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ELECTROCHEMICAL PREANODIZATION OF GLASSY CARBON ELECTRODE AND APPLICATION TO DETERMINE CHLOROAMPHENICOL

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Abstract

This paper suggested a method to enhance the performance of carbon electrodes for the determination of chloramphenicol (CAP). The sensitivity and the reproducibility of the carbon electrodes could be enhanced easily by electrochemical pretreatment. Some kinds of carbon material were studied including glassy carbon, graphite carbon and pyrolytic carbon. Numerous kinds of supporting electrolyte have been tried. For glassy carbon electrode, the acidic solution, H_2SO_4 5mM, resulted in best performance at pretreated voltage of +2.1V (vs. Ag/AgCl) in duration of 250 second. However, for graphite and pyrolytic carbon electrodes, the phosphate buffer solution pH 6.0 gave the best performance at +1.7V (vs. Ag/AgCl) in duration of 20 seconds. The detection limit could be at very low concentration of CAP: 0.8ng/ml for glassy carbon electrode, 3.5ng/ml for graphite carbon electrode. The method was successful applied to aqua-agriculture water sample and milk sample with simple extraction as well as direct ointment sample analysis.

Keywords : Chloramphenicol, electrochemical pretreatment, voltammetry, glassy carbon electrode

Introduction

CAP is a wide-spectrum antibiotic used for domestic animals, poultry, as well as aqua-agriculture farming. Because of its risk to cause cancer, it has been banned in food-producing animals. According to EU commission decisions, the determination of CAP has to be *screening; identifying and quantifying* with complex and strict protocol have to be obeyed [2002/657/EC, 2002]. This requires LG/MS or GC/MS as unique analytical methods literated in references [Sandra Impens 2003, Maciej J. Bogusz 2004, Hao-Yu Shen 2005, Santos, Lucia 2005]. However, these methods are very time-consumed and expensive ones which are not suitable for routine check in farm and food-producing factories, especially in developing countries. Voltammetric method has been known world-wide due to its low cost instrument, simple sample preparation, and available for routine check. The problem accompanied with the technique is the usage of mercury electrode. Voltammetric assay using mercury electrode for CAP was described elsewhere [J.J. V. Der Lee, 1980]**.** However, the voltammetric assay for CAP on carbon electrodes just gains poor detection limit [M. Feng 1998, L. Agüí 2002].

Normal carbon electrodes are not sensitive. In order to improve this, surface pretreatment seem to be the most essential matter. Chemical pretreatment [R. J. Taylor, 1973], vacuum heating [D. T. Fagan, 1985], heating at low pressure [K. J. Stutts, 1983], and laser irradiation [E. Hershenhart, 1984]… can be used. Another simple method of surface pretreatment is electrochemical pretreatment [R.C. Engstrom 1982, A. L. Beilby 1995, T. Nagaoka 1986, R. C. Engstrom 1984, A. Dekanski 2001, D. Jürgen, E. Steckhan 1992]. It just simply requires treating the carbon electrode at high potential in a given solvent and electrolyte. Such electrochemical pretreatment generates surface functional groups like phenol, carboxyl, carboxylic... These functional groups are believed to be the mediators to speed up electron transfer rate so enhance the sensitivity and reproducibility of the electrode. The mechanism of electrochemical pretreatment was proposed by A. Dekanski with three-stage model [A. Dekanski, 2001].

Our current work is to use electrochemical pretreatment to facilitate the determination of CAP on carbon electrodes by voltammetry. We set out to investigate different types of carbon material such as glassy carbon, graphite carbon, and pyrolytic carbon in order to get the overall picture of electrochemical applications of each,

Experimental

Chemicals and Reagents

All chemicals were analytical grade, organic solvents were HPLC grade, a stock solution of CAP (Aldrich-Sigma) 1000mg/l was prepared by dissolving the solid compound in acetonitril (ACN). Standard solution was prepared by serial dilution of the stock solution with ACN. Stock solution of CH_3COOH 1.0M, H_3PO_4 1.0M, H_2SO_4 1.0M, NaOH 1.0M, $LiClO_4$ 1.0M were prepared in distilled water.

Instruments

Electrochemical pretreatment and voltammetric analysis were done with PAR 264 potentiostat, Ag/AgCl saturated KCl (Metrohm EA285) was used as reference electrode, glassy carbon electrode was 6.0mm i.d.; graphite carbon was 3.0mm i.d; pyrolytic carbon electrode was prepared from graphite tube used for ET-AAS method, both new and disposal tube were used. The tube was broken; a spice was sharpened into 1.0 x 3.0 mm shape, and then made one head of the electrode connect to copper wire; finally the carbon part was placed in clinical syringe, the copper wire was made contact to metal connector to rotating electrode. All the electrodes were polished on cotton cloth with slurry Al_2O_3 powder 0.3 µm until bright surface, and then washed with a stream of double distilled water to remove any remains of Al_2O_3 particles.

Electrode pretreatment

10ml of electrolyte was bubbled with pure nitrogen in 10 minute to remove oxygen, during all experiments, a stream of nitrogen was covered the surface to protect the solution from oxygen in air. The electrode was held at +2.1V in 250 seconds for glassy carbon electrode, or +1.7V in 20 seconds for graphite and pyrolytic carbon electrode. After that, the solution was bubbled again to remove the generated oxygen in 2 minutes and the electrodes were ready for analysis.

Result And Discussion

Electrochemical pretreatment

The electrodes with different carbon types were preanodized at potentials from 1.7V to 2.5V (vs. Ag/AgCl). For each electrode, several electrolytes with pH ranging from acidic to basic were tried to find out the best match for each. The sensitivity of reduction peak of CAP was used to evaluate the improvement of the pretreatment procedure. The result showed that glassy carbon electrode needed strong acidic solution (pH = 2) with fairly high potential (+2.1V) to get the best sensitivity. However, graphite carbon electrode, and pyrolytic carbon electrode required fairly low potential (+1.7V) in neutral media (pH = 6.0). After preanodization, the signal of CAP on each electrode was facilitated due to the adsorption behavior (Fig. 1).

In addition, from the figure 1, the preanodization process also raised the background current. This was noted before because of the formation of non-conductive graphite oxide film on the surface of carbon electrode [R. C. Engstrom 1982, A. L. Beilby 1995]. The appearance of glassy carbon surface turned to green color and that of graphite carbon and pyrolytic carbon were rough and easy to pill off.

While preanodization could enhance the signal of CAP through the adsorption of the analyte, precathodization, however, gave back drawn results in all type of electrode as can be seen in Figure 2 in the case of glassy carbon electrode with the changing of the electrochemical behavior of CAP just by changing pretreatment potential. This result has not been reported before. In our previous report, we pointed out that the electrochemical reduction of CAP on bare glassy carbon electrode underwent two steps to radical anion then to hydroxylamine derivative, but the reduction on preanodized glassy carbon electrode went directly to hydroxylamine derivative with 4 electrons transfer [N.M. Truc, 2005]. According to G. Ilangovan (1999), the fraction of alcohol group generated by precathodization was more than it was by preanodization. We thought that the electrochemical change might be due to the different in functional group type generated after preanodization and precathodization. This result provided strong proof of the important role of the functional groups on electrochemical characteristics of analyte under interest.

Because the performance of preanodization strongly depends on the pH as well as type and concentration of electrolyte [A. L. Beilby 1995], we set out to try with some kinds of electrolyte, and pH. The result showed that glassy carbon required strong acidic media, while graphite carbon and pyrolytic carbon preferred neutral media. The color of the surface of glassy carbon electrode changed when changing from this electrolyte to others: green in H_2SO_4, pink in H_3PO_4, brown in $LiClO_4$, and original black in CH_3COOH and NaOH, for example. While mineral acidic medium gave good performance, acetic acid solution didn't. It seemed that the functional groups *were locked* through the acetylation or esterization reaction with acetic acid. Like former research, the preanodization in alkaline did not support the analytical purpose [A. Dekanski, 2001] (Figure 3).

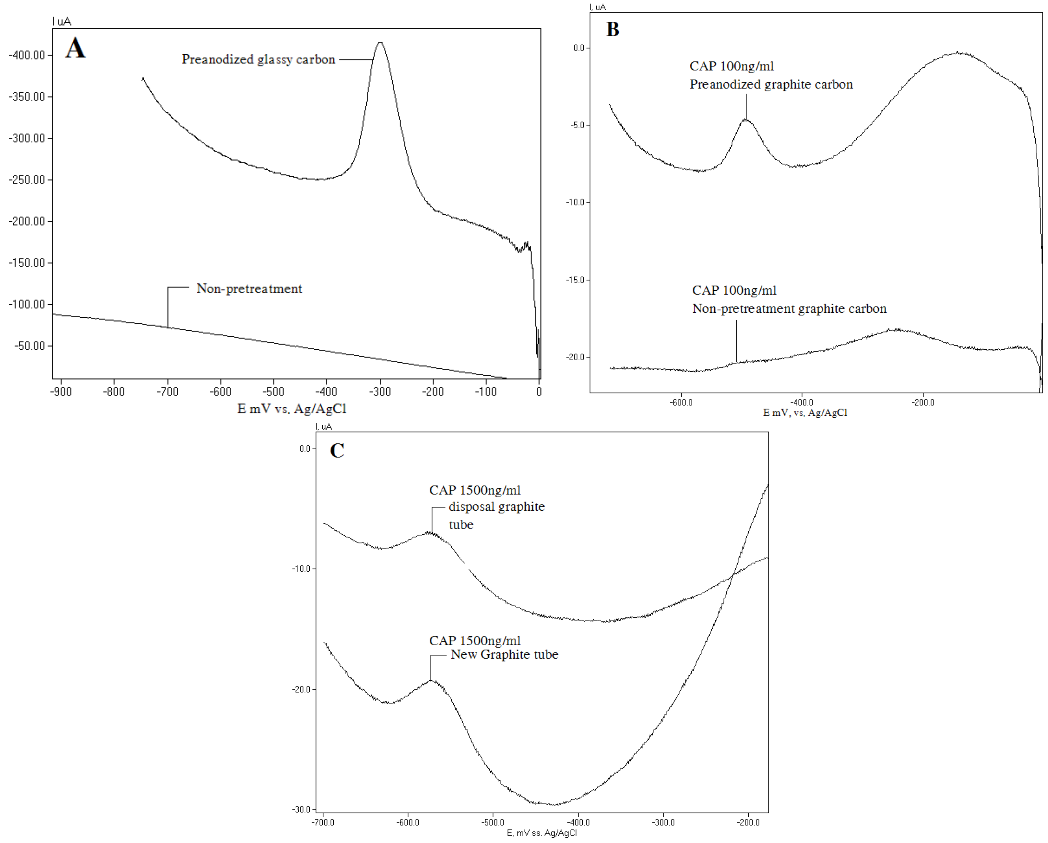

Fig. 1: Adsorptive voltamogram of CAP on A: glassy carbon electrode, B: graphite carbon electrode, C: pyrolytic carbon electrode. (Experimental sets is in the test).

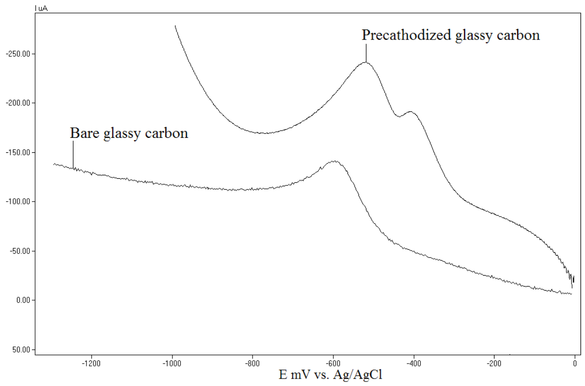

Fig. 2: Linear voltamogram of CAP (0.4mM) on bare and precathodized glassy carbon electrode in H_2SO_4 1.0M (scan rate 100mv/s, adsorp. time 0sec)

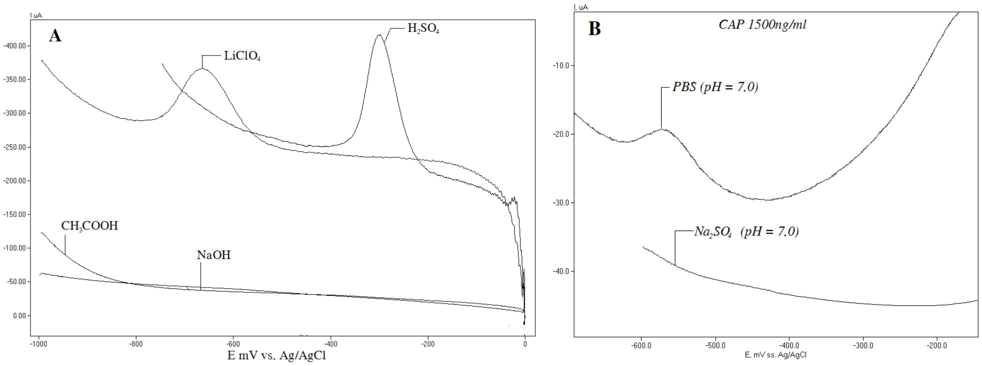

Fig. 3: The influence of type of electrolyte on performance of preanodized glassy carbon electrode (A) and graphite carbon electrode (B) (CAP 300ng/ml, adsorption time 100sec, linear scan rate 100mv/s), each electrolyte had conc. of 1.0M.

For graphite and pyrolytic carbon electrodes, both acidic and basic electrolyte resulted in very poor sensitivity. Beside that, with the same pH solution but phosphate buffer solution (PBS) gave better performance than Na_2SO_4 did (fig 3B). This was an unanswered question, but we thought that may be due to the protonated phosphate ions that may support through exchanged reaction for the formation of functional groups on the surface of graphite so enhanced the signal.

The concentration of appropriate electrolyte was optimized and was summarized in Table 1.

Table 1: Some typical preanodization conditions for carbon electrode with the detection limit for CAP in different adsorption time.

Carbon type	Preanodized condition	Adsorptive time (sec)	LOD (ng/ml)
Glassy carbon	+2.1V, 250sec, H_2SO_4 5mM	150	0.8
Glassy carbon	+2.1V, 250sec, H_2SO_4 5mM	60	3.0
Graphite carbon	+1.7V, 20sec, PBS pH 6.0, 0.10M	150	3.5
Pyrolytic carbon	+1.7V, 25sec, PBS pH 6.0, 0.10M	150	~200

Other instrument parameters were optimized. For glassy carbon electrode, linear stripping mode was used with linear scan rate of 10mV/s, 15sec resting time, starting potential was 0V and final potential was -0.70V, rotating speed of 1600rpm. For graphite and pyrolytic carbon electrode, differential stripping mode was used with scan rate of 10mV/s, pulse amplitude was 50mV, 15sec of resting time, initial potential was 0V, and final potential was -0.75V, rotating speed of 1600rpm.

Samples analysis

Eyes drop liquid

From the Table 1, we can see that the pyrolytic carbon electrode has very poor sensitivity, but available for direct analysis of eyes drop liquid with simple diluting the sample (Fig. 4).

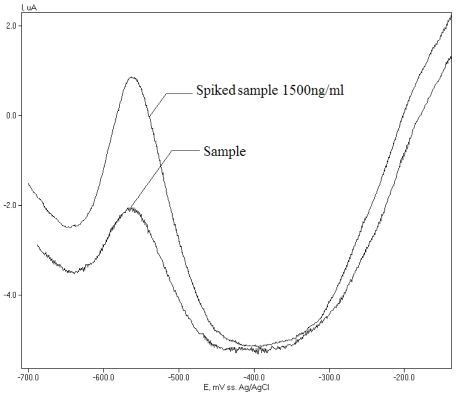

Fig. 4: Direct analysis of diluted eyes drop liquid sample with preanodized pyrolytic carbon electrode (adsorption time 150s, sample was diluted to 20 times)

Water sample

The water sample was collected in a shrimp keeping farm, filter with 0.45µm filter. 100ml of filtered sample was extracted with 15ml ethyl acetate. The organic portion was then taken out and dried under stream of nitrogen. The residue was redissolved with 10ml H_2SO_4 as electrolyte, and put to three electrode cell with glassy carbon electrode had been preanodized with the above conditions. Recovery of the extraction was interpreted in Table 2.

Table 2: Percentage recovery of water sample extraction

Spiked amount CAP (ng/ml)	Recovery (%)
2.0	99 ± 17 (n = 3)
5.0	96 ± 11 (n = 3)
10.0	100.8 ± 7.0 (n = 3)

The calibration curve can be set up from 10ng/ml to 70 ng/ml with adsorption time was 60sec, The longer adsorption time the poorer sensitivity due to matrix interference. In our experiment, 60 sec adsorption was optimal, and the detection limit in extracted sample can be estimated ca. 3 ng/ml. With the preconcentrated factor up to 10, the method on glassy carbon electrode can be used to detect as low as 0.3ng/ml CAP in water sample. Other antibiotics and chemical such as ciprofloxacin, ampicillin, malachite green cypermethrin, deltamethrin were checked with no interference. Penicillin was interfered but in very high concentration. However, nitrophenols like ortho-nitrophenol or para-nitrophenol had the same behavior like CAP on preanodized glassy carbon electrode and could not be removed (Figure 5).

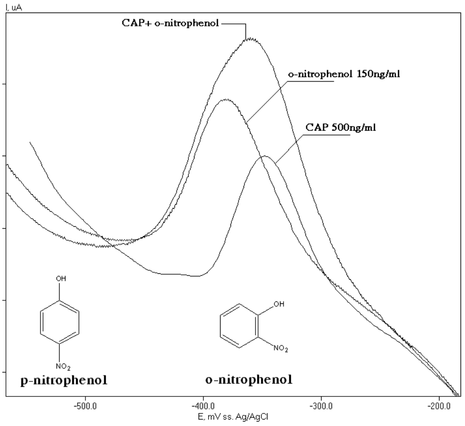

Fig. 5: The interference of nitrophenols on preanodized glassy carbon electrode.
Experimental set was like above.

Milk sample

The sample was collected in local market. 50ml of sample was extracted with 20ml ethyl acetate in ultra sonic bath in 15 minutes. The organic portion was taken out and dried under stream of nitrogen. The residue was diluted with 5ml mixture of methanol: water (5:95, v/v), and defatted with 3ml n-hexane, discarded the upper organic portion. The remaining water portion was re-extracted with 10ml ethyl acetate. Finally, the ethyl acetate extractant was colleted and dried under stream of nitrogen, and redissolved the residue with 10ml phosphate buffer pH 6.0. The extracted sample was ready for analysis with already preanodized graphite carbon electrode as described above. In our previous research, this extraction procedure can have the recovery of 88%. Standard addition method could be set up from 5ng/ml to 50ng/ml with linear coefficient was 0.995, and the detection limit could be estimated as low as 3.5ng/ml. Vitamin B2, as well as other antibiotics was checked with no interference. So the method was appropriated for milk sample.

Conclusion

A simple and sensitive method based on preanodized carbon electrodes and adsorptive stripping voltammetric assay for CAP was developed. However, in water analysis the interference of nitrophenols was not removed. But this assay can be used to evaluate the contaminants of aromatic nitro compounds because all of them are potential carcinogen. The pyrolytic carbon electrode had poorest sensitivity and can be used to test directly eyes drop liquid sample. Although preanodization could facilitate the analytical signal, precathodization did not give any out come. It should be noted that the existing of functional group on the surface of carbon electrode has an important role in the study of electrochemical mechanism. In addition, the influence of acetic acid in the preanodization and the nature of functional groups generated in this media is what needs more in sight research in the future. This opens an idea of controlling the type and the structure of graphite oxide film on the surface of electrode by adding to the solution a controlled or modified agent to get a high selectivity for a given analyte. Eventually, preanodization is a topic that is worth to paid more research in the future.

References

1. A. Dekanski, J. Stevanovíc, R. Stevanovíc, B. Ž. Nikolíc, V. M. Jovanovíc (2001), *Glassy carbon electrodes I. Characterization and electrochemical activation, Carbon* 39, 1195–1205.
2. A. L. Beilby, H. Y. Stem (1995), *Electrochemical Pretreatment of Carbon Electrodes as a Function of Potential, pH, and Time,* Anal. Chem. 67, 976 – 980.
3. D. Jürgen, E. Steckhan (1992), *Influence of the supporting electrolyte and the pH on the electrooxidative activation of glassy carbon electrodes, J. Electroanal. Chem.*, 333, 177 – 193.
4. D. T. Fagan, I. Hu, and T. Kuwana (1985), *Vacuum Heat Treatment for Activation of Glassy Carbon Electrodes, Anal. Chem.* 57, 2759-2763.
5. E. Hershenhart, R. L. McCreery, R. D. Knight (1984), *In situ cleaning and activation of solid electrode surfaces by pulsed laser light, Anal. Chem.* 56, 2256-2257.

6. European Commission Decision 2002/657/EC of 12 August 2002 *implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC) L221/8* (2002).
7. G. Ilangovan, K. Chandrasekara Pillai (1999), *Machenism of activation of glassy carbon electrodes by cathodic pretreatment*, J Solid State Electrochem. 3, 357 – 360.
8. Hao-Yu Shen, Hai-Liang Jiang (2005), *Screening, determination and confirmation of chloramphenicol in seafood, meat and honey using ELISA, HPLC–UVD, GC–ECD, GC–MS–EI–SIM and GCMS–NCI–SIM methods*, Anal. Chim. Acta 535 33–41.
9. J.J. V. Der Lee, W. P. Van Bennekom, H. J. De Jong (1980), *Determination of chloramphenicol at ultra-trace levels by high-performance differential polarography. Application to milk and meat,* Anal. Chim. Acta, 117, 171 – 182.
10. K. J. Stutts, P. M. Kovach, W.G. Kuhr, R. M. Wightman (1983), *Enhanced electrochemical reversibility at heat-treated glassy carbon electrodes, Anal. Chem.* 55; 1632-1634.
11. L. Agüí, A.Guzmán, P.Yáñez-Sedeño, J.M.Pingarrón (2002), *Voltammetric determination of chloramphenicol in milk at electrochemically activated carbon fibre microelectrodes,* Anal. Chim. Acta 461 65–73.
12. L. J. Kepley, A. J. Bard (1988), *Ellipsometric, Electrochemical, and Elemental Characterization of the Surface Phase Produced on Glassy Carbon Electrodes by Electrochemical Activation, Anal. Chem.* 60, 1459-1467.
13. M. Feng, D. Long, Y. Fang (1998), *Parallel incident spectroelectrochemistry study of chloramphenicol*, Anal. Chim. Acta. 363: 67-73
14. Maciej J. Bogusz, Huda Hassan, Eid Al-Enazi, Zuhour Ibrahim, Mohammed Al-Tufail (2004), *Rapid determination of chloramphenicol and its glucuronide in food products by liquid chromatography–electrospray negative ionization tandem mass spectrometry*, J. Chromatography B, 807 343–356.
15. Nguyen Minh Truc, John Mortensen, Nguyen Ba Hoai Anh (2005*), Proceedings of The second national conference on Analytical Sciences, Hanoi, Viet Nam, 222-225.*
16. R. C. Engstrom (1982), *Electrochemical Pretreatment of Glassy Carbon Electrodes,* Anal. Chem. 54, 2310-2314.
17. R. C. Engstrom, V. A. Strasser (1984), *Characterization of Electrochemically Pretreated Glassy Carbon Electrodes , Anal. Chem.* 56, 136-141.
18. R. J. Taylor, A. A. Humffray (1973), *Electrochemical studies on glassy carbon electrodes: I. Electron transfer kinetics J. Electroanal. Chem.* 42, 347.
19. Sandra Impens, Wim Reybroeck, Jan Vercammenc, Dirk Courtheyn, Sigrid Oogheb, Katia De Wasch, Walter Smedts, Hubert De Brabander, (2003) *Screening and confirmation of chloramphenicol in shrimp tissue using ELISA in combination with GC–MS and LC–MS,* Anal. Chim. Acta 483 153–163.
20. Santos, Lucia; Barbosa, Jorge; Castilho, M. Conceicao; Ramos, Fernando; Ribeiro, Carlos A. Fontes; Noronha da Silveira, M. Irene (2005). *Determination of chloramphenicol residues in rainbow trouts by gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry*. Anal. Chim. Acta, 529, 249-256.
21. T. Nagaoka, T. Yoshino (1986), *Surface Properties of Electrochemically Pretreated Glassy Carbon, Anal. Chem.* 58, 1037-1042.