

Diagnostic Assay of Phenol Ions in Human Tissue

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Abstract : A voltammetric assay of phenol ions was investigated using three electrode systems of graphite pencil working, reference and counter electrodes. Under optimum analytical parameters, square wave stripping working ranges were attained at a mili range of 10~80 mg/L and a micro range of 20~90 ug/L using seawater electrolyte. The developed sensor was applied to tap water and the human body system of a smoker. It was found that the methods can be applied to *in vivo* fluid or medicinal diagnosis.

Keywords : Phenol, voltammetry, Human body, Diagnosis, seawater

1. INTRODUCTION

Phenol is a pollutant that is widely found in water and in the atmosphere as well as in chemical productions. This compound is an object of interest in many applications including environmental, clinical neurochemistry[1,2] and food processes[3]. Considering biologically active molecules[4] and their toxicity and persistency, many methods for spectrophotometry and separation techniques have been developed. These include gas chromatographic mass spectrometry[5], piezoelectric crystal sensor[6], capillary electro-chromatography[7], coupled column HPLC with fluorimetric detection[8], electro catalytic oxidation[9], FTIR studies [10], SPME GC MS analysis[11], GC MS[12]

and LC FD. However, these are not useful for nano range and *in vivo* detection. Recently, simple and sensitive voltammetric methods have also been developed, such as glassy carbon disk electrode[13], zeolite graphite composite electrodes[14], gas diffusion electrode[15], boron-doped diamond electrode[16,17], sodium montmorillonite anthraquinone chemically modified glassy carbon electrode[18], oxidized multiwalled carbon nanotubes[19] and mixed binder carbon paste electrode[20]. Some electrodes are expensive and require difficult modification. Moreover, all the electrochemical methods are used with expensive Ag/AgCl reference and counter platinum wire type electrodes, respectively. In this study, low cost graphite electrodes(GE) were used as working, reference and counter electrodes, which successfully attained low detection ranges. Also, the acid and base electrolyte is

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expensive for electrochemical reagent solutions and can contaminate the water systems. In this paper, sea water was used as the electrolyte solution. Seawater is inexpensive and is widely available for environmental considerations.

2. EXPERIMENTAL PROCEDURE

2.1. Systems, Reagents, Electrode Preparation

Electrochemical instruments were used with the new Bioelectronics-1 system, which was first constructed at the authors' institute. The new version is a computerized handheld voltammetric system with a 2.4 V potential range, a 2 mA current range, a 10 pico A measuring current, and uses a rechargeable battery or external power and has a USB port interface with a computer. The instrument's size is similar to that of a typical cellular phone and can be used for the bio assay and sensor techniques for individual and laboratory applications. The graphite (DongA XQ ceramic 60mm 0.9B) GE was used as the working electrode, reference electrode and auxiliary electrode. All chemical reagents used were of standard grade, and the electrolyte solution was used with deep sea water.

3. RESULTS AND DISCUSSION

First, sea water is used as the electrolyte blank solution. Three graphites were used as the working, reference and counter electrodes. With the sea water electrolyte, no peak and linear curves were obtained. Thus, the voltammetric peak potential was determined using the CV(cyclic voltammetry) variation by anodic and cathodic scans with a conc phenol ion. Figure 1 (A) shows the CV effect using GE and variation from 0 to 50 mg/L additional. Peak current appeared at 0.5 V.

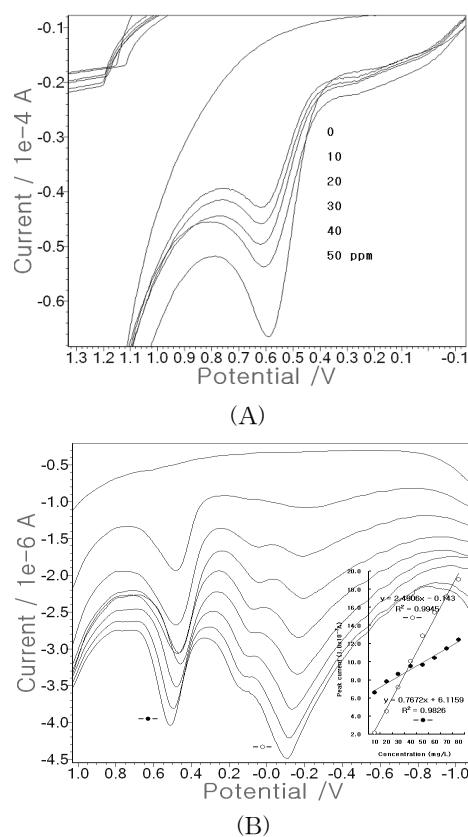


Fig. 1. (A) CV for phenol variations from 0–50 mg/L using PE, $-2.0\sim 2.0$ V potential range, 0.5 mV/s scan rate. (B): SW for phenol varied in range from 0–80 mg/L using graphite three-electrode cell under optimum parameters.

The current obtained 2.141 , 3.200 , 1.229 , 1.929 and 1.279×10^{-5} A under oxidation scan and did not appear in the reduction scan. Here, the anodic SW(square wave voltammetry) was performed with the same direction. Figure.1(B) shows the SWeffects using GE variation of 0–80mg/L additional, under 30sec accumulation times. The peak current increased from 8.331 to 12.410×10^{-7} A. The peak height was sensitive and -0.1 V peak potential was obtained linearly. Both equations were of $y=2.4906x-0.143$, and the precision was $R^2=0.9945$. At $y=7672x+6.1159$,

the precision was $R^2=0.9826$. Both curves can be applied to the analytical application.

3.1. SW Optimizations of FPE

Figure 2(A) shows the peak current in the 10 mg/L phenol concentration as a function of the varying square wave amplitude for the ranges of 0.005~0.04V. In this study, two peaks appeared. When 0.005, 0.01, 0.015, 0.02, 0.025, 0.03, 0.035 and 0.04V amp were given, one peak current obtained 1.864, 3.770, 5.839, 7.861, 9.791, 10.960, 13.530 and 13.900×10^{-6} A. The other peak current obtained 1.353, 3.419, 5.814, 8.211, 10.63, 12.9, 16.12 and 18.39×10^{-6} A. The peak current went up as the higher amplitude was given. When 0.04V amp was given, the maximum current was obtained. Therefore, 0.04V amp was chosen as an optimum condition. Under the second conditions, Figure 2(B) shows the peak current in 10mg/L phenol concentration as a function of the square wave frequency for 8 points. 15, 20, 25, 30, 35, 40, 45 and 50Hz were given. One peak current obtained 1.524, 1.712, 1.667, 1.636, 1.605, 1.582, 1.505, 1.549×10^{-5} A and the other peak current obtained 1.865, 2.003, 2.336, 2.628, 2.692, 2.554, 2.541, 2.604×10^{-5} A. The peak current increased from 15V to 35V amp. However, it started to drop from 40V amp. The maximum current was obtained at 35Hz. It was chosen as an optimum condition. Under the separameters, Figure 2(C) illustrates the accumulation time effects of 30~240s variations. When 30, 60, 90, 120, 150, 180, 210 and 240s were given, peak current obtained 2.167, 2.410, 2.712, 2.927, 3.119, 3.289, 3.458 and 3.652×10^{-5} A. The other obtained 3.164, 3.473, 3.816, 3.996, 4.042, 4.049, 4.03 and 4.086×10^{-5} A. The peak current increased slightly as more time was given. Therefore, 240s was chosen as an optimum condition. Under these conditions, analytical working ranges were examined.

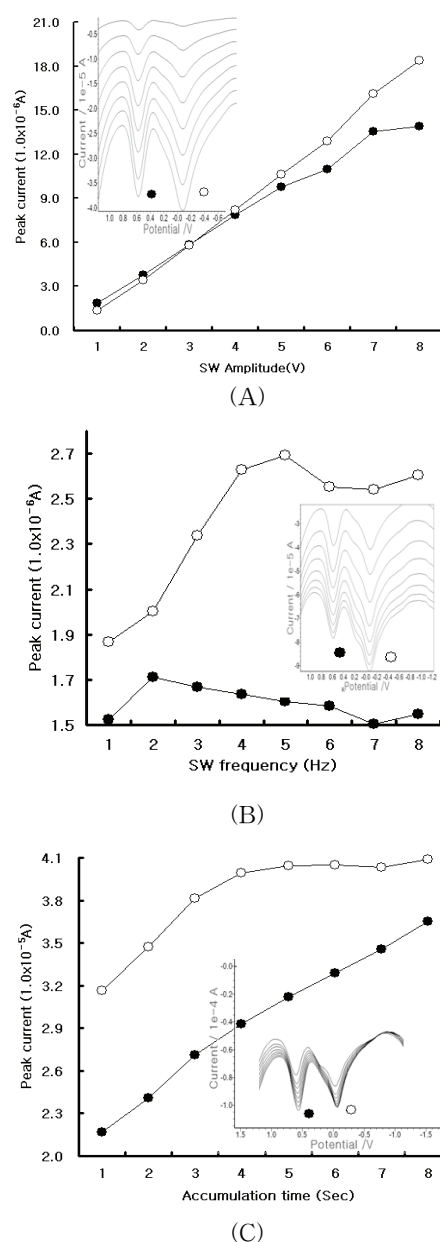


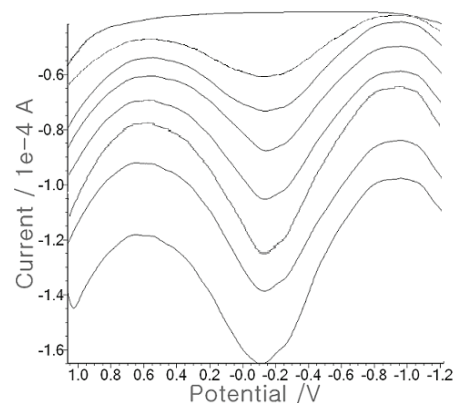
Fig. 2. (A) Variations in the SW amplitude range for 0.005, 0.01, 0.015, 0.02, 0.025, 0.03, 0.035 and 0.04 V. (B) SW frequency variations of 15, 20, 25, 30, 35, 40, 45 and 50 Hz for the 10 mg/L phenol ion add. (C) SW accumulation time range for 30, 60, 90, 120, 150, 180, 210 and 240 s. Using sea water electrolyte.

3.2. Analytical Working Range and Statistics

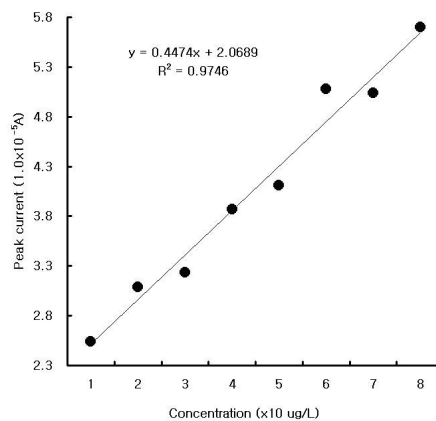
Under optimum SW conditions, various working ranges were examined using anodic stripping. The final results were obtained in milligrams and microgram ranges. Figure 3(A) shows the SW result in the micro variation from 10 to 80 ug/L. When 20, 30, 40, 50, 60, 70, 80 and 90 ug/L was spiked, the peak current obtained 2.542, 3.089, 3.237, 3.872, 4.107, 5.076, 5.038 and 5.697×10^{-5} A. The more phenol was spiked, the deeper the curve was. Figure 3(B) shows the linear equation of the SW result. The curve increased linearly. The equation of the SW statistic was $y = 0.447x + 2.068$ and the precision was $R^2 = 0.974$. This result means that GE is highly sensitive in detecting phenol despite the low concentration. These ranges can be applicable to *in vivo* or *in vitro* assay.

After achieving the optimum condition, the analytical application was performed on the nails of a smoker and a nonsmoker. 0.026g nail was dissolved in conc 5 ml nitric acid. Subsequently, it was diluted with distilled water. Figure 4(A) shows the SW result of the linear equation. The black circle represents the smoker's nail. The linear equation was $y = 58.16x + 20.064$ and the precision was $R^2 = 0.9836$. The white circle represents the SW result of the nonsmoker's nail. The linear equation was $y = 52.202x - 0.4042$ and the precision was $R^2 = 0.8585$. The black smoker was more concentrated compared to that of the nonsmoker. This indicates that phenol is detected in the smoker's cell. Figure 4(B) shows the SW voltammograms of the smoker's nail. When 0.1 mg/L of the sample solution was added, a peak current of 5.78×10^{-6} A was obtained. When 0.2, 0.3, 0.4 and 0.5mg/L standard was spiked, it obtained 10.99, 5.81, 18.68 and 23.63×10^{-6} A peak current. It can be applicable to *ex vivo* detection. More advanced application was performed in pond water. Figure 4(C) shows

the SW standard addition methods. In the blank solution, no peak was obtained, then water sample of 1ug/L was added and the peak current of 1.143×10^{-6} A was obtained. Under this condition, the standard was added, obtaining 7.767, 11.45 and 13.79×10^{-6} A. The peak current increased linearly. This result shows that phenol exists in this water and can be used in low cost *in vivo* diagnosis.

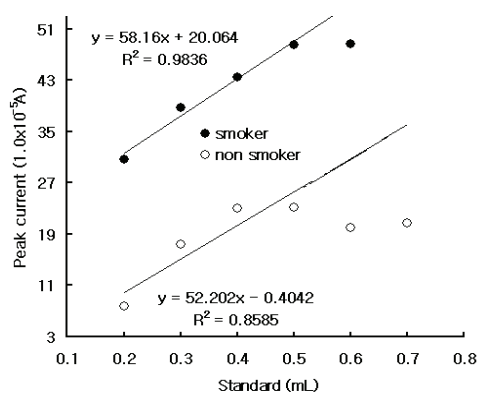


(A)

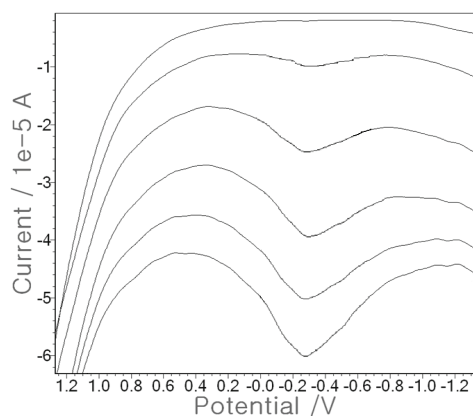


(B)

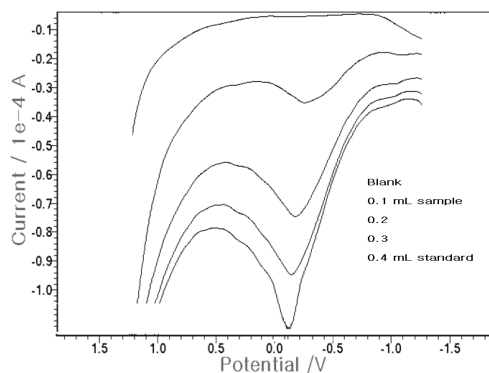
Fig. 3. (A) Analytical working ranges of 0, 20, 30, 40, 50, 60, 70 and 80 ug/L phenol ion added using PE. (B): The linear curve of the SW results in low concentration with optimum parameters.



(A)



(B)



(C)

Fig. 4. (A): Application to smoker's and nonsmoker's nail comparison with SW. (B): The smoker's SW voltammograms. (C) Application to tap water.

4. CONCLUSION

Results of the experiment under the optimum condition are as follows: 0.005 V increment, 0.04 V amplitude, 35 Hz frequency, -1.9 V initial potential, 1.6 V final potential and 240s accumulation time. These conditions were applied in the detection of low concentration and smoker's nail and pond water. Despite the use of inexpensive and renewable PE as the working, reference and auxiliary electrodes, the setup was found to detect micro and nano ranges. It also used seawater instead of acid and base electrolyte for environmental considerations.

References

1. H. Yi, K. Wu, S. Hu and D. Cui, Adsorption stripping voltammetry of phenol at Nafion-modified glassy carbon electrode in the presence of surfactants, *Talanta* 55, 1205-1210(2001).
2. R. M. de Carvalho, C. Mello, Lauro T. Kubota, Simultaneous determination of phenol isomers in binary mixtures by differential pulse voltammetry using carbon fibre electrode and neural network with pruning as a multivariate calibration tool, *Analytica Chimica Acta* 420, 109-121(2000).
3. Silvana Andreescu, Daniel Andreescu, Omowunmi A. Sadik, A new electrocatalytic mechanism for the oxidation of phenols at platinum electrodes, *Electrochemistry Communications* 5, 681-688(2003).
4. M. Saeed, M. Depala I, D. H. Craston and I. G. M. Anderson, Application of Capillary Electrochromatography (CEC) for the Analysis of Phenols in Mainstream and Sidestream Tobacco Smoke, *Chromatographia*, 49(7/8), 391-398(1999).
5. E. Baltussen, F. David, Pat Sandra, H.-G. Janssen, C. Cramers, Automated

- Sorptive Extraction-Thermal Desorption-Gas Chromatography-Mass Spectrometry Analysis: Determination of Phenols in Water Samples, *J. Microcolumn Separations*, **116**, 471-474(1999).
6. T. N. Ermolaeva, T. L. Lavrent'eva, A. E. Seredkin and Ya. I. Korenman, A Sensitive Piezoelectric Crystal Sensor for Analyzing Phenols in Air, *Russian Journal of Applied Chemistry*, **74**(2), 1993204(2001)
 7. V. Andrei, O. Pirogov and A. Shpigun, Application of water-soluble polymers as modifiers in electrophoretic analysis of phenols, *Electrophoresis*, **24**, 2099-2105(2003).
 8. G. Marrubini, E. Calleri, T. Coccini, A. F. Castoldi and L. Manzo, Direct Analysis of Phenol, Catechol and Hydroquinone in Human Urine by Coupled-Column HPLC with Fluorimetric Detection, *Chromatographia*, **62**, July 25-31(2005).
 9. Y.J. Feng and X.Y. Li, Electro-catalytic oxidation of phenol on several metal-oxide electrodes in aqueous solution, *Water Research* **37**, 2399-2407(2003).
 10. M. Pakuła, M. Walczyk, S. Biniak and A. S. tkowski, Electrochemical and FTIR studies of the mutual influence of lead(II) or iron(III) and phenol on their adsorption from aqueous acid solution by modified activated carbons, *Chemosphere*, **69**, 209-219(2007).
 11. N. G. Simoes, V. V. Cardoso, E. Ferreira, M. J. Benoliel and C. M. M. Almeida, Experimental and statistical validation of SPME-GC-S analysis of phenol and chlorophenols in raw and treated water, *Chemosphere* **68**, 501-510(2007).
 12. S. C. Moldoveanu and M. Kiser, Gas chromatography/mass spectrometry versus liquid chromatography/fluorescence detection in the analysis of phenols in mainstream cigarette smoke, *Journal of Chromatography A*, **1141**, 90-97(2007).
 13. D. A. Palnero and J. V. Arean, Analysis of Phenol in an Over-the-Counter Sore Throat Spray by Cyclic Voltammetry, *Journal of pharmaceutical sciences*, **90**, 2, 165- 171(2001).
 14. R.H. Carvalho, M.A.N.D.A. Lemos, F. Lemos, J.M.S. Cabral, F and R. Ribeiro, Electro-oxidation of phenol on zeolite/graphite composite electrodes Part 2. Influence of zeolite type and composition, *Journal of Molecular Catalysis A: Chemical*, **253**, 170-75(2006).
 15. H. Wang, J. Wang, Electrochemical degradation of 4-chlorophenol using a novel Pd/C gas-diffusion electrode, *Applied Catalysis B: Environmental*, **77**, 58-5(2007).
 16. J. Iniesta, P.A. Michaud, M. Panizza, G. Cerisola, A. Aldaz,Ch. Comminellis, Electrochemical oxidation of phenol at boron-doped diamond electrode, *Electrochimica Acta*, **46**, 3573-3578(2001).
 17. M. A. Ghanem, R. G. Comptob, B. A. Colesb, E. Psillakis, M. A. Kulandainathan and F. Marken, Microwave activation of electrochemical processes: High temperature phenol and triclosan electro-oxidation at carbon and diamond electrodes, *Electrochimica Acta*, **53**, 1092-1099(2007).
 18. S. Hu, C. Xu, G. Wang and D. Cui, Voltammetric determination of 4-nitrophenol at a sodium montmorillonite-anthraquinone chemically modified glassy carbon electrode, *Talanta*, **54**, 115-123(2001).
 19. X. Liu, Y. Ji, Y. Zhang, H. Zhang , M. Liu, Oxidized multiwalled carbon nanotubes as a novel solid-phase micro extraction fiber for determination of phenols in aqueous samples, *Journal of Chromatography A*, **1165**, 10-17(2007).
 20. Z. Q. Zhang, H. Liu, H. Zhang and Y. F. Li, Simultaneous cathodic stripping voltammetric determination of mercury, cobalt, nickel and palladium by mixed binder carbon paste electrode containing dimethylglyoxime, *Analytica Chimica Acta*, **333**, 119-124(1996).