using 5.0 mM phosphate solutions. No peaks were obtained for phenol and PCP with solution pH between 10 and 11 at the studied concentrations. Additionally, as can be observed in Fig. 3, poor resolution (R = 0.33, phenol-PCP, pH = 11.6) was achieved between pH 11 and 12. However, at pH > 12 a clear separation of the three peaks was achieved. The migration order reflects a clear dependence of the electrophoretic mobility with respect to the molecular weight of the selected compounds.

The potential for the onset of gold oxide formation has been observed to decrease as pH increases, so it is easier to form surface oxide as the pH increases.[53] The effect of the pH of run buffer upon the amperometric response is also shown in Fig. 3. As can be observed, the response for phenol increases as the pH increases, reaching a maximum at pH = 12.2. At higher pH values (pH > 12.2) a slight decrease in the electrochemical response was observed for phenol. This decrease could be attributed to the formation of a passivating layer of gold oxide on the electrode surface. The response for PCP and DNOC was less affected by changes in the solution pH. It is worth noting that by lowering the pH, the formation of surface oxide

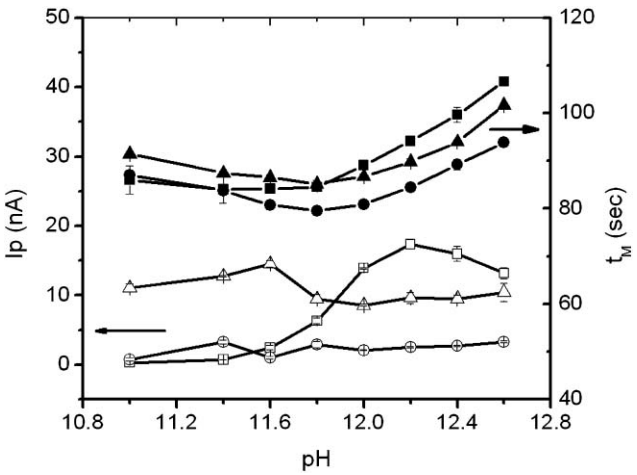

Fig. 3 Effect of run buffer pH on the migration time (solid symbols) and electrochemical response (open symbols) of phenols. 45 μM phenol (-■-)(-□-), 26 μM pentachlorophenol (-●-)(-○-), 11 μM 4,6-dinitro-*o*-cresol (-▲-)(-△-). Other conditions as in Fig. 2.

(helpful for stripping off the adsorbed material) was inhibited, and therefore the signal stability was also compromised (data not shown). An optimum pH of 12.4 was selected in order to balance signal magnitude and stability. It is worth noting that the solution pH does not represent a limitation for the conditions required for the separation and detection of phenols. If lower pH values are required for the separation of a particular set of phenols, the detection pH can be easily adjusted after the separation.[60]

3.4 Effect of organic additives

The use of organic solvents has been reported to improve separations by CE.[70] The resolution enhancement results from a combination of a change in the viscosity of the electrolyte and a change in the electroosmotic flow. However, the addition of 5% v/v of different alcohols (methanol, ethanol, 1-propanol, 1-butanol) or acetonitrile to the running solution did not show any impact on the separation efficiency. Moreover, as it was previously reported,[58] the use of organic solvents as additives produced a decrease in the signal magnitude and an increase in the baseline noise (data not shown). This increase in noise can be attributed to the adsorption process of the organic compounds onto the electrode surface. Surfactants such as sodium dodecyl sulfate (SDS) have also been used not only to control EOF but also to improve the separation of organic molecules. However, the use of SDS in the 1–20 mM range did not show a significant improvement in either the resolution or oxidation current of phenolic compounds, probably as a result of the highly alkaline conditions selected. For these reasons, the use of organic additives was not pursued further.

3.5 Effect of the injection time

Different injection methods have been designed for micro-fluidic devices using both electroosmotic and hydrodynamic flow.[71] The present device uses a pinched (volume-based) injection which allows a better control of sample volume (with respect to gated injection). However, because the detector electrodes are in the waste reservoir (and no potential can be applied), a small amount of sample diffuses to the separation channel increasing the size of the sample plug. To evaluate the effect of injection time on the peak height and resolution, injections ranging from 1 to 30 s were performed. As can be observed in Fig. 4, a minimum of 2 s are necessary to introduce the sample in to the separation channel. In general, the higher the injection time, the more sample is introduced and therefore the higher the peaks are. However, as can be expected, when larger sample plugs are injected, a decrease in resolution was observed. For example, the resolution between PCP and DNOC was 1.6, 1.2, and 0.8 using 3, 10 or 30 s, respectively. From these results, and as a compromise between resolution and sensitivity, an injection time of 10 s was selected as optimum and used for all the experiments.

3.6 Separation and PAD of phenols

Fig. 5 shows a series of electropherograms for two mixtures of pentachlorophenol, 4,6-dinitro-*o*-cresol, and phenol. Using the

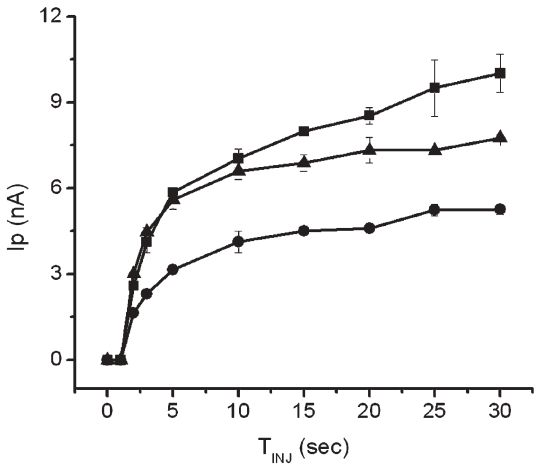

Fig. 4 Effect of the injection time on the electrochemical response. 27 μM phenol (-■-), 16 μM pentachlorophenol (-●-), 8.3 μM 4,6-dinitro-*o*-cresol (-▲-). Other conditions as in Fig. 2.

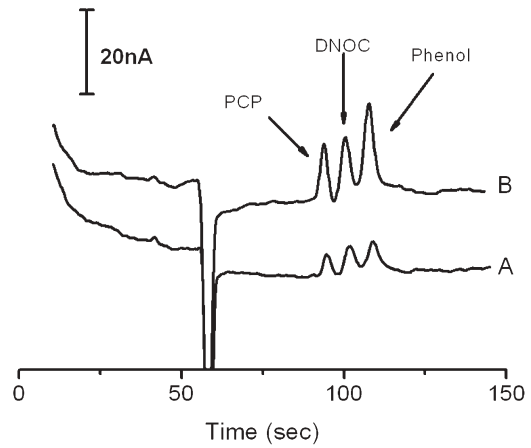

Fig. 5 Separation and PAD of phenols using the PDMS microchip. (A) 22.5 μM phenol, 13.1 μM pentachlorophenol, and 8.3 μM 4,6-dinitro-*o*-cresol. (B) 67.7 μM phenol, 39.4 μM pentachlorophenol, and 25.0 μM 4,6-dinitro-*o*-cresol. Other conditions as in Fig. 2.

optimized conditions for the separation and detection (5 mM phosphate, pH = 12.4, separation potential = 1200 V, detection potential = 0.7 V, injection time = 10 s) the three phenols were baseline separated within 2 min ($t_{M\text{-}PCP}$ = 94 ± 1 s, $t_{M\text{-}DNOC}$ = 101 ± 1 s, $t_{M\text{-}Ph}$ = 108 ± 1 s) (the average t_M were calculated using five different concentrations and five injections at each concentration). These conditions, however, do not resolve 2-nitrophenol, 2,4-dinitrophenol, 2-chloro-5-methylphenol and *o*-aminophenol (data not shown). A slight chip-to-chip difference in EOF (affecting the migration time) was observed and has been attributed to small variations during the plasma oxidation.

3.7 Stability, linearity and limit of detection

Amperometric detection of phenols is commonly prone to surface fouling, due to the formation of inhibitory polymeric films. No such passivation problem was observed using the CE/pulsed amperometric detector microsystem when a series

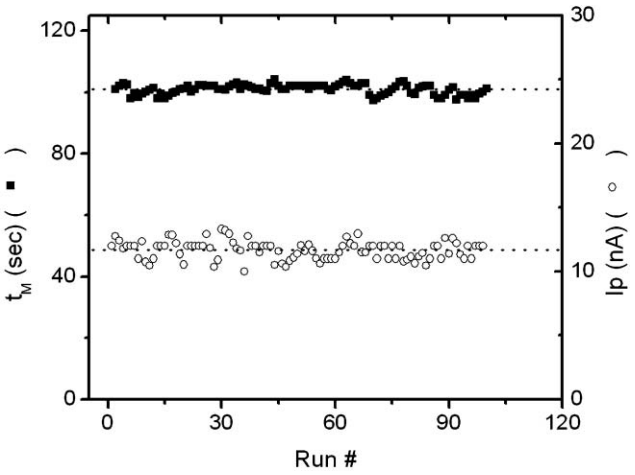

Fig. 6 Electrochemical response and migration time obtained with repetitive injections of 90 μM phenol. Other conditions as in Fig. 2. The dotted line represents a linear fit with a fixed slope equal to 0.

of more than 100 repetitive injections of a 90 μM phenol solution were performed over 8 h (Fig. 6). The relative standard derivations of migration time and response were 1.6% and 6.1%, respectively. Such high stability is attributed to the successful use of PAD for electrochemically cleaning the working electrode. It is worth noting that no peak was obtained for phenol under the same conditions using DC amperometry. However, a significant signal decrease was observed when catechins (natural polyphenols) were analyzed using gold[11] or carbon[9,48,49,69] surfaces by DC amperometry.

Using the optimized conditions for separation and detection (5 mM phosphate, pH = 12.4, separation potential = 1200 V, detection potential = 0.7 V, injection time = 10 s), a linear relationship between the concentration and the peak current was obtained for the three phenols analyzed (see Fig. 5). The corresponding slopes for the calibration curves were 0.43 ± 0.01 nA μM^{-1} (R^2 = 0.9995) for pentachlorophenol, 0.71 ± 0.03 nA μM^{-1} (R^2 = 0.9992) for 4,6-dinitro-*o*-cresol, and 0.330 ± 0.005 nA μM^{-1} (R^2 = 0.9994) for phenol. Using the same conditions and a signal/noise ratio of at least 3, the measured limits of detection for phenol, 4,6-dinitro-*o*-cresol and pentachlorophenol were 2.25 μM (2.81 fmol), 0.87 μM (1.06 fmol) and 1.3 μM (1.6 fmol), respectively. These measured values are of the same magnitude than previously reported for similar devices and analytes.[40,72,73]

3.8 Real sample analysis

The suitability of the CE microchip for measuring low levels of phenolic compounds in real samples was demonstrated by analyzing a water sample and two pharmaceutical formulations. As shown in Fig. 7, no phenols were detected in a local city water sample (Fig. 7A). The three characteristic peaks were observed when the sample was spiked with pentachlorophenol, 4,6-dinitro-*o*-cresol, and phenol (Fig. 7B). Additionally, a clear peak matching with the peak corresponding to phenol was observed for both throat medicines (Fig. 7B and 7C). The identity of these peaks was confirmed by the injection of samples spiked with phenol. An additional two

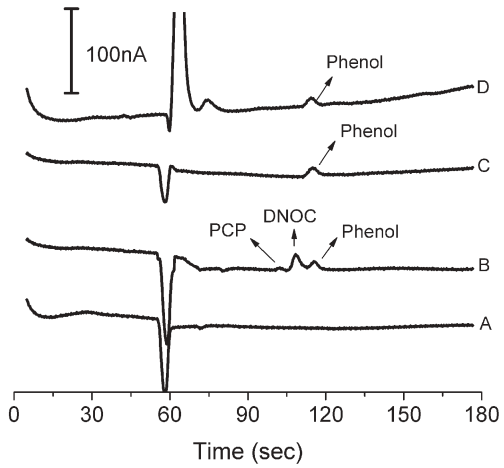

Fig. 7 Electropherograms corresponding to the analysis of a San Antonio water sample (A), a spiked city water sample (final concentrations: 7.9 μM pentachlorophenol, 8.3 μM 4,6-dinitro-*o*-cresol, and 22.6 μM phenol) (B), a dilution of the CVS sore-throat spray (C), and a 1/400 dilution of the sore-throat lozenges (D).

peaks were observed in the lozenge sample, probably corresponding to glucose and flavoring compounds. The results obtained were compared with the declared contents described in their label and represent 99.6 ± 0.5% for the spray and 96 ± 2% for the lozenge.

Conclusions

The utility of PDMS microchips with pulsed amperometric detection for the determination of phenolic compounds has been demonstrated. Such coupling offers great promise for screening of toxic phenolic compounds. Similar to other reports, these results show that solution pH is one of the most critical parameters controlling the separation of phenols by CE.[74] Alkaline conditions are also required to achieve PAD of phenols. Under optimum conditions (5 mM phosphate, pH = 12.4, separation potential = 1200 V, detection potential = 0.7 V, injection time = 10 s) the separation of PCP, DNOC and phenol was achieved in 2 min with the highest signal stability (migration time and peak height) reported to date using CE and electrochemical detection. The negligible waste production compared to current liquid chromatographic protocols results in a very attractive method for the quantification of phenols in environmental and pharmaceutical matrices.

Acknowledgements

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