Selenomethionine-catalyzed nickel ion reduction at a mercury electrode: applications in the analysis of nutritional supplements

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Abstract

Selenomethionine (SeMet) is a catalyst for Ni^{2+} reduction at a mercury electrode in a borax buffer at pH around 9 and gives rise to a differential pulse voltammetric peak, A, at -0.74 V vs. the Ag|AgCl, (3 M KCl) reference electrode. Peak current is directly proportional to SeMet over the concentration range 0.4–10 μM. Alkali and alkali-earth ions depress to some extent the sensitivity but the current-concentration relationships remains linear even under these conditions. Differential pulse cathodic stripping voltammetry (DPCSV) in 0.01 M borax results in two partially overlapped peaks. The more negative (A, at about -0.74 V) is similar to that recorded with no deposition and is due to the catalysis by non-adsorbed SeMet, whereas the more positive one (B, at about –0.60 V) results from the catalysis by adsorbed SeMet. Only the DPCSV peak A appears if 0.1 M KNO_3 is also present along with 0.01 M borax. Stearic acid, which is present in nutritional supplement tablets, improves the separation of the DPCSV peaks. Consequently, the peak B recorded with 0.01 M borax buffer allows determining SeMet in nutritional supplement tablets by the standard addition method.

Keywords: Selenomethionine, Electrocatalysis; Nickel ion reduction; Nutritional supplements.

Selenomethionine (2-amino-4-methylselanyl-butanoic acid, SeMet) is employed as a dietary supplement for avoidance of Se deficiency in humans and ruminants [1] and is a potential therapeutic and cancer preventive agent [2, 3]. Quantification of SeMet relies on various spectrometric methods for Se determination [4] or liquid chromatography with detection by atomic or mass-spectrometry (Refs. [5, 6] and references therein). SeMet undergoes an anodic reaction at gold electrodes [7, 8] but no SeMet electrochemical reaction at mercury electrodes was till now reported. This paper presents an indirect electrochemical method for SeMet determination which is based on its catalytic effect in Ni^{2+} reduction at the hanging mercury drop electrode (HMDE) and can be applied for the analysis of nutritional supplements tablets.

SeMet does not induce a cathodic stripping voltammetric (CSV) peak at the HMDE as methionine (Met) [9] and does not give rise to a CSV peak in the presence of Cu^{2+} like cysteine (Cys) and cystine [10]. However, differential pulse voltammetry (DPV) in a Ni^{2+} and SeMet containing borax solution results in a peak A at -0.74 V (Fig. 1). It precedes the main Ni^{2+} peak M (partially shown in Fig. 1) which also occurs in the absence of SeMet. Linear scan voltammetry (LSV) produced *i-E* curves with a sigmoid shape that is typical of a steady state electrode process (as confirmed by the linear shape of the logarithmic plot). The LSV current is independent on the scan rate. Consequently, the process A was assigned to the catalytic reduction of nickel according to a mechanism [11, 12] which involves two main steps (1) formation of a reducible Ni^{2+}-SeMet complex as the rate determining step and, (2) Ni^{2+} reduction in this complex (with a lower overvoltage relative to the reduction of the hydrated Ni^{2+} ion) and release of a free SeMet molecule that enters again the step (1). The relatively high excess of Ni^{2+} in solution prevents Ni^{2+} depletion in the reaction layer by the above process and rules out diffusion effects on the overall reaction rate, as proved by the effect of the scan rate and the logarithmic analysis of the LSV curve. Moreover, the catalytic character of the process

A is proved by the fact that the LSV signal A current is much higher that that expected for the simple reduction of a Ni^{2+}-SeMet complex. For analytical purposes, DPV (which minimizes the interference of the main Ni^{2+} peak) is more convenient than LSV. Square wave voltammetry is inferior in terms of sensitivity and accuracy, probably due to the irreversible character of Ni^{2+} reduction.

Fig. 2 shows that the catalytic current displays a maximum value at pH around 9. At lower pH values the protonation of the amino group in SeMet (pK = 9.05 [13]) hampers the formation of the Ni^{2+} complex, whereas greater pH values arouse Ni^{2+} hydrolysis. The best sensitivity occurs with a 0.01 M borax buffer (curve 1) and the presence of additional salts (curves 2 and 3) result in lower peak currents. An ammonia buffer (0.1 M NH_4NO_3, 0.1 M NH_3, 0.1 M $NaNO_3$, pH 9.01) was also checked and found as not suitable because of the poor separation of the catalytic peak A from the main Ni^{2+} peak.

Ni^{2+} concentration determines the reaction rate of the complex formation in the reaction layer and, therefore, the peak current at a given SeMet concentration. An investigation of the Ni^{2+} concentration effect (from 10 to 100μM) at pH 9.2 and SeMet concentrations of 1.2 and 8 μM proved that the peak A current increases with the Ni^{2+} concentration and attains a limiting value at a SeMet/Ni^{2+} concentration ratio above 50.

Summing up, the best sensitivity in DPV was obtained with a 0.01 M borax buffer (pH 9.2), 100 μM Ni^{2+}, scan rate 0.01V/s, pulse amplitude 0.02V, and pulse application interval 0.1s. Under these conditions, peak A current is directly proportional to SeMet concentration over the range 0.4–10 μM, with a slope of 3.6 nA/μM. Detection and quantification limits are 0.13 and 0.4 μM, respectively. For spiked synthetic samples with SeMet concentrations between 0.8 and 8 μM, the recovery varied between 95 and 102 %.

Alkali and alkali-earth cations induce a moderate depression of the peak A current due to their effect on the double layer structure (Fig. 3). This effect increases with ion charge and

levels off at metal ion concentrations over 0.04 M. Transition metal ions like Cu^{2+} and Co^{2+} have a stronger effect because of the interference with the formation of the Ni^{2+}-SeMet complex (Fig. 3). Actually, Co^{2+} reduction is also catalyzed by SeMet, but the emerging catalytic peak shows a very low sensitivity and is of no analytical use. Zn^{2+} also produces a slight modification of the peak A current but does not interfere as long as its concentration does not overcomes that of Ni^{2+}. The nature of the anion (NO_3^-, Cl^-, or SO_4^{2-}) has almost no effect on the peak A current. Ascorbic acid causes a moderate increase of the peak current, most likely by inclusion in a ternary complex with Ni^{2+} and SeMet. Except for Cu^{2+} and Co^{2+}, any of the above-mentioned interferences can be tackled by the standard addition method, because the calibration graph remains linear and the sensitivity only is more or less affected.

Selenious acid has no effect on Ni^{2+} reduction and does not interfere with SeMet up to an H_2SeO_3/SeMet concentration ratio of 5. The catalytic reduction of Ni^{2+} enables therefore discriminating between inorganic and organic Se forms. Met induce a similar Ni^{2+} catalytic reduction process and can interfere with SeMet determination but the simultaneous occurrence of SeMet and Met in nutritional supplement tablets is unlikely.

Differential pulse cathodic stripping voltammetry (DPCSV) with adsorptive pre-concentration in 0.01 M borax results in two peaks (Fig. 4, curve 1). The second one (A) lies at the same potential than that obtained with no pre-concentration and is ascribed to Ni^{2+} reduction by SeMet in non-adsorbed state. The first peak (B in Fig 3, curve 1) is assigned to Ni^{2+} reduction catalyzed by adsorbed SeMet. A peak overlapping prevents analytical applications. If 0.1 M KNO_3 is also present (Fig 4, curve 2), only the peak A occurs proving that a high concentration of K^+ in the diffuse part of the double layer restrains the adsorption of the positive Ni^{2+}-SeMet complex. A linear calibration graph results under these conditions, but the sensitivity is almost the same as in the absence of the pre-concentration. Peak current vs. pH curves under the DPCV conditions shows the same trend as in Fig. 2. The fact that the pre-

concentration does not bring about a major improvement in sensitivity proves that SeMet adsorbs to a low extent at the mercury electrode. This contrasts the behavior of selenocysteine [14] and Cys [12, 15] that can act as catalysts for Ni^{2+} reduction in the adsorbed state. Ionized selenol and thiol groups in such compounds form strong covalent bond with metals like mercury and gold [16]. Such a strong interaction cannot be expected with a double-substituted selenium derivative like SeMet.

A good separation of peaks A and B occurs if stearic acid is also present (Fig. 4, curve 3). SeMet co-adsorbs with stearic acid and operates in this form as a catalyst in the peak B process. SeMet tablets contain stearic acid and a tablet extract (Fig. 5 curve 2) gives rise to voltammetric patterns alike to curve 3 in Fig. 4. Peak B current recorded under these conditions is directly proportional to SeMet concentration and allows determining SeMet by DPCSV using the standard addition method (Fig. 5, curves 2–5). Results thus obtained were in good agreement with the claimed SeMet content and the results of SeMet determination by electro-thermal atomization-atomic absorption spectrometry (ET-AAS).

In conclusion, SeMet is a catalyst for Ni^{2+} reduction at mercury electrodes at pH around 9 in a 0.01 M borax solution. This electrode process allows determining SeMet in nutritional supplement products by DPCSV.

Experimental

L-(+)-Selenomethionine (99+%, Acros Organics), L-methionine (Sigma Aldrich, 98%), stearic acid (Fluka, 99.5%), and other reagents (of p.a. degree) were used as received. Voltammetric measurements were carried out with an Autolab PG STAT 30 electrochemical system (EcoChemie, Netherlands) combined with a Metrohm 663 VA stand. The three electrode cell was fitted with a HMDE, an Ag|AgCl (3 M KCl) as reference and a glassy carbon rod as counter electrode. Oxygen was removed by a pure nitrogen stream. ET-AAS

analysis was performed by a Perkin Elmer AAanalyst 600 instrument according with the supplier's recommendations for selenium determination. Food supplement tablets (Solary Selenium product containing 100 μg SeMet per tablet and non-specified amounts of silicon dioxide, microcrystalline cellulose, stearic acid and magnesium stearate) were grinded using mortar and pestle. A weighed portion of powder (containing about 0.100 μg SeMet) was sonicated with 20 ml of 0.01M borax for 60 min. The mixture was centrifuged for 2 hours at 4000 rpm, the supernatant was mixed with 0.01 M borax solution so as to adjust SeMet concentration to 1.5-2 μM and SeMet was then determined by DPCSV in the presence of 200 μM Ni^{2+} (5 min pre-concentration at -0.3 V) using the standard addition method.

Figure captions

Fig. 1. DP voltammograms for Ni^{2+} reduction catalyzed by SeMet. 0.01 M borax, 0.1 M KNO_3, 80 μM Ni^{2+}. SeMet (μM): 1) 0.0; 2) 0.5; 3) 1.3; 4) 3.1; 5) 5.1. Inset: peak current vs. SeMet concentration.

Fig. 2. pH effect on the DPV catalytic peak current. SeMet, 4 μM, Ni^{2+}, 100 μM. Electrolyte: 1) 0.01 M borax; 2) 0.01 M borax, 0.1 M KNO_3; 3) 0.01 M borax, 0.1 M KNO_3, 0.01M KH_2PO_4. pH adjusted by means of $HClO_4$ (pH>9.2) or NaOH (pH<9.2).

Fig. 3. The influence of cations (as nitrates) on the catalytic peak A current. 0.01 M borax, 100 μM Ni^{2+}, 8 μM SeMet. I_p and $I_{p,o}$ stand for the peak current in the presence and in the absence of the added cation, respectively.

Fig. 4. DPCSV for Ni^{2+} reduction catalyzed by SeMet. Curves 1 and 2: 2 μM SeMet, 100 μM Ni^{2+}, pre-concentration for 5 min at -0.4 V. Background electrolyte: 1) 0.01 M borax; 2) 0.01 M borax and 0.1 M KNO_3. Curve 3: DPCSV in the presence of stearic acid (half saturation concentration). 0.01 M borax, 200 μM Ni^{2+}, 8 μM SeMet.

Fig. 5. SeMet determination by DPCSV (standard addition method) in nutritional supplement tablets. 0.01 M borax, 1) Tablet extract alone; 2) 200 μM Ni^{2+} added; 3–5) with SeMet additions: 0.8, 2.6, and 3.6 μM, respectively. Pre-concentration: 5 min at -0.3 V.

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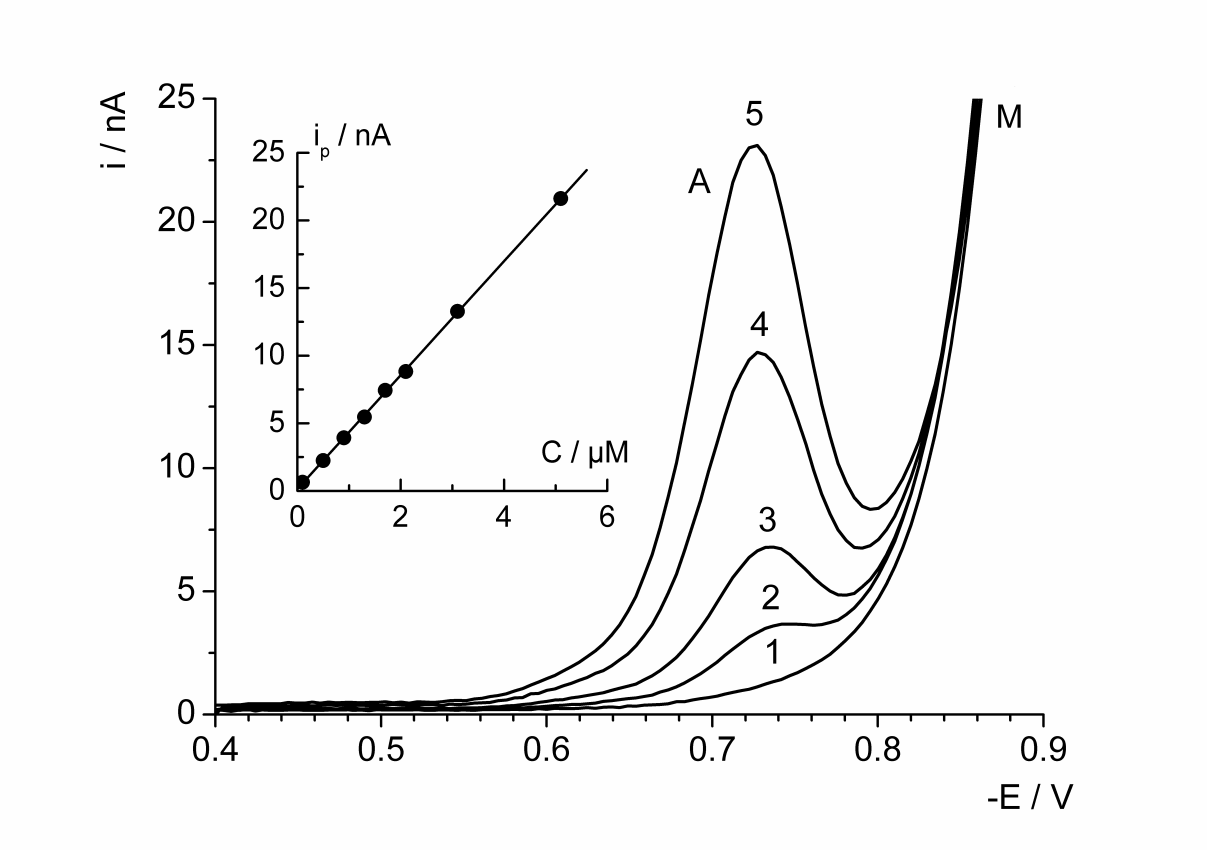

Fig. 1

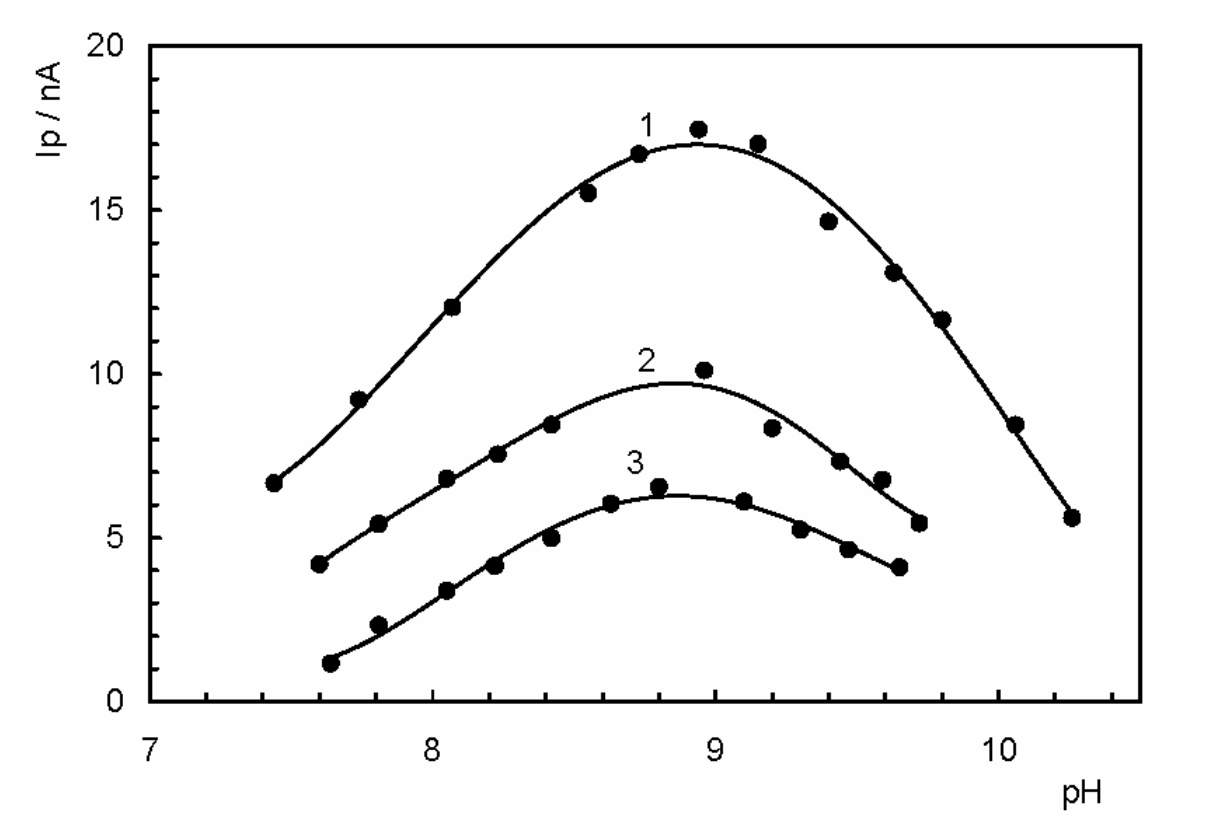

Fig. 2

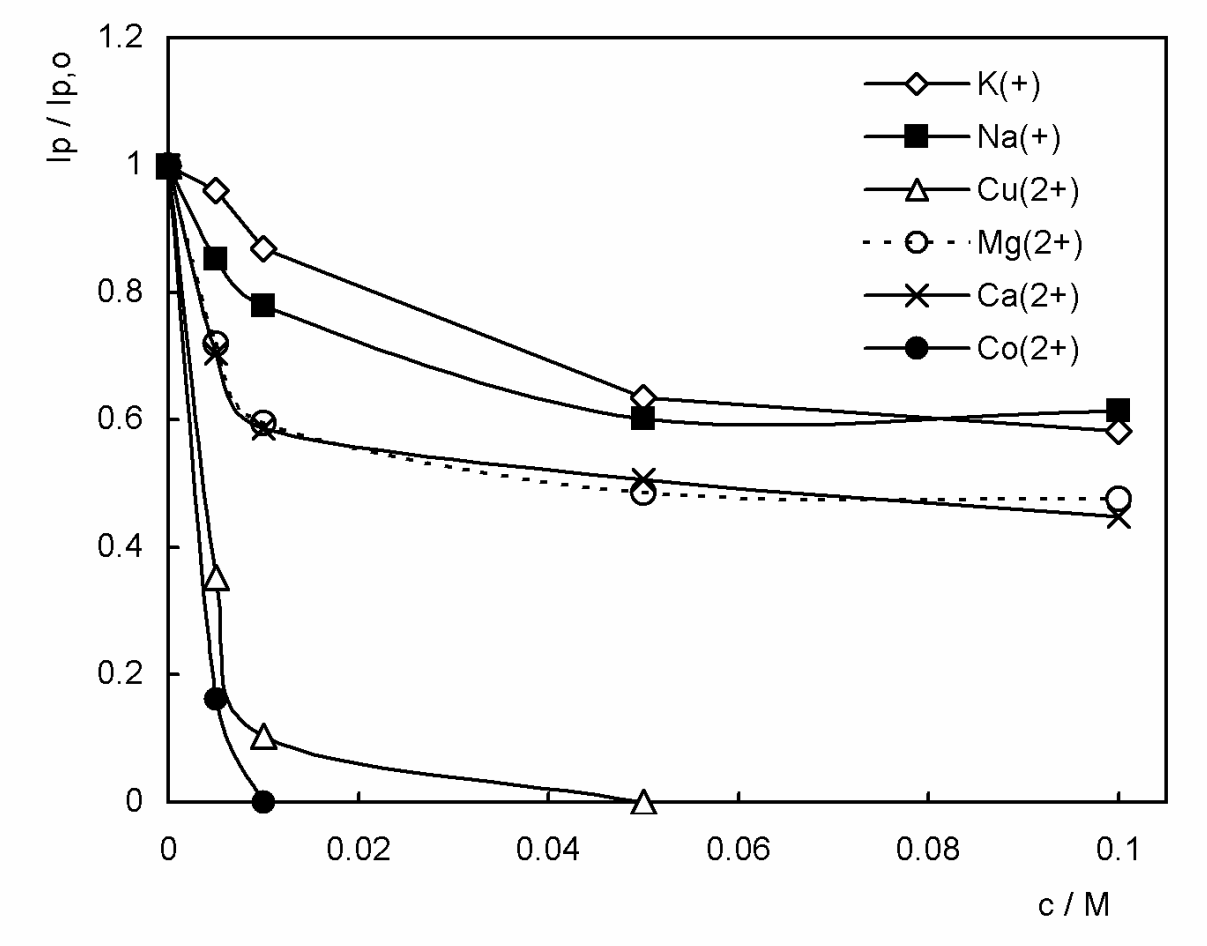

Fig. 3

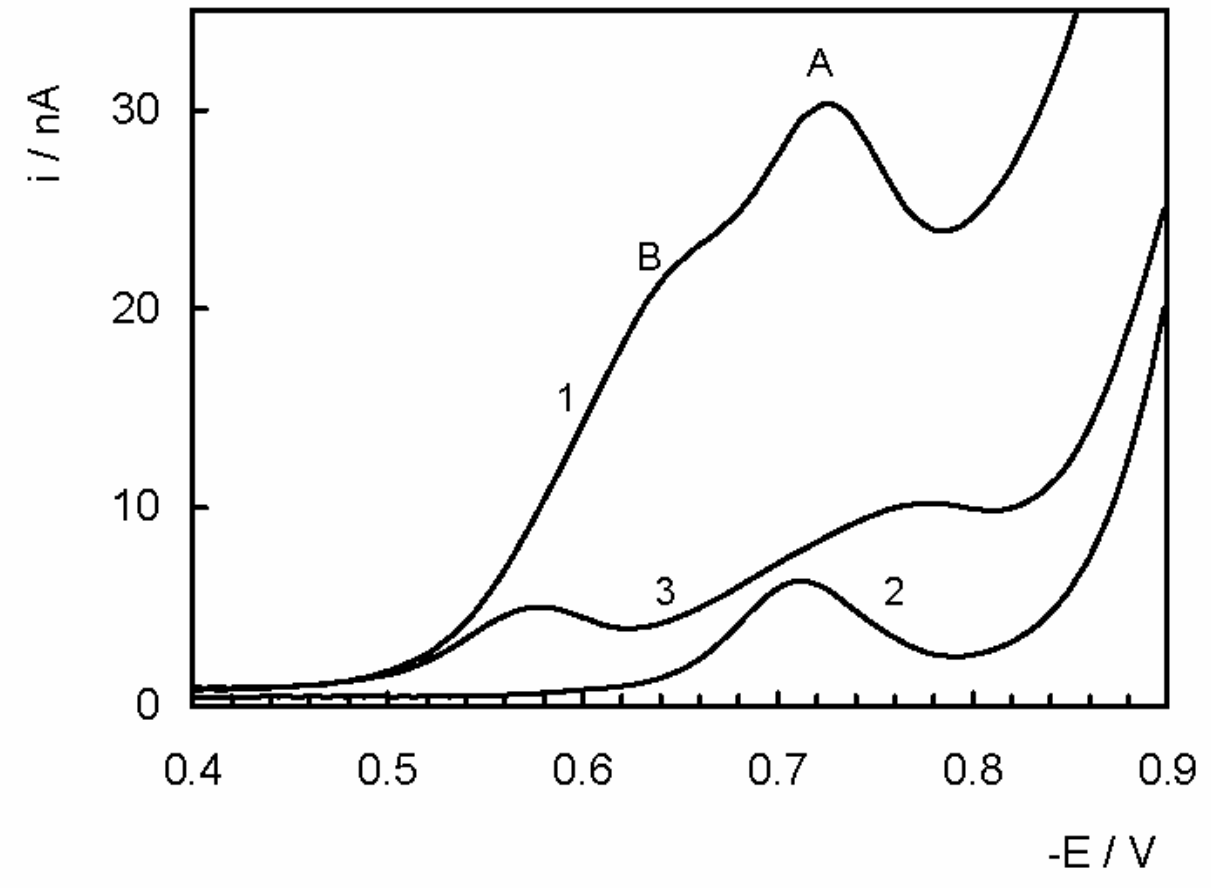

Fig. 4

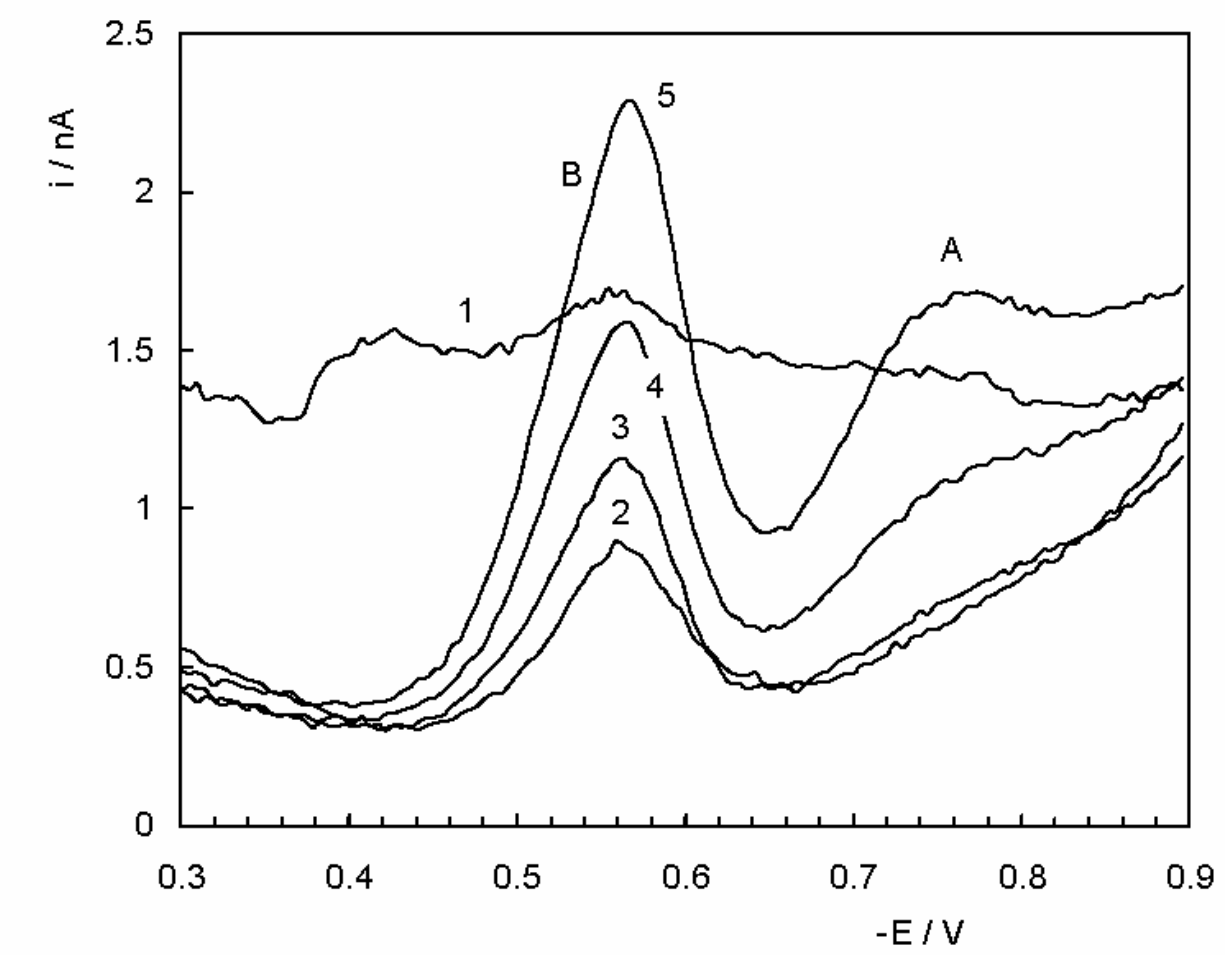

Fig. 5