

## **α-Cyclodextrin Modified Screen Printed Graphite Electrodes for Detection of Phenols**

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A screen printed graphite electrode has been developed for a simple and sensitive determination of phenolic compounds in an aqueous solution. The electrode developed uses a simple and effective screen printing technique with  $\alpha$ -Cyclodextrin( $\alpha$ -CD) modified graphite ink. Phenols were captured on the surface of the  $\alpha$ -CD modified electrode through complex formation. The phenol/ $\alpha$ -CD complex was deposited and quantified electrochemically using cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV). The optimization of the experimental parameters was performed in regard to electrode composition, pH, temperature, sample preconcentration time. Interferences from other organic compounds were investigated. The detection limit for phenols was  $500 \pm 7$  nM for DPV, with the linear range of  $0.5 \mu\text{M} - 25.0 \mu\text{M}$  and  $30 \pm 2$  nM for SWV, with the linear range of  $30 \text{ nM} - 50 \mu\text{M}$ , respectively.

**Keywords :**  $\alpha$ -Cyclodextrin, Phenols, Screen printed electrode, Voltammetry.

### **Introduction**

Phenolic compounds are useful chemicals, but they are toxic starting materials in a broad range of chemical and pharmaceutical manufacturing process.<sup>1</sup> Particularly in the case of coal conversion, phenolic residues are considered an acute environmental problem.<sup>2</sup> Soil and surface water from sites around coal production and processing plants were frequently contaminated by phenols, which may cause considerable pollution of ground water resources. Therefore, great importance has been placed on screening, monitoring, and controlling of these pollutants.<sup>3</sup> However, the requirement of a multi-stepped sample pretreatment of conventional photometric methods<sup>4</sup> demands for more sophisticated and simple analytical techniques. To meet this need, a sensor-based system that is simple to use, inexpensive, disposable and highly sensitive to phenols is becoming increasingly important in environmental analysis. Most sensor-based systems for this purpose have been developed using immobilized enzymes, such as tyrosinase or peroxidase.<sup>5-8</sup>

Screen-printing technology has increasingly been used for the mass production of inexpensive, reproducible and sensitive disposable electrochemical sensors for the determination of trace levels of pollutants and toxic compounds in environmental and biological fluids samples.<sup>9</sup> A disposable sensor has several advantages, such as preventing contamination between samples, constant sensitivity and high reproducibility of different printed sensors.<sup>10</sup> Although there are numerous screen printing inks commercially available, some of them may require a high firing temperature to print. As an example, inks based on noble metals, such as Au, Pt, etc., require a firing temperature of more than 1000 °C. Therefore, apparently, the most useful materials for printing electrochemical sensors could be carbon-based inks. This is because they have a very low firing temperature (20-120 °C) and they can be printed on plastic substrates.<sup>10</sup> Carbon can also be directly mixed with different compounds, such as

mediators and enzymes, and such systems can be easily mass-produced.<sup>9</sup>

Cyclodextrins (CDs) exist in three significant forms:  $\alpha$ -,  $\beta$ - and  $\gamma$ -CDs. CDs have selective interaction sites for the inclusion with guest species in their hydrophobic cavities. The binding forces associated with the CD-guest inclusion formation are attributed to a number of factors, such as hydrophobic forces, hydrogen binding, size of the cavity, shape of the guest molecule, and electrostatic interactions.<sup>11</sup> Some studies for the direct analysis of phenol derivatives using CDs by electrochemical methods have been introduced.<sup>12-16</sup> Matsue *et al.* report the determination of o-nitrophenol in the presence of p-nitrophenol with the addition of  $\alpha$ -CD, using cyclic voltammetric method.<sup>17</sup> Nagase *et al.* developed voltammetric anion responsive sensors based on ordered membrane assemblies containing CD polyamine deposited on a glassy carbon electrode by the Langmuir-Blodgett method.<sup>18</sup> Maheswaran *et al.* investigated for inclusion compounds formed between  $\beta$ -CD and some substituted phenols by the potentiometric method.<sup>19</sup> Kim *et al.* reported the detection of phenol derivatives, using  $\beta$ -CD containing chemically modified electrodes.<sup>20</sup>

In the present study,  $\alpha$ -CD modified screen-printed electrodes were manufactured using  $\alpha$ -CD modified graphite ink for phenol detection in aqueous samples. The electrodes were characterized and optimized with regard to the main experimental parameters affecting the electrode responses, such as composition of ink, pH, deposition time, temperature, and interferences. Determination of phenols was carried out using cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV) at the optimized experimental condition.

### **Experimental Section**

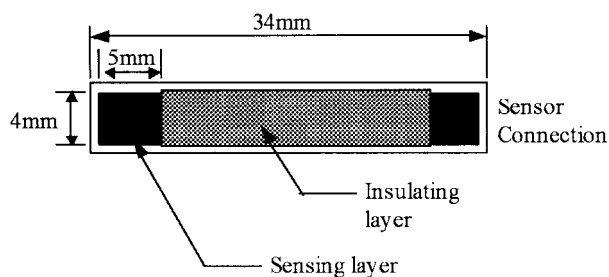
**Reagents.**  $\alpha$ -Cyclodextrin and polyvinyl chloride (PVC) were obtained from Tokyo Kasei Corporation in Japan.

Graphite powder (grade #38) was purchased from Fisher Scientific Corporation. Potassium nitrate ( $\text{KNO}_3$ ), tetrahydrofuran (THF), phenol, cresols and all other chemicals were obtained from Sigma Chemical Corporation. All chemicals used were extra pure grade and used as received. All solutions were prepared using water purified with a Millipore Milli-Q system ( $18 \text{ M}\Omega\text{cm}^{-1}$ , USA).

**Sensor preparation.** The  $\alpha$ -CD modified screen-printed electrodes were manufactured using a graphite-based ink prepared by the following procedure: 10 g of graphite powder was put into a nitrogen purged 50% ethanol solution. The graphite powder was treated in this manner three times then dried in a vacuum oven at  $100^\circ\text{C}$  for 24 hours. The mixtures of  $\alpha$ -CD and graphite powder were prepared in weight ratios of 0, 2, 5, 7, and 10%(w/w) of  $\alpha$ -CD. To print sensors, the graphite inks and screens were manufactured. 0.1 g of the mixture of graphite and  $\alpha$ -CD was mixed with 0.2 mL of 2% PVC dissolved in THF. The screens, which were designed by CAD and manufactured by a local company, had a  $20 \times 20$  cm printable area. The sensing layers of  $\alpha$ -CD modified electrodes were printed on  $1.0 \times 3.4$  cm alumina ceramic plates by hand-printing using the inks, screens and rubber squeezer then dried in a vacuum oven at  $65^\circ\text{C}$  for 24 hours. After drying, electrodes were reprinted for insulation, using a commercial silicon resin epoxy, and dried at room temperature over night. A diagram of the electrode prepared is shown in Figure 1. As can be seen, each electrode consisted of two layers: a sensing and connecting layer and an insulating layer.

**Apparatus.** A Pine Instrument AFRDE4 Series Potentiostat/Galvanostat (USA) was used for voltammetric experiments. Voltammetric measurements were performed using a conventional three-electrode cell with a screen-printed electrode as a working electrode, a  $\text{Ag}/\text{AgCl}$  electrode as a reference electrode and a Pt wire as a counter electrode. Voltammograms were recorded on a X-Y chart recorder (BD90, Kipp & Zonen, Netherlands).

**Measurement procedure.** An aliquot of 100 mM phenol stock solution was prepared daily, using a standard method<sup>21</sup> and was diluted to the desired concentrations with a 2.0 M citric acid/ $\text{NaOH}$  buffer solution. The preconcentration of samples was accomplished by immersing the electrode into a stirred 30 mL of the sample solution for desired time 5 to 20 min. After preconcentration, the electrodes were removed from the solution and rinsed thoroughly with the buffer then

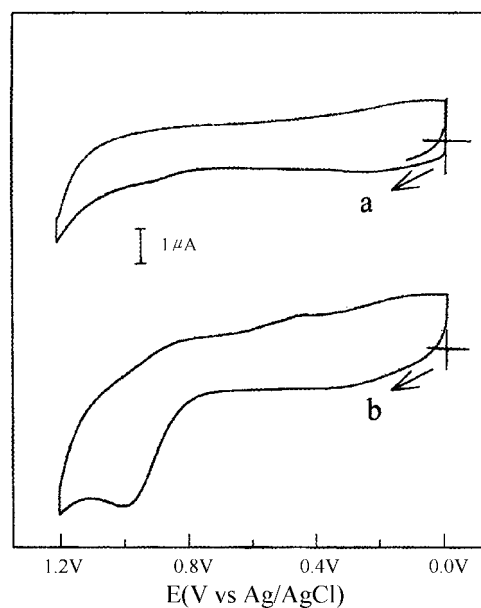


**Figure 1.** A representative diagram of  $\alpha$ -CD modified SPE, not to scale

immediately transferred into a separate measuring solution containing a supporting electrolyte ( $0.1 \text{ M KNO}_3$ ) only. Cyclic voltammograms were obtained at potentials scanning from  $0.0 \text{ V}$  to  $+1.2 \text{ V}$  at a scan rate of  $100 \text{ mVs}^{-1}$ . A series of voltammetric experiments such as, DPV and SWV, were carried out. DPV was performed at potentials sweeping from  $0.6 \text{ V}$  to  $1.2 \text{ V}$  at a scan rate of  $20 \text{ mVs}^{-1}$  at the following parameters: pulse repetition time ( $\tau$ ) was 200 ms, pulse width ( $t_p$ ) was 50ms and change in pulse potential ( $\Delta E_p$ ) was 50 mV. SWV was also carried out at potentials sweeping from  $0.6 \text{ V}$  to  $1.2 \text{ V}$ , where  $\tau$  was 4 ms,  $\Delta E_p$  was 25 mV and the step height ( $\Delta E_s$ ) was 4 mV.

## Results and Discussion

**Electrochemical detection of phenols.** Cyclic voltammograms recorded for a bare SPE without  $\alpha$ -CD showed no significant differences between CVs recorded in a  $0.1 \text{ M KNO}_3$  solution after 10 min preconcentration in the blank electrolyte solution and a  $20 \mu\text{M}$  phenol solution (pH 3.0, CVs are not shown). Although a previous study using  $\beta$ -CD modified carbon paste electrodes (CPEs) by Kim *et al.*<sup>20</sup> demonstrated that  $\beta$ -CD itself can produce an anodic peak near  $+0.5 \text{ V vs Ag}/\text{AgCl}$ , in the present system, the peak was not detected. The CVs in Figure 2 were recorded using the modified SPE printed using ink containing 7%  $\alpha$ -CD with respect to graphite in weight percent. Figure 2a is CV recorded for the modified electrode in a  $0.1 \text{ M KNO}_3$  solution, followed by transferring the electrodes from a blank buffer solution without phenol. Figure 2b is the CV recorded for the modified electrode in a  $\text{KNO}_3$  solution, followed by the preconcentration in  $20 \mu\text{M}$  phenol solution. As can be seen in Figure 2b, a well-defined anodic peak

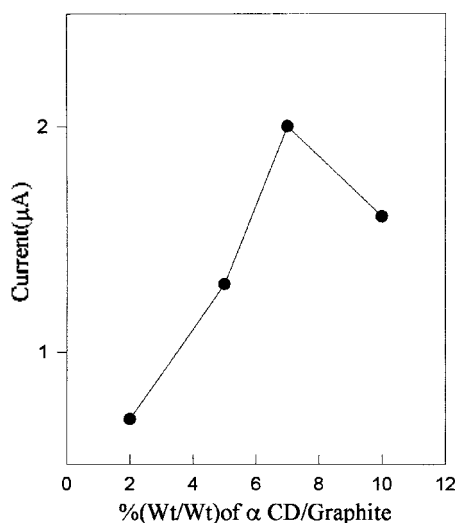


**Figure 2.** Cyclic voltammograms of phenol in a  $0.1 \text{ M KNO}_3$  solution after 10 min preconcentration in  $20 \mu\text{M}$  phenol containing solution using (a) bare and (b)  $\alpha$ -CD modified SPE. Potential scanning range was from  $0 \text{ V}$  to  $1.2 \text{ V}$  at a scan rate of  $100 \text{ mVs}^{-1}$ .

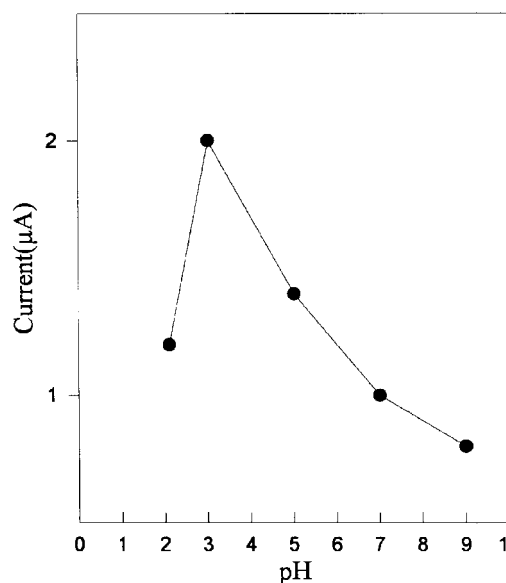
appeared with a peak current of +0.9 V vs. Ag/AgCl, whereas the CV recorded for an undeposited SPE shows no significant current responses (Figure 2a). This result suggests that active sites of  $\alpha$ -CD modified formed a complex with phenol during pre-deposition and produced the anodic peak shown in Figure 2b. The complex formation of  $\alpha$ -CD with phenolic compounds and their electrochemical determination is a well-known mechanism that has been described in previous studies.<sup>12-16</sup> Therefore, the present system seems suited for the analysis of phenols.

**Electrode optimization.** To determine the optimum composition ratio of  $\alpha$ -CD to graphite in ink, the response of the electrode prepared in different compositions of  $\alpha$ -CD and graphite powder, in ratio of 1-10%(wt/wt)  $\alpha$ -CD, was evaluated. The anodic peak current ( $i_p$ ) of each electrode was measured at 0.9 V vs. Ag/AgCl in 0.1 M KNO<sub>3</sub> after a 10 min pre-concentration in a 20  $\mu$ M phenol solution (pH 3.0). As can be seen in Figure 3, the anodic peak current gradually increased as the  $\alpha$ -CD content increased, reaching a maximum response at 7%(wt/wt)  $\alpha$ -CD. After reaching the maximum value, the peak current decreased. This suggests that the decline of the current responses may be caused by an increase in electrode resistance due to the large amount of  $\alpha$ -CD compared with graphite content. Thus, 7%(wt/wt)  $\alpha$ -CD was chosen as the optimum composition, and all the electrodes used in this study were manufactured to meet this specification.

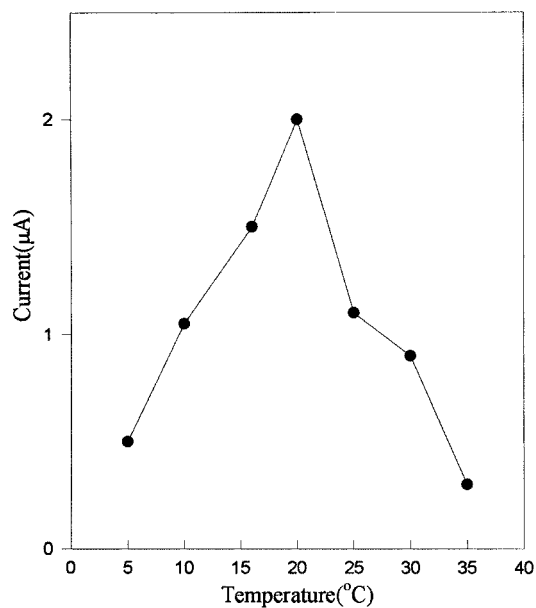
The pH and temperature dependency of the anodic peak currents of  $\alpha$ -CD modified were assessed. As shown in Figure 4, the optimum pH of the pre-concentration solution was assessed to be pH 3.0. The dependency of the anodic peak height on the temperature of the pre-concentration solution is shown in Figure 5. The CVs were recorded in a 20  $\mu$ M phenol solution (pH 3.0) at a deposition temperature ranging from 5  $^{\circ}$ C to 35  $^{\circ}$ C. Maximum current response was



**Figure 3.** Plot for the peak current vs. weight ratios (%w/w) of the  $\alpha$ -CD and graphite in ink. The concentration of phenol was 10  $\mu$ M, the pre-concentration time was 15 min at pH 3.0 and scan rate was 100 mV/sec.



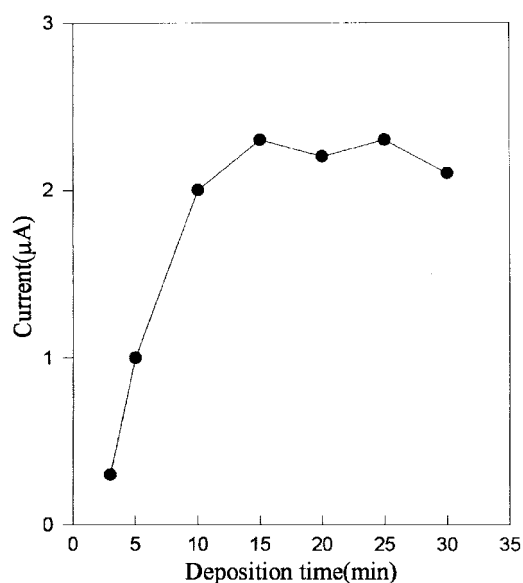
**Figure 4.** Peak current responses of  $\alpha$ -CD modified SPE to 20  $\mu$ M phenol, as a function of pH



**Figure 5.** Peak current responses of  $\alpha$ -CD modified SPE to 20  $\mu$ M phenol, as a function of temperature.

obtained at 20  $^{\circ}$ C. These combined results revealed that the inclusion complex formation between phenol and  $\alpha$ -CD effectively occurred at pH 3.0 and 20  $^{\circ}$ C. This is due to the instability of CDs in a strongly acidic medium. Although CDs are stable in basic aqueous solution the reactivity of CDs increases in acidic condition.<sup>21</sup> This also indicates that the phenol/ $\alpha$ -CD complex formed has lower stability in weak acidic conditions at higher temperature because of the deprotonation of the CDs. Thus, all measurements for the determination of phenol were performed at pH 3.0 and at 20  $^{\circ}$ C.

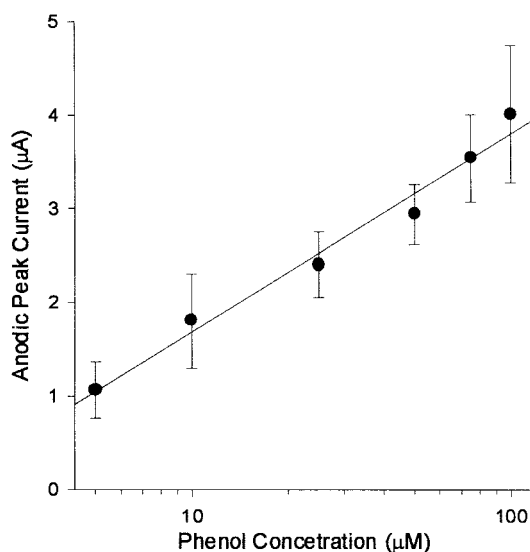
The effect of pre-concentration time on the change in the



**Figure 6.** The plot of anodic peak current obtained for 50  $\mu\text{M}$  phenol vs. the preconcentration time. pH of the preconcentration solution was 3.0 and scan rate was 100 mV/sec.

peak current was obtained using a 50  $\mu\text{M}$  phenol solution (pH 3.0), according to the preconcentration time between 3 and 30 min. As can be seen in Figure 6, increasing the deposition time led to a larger anodic peak current at the given concentration until the time reached 15 min. The elevation of the peak current slowed and reached a plateau after 15 min. The reason for this could be that the active sites of  $\alpha$ -CD modified were fully saturated by the phenol after 15 min preconcentration.

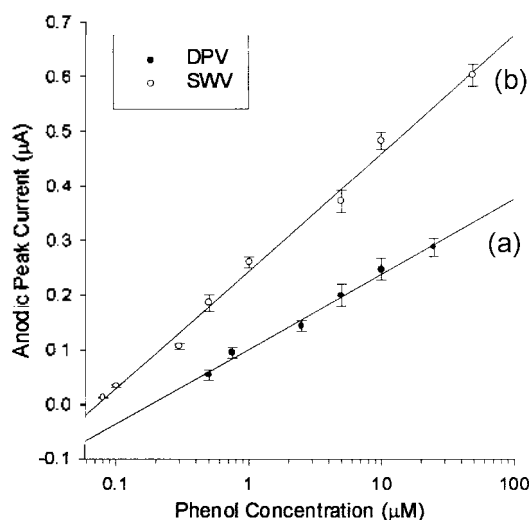
**Electrode calibration for phenol analysis.** The calibration plots for phenol were obtained from voltammograms recorded using CV, DPV, and SWV as shown in Figure 7. The equation of the regression line of the plot obtained using CV was determined to be  $I(\mu\text{A}) = 2.11 \times \log C(\mu\text{M}) - 0.43$



**Figure 7.** Calibration curve for phenol obtained using CV.

( $r^2 = 0.98$ ). Responses to phenol could be detected at a concentration as low as  $5.0 \pm 0.4$ ,  $\mu\text{M}$  using CV. The responses show good linearity up to phenol concentration of 100  $\mu\text{M}$ . All the detection limits of the electrodes in the present study were taken as six times the standard deviation of the current change due to the addition of a blank solution. The reproducibility of the electrode was investigated between the concentrations of 10  $\mu\text{M}$  and of 100  $\mu\text{M}$  phenol. The electrode performance demonstrated the maximum %CV (coefficient of variation) of 17.7% across the concentration range. Calibration plots for phenol, using DPV, are shown in Figure 8a. Each point represents the mean value of five measurements and the error bars represent standard deviation. In the curve obtained, the equation of the regression line was calculated to be  $I(\mu\text{A}) = 0.14 \times \log C(\mu\text{M}) + 0.10$  ( $n = 5$ ,  $r^2 = 0.99$ ) over the phenol concentration from 0.5  $\mu\text{M}$  to 50  $\mu\text{M}$ . The current responses were linear up to 25  $\mu\text{M}$  with good reproducibility (%CV < 10.5%), and the detection limit was  $0.50 \pm 0.07$   $\mu\text{M}$ . From the results, the best performance was determined in the case using SWV. Curve b in Figure 8 gives the equation of the regression line, which was calculated to be  $I(\mu\text{A}) = 2.11 \times \log C(\mu\text{M}) - 0.24$  ( $n = 5$ ,  $r^2 = 0.99$ ) over the phenol concentration from 30 nM to 50  $\mu\text{M}$ , and the detection limit was  $30 \pm 2$  nM. Although the sensitivities of the electrode obtained from DPV (slope in Figure 8a = 0.14) and SWV (slope in Figure 8b = 0.21) are lower than 10% of the value obtained from CV (slope in Figure 7 = 2.11), the values of reproducibility of the electrode obtained from DPV (%CV < 10.5%) and SWV (%CV < 6.5%) show much greater performance than the value obtained from cyclic voltammetry (%CV < 17.7%) with lower detection limits as described. The performance characteristics of the electrodes obtained using CV, DPV, and SWV are summarized in Table 1.

**Detection of other phenolic compounds and interferences.** Because of its higher performance characteristics, SWV was used for the detection of other common phenolic



**Figure 8.** Calibration curves for phenol obtained using DPV (a) and SWV (b).

**Table 1.** Performance characteristics of  $\alpha$ -CD modified SPE using CV, DPV, and SWV

Method of voltammetry	CV	DPV	SWV
Detection limit	$5.0 \pm 0.4 \mu\text{M}$	$500 \pm 7 \text{nM}$	$30 \pm 2 \text{nM}$
Linear range	$5.0\text{-}100.0 \mu\text{M}$	$0.5\text{-}25.0 \mu\text{M}$	$30 \text{nM}\text{-}50 \mu\text{M}$
Reproducibility (%CV)	< 17.7%	< 10.5%	< 6.5%

compounds, *p*-nitrophenol and *o*-, *m*-, *p*-cresol. Voltammetric responses to each analyte were assessed and calibration plots for each analyte were obtained. The equations of the regression lines were calculated for all phenolic compounds over concentrations ranging from 0  $\mu\text{M}$  to 25  $\mu\text{M}$ . All cresols show higher sensitivities compared with phenol:  $275 \pm 12\%$  for *o*-cresol,  $150 \pm 8\%$  for *m*-cresol, and  $135 \pm 6\%$  for *p*-cresol. However, the electrode showed only one third of the relative responses for *p*-nitrophenol ( $34 \pm 3\%$ ). To investigate interference from other organic compounds, the peak currents from the aliquots of 25  $\mu\text{M}$  for each interference was assessed using SWV and compared with the value obtained from the same concentration of phenols. The result shows that some chemicals such as ascorbic acid ( $81 \pm 5\%$ ) and cyclohexanol ( $79 \pm 4\%$ ), are highly responsive, all other organic compounds examined, showed less than 22% of the relative responses: benzoic acid ( $10 \pm 1\%$ ), benzaldehyde ( $9 \pm 1\%$ ), benzylalcohol ( $15 \pm 1\%$ ), hippuric acid ( $15 \pm 2\%$ ), and 2-methylcyclohexanol ( $22 \pm 1\%$ ).

The storage stability of the electrodes was evaluated by tracing the sensitivity of the electrodes response to 10  $\mu\text{M}$  phenol during extended use.  $\alpha$ -CD modified SPEs were stored at room temperature in the dark and subjected to week-by-week measurements. Electrodes showed a slight decrease in sensitivity after three-weeks-storage. However, all the electrodes showed that more than 91% of their initial sensitivity was retained after 60 days.

### Conclusion

$\alpha$ -CD modified SPEs for incorporation into compact in-field analytical devices have been developed for the detection of phenols in water resources. The simple screen-printing technique and the effective  $\alpha$ -CD immobilization procedure, using direct formulation into graphite ink, allow inexpensive and disposable electrode manufacturing, and can readily be applied to mass-production. A new concept for a non-enzyme electrode for phenol detection with high sensitivity and the lower nanomolar range detection limit

achieved high stability and easy storage compatibility. These combined results suggest that the system can be applied to high sensitivity monitoring of phenols in environmental samples. We are investigating the application of this approach to clinical analytes, such as epinephrine, dopamine, and paracetamol, and minimizing interference from some organic contaminants as well as integrated format of electrode designs-miniaturized all-in-one format, including reference and counter electrodes.

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