ANALYTICAL SCIENCES APRIL 2008, VOL. 24

Electrochemical Analysis of Chloramphenicol Using Boron-doped Diamond Electrode Applied to a Flow-Injection System

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The electrochemical properties of chloramphenicol at a boron-doped diamond thin-film (BDD) electrode were studied using cyclic voltammetry. The highest current response of chloramphenicol was obtained with phosphate buffer, pH 6 (0.1 M) in 1% ethanol. The relationship between the concentration of chloramphenicol and the current response was linear over the range of 0.1 – 10 mM (R^2 = 0.9990). The amount of chloramphenicol was analyzed by flow-injection analysis. A thin-layer flow cell equipped with a BDD electrode was used as an amperometric detector, and experiments were carried out at –0.7 V (*vs.* Ag/AgCl). The linear relationship between the current response and the concentration of chloramphenicol in the range of 0.1 – 50 μM (R^2 = 0.9948) and the limit of detection of 0.03 μM (S/N = 3) were obtained. This method has been successfully applied to the determination of chloramphenicol in sterile eye drops and milk sample by the standard addition method. The average recoveries of chloramphenicol in eye drops were 98.0%, and the average recoveries of chloramphenicol from spiked milk were 93.9 – 103%.

(Received July 3, 2007; Accepted November 12, 2007; Published April 10, 2008)

Introduction

Chloramphenicol is a broad-spectrum antibiotic active against Gram-positive and Gram-negative bacteria. It is produced naturally by the soil bacterium *Streptomyces venezuelae*, but is presently mainly produced by chemical synthesis.[1-3] Chloramphenicol is currently used in ophthalmic solutions to treat superficial ocular infections, in topical ointments to treat the external ear or skin, in various tablets for oral administration and in intravenous suspensions to treat internal infections.[1] Moreover, it has been used in veterinary practice for prevention and treatment of many bacterial infections because of its efficiency, easy availability and low cost.[4,5] Due to its genotoxic effect and severe side effects, such as anemia, leucopenia, agranulocytosis and aplastic anemia in some people, its use is limited to the therapy of serious infections (*e.g.* typhoid fever, meningitis). Furthermore, its use in food production, such as aquaculture farming, has been banned worldwide.[1,6] No maximum residue limit (MRL) of chloramphenicol in animal-derived food has been established, because its toxic effects are not dose-dependent, but rather related to the hypersensitivity of certain individuals.[7]

Several analytical methods have been reported for the determination of chloramphenicol in various samples, such as shrimp,[3,8-11] seafood, meat,[7,12-15] eggs,[13] milk,[4,13] honey,[12,13,15] animal feeds,[5] urine, serum[14-16] and pharmaceutical formulations[17-22] based on liquid chromatography (LC),[5,12] liquid chromatography–mass spectrometry (LC-MS),[3,7-11,14,15] gas chromatography (GC), gas chromatography–mass spectrometry (GC-MS),[3,12,14] capillary zone electrophoresis,[16,17] enzyme-linked immunosorbent assay (ELISA),[3,13] spectrophotometry,[18,19] and chemiluminescence.[20-22] LC-MS is a common method that is used to determine chloramphenicol, because of its high sensitivity, and low limit of detection. However, it needs expensive apparatus and reagents, and is time-consuming. A sensitive, rapid and cheap method for analysis is still needed.

Electrochemical methods are widely used in many applications because they are simple, fast, involve no more reagents for derivatization and low cost. Several methods have been developed for the determination of chloramphenicol using electrochemical detection, such as voltammetry at electrochemically activated carbon fiber microelectrodes[4] and capillary-zone electrophoresis with amperometric detection at a carbon disk electrode[17] and a carbon fiber micro-disk array electrode.[16] Boron-doped diamond thin film (BDD) electrodes have many advantages for electroanalytical applications, due to their unique characteristics, which include a very low background current,[23,24] a wide electrochemical potential window in aqueous solutions,[25,26] a long-term stability of response,[27-30] a slight adsorption of polar organic molecules[28] and low sensitivity to dissolved oxygen.[31] Because of these attractive properties, BDD electrodes have been successfully used for the determination of various compounds, such as tiopronin,[30] acetaminophen,[32] D-penicillamine,[33] captopril,[34] lincomycin,[35] sulfonamides,[36] malachite green and leucomalachite green.[37] No reports have described the use of BDD electrodes for the determination of chloramphenicol.

The present report describes the use of the BDD electrode to study chloramphenicol using cyclic voltammetry and hydrodynamic voltammetry. In addition, flow-injection analysis

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with amperometric detection was used to determine chloramphenicol in the standard chemical form, eye drops and milk sample.

Experimental

Reagents and chemicals

All solutions were prepared with analytical-grade reagents and ultrapure water from a Milli-Q® Ultrapure Water Purification System by Millipore (USA). Phosphate buffer solutions (pH 2 – 4), 0.1 M, were prepared from 0.1 M potassium dihydrogenphosphate (BDH Chemicals, England), and the pH values were adjusted with 0.1 M orthophosphoric acid (85%, Carlo Erba Reagents, Italy). Phosphate buffer solutions (pH 5 – 8), 0.1 M, were prepared from 0.1 M potassium dihydrogenphosphate and 0.1 M disodium hydrogenphosphate (BDH Chemicals, England). The pH was measured with a 744 pH Meter (Metrohm, Switzerland) and the combined glass electrode at room temperature.

Standard chloramphenicol (Sigma-Aldrich, USA) solutions were freshly prepared in a 0.1 M phosphate buffer solution with 1% ethanol (Merck, Germany). Solid phase extraction (SPE) cartridges (Oasis® HLB, 30 µm, Waters, USA) were conditioned sequentially with 5 mL of methanol (Merck, Germany), 5 mL of ultrapure water and 5 mL of 0.1 M phosphate buffer (pH 6).

Cyclic voltammetry

Electrochemical experiments were carried out in a single-compartment three-electrode glass cell. BDD electrodes were obtained from CSEM Centre Suisse d'Electronique et de Microtechnique SA (Switzerland), and used as received without any further modifications. They were rinsed with ultrapure water prior to use. The BDD electrode was pressed against a smooth ground joint at the bottom of the cell, isolated by an O-ring (area 0.07 cm^2) and served as the working electrode. Electrical contact was made by placing the backside of the conducting silicon substrate of the BDD electrode onto a brass holder. A silver/silver chloride (Ag/AgCl) electrode with a salt bridge and a platinum wire were used as reference and counter electrodes, respectively. Cyclic voltammetric measurements were performed with the three-electrode system using an Autolab PGSTAT100 Potentiostat (Eco Chemie, The Netherlands). The electrochemical equipments were housed in a copper Faraday cage to reduce any electrical noise. All experiments were performed at room temperature.

Flow injection analysis with amperometric detection

The flow-injection analysis system consisted of a thin-layer flow cell (Bioanalytical Systems, USA), an injector port (Rheodyne 7725, USA) with a 20 µL injection loop, a peristaltic pump (Ismatec, Switzerland), and an electrochemical detector (Autolab PGSTAT100 Potentiostat, Eco Chemie, The Netherlands). The carrier stream, 0.1 M phosphate buffer (pH 6), was regulated by a reagent delivery module at a flow rate of 1 mL min^{-1}. A pulse dampener was used in series to reduce the pump noise. The thin layer flow-cell consisted of a gasket as a spacer, a BDD electrode as a working electrode, an Ag/AgCl electrode as a reference electrode, and a stainless-steel tube as a counter electrode and an outlet of the flow cell. The experiments were performed at room temperature in a copper Faraday cage to reduce electrical noise.

Before amperometric determination was performed, hydrodynamic voltammetry was carried out. The peak current after each injection was recorded, together with the corresponding background current. The average values of the peak current of three injections were plotted as a function of the applied potential to obtain hydrodynamic voltammograms. Amperometric measurements were carried out at the potential giving the maximum signal-to-background ratio (*S*/*B*) in the hydrodynamic voltammograms.

Sample preparation

Chloramphenicol eye drops. A 323 µL portion of chloramphenicol eye drops was transferred to a 10 mL volumetric flask and diluted to 10 mL with 0.1 M phosphate buffer (pH 6) in 1% ethanol. A 250 µL portion of this sample solution was transferred to each of six 10 mL-volumetric flasks, and a chloramphenicol standard solution was added. Then, each flask was made up to the mark with 0.1 M phosphate buffer (pH 6) in 1% ethanol. The concentrations of the standard chloramphenicol added were 0, 5, 10, 20 and 30 µM, respectively.

Milk. Five 5 mL portions of blank milk samples were transferred to centrifuge tubes and spiked with a chloramphenicol standard solution at concentrations of 0, 5, 10, 20 and 30 µM, respectively. To each spiked sample, 2.5 mL of a 20% aqueous trichloroacetic acid solution was added for protein precipitation.[38] The mixture was vortexed for 1 min, and then centrifuged at 4000 rpm for 10 min. The supernatant was collected and filtered through a 0.45 µm membrane filter. Two milliliters of extract were loaded onto the preconditioned SPE cartridge. After the extract was passed through, the cartridge was dried for 3 min under a vacuum condition, and then washed with 2 mL of 5% ethanol and 2 mL of ultrapure water. The retained chloramphenicol was eluted from the SPE cartridge with 2 mL of methanol. The eluate was then dried under a nitrogen stream in a water bath at 35°C. The residue was dissolved in 5.0 mL of 0.1 M phosphate buffer (pH 6) and the resulting solution was filtered through a 0.45 µm membrane filter.

Results and Discussion

Cyclic voltammetry

Figure 1 shows cyclic voltammograms for 1 mM chloramphenicol in 0.1 M phosphate buffer (pH 6) in 1% ethanol, together with the background cyclic voltammogram for 0.1 M phosphate buffer (pH 6) in 1% ethanol at a BDD electrode. The potential was scanned in two cycles from −1.0 to 1.0 V at a scan rate of 50 mV s^{-1}. In the first scan, two peaks were observed at −0.85 V *vs.* Ag/AgCl and 0.44 V *vs.* Ag/AgCl. In the second scan, a cathodic peak current at −0.85 V *vs.* Ag/AgCl had slightly decreased, whereas the anodic peak current had slightly increased; a new cathodic peak was observed at −0.39 V *vs.* Ag/AgCl. This revealed the presence of intermediates in oxidation/reduction reactions of chloramphenicol.[38] The first cathodic peak (−0.85 V *vs.* Ag/AgCl) corresponds to an irreversible reduction of the nitro group to the hydroxylamine derivative. The anodic peak (0.44 V *vs.* Ag/AgCl) arises from an oxidation of the hydroxylamine derivative to a nitroso derivative during a reverse scan. Also, the second cathodic peak (−0.39 V *vs.* Ag/AgCl) results from a reduction of the nitroso compound to the hydroxylamine derivative.[38,39] The electrode reactions are:

$$R\text{-}NO_2 + 4H^+ + 4e^- \longrightarrow R\text{-}NHOH + H_2O, \quad (1)$$

$$R\text{-}NO + 2H^+ + 2e^- \rightleftharpoons R\text{-}NHOH. \quad (2)$$

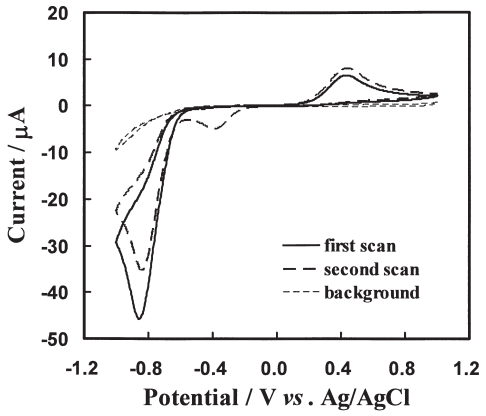

Fig. 1 Cyclic voltammograms for 1 mM chloramphenicol in 0.1 M phosphate buffer (pH 6) in 1% ethanol together with the background cyclic voltammogram for 0.1 M phosphate buffer (pH 6) in 1% ethanol at a BDD electrode. Potential was scanned in two cycles from −1.1 to 1.1 V at a scan rate of 50 mV s^{-1}.

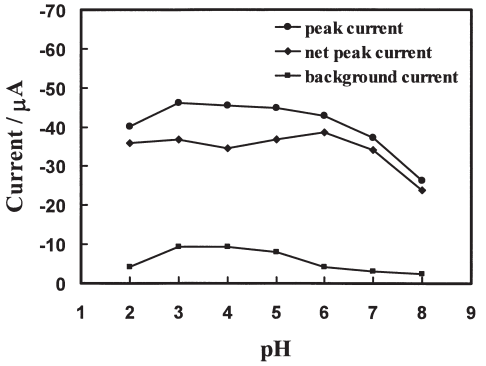

Fig. 2 Effect of the pH on the cathodic peak current of cyclic voltammograms for 1 mM chloramphenicol in 0.1 M phosphate buffer in 1% ethanol at a BDD electrode. The effect of the pH on the corresponding background current and the net cathodic peak current (equal to the cathodic peak current minus the background cuurent) are also shown.

Because a much higher peak current value was found at the first cathodic peak, it was chosen to be used in the electrochemical detection of chloramphenicol.

Effect of the presence of ethanol and the pH

Because chloramphenicol is slightly soluble in water, but it is readily soluble in alcohol, chloramphenicol solutions used in this work were prepared by dissolving chloramphenicol in a small amount of ethanol, and diluting with 0.1 M phosphate buffer solutions. The effect of the presence of ethanol in the chloramphenicol solution was studied using 1 mM chloramphenicol in 0.1 M phosphate buffer with 0, 1, 2, 5 and 10% of ethanol. It was found that increasing the amount of ethanol decreased the peak current. Therefore, 0.1 M phosphate buffer in 1% ethanol was chosen for the maximum sensitivity of the next experiments.

The effect of the pH on the electrochemical reduction of chloramphenicol was studied using 1 mM chloramphenicol in a 0.1 M phosphate buffer solution, pH 2 to 8, in 1% ethanol. As shown in Fig. 2, the cathodic peak current depended on the pH, since it was expected for a reduction process involving protons, such as that of the nitro group of the chloramphenicol molecule.[40]

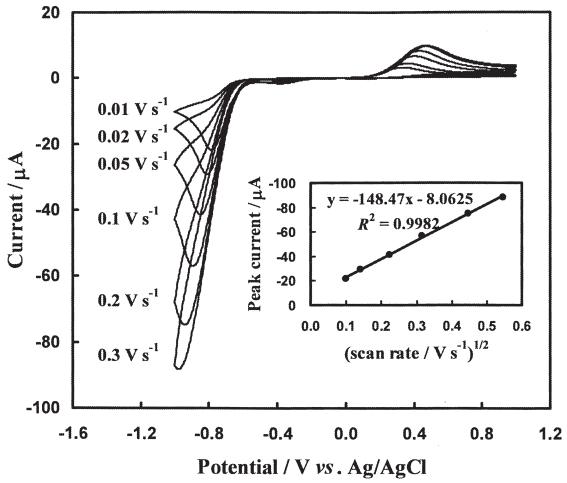

Fig. 3 Cyclic voltammograms for 1 mM chloramphenicol in 0.1 M phosphate buffer (pH 6) in 1% ethanol at a BDD electrode. The potential scan rate was varied from 0.01 to 0.3 V s^{-1} (10 to 300 mV s^{-1}). The graph of the relationship between current and $(\text{scan rate})^{1/2}$ is shown in the inset.

However, the background current at the corresponding potential also depended on the pH. Therefore, the net cathodic peak current, which is equal to the cathodic peak current minus the background current, was used to determine the optimum pH. The highest net cathodic peak current was obtained when 0.1 M phosphate buffer, pH 6, in 1% ethanol was used. Therefore, the chloramphenicol solutions in the following experiments were prepared with 0.1 M phosphate buffer, pH 6, in 1% ethanol.

Scan-rate dependence study

Figure 3 shows cyclic voltammograms of 1 mM chloramphenicol in 0.1 M phosphate buffer (pH 6) in 1% ethanol at the BDD electrode with the potential scan rate in the range of 10 to 300 mV s^{-1} (0.01 to 0.3 V s^{-1}). The cathodic peak potential shifted to more negative values as the scan rate increased. The net cathodic peak current has a linear relationship with the square root of the scan rate ($\nu^{1/2}$) with a correlation coefficient (R^2) of 0.9982. The results indicated that the electrochemical reaction of chloramphenicol is a diffusion-controlled process.

Concentration dependence study

Figure 4 shows cyclic voltammograms of chloramphenicol solutions in the concentration range of 0.1 to 10 mM in 0.1 M phosphate buffer (pH 6) in 1% ethanol at the BDD electrode with a scan rate of 50 mV s^{-1}. In the range of 0.1 to 10 mM, the peak current has a good linear relationship with the concentration of chloramphenicol (R^2 = 0.9990), as shown in the inset of this figure.

Flow injection analysis with amperometric detection

From previous work, the effect of the flow rate of the carrier solution was studied, and it was found that a flow rate of 1 mL min^{-1} provided a compromise between the sensitivity and the consumption of the carrier solution. Therefore, a flow rate of 1 mL min^{-1} was selected for all experiments.

Hydrodynamic voltammetry

Figure 5(a) shows a hydrodynamic voltammetric *i–E* curve obtained at the BDD electrode for 20 μL injections of 100 μM chloramphenicol in 0.1 M phosphate buffer (pH 6) in 1%

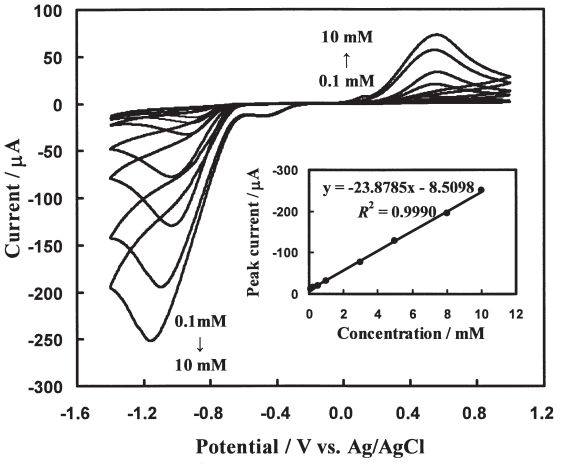

Fig. 4 Cyclic voltammograms of chloramphenicol solutions in the concentration range of 0.1 to 10 mM in 0.1 M phosphate buffer (pH 6) in 1% ethanol at the BDD electrode with a scan rate of 50 mV s^{-1}. The calibration graph is also shown in the inset.

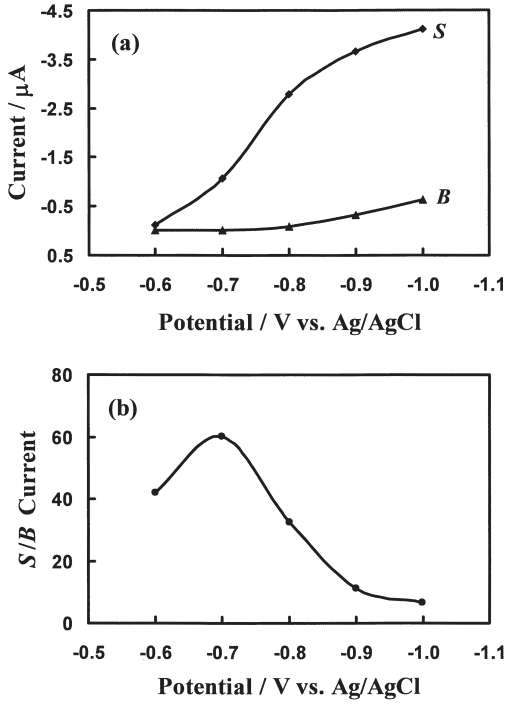

Fig. 5 (a) Hydrodynamic voltammograms of 100 μM chloramphenicol in 0.1 M phosphate buffer (pH 6) in 1% ethanol (signal, *S*) and 0.1 M phosphate buffer (pH 6) in 1% ethanol (background, *B*), using 0.1 M phosphate buffer (pH 6) in 1% ethanol as a carrier solution with a flow rate of 1 mL min^{-1}. (b) Corresponding hydrodynamic voltammetric signal-to-background ratio (*S*/*B* current) *versus* potential curve.

ethanol, using 0.1 M phosphate buffer (pH 6) in 1% ethanol as the carrier solution. The absolute magnitude of the background current at each potential is also shown for a comparison. The hydrodynamic voltammogram for chloramphenicol does not have a sigmoidal shape. To obtain the optimum potential, the signal-to-background ratio (*S*/*B*) was calculated, and plotted as a function of the potential to obtain the hydrodynamic voltammetric *S*/*B* ratio *versus* the potential, as shown in Fig. 5(b).

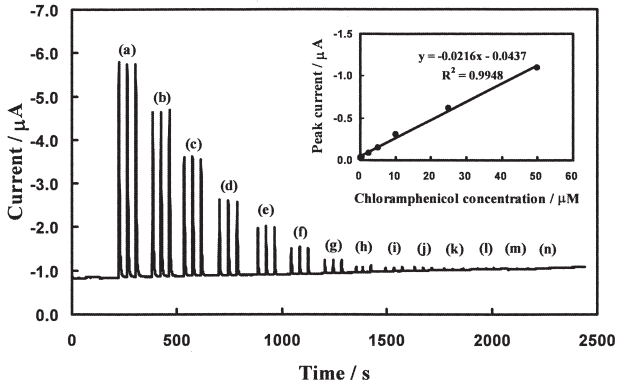

Fig. 6 Flow-injection analysis with amperometric detection results for various concentrations of chloramphenicol in 0.1 M phosphate buffer (pH 6) in 1% ethanol (a) 1000, (b) 500, (c) 250, (d) 100, (e) 50, (f) 25, (g) 10, (h) 5, (i) 2.5, (j) 1, (k) 0.5, (l) 0.25, (m) 0.1, and (n) 0.05 μM. The calibration graph is also shown in the inset.

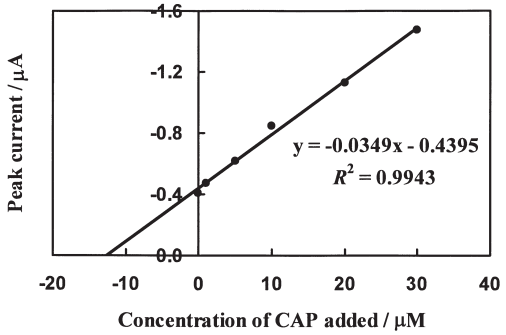

Fig. 7 Typical standard addition plot for the determination of chloramphenicol in eye drops by flow-injection analysis with amperometric detection using a BDD electrode.

The maximum *S*/*B* ratio is observed at −0.7 V *vs.* Ag/AgCl; therefore, this potential was chosen for the amperometric detection of chloramphenicol in flow-injection analysis.

Linear range, detection limit and reproducibility

Figure 6 shows a series of triplicate 20 μL injections of chloramphenicol standard solutions in 0.1 M phosphate buffer (pH 6) in 1% ethanol at a detection potential of −0.7 V *vs.* Ag/AgCl. Well-defined signals without peak tailing were obtained at all concentrations from 1000 to 0.05 μM. The peak current has a good linear relationship (R^2 = 0.9948) with the concentration of chloramphenicol in the range of 0.1 to 50 μM. The sensitivity of this method, which is the slope of the calibration graph between the current and the concentration over the linear range, is −21.6 nA μM^{-1}. The limit of detection (LOD, the concentration corresponding to three-times the standard deviation of blank divided by the slope of the calibration graph) was obtained at concentrations as low as 0.03 μM of chloramphenicol. The reproducibility of the current response was also examined. The relative standard deviation (RSD) of 10 measurements for 5 and 10 μM chloramphenicol was 3.5 and 3.0%, respectively, indicating a high reproducibility of the BDD electrode.

Determination of chloramphenicol in eye drops

The proposed flow-injection analysis with amperometric

Table 1 Recoveries and precision for the determination of chloramphenicol in spiked milk by flow-injection analysis with amperometric detection using the BDD electrode

Spiked concentration/ μM	Intra-assay (n = 3)			Inter-assay (n = 6)		
	Average concentration found/μM	Average recovery, %	RSD, %	Average concentration found/μM	Average recovery, %	RSD, %
5	4.8	95.6	4.9	4.7	93.9	4.6
10	10.3	103	2.4	10.1	101	4.1
20	21.4	96.9	2.9	19.3	96.7	3.3
30	30.4	101	1.0	30.5	102	1.4

Table 2 Comparison of electroanalytical data for the determination of chloramphenicol

Method	Electrode	Linear dynamic range/M	Detection limit/M	Precision (RSD, %)	Ref.
Amperometry applied to flow injection analysis	Boron-doped diamond thin film electrode	1.0×10^{-7} – 5.0×10^{-5}	3.0×10^{-8} (S/N = 3)	3.5 and 3.0 for 5 and 10 μM	This method
Voltammetry	Electrochemically activated carbon fiber microelectrodes	1.0×10^{-7} – 1.0×10^{-5}	4.7×10^{-8} (S/N = 3)	—	4
Capillary zone electrophoresis with amperometric detection	Carbon fiber micro-disk array electrode	5×10^{-6} – 1×10^{-3}	4.7×10^{-8} (S/N = 2)	2.3	16
Capillary zone electrophoresis with amperometric detection	Carbon disk electrode	2.0×10^{-6} – 5.4×10^{-4}	1.6×10^{-6} (S/N = 3)	—	17

detection using a BDD electrode at −0.7 V *vs.* Ag/AgCl was applied to determine chloramphenicol in eye drops by the standard addition method. A typical standard addition plot is shown in Fig. 7. The amount of chloramphenicol obtained, 0.49% chloramphenicol, was in good agreement with the labeled value (0.5% chloramphenicol) with RSD of 8.3% (n = 3).

Determination of chloramphenicol in spiked milk samples

The proposed method was also applied to determine chloramphenicol in milk by the standard addition method. Blank milk samples were spiked with a chloramphenicol standard solution prior to applying the extraction procedure. Recovery studies were carried out by intra- and inter-assays at levels of 5, 10, 20 and 30 μM of chloramphenicol, and run along with both reagent and sample blanks. As shown in Table 1, the average recoveries of the intra-assay were 95.6 – 103% with RSD values at 1.0 – 4.9%; the average recoveries of the inter-assay were 93.9 – 102% with RSD values at 1.4 – 4.6%. These results indicate that the recoveries and reproducibility of chloramphenicol in the milk sample were highly satisfactory.

Comparison with other methods

Table 2 summarizes the electroanalytical method for chloramphenicol from this study compared with other methods. It can be observed that using the BDD electrode with flow injection with amperometric detection provides a wide linear range, low detection limit, high sensitivity and reproducible response.

Conclusions

The BDD electrode can be used for the electrochemical analysis of chloramphenicol. This electrode exhibited excellent performance for the reductive detection of chloramphenicol. Well-defined voltammograms were obtained at the BDD electrode, which exhibited high sensitivity. The determination of chloramphenicol by flow-injection analysis with amperometric detection using the BDD electrode provides a calibration graph with a wide dynamic range from 0.1 to 50 μM, and a remarkably low detection limit (0.03 μM). The application of the proposed method for the determination of chloramphenicol in eye drops and milk samples shows that this method is sensitive, precise and accurate. Moreover, the use of the BDD electrode applied to flow injection with amperometric detection is simple, rapid, and provides high sample throughput because no chemical modification is required. Cleaning the electrode is also not necessary, due to the long-term stability of the BDD electrode response.

Acknowledgements

The authors gratefully acknowledge Thailand Research Fund for financial support. Special thanks are extended to Wanida Wonsawat, and Haruthai Sangjarusvichai for their experiments in some parts of this work.

References

1. Database of National Toxicology Program (NTP), U. S. Department of Health and Human Services, Substance Profiles Report on Carcinogens, 11th ed., http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s032chlo.pdf.
2. P. A. Guya, D. Royerb, P. Mottiera, E. Gremauda, A. Perisseta, and R. H. Stadlerc, *J. Chromatogr., A,* **2004**, *1054*, 365.

3. S. Impens, W. Reybroeck, J. Vercammen, D. Courtheyn, S. Ooghe, K. De Wasch, W. Smedts, and H. De Brabander, *Anal. Chim. Acta*, **2003**, *483*, 153.
4. L. Agüí, A. Guzmán, P. Yáñez-Sedeño, and J. M. Pingarrón, *Anal. Chim. Acta*, **2002**, *461*, 65.
5. P. Viñas, N. Balsalobre, and M. Hernández-Córdoba, *Anal. Chim. Acta*, **2006**, *558*, 11.
6. R. J. Shakila, S. A. Vyla, R. S. Kumar, G. Jeyasekaran, and G. I. Jasmine, *Food Microbiology*, **2006**, *23*, 47.
7. P. Mottier, V. Parisod, E. Gremaud, P. A. Guy, and R. H. Stadler, *J. Chromatogr., A*, **2003**, *994*, 75.
8. M. Ramos, P. Muñoz, A. Aranda, I. Rodriguez, R. Diaz, and J. Blanca, *J. Chromatogr., B*, **2003**, *791*, 31.
9. J. Storey, A. Pfenning, S. Turnipseed, G. Nandrea, R. Lee, C. Burns, and M. Madson, *Lab. Inform. Bull*., **2003**, 4306.
10. B. K. Neuhaus, J. A. Hurlbut, and W. Hammack, *Lab. Inform. Bull*., **2002**, 4290.
11. A. Pfenning, S. Turnipseed, J. Roybal, C. Burns, M. Madson, J. Storey, and R. Lee, *Laboratory Information Bulletin*, **2002**, 4284.
12. H. Y. Shen and H. L. Jiang, *Anal. Chim. Acta*, **2005**, *535*, 33.
13. G. Scortichini, L. Annunziata, M. N. Haouet, F. Benedetti, I. Krusteva, and R. Galarini, *Anal. Chim. Acta*, **2005**, *535*, 43.
14. A. Gantverg, E. Shishani, and M. Hoffmana, *Anal. Chim. Acta*, **2003**, *483*, 125.
15. M. J. Bogusz, H. Hassan, E. Al-Enazi, Z. Ibrahim, and M. Al-Tufail, *J. Chromatogr., B*, **2004**, *807*, 343.
16. W. Jin, X. Ye, D. Yu, and Q. Dong, *J. Chromatogr., B*, **2000**, *741*, 155.
17. A. B. Wang, L. Zhang, and Y. Z. Fang, *Anal. Chim. Acta*, **1999**, *394*, 309.
18. M. S. Collado, V. E. Mantovani, H. C. Goicoechea, and A. C. Olivieri, *Talanta*, **2000**, *52*, 909.
19. C. J. Eboka, J. Smart, and S. A. Adelusi, *Tropical J. Pharm. Res*., **2003**, *2*, 215.
20. C. A. Lindino and L. O. S. Bulhões, *J. Braz. Chem. Soc.*, **2004**, *15*, 178.
21. M. C. Icardo, M. Misiewicz, A. Ciucu, J. V. Mateo, and J. M. Calatayud, *Talanta*, **2003**, *60*, 405.
22. V. David, R. M. Saèz, J. V. Mateo, and J. M. Calatayud, *Analyst*, **2000**, *125*, 1313.
23. S. Jolley, M. D. Koppang, T. M. Jackson, and G. M. Swain, *Anal. Chem.*, **1997**, *69*, 4099.
24. T. Yano, D. A. Tryk, K. Hashimoto, and A. Fujishima, *J. Electroanal. Chem.*, **1998**, *145*, 1870.
25. G. M. Swain and R. Ramesham, *Anal. Chem.*, **1993**, *65*, 345.
26. J. W. Strojek, M. C. Granger, G. M. Swain, T. Dallas, and M. V. Holtz, *Anal. Chem.*, **1996**, *68*, 2031.
27. E. Popa, H. Notsu, T. Miwa, D. A. Tryk, and A. Fujishima, *Electrochem. Solid-State Lett.*, **1999**, *2*, 49.
28. J. S. Xu, Q. Y. Chen, and G. M. Swain, *Anal. Chem.*, **1998**, *70*, 3146.
29. B. V. Sarada, T. N. Rao, D. A. Tryk, and A. Fujishima, *Anal. Chem.*, **2000**, *72*, 1632.
30. W. Siangproh, N. Wangfuengkanagul, and O. Chailapakul, *Anal. Chim. Acta*, **2003**, *499*, 183.
31. T. N. Rao, I. Yagi, T. Miwa, D. A. Tryk, and A. Fujishima, *Anal. Chem.*, **1999**, *71*, 2506.
32. N. Wangfuengkanagul and O. Chailapakul, *J. Pharm. Biomed. Anal.*, **2002**, *28*, 841.
33. N. Wangfuengkanagul and O. Chailapakul, *Talanta*, **2002**, *58*, 1213.
34. W. Siangproh, P. Ngamukot, and O. Chailapakul, *Sens. Actuators, B*, **2003**, *91*, 60.
35. K. Boonsong, S. Chuanuwatanakul, N. Wangfuengkanagul, and O. Chailapakul, *Sens. Actuators, B*, **2005**, *108*, 627.
36. A. Preechaworapun, S. Chuanuwatanakul, Y. Einaga, K. Grudpan, S. Motomizu, and O. Chailapakul, *Talanta*, **2006**, *68*, 1726.
37. P. Ngamukot, T. Charoenraks, O. Chailapakul, S. Motomizu, and S. Chuanuwatanakul, *Anal. Sci.*, **2006**, *22*, 111.
38. D. A. Skoog, D. M. West, F. J. Holler, and S. R. Crouch, "*Fundamentals of Analytical Chemistry*", 8th ed., **2004**, Thomson Learning, Belmont, 694.
39. M. D. Corbett and B. R. Chipko, *Antimicrob. Agents Chemother.*, **1978**, *13*, 193.
40. F. C. de Abreu, P. A. de L. Ferraz, and M. O. F. Goulart, *J. Braz. Chem. Soc.*, **2002**, *13*, 19.