



# International Journal of Pharmacology

ISSN 1811-7775

**science**  
alert

**ansinet**  
Asian Network for Scientific Information

## Determination of Kanamycin by Square-wave Cathodic Adsorptive Stripping Voltammetry

Jin-Long Yan

School of Chemical and Biological Engineering,  
Yancheng Institute of Technology, Yancheng 224003, People's Republic of China

**Abstract:** A square-wave cathodic adsorptive stripping voltammetric (SWCASV) method for the determination of kanamycin was developed on a Thin-film Mercury Electrode (TFME). The optimal working conditions for the application of the method were found to be pH 8.0, provided by a Britton-Robinson (BR) buffer and a adsorption potential of +0.30 V during 300 sec. The equilibration time was applied during 10 sec and potential scans were performed at a scan rate of 40 mV sec<sup>-1</sup>, with a square-wave frequency of 100 Hz. The measuring-system response was linear over the kanamycin concentration range from 1.2×10<sup>-9</sup> to 5.0×10<sup>-8</sup> mol L<sup>-1</sup> and the detection limit achieved was 4.8×10<sup>-10</sup> mol L<sup>-1</sup>. The relative error and relative standard deviation obtained were 1.20 and 4.67%, respectively. The voltammetric procedure was applied successfully to give a rapid and precise assay of kanamycin in kanamycin sulfate injection form.

**Key words:** square-wave voltammetry, adsorption, kanamycin, cathodic stripping voltammetry

### INTRODUCTION

Kanamycin is a member of the amino glycoside family of antibiotics. These antibiotics have the ability to kill a wide variety of bacteria. Kanamycin binds to components in the bacterial cell which result in the production of abnormal proteins. These proteins are necessary for the bacteria's survival. Therefore the production of these abnormal proteins is ultimately fatal to the bacteria. It has been used to treat infections caused by *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* sp. and *Proteus* spp., as well as other bacteria. Since kanamycin has a narrow therapeutic range, proper dosage administration was necessary to achieve maximum therapeutic efficacy and to avoid adverse effects, including nephrotoxicity and ototoxicity (Aurora *et al.*, 1986; Hiroaki *et al.*, 1985). Interpatient variability was also evident in response to a given dose of an amino glycoside antibiotic (Essers, 1984; Hiroaki *et al.*, 1985). Therefore, monitoring of kanamycin levels in injections was necessary for safe and effective therapy. People taking this medicine should have regular blood tests. Monitoring of kidney and ear function should be carried out regularly whilst taking this medicine. One of the more widely used analytical procedure for the determination of kanamycin was chromatography (Yeh *et al.*, 2005; Kim *et al.*, 2001; Essers, 1984). At present, no studies dealing with kanamycin electrochemical behavior has been reported.

Cathodic stripping voltammetry was a well-known electro-analytical method for determination of various species capable to create sparingly soluble compounds

with mercury or silver ions (Ghoneim, 2004; Zaitsev *et al.*, 2004; Mlakar, 2003). In the course of the deposition step, the working electrode was polarized anodically at a constant potential. Consequently, a film of insoluble compound formed during the deposition step covers the working electrode surface. During the subsequent stripping step, the deposited film was stripped off from the electrode surface by cathodic polarization of the electrode. Among the numerous factors affecting the overall method, its sensitivity depends considerably on the voltammetric technique employed in the stripping step. Most frequently, square-wave voltammetry was a technique of choice (Mirceski, 2004; Fernando, 2003). This technique possesses unique advantages, since it provides an insight view into the mechanism of the electrode process and moreover, it exhibits the highest sensitivity to the reacting compound.

In this study the electrochemical behavior of the kanamycin has been examined in Thin-film Mercury Electrode (TFME) and an electrochemical method for the determination of kanamycin was described in a buffer solution of Britton-Robinson. The adsorption and preconcentration studies were followed by the application of SWCASW techniques for the trace determination of Kanamycin. The step of determination was based on a strong interaction of kanamycin with the mercury electrode surface forming the Hg(II)-Kanamycin compound. The present method was simple, convenient and more sensitive. There was a good linear relationship within the range of 1.2×10<sup>-9</sup> to 5.0×10<sup>-8</sup> mol L<sup>-1</sup> and the detection limit achieved was 4.8×10<sup>-10</sup> mol L<sup>-1</sup>.

**MATERIALS AND METHODS**

**Apparatus:** All measurements were carried out with a Model CHI832 multifunction voltammetric analyzer system (Shanghai Chenhua Electroanalysis Instruments Corporation, China). A Thin-film Mercury Electrode (TFME) using a Ag substrate with area 0.034 cm<sup>2</sup> was used as working electrode. Mercury films were deposited from solutions 1×10<sup>-3</sup> mol L<sup>-1</sup> Hg (II) and 0.1 mol L<sup>-1</sup> HClO<sub>4</sub> at -0.5 V vs. SCE. Film thickness was calculated from the charge consumed during deposition and also from the current on stripping the film into 1.0 mol L<sup>-1</sup> KSCN. A Saturated Calomel Electrode (SCE) was used as a reference electrode together with a platinum wire as the counter-electrode. The pH measurements were carried out with a 25 pHs-2C model acidity meter (Leici Instrumental Factory, Shanghai, China), using a combination electrode. The electrolytic cell was a 50 mL beaker. A SRD-1 Model magnetic stirrer and a stirring bar (2.5 cm in length) provided the convective transport during the pre-concentration. All experiments were performed at room temperature and dissolved oxygen was removed from the solutions by bubbling oxygen-free nitrogen through the cell for 10 min.

**Reagents:** Kanamycin was obtained from Sigma and was used without further purification. Solution containing different concentration of the investigated compound was prepared by dissolving a known amount of the chemically pure product in a specific volume of twice-distilled water. Pure drugs and their formations were purchased on the market. Kanamycin sulfate for injection containing nominal 0.25 g mL<sup>-1</sup> was dilute by water for analysis. Britton-Robinson (BR) buffer was brought to a constant ionic strength of 0.5 mol mL<sup>-1</sup> by the addition of NaNO<sub>3</sub> and adjusted to the desired pH. All of the chemicals were of reagent grade (Merck, Darmstadt). Twice-distilled deionized water served as a solvent.

**Procedure:** Transferred of the stock solution or the kanamycin sulfate for injection needed for assayed into a 50 mL standard flask, followed by the addition 5.0 mL of Britton-Robinson (BR) buffer and make up to volume with distilled water. Transferred the solution into the electrolytic cell, then the pre-concentration step was performed in a stirred (ca. 500 rev min<sup>-1</sup>) solution for a giving time period. During this period the TFME was held at +0.30 V. The stirring was then stopped and after 10 sec the voltammogram was recorded by applying the square wave voltammetry from +0.30 to -0.50 V (vs. SCE) and measured the peak height of +0.056 V.

**RESULTS**

**Square-wave cathodic adsorptive stripping voltammetric determination of kanamycin:** The SWCASW of various concentration of kanamycin were recorded in a solution of pH 8.0 represented in Fig. 1. The observed voltammetric peaks were mainly due to the reduction of Hg (II) bound in the adsorbed Hg-Kanamycin film. This SWCASW peak was pH dependent and no peaks were seen at pHs <6.2 indicating that the protonated form of the investigated compounds weakly interacts with the positively charged Hg surface. However, a well-defined peak was observed in neutral and slightly alkaline solutions due to a strong chemical interaction of the neutral forms with Hg forming the Hg (II)-Kanamycin film. The more sensitive peaks were observed at pH 8.00 for Kanamycin. These peaks were recorded in borate and B.R the buffer solutions containing indifferent anions. The maximum response was reported at pH 8.0 for Kanamycin in a B.R the buffer solution containing 0.5 mol L<sup>-1</sup> NO<sub>3</sub><sup>-</sup>. Therefore, the later media was chosen for the trace determination of Kanamycin. The effects of other operational conditions on the response of the more sensitive SWCASW peak were considered. The SWCASW peak of Kanamycin was recorded at various frequency (10-100 Hz) and scan rates (10-100 mV/sec) at pH 8.0 for Kanamycin. A well-defined peak and the maximum current response was recorded at a 100 Hz frequency. The maximum response and well-defined peaks

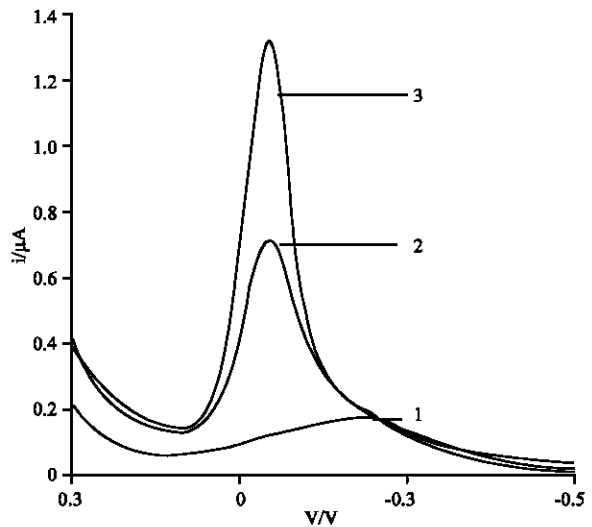


Fig. 1: Squere-wave cathodic adsorptive stripping voltamto grams of kanamycin. Adsorption time, 120 sec; adsorption potential, +0.30 V and scan rate, 100 mV/sec and pH 8.0. 1) c<sub>kanamycin</sub> = 0; 2) c<sub>kanamycin</sub> = 2.0×10<sup>-9</sup> mol L<sup>-1</sup>; 3) c<sub>kanamycin</sub> = 4.0×10<sup>-9</sup> mol L<sup>-1</sup>

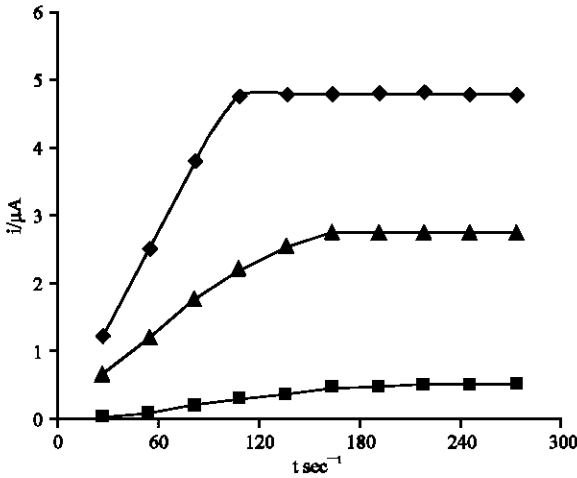


Fig. 2: Effect of the deposition time on the maximum reduction peak height. ■  $c_{\text{kanamycin}} = 1.2 \times 10^{-9} \text{ mol L}^{-1}$ ; ▲  $c_{\text{kanamycin}} = 8.4 \times 10^{-9} \text{ mol L}^{-1}$ ; ◆  $c_{\text{kanamycin}} = 1.5 \times 10^{-8} \text{ mol L}^{-1}$

Table 1: Effects of several types of interfering species on the determination of  $1.0 \times 10^{-8} \text{ mol L}^{-1}$  Kanamycin

Interfering species	Concentration ( $\text{mol L}^{-1}$ )	Degree of discovery (%)
Zn(II)	$1.0 \times 10^{-6}$	72.86
Co(II)	$1.0 \times 10^{-6}$	85.24
Cu(II)	$1.0 \times 10^{-6}$	70.15
Pb(II)	$1.0 \times 10^{-6}$	91.24
EDTA	$1.0 \times 10^{-4}$	92.88
Triton X-100	(4 mg)	68.20
Ascorbic acid	$1.0 \times 10^{-4}$	100.00
Alanine	$1.0 \times 10^{-4}$	99.86

were obtained at a  $40 \text{ mV sec}^{-1}$  scan rates, whereas at  $100 \text{ mV sec}^{-1}$  the response was relatively high, but the Kanamycin peak was shifted towards the mercury dissolution potential. So a 100 Hz frequency and a  $40 \text{ mV sec}^{-1}$  scan rate were therefore selected for subsequent experiments.

The peak-height deposition potential dependence was investigated at the optimum pH, frequency and scan rate. The peak current was highly sensitive to the deposition potential and the maximum peak height was obtained when the deposition potential was controlled at +0.3 V. At more negative potential values, the chemical interaction of the adsorbed species of Kanamycin was weak and there was no chance for the formation of the Hg-kanamycin film.

The effect of the deposition time on the maximum reduction peak height was examined under the aforementioned optimum solution and operational conditions, over a wide concentration range (Fig. 2). The peak height increased with the adsorption time in the form of the adsorption isotherm. At relatively longer adsorption times, an equilibrium surface concentration was reached and the peak height became almost constant.

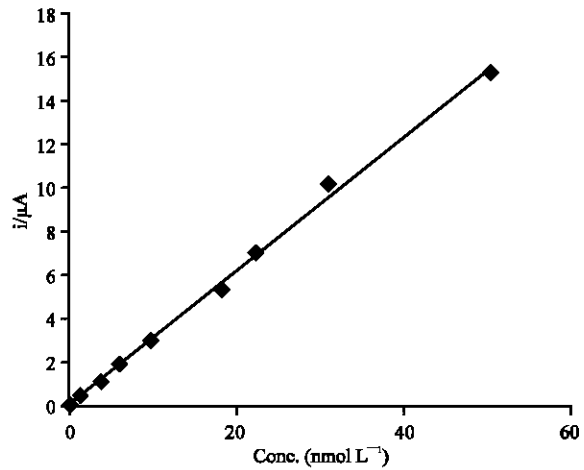


Fig. 3: Calibration plot of the determination of kanamycin by SWCASW

Preconcentration times of 300s was arbitrary adopted at the concentration ranges of  $1.2 \times 10^{-9}$ - $5.0 \times 10^{-8} \text{ mol L}^{-1}$ , respectively.

Under the optimum conditions and over a concentration range of  $1.2 \times 10^{-9}$ - $5.0 \times 10^{-8} \text{ mol L}^{-1}$  for kanamycin, the SWCASW peak height varied linearly with concentration of kanamycin and the calibration plot was shown in Fig. 3. Each point represents the mean value of six measurements, the equation of the regression line of the plot obtained was calculated to be  $I(\mu\text{A}) = 0.14 + 0.31 c \text{ (nmol L}^{-1})$ ,  $n = 6$ ,  $r^2 = 0.988$  and the determination limit was  $4.8 \times 10^{-10} \text{ mol L}^{-1}$ .

The effects of several types of interfering species (cations, anions and neutral organic species) on the determination of  $1.0 \times 10^{-8} \text{ mol L}^{-1}$  Kanamycin were examined and were represented in Table 1. At the micromol concentration range of some metal ions e.g., Zn(II), Co(II), Cu(II) and Pb(II), the degree of recovery of the Kanamycin response was lowered by 11-30%. This indicates that Hg(II)-Kanamycin compound at the mercury electrode surface as a result of their tendency for complexation with Kanamycin under these conditions. The interference as the result of diminished sensitivity was caused by the presence of EDTA or a surface-active substance e.g., Triton X-100 due to their competitive effect on the adsorption and interaction of kanamycin with the mercury surface, but the other organic additives did not highly affect the adsorption and interaction of Kanamycin with the mercury-electrode surface due to their chemical interaction with Hg(II) at the mercury-electrode surface.

**Analysis of kanamycin sulfate for injection:** The determination of kanamycin in kanamycin sulfate for injection was performed by the standard-addition

method. The excipients in the injection did not affect the voltammogram. The determination result of the Kanamycin injection (nominal 25 g per mL) was  $24.70 \text{ g mL}^{-1}$ , the relative error and the relative standard deviations were 1.20 and 4.67%, respectively. The result confirmed the utility of this proposed method in the determination of kanamycin.

### DISCUSSION

**Study of voltammetric behavior:** The surface activity and the redox behavior of kanamycin under investigation were studied at different pH values (6.0-10.0) using Cyclic Voltammetry (CV) at (TFME). In order to enhance the faradaic response of the adsorbed species only the CV voltammograms were recorded at a relatively higher scan rate ( $100 \text{ mV/s}$ ) using very dilute concentrations of Kanamycin (micromoles level).

Over the studied pH range, the CV voltammograms showed one anodic peak in Fig. 4. The morphology and the position of these redox peaks were mainly dependent on the solution and operational conditions e.g., the adsorption time and potential and the scan rate.

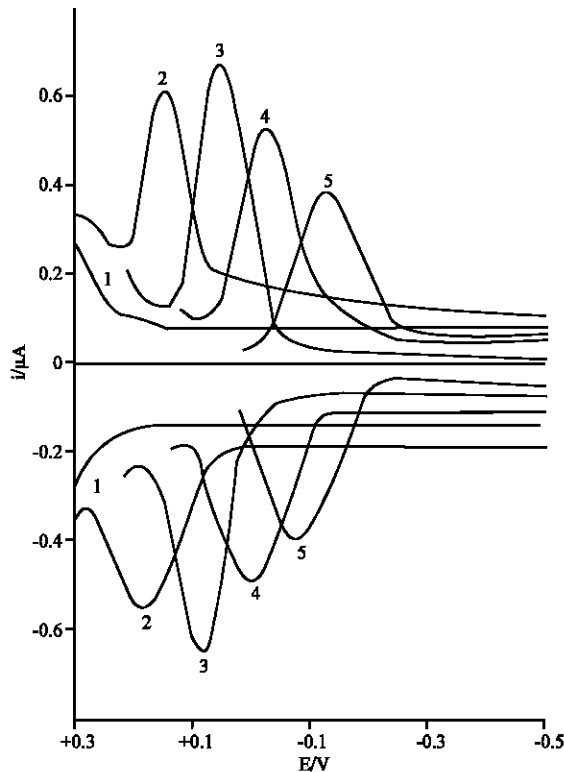


Fig. 4: Cyclic voltammograms of  $8.0 \times 10^{-7} \text{ mol L}^{-1}$  kanamycin at different pH values. Adsorption time, 120 sec; adsorption potential, +0.30 V and scan rate,  $100 \text{ mV/sec}$ . 1) pH 6.0; 2) pH 7.0; 3) pH 8.0; 4) pH 9.0; 5) pH 10.0

The strongest peak was found at about pH 8.0, while in acid and strong alkaline solutions the peak current decreased or disappeared. The results showed that the neutral forms of kanamycin were strongly adsorbed at the mercury electrode surface. This was to be expected if the association of the adsorbed molecules depends predominantly on a stacking interaction of the neutral species of kanamycin under investigation. The negative charge on the anionic forms of the adsorbed molecules decreases the intermolecular association and the stacking interaction between molecules. Moreover, there were repulsion forces between the net negative charge of the adsorbed species and the negatively charged mercury electrode surface.

Kanamycin molecule contains lots  $\text{-NH}_2$  radical (Fig. 5), which was easily coordination with  $\text{Hg(II)}$ . According to the previous work the cathodic peak corresponds to the reduction of  $\text{Hg(II)}$  bound in the adsorbed and accumulated  $\text{Hg(II)}$  film, which could be formed as the result of a strong chemical interaction of the adsorbed molecule with the positively charged mercury surface via the nitrogen atom. The anodic peak was due

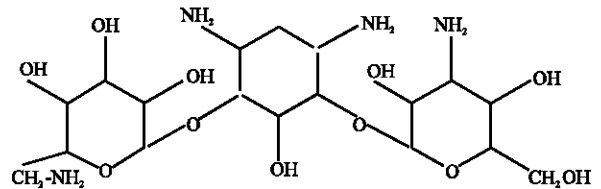


Fig. 5: Structure of kanamycin

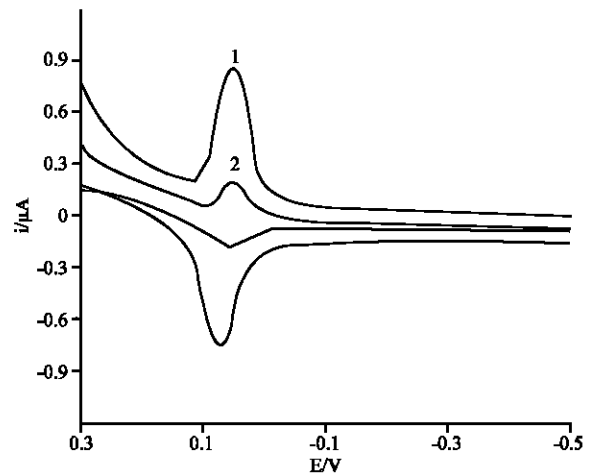
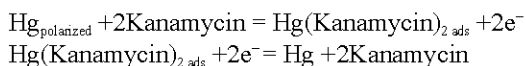


Fig. 6: Repetitive cyclic voltammetric curves of  $8.0 \times 10^{-7} \text{ mol L}^{-1}$  kanamycin. Adsorption time, 120 sec; adsorption potential, +0.30 V and scan rate,  $100 \text{ mV sec}^{-1}$  and pH 8.0. 1) first scan; 2) second scan

to the regeneration and formation of a Hg(II) film at the electrode surface. The above-mentioned cathodic and anodic processes could be represented by the following equations:



The typical repetitive cyclic voltammetric curves were shown in Fig. 6. The reduction peak in the first scan after an accumulation time of 180 sec was much higher than in the second scan. The aforementioned results supported the strong adsorption and accumulation of Kanamycin at the Hg surface, which was a prerequisite step for applications of SWCASW voltammetric techniques for the ultra trace determination of kanamycin.

### CONCLUSIONS

The result cyclic voltammetric measurement indicates that Kanamycin was strongly adsorbed and chemically interacted with a polarized mercury-electrode surface. The formation of the Hg-kanamycin at the TFME surface was a prerequisite step for the application of SWCASV techniques for the determination of Kanamycin. The optimal working conditions for the application of the method were found to be pH 8.0, provided by a Britton-Robinson (BR) buffer and a adsorption potential of +0.30 V during 300 sec. The equilibration time was applied during 10 sec and potential scans were performed at a scan rate of 40 mV s<sup>-1</sup>, with a square-wave frequency of 100 Hz. The measuring-system response was linear over the kanamycin concentration range from 1.2×10<sup>-9</sup> to 5.0×10<sup>-8</sup> mol L<sup>-1</sup> and the detection limit achieved was 4.8×10<sup>-10</sup> mol L<sup>-1</sup>. The relative error and relative standard deviation obtained were 1.20 and 4.67%, respectively.

### ACKNOWLEDGEMENT

This study was generously supported by Jiangsu Education Department of China (Granted No. 03 KJD 150277) and Excellent Youth Fund of Yancheng Institute of Technology (Granted No. YQ 2001003).

### REFERENCES

Aurora, F.D., D.P. Janet, T.L. Charles and P. Chittaranjan, 1986. Determination of kanamycin concentration in serum by substrate-labeled fluorescent immunoassay. *Antimicrob. Agents Chemother.*, 29: 961-964.

Essers, L., 1984. An automated high-performance liquid chromatographic method for the determination of amino glycosides in serum using pre-column sample clean-up and derivatization. *J. Chromat. B, Anal. Technol. Biomed. Life Sci.*, 305: 345-352.

Fernando, G., 2003. Adsorptive square-wave voltammetry of metal complexes. Effect of ligand concentration Part III. Theory. *J. Electroanal. Chem. Interfacial Electrochem.*, 548: 1-9.

Ghoneim, M.M., 2004. A. Tawfik. Assay of anti-coagulant drug warfarin sodium in pharmaceutical formulation and human biological fluids by square-wave adsorptive Cathodic stripping voltammetry. *Analytica Chimica Acta*, 511: 63-69.

Hiroaki, K., K. Yoshie and N. Takashi, 1985. Rapid determination of kanamycin and dibekacin in serum by use of high-pressure liquid chromatography. *Antimicrob. Agents Chemother.* 28:5 21-523.

Kim, B.H., J.H. Ok and Y.K. Kim, 2001. Development of liquid chromatographic method for the analysis of kanamycin residues in varicella vaccine using phenylisocyanate as a derivatization reagent. *J. Chromat.B*, 752: 173-177.

Mirceski, V., 2004. Charge transfer kinetics in thin-film voltammetry. Theoretical study under conditions of square-wave voltammetry. *J. Physical Chem. B, Condensed Matter, Materials, Surfaces, Interfaces Biophysical*, 108: 13719-13725.

Mlakar, M., 2003. Determination of ytterbium traces by Cathodic stripping voltammetry. *Electroanalysis*, 15: 27-32.

Yeh, H.H., S.J. Lin, J.Y. Ko, C.A. Chou and S.H. Chen, 2005. Rapid and selective micellar electrokinetic chromatography for simultaneous determination of amikacin kanamycin A and tobramycin with UV detection and application in drugformulations. *Electrophoresis*, 26: 947-953.

Zaitsev, N.K., E.A. Osipova, D.M. Fedulov and A.G. Dedov, 2004. Electrochemical preconcentration of iodide and its determination by cathodic stripping voltammetry. *J. Anal. Chem.*, 59: 474-480.