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Colloidal-gold cysteamine-modified carbon paste electrodes as suitable electrode materials for the electrochemical determination of sulphur-containing compounds
Application to the determination of methionine

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Abstract

The suitability of colloidal-gold cysteamine-modified carbon paste electrodes (nAu-Cyst-CPE) for the electrochemical determination of sulphur-containing compounds is illustrated in this work by determining the amino acid methionine in real samples, as well as a methionine-based peptide. Voltammograms from methionine solutions at nAu-Cyst-CPE exhibited improved electroanalytical characteristics when compared with colloidal-gold cysteamine-modified Au disk electrodes (nAu-Cyst-AuE). Differential pulse voltammetry in phosphate buffer of pH 7.0 was used for the determination of methionine, with a range of linearity of (1.0–100) $\times 10^{-6}$ mol l^{-1} and a detection limit of 5.9×10^{-7} mol l^{-1}. This detection limit is remarkably lower than those reported previously using other modified electrodes or amperometric detection. Methionine peptides also exhibited anodic peaks suitable to detect this kind of molecules. Methionine was determined with good results in a pharmaceutical product containing several vitamins, amino acids and other compounds, and in spiked meat peptone, a complex sample containing enzimatically digested protein.

Keywords: Colloidal-gold cysteamine-modified electrodes; Thiocompounds; Methionine

1. Introduction

Metal nanoparticles play an important role in modern bioanalytical chemistry due to their usefulness for the preparation of sensors giving rise to improved responses from biological compounds with respect to those observed at conventional metal surfaces. In particular, gold nanoparticles have demonstrated to be very appropriate for the development of modified electrodes. Thus, many biological molecules are physically adsorbed on gold nanoparticles without loss of their biological activity, producing nanoparticles functionalization and neutralizing their negative charge [1]. Moreover, gold nanoparticles allow the construction of electrode nanoarrays in one or several dimensions, with signal-to-noise rations several orders of magnitude higher than those achieved at conventional electrodes.

Colloidal gold-modified electrodes have been prepared using glassy carbon [2,3], metal Au [4,5] and carbon paste [6,7] as electrode substrates. They permit analytical applications exhibiting very high sensitivity for the determination of proteins, nucleic acids or carbohydrates [2]. As an example, a DNA sensor with a limit of detection of 5×10^{-10} mol l^{-1} has been recently reported [5]. Enzyme biosensors based on gold nanoparticle modification is another exciting field of work. Some examples recently reported are the construction of a xanthine oxidase biosensor by adsorption of the enzyme on a colloidal gold-modified glassy carbon electrode [8], or a peroxidase biosensor based on a Au electrode modified with a cysteamine self-assembled monolayer coated with colloidal gold [4]. Other applications of colloidal gold-modified electrodes involve determination of neurotransmiters and drugs [3,9].

Very recently, we have demonstrated that carbon paste electrodes (CPEs) modified with cysteamine (Cyst) constitute excellent electrode surfaces for the preparation

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of colloidal-gold electrodes [10]. These colloidal-gold cysteamine-modified carbon paste electrodes (nAu-Cyst-CPEs) are prepared by following an extremely simple procedure consisting of adsorption of Cyst on the CPE surface, and immersion of the Cyst-CPE in a colloidal-gold suspension 24 nm in diameter. The so prepared nAu-Cyst-CPEs exhibited good analytical characteristics towards the electrochemical responses of different analytes containing sulphur atoms compared with conventional CP, glassy carbon and Au electrodes, as well as with a colloidal gold-modified CPE prepared by mixing the metal nanoparticles with graphite powder following the procedure described by Ju et al. [6]. No antecedents are found in the literature on gold nanoparticles sensors on CP modified with Cyst, except our above-mentioned work [10], although similar designs using metal gold electrode substrates have been cited above [4,5].

Methionine is an essential amino acid with an important role in biological methylation reactions. It constitutes the main supply of sulphur in the diet, preventing disorders in hair, skin or nails. Moreover, it helps to reduce cholesterol levels by increasing the lecitin production in liver, being also a natural chelating agent for heavy metals. Methionine can be present in real samples at different concentration level, from near 0.1% (w/v) in some drugs to 10^{-5} mol l^{-1} in blood plasma.

Chromatographic techniques such as GC-MS or LC with UV or fluorimetric detection have been used for the determination of methionine and related compounds in body fluids [11–18]. Regarding electrochemical techniques, flow injection methods with amperometric detection at composite electrodes modified with Ru or RuO_2 [19], or at carbon paste electrodes modified with Ru complexes [20] have been reported. Indirect methods based on the amperometric detection of bromide [21] or iodide [22], which are related with the amount of methionine by a redox reaction, were also developed. Moreover, pulse amperometric detection (PAD) at gold electrodes has been used for the detection of methionine and other amino acids after separation by liquid chromatography [23,24]. Voltammetric methods at CuO-carbon paste electrodes [25] or HMDE [26] were also reported.

In this article, the suitability of nAu-Cyst-CPEs for the electrochemical determination of sulphur-containing compounds is demonstrated by using methionine as the analyte probe. Thus, methionine was determined in real samples, and the analysis of a methionine-based peptide is also reported.

2. Experimental

2.1. Apparatus and electrodes

Voltammetric measurements were carried out with a BAS (West Lafayette, IN, USA) 100 B potentiostat provided with a BAS C2 EF-1080 cell stand. Working electrodes were colloidal-gold cysteamine-modified carbon paste electrodes prepared as reported previously [10]. Colloidal-gold cysteamine-modified Au electrodes (nAu-Cyst-AuE) were also prepared for comparison purposes. A Metrohm 6.1204.020 Au disk electrode (3 mm in diameter) was cleaned using a "piranha" solution at 60 °C for 5 min. Then, it was washed with ethanol and water, alternatively, three times each and dried with a nitrogen stream. Then, the same procedures used to modify CPEs with Cyst and to deposit colloidal gold on the Cyst-modified electrodes [10] were employed. The reference electrode was a BAS MF 2063 Ag/AgCl electrode and a BAS MW-1032 Pt wire was used as the auxiliary electrode. BAS VC-2 10-ml electrochemical cells were also used.

2.2. Reagents and solutions

Aqueous 1% $HAuCl_4.3H_2O$ (Sigma, >49.0% as Au), 1% sodium citrate (Panreac, 98%) and 0.40 mol l^{-1} cysteamine hydrochloride (2-aminoethanethiol) (Aldrich, 98%) were used. Stock 1.0×10^{-2} mol l^{-1} aqueous solutions of L-methionine (Sigma, >99% TLC), thiamine hydrochloride (Vitamin B_1) (Sigma) and the amino acids Glu, His, Cys, Lys, Gly, Trp, Tyr, Leu, Arg and Val (Sigma or Aldrich, 99%) were also used. A 0.10 mol l^{-1} $H_2PO_4^-/HPO_4^{2-}$ (Scharlab, 99%) buffer solution of pH 7.0 and a Britton–Robinson buffer solution containing each component acid at 0.2 mol l^{-1}, whose pH values were adjusted by suitable addition of a 2 mol l^{-1} NaOH solution, were used. "Piranha" solution containing 4:1 concentrated H_2SO_4 and 30% H_2O_2 was also used. (CAUTION: "Piranha" solution is a very strong oxidizing agent and very dangerous to handle in the laboratory. Protective equipment including gloves and face shields should be used all times). All solvents and chemicals were of analytical reagent grade. The water used was obtained from a Millipore Milli-Q system.

2.3. Samples

The pharmaceutical product "Aminoveinte" (Laboratories Madariaga, Madrid, Spain), a white suspension containing 66.6 mg methionine per 100 ml (label concentration), B_1, B_2, B_6, D_3 and H Vitamins, Lys, Gly, His, Glu amino acids, nicotinamide, folic acid, inositol and excipients, was analysed. Meat peptone in powder (Scharlab), prepared from animal tissues by pepsin hydrolysis, containing peptides of Ala, Arg, Phe, Gly, Glu, His, Ile, Leu, Lys, Met, Pro, Ser, Thr and Trp, as well as phosphate, chloride, calcium and sodium ions, was also analysed.

2.4. Procedures

2.4.1. Preparation of colloidal gold

Colloidal gold (d 24 nm) was prepared as described previously [10], by adding 0.66 ml of sodium citrate solution to a boiling 50-ml solution of 0.01% $HAuCl_4$. The mixture was maintained boiling for 15 min and stirred for other 15 min until room temperature was reached. The colloidal suspension obtained was stored in a brown glass bottle at 4 °C.

2.4.2. Determination of methionine in the pharmaceutical product "Aminoveinte"

The homogeneized sample suspension was filtered through a 0.45 μm nylon mesh. The filtered sample was passed through a C_{18} cartridge (Varian, Mega Bond Elut, 6 ml, 1 g), previously conditioned with 3 ml of Milli-Q water. The eluate was filtered through a 0.22 μm nylon mesh and 11 μl of aliquot was transferred to a 5-ml flask and diluted to the mark with 0.1 mol l^{-1} $H_2PO_4^-/HPO_4^{2-}$ buffer solution of pH 7.0. This solution was transferred to the electrochemical cell and differential pulse voltammetry at the nAu-Cyst-CP electrode was performed. Methionine determination was carried out by applying the standard additions method, which implied the addition of methionine in the range (1.0–10.0) $\times$ 10^{-5} mol l^{-1} from a standard 5.0 $\times$ 10^{-3} mol l^{-1} methionine solution.

2.4.3. Determination of methionine in meat peptone

About 1 g of sample previously homogeneized in a mortar and spiked with methionine at the 15 μg g^{-1} level, was let to equilibrate for at least fifteen minutes and then it was accurately weighed into a 25 ml centrifuge tube. Next, 10.0 ml of Milli-Q water were added, and the mixture was sonicated for 2 min. A 10 μl aliquot of this solution was transferred to a 5-ml flask and diluted to the mark with 0.1 mol l^{-1} $H_2PO_4^-/HPO_4^{2-}$ buffer solution of pH 7.0. This solution was filtered through a 0.22 μm nylon mesh, and then transferred to the electrochemical cell. Differential pulse voltammetry at the nAu-Cyst-CPE was carried out. The determination of methionine was performed by applying the standard additions method, which implied four additions of 10 μl from a standard 5.0 $\times$ 10^{-3} mol l^{-1} methionine solution.

3. Results and discussion

As it has been pointed out in the Section 1, we have demonstrated recently that sulphur-containing compounds have been shown to exhibit improved voltammetric responses at nAu-Cyst-CPEs when compared with conventional CP, glassy carbon and Au electrodes, as well as with a colloidal gold-modified CPE prepared by mixing the metal nanoparticles with graphite powder [10]. Modification of the CPE with Cyst self-assembled monolayers allowed the subsequent bond of gold nanoparticles by following an extremely simple procedure consisting of immersion of Cyst-CPEs in a colloidal-gold suspension 24 nm in diameter [10]. This led us to develop a methodology for the determination of the amino acid methionine in real samples. Fig. 1 shows cyclic voltammograms from a 1.0 $\times$ 10^{-4} mol l^{-1} methionine solution obtained at a nAu-Cyst-CPE and at a colloidal-gold Cyst-modified Au disk electrode (nAu-Cyst-AuE), prepared following a similar procedure to that used to modifiy CPEs with Cyst and to deposit colloidal gold on the Cyst-modified electrodes (see Section 2). Methionine shows an oxidation peak at approximately +0.95 V at both colloidal-gold Cyst-modified electrodes. This oxidation is produced at potential values close to those corresponding to the formation of gold oxides (see dotted voltammograms obtained from the background solution). It is well known [24] that the anodic response of thiocompounds at Au electrodes is initiated with the thiocompound preadsorption onto the oxide-free electrode. Then, the oxidation occurs concomitantly with the formation of the surface oxide and, in this step, the transfer of oxygen to the thiocompound is facilitated by the labile surface oxides thus occurring an oxide-catalyzed process. The electrochemical oxidation of thiocompounds containing a sulfur atom in an intermediate position, such as methionine, gives rise to a sulfone as the final product, via the reaction: $R - S - R' + 2H_2O \rightarrow R-SO_2-R'+4H^+ +4e^-$ [27]. The cathodic peak observed in Fig. 1 at approximately +500 mV is attributed to the reduction of the gold oxides formed previously in the anodic scan.

As it can be observed in Fig. 1, the methionine oxidation signal at the nAu-Cyst-CPE was much better defined than

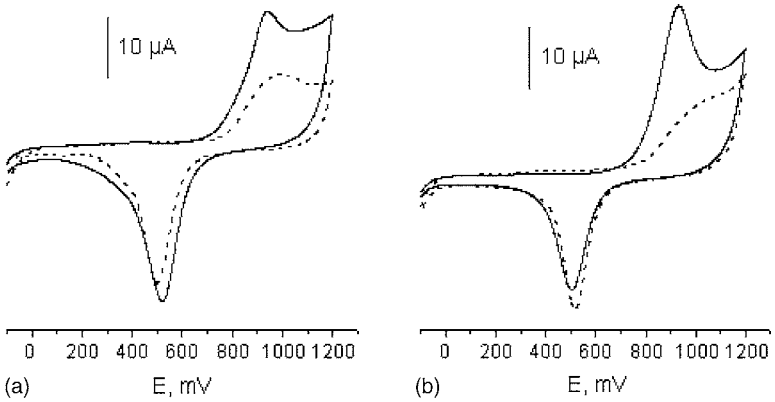

Fig. 1. Cyclic voltammograms of 1.0 $\times$ 10^{-4} mol l^{-1} methionine at: (a) nAu-Cyst-AuE; (b) nAu-Cyst-CPE; (---) background solution. 0.1 mol l^{-1} $H_2PO_4^-/HPO_4^{2-}$ buffer solution, pH 7.0; $v = 50$ mV s^{-1}.

that obtained at the nAu-Cyst-AuE. Also a higher peak current was measured at the nAu-Cyst-CPE, thus confirming that CPEs modified with Cyst constitute excellent electrode surfaces for the preparation of colloidal-gold electrodes.

Different aspects concerning the stability of the nAu-Cyst-CPEs and the reproducibility of the methionine voltammetric responses were tested and compared with the results obtained also at nAu-Cyst-AuEs.

Thus, the voltammetric response of a 1.0×10^{-4} mol l^{-1} methionine solution in 0.1 mol l^{-1} $H_2PO_4^-/HPO_4^{2-}$ buffer of pH 7.0 was evaluated using three different nAu-Cyst-CPEs constructed in the same manner. The measurements were carried out every 24 h in three different days, and the modified electrodes were stored at 4 °C after the measurements. Similar experiments were made with three different nAu-Cyst-AuEs. A slight decrease of the methionine oxidation peak currents with time, more pronounced at the nAu-Cyst-AuEs, was observed. Thus, a relative standard deviation (R.S.D.) of 12% was obtained for the i_p values measured with all the three nAu-Cyst-CPEs for the three days, whereas a RSD of 26% was found for nAu-Cyst-AuEs. This difference suggests that the nature of the electrode substrate employed can affect the stability of the Cyst-colloidal gold coating. Moreover, peak current mean values of 20.5 ± 0.4 μA and 15 ± 2 μA were obtained for the three nAu-Cyst-CPEs and the three nAu-Cyst-AuEs during the first working day, respectively. Finally, repetitive measurements with one electrode yielded R.S.D. values of 2.4 and 11.5% ($n = 5$) for the nAu-Cyst-CPEs and the nAu-Cyst-AuEs, respectively.

Additional advantages of the preparation of colloidal-gold electrodes onto carbon paste substrates compared with gold, are that an exhaustive cleaning pretreatment of the substrate surface prior the adsorption of the Cyst layer is not necessary, and that the substrate material is much cheaper.

3.1. Voltammetric determination of methionine at nAu-Cyst-CPEs

Fig. 2 shows differential pulse (DP) and net-square wave (SW) voltammograms, recorded with background substraction, at a nAu-Cyst-CPE for methionine concentrations between 1.0 and 5.0×10^{-4} mol l^{-1}. As expected for an irreversible electrochemical system, the use of SWV did not produce a significant increase of the peak current with respect to that obtained by DPV. Moreover, the slope values of the calibration graphs obtained in this short concentration range were similar by using both techniques, although a higher intercept was observed with SWV, Finally, 25 successive peak current measurements from 5.0×10^{-5} mol l^{-1} methionine solutions gave R.S.D. values of 2.4% by DPV and 4.5% by SWV. All these results led us to choose DPV as the technique to implement the voltammetric determination of methionine.

The influence of pH on the peak current and the peak potential of 1.0×10^{-5} mol l^{-1} methionine was checked in

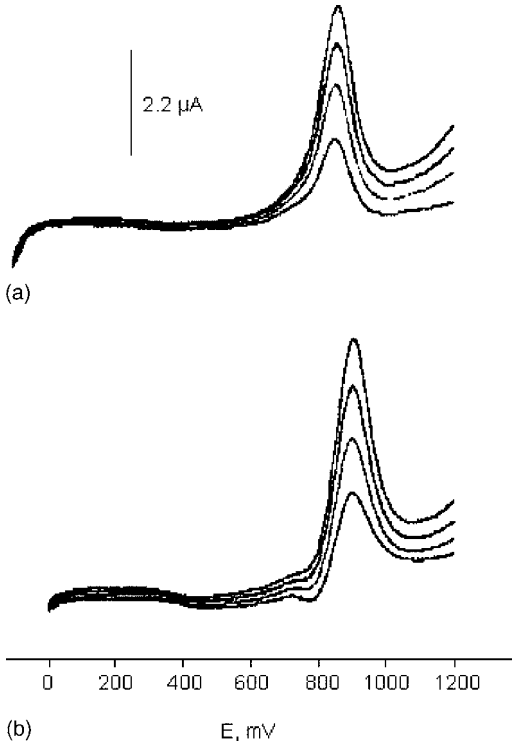

Fig. 2. DP (a) and SW (b) voltammograms from $(1.0–5.0) \times 10^{-4}$ mol l^{-1} methionine solutions at nAu-Cyst-CPE. (0.1 mol l^{-1} $H_2PO_4^-/HPO_4^{2-}$ buffer solution, pH 7.0; $v = 50$ mV s^{-1}. DPV: $\Delta E = 50$ mV; $v = 20$ mV s^{-1}; SWV: $E_{SW} = 25$ mV; $\Delta E_s = 4$ mV; $f = 15$ Hz.

the 2.0–10.0 range using a 0.05 mol l^{-1} Britton–Robinson buffer solution as supporting electrolyte. A single oxidation peak for methionine was observed over the whole pH range, whose E_p and i_p values decreased as the pH increased. Furthermore, the background oxidation signal corresponding to the formation of gold oxides, appearing at potentials close to those of methionine, showed a similar potential shifting with pH. Fig. 3A shows the dependence of E_p with pH, exhibiting a linear plot ($r = 0.998$) over the 4–9 pH range. The slope of this linear plot is −60 mV, indicating that the same number of protons and electrons are involved in the electrochemical reaction, in good agreement with that reported in the literature ($nH^+ = ne = 4$) [27]. Deviations from linearity observed at lower and higher pH values than those of the linear range are related to the pK_a values of methionine (2.3 and 9.2) [28]. Concerning the i_p dependence with pH, Fig. 3B shows some of the voltammograms obtained at different pH values. As can be seen, higher i_p values were obtained in acidic and neutral media. Considering both the signal-to-background current ratio and the peak potential, pH 7.0 was selected for further work as a compromise between good sensitivity and selectivity. Regarding the composition of the buffer solution, methionine DPV responses in 0.1 mol l^{-1} phosphate, citrate and Britton–Robinson buffers were compared. The best peak current-to-background current ratio was observed in the $H_2PO_4^-/HPO_4^{2-}$ solution, and then it was selected for further work.

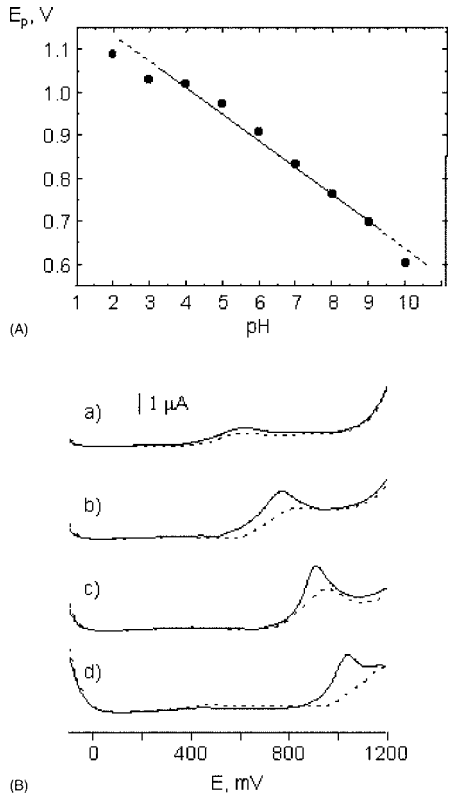

Fig. 3. (A) Effect of pH on the methionine oxidation peak potential obtained by differential pulse voltammetry; 1.0×10^{-5} mol l^{-1} methionine in 0.05 mol l^{-1} Britton–Robinson buffer. (B) Differential pulse voltammograms obtained at pH 10.0 (a); 8.0 (b); 6.0 (c) and 3.0 (d) at a nAu-Cyst-CPE; $\Delta E = 50$ mV; $\nu = 20$ mV s^{-1}.

Under these experimental conditions, repetitive voltammograms from 20 different 1.0×10^{-5} mol l^{-1} methionine solutions, obtained with the same electrode, yielded a R.S.D. for i_p of 2.3%, thus, demonstrating a very good precision in the electroanalytical measurements. As a comparison, a similar set of voltammograms at a bare Au disk electrode gave a mean peak current value for methionine 40% than that obtained at the nAu-Cyst-CPE, with a R.S.D. of 10%. Furthermore, a R.S.D. for i_p of 4.2% was obtained from repetitive DP voltammograms with four different nAu-Cyst-CPEs, indicating a good reproducibility of the measurements when different colloidal gold-modified electrodes were employed.

A linear calibration graph ($r = 0.997$) for methionine was obtained by DPV in the (1.0–100) $\times 10^{-6}$ mol l^{-1} concentration range, with a slope of (3.16 ± 0.06) $\times 10^4$ μA mol^{-1} l. A limit of detection of 5.9×10^{-7} mol l^{-1} was calculated from the $3s_b$/m criterion, where s_b was estimated as the standard deviation of the signals from 1.0×10^{-6} mol l^{-1} solutions ($n = 10$), and m was the slope value of the calibration plot. This detection limit, which corresponds to 88 μg l^{-1} methionine, was remarkably lower than those reported previously using other modified electrodes [19,20] or amperometric detection [22]. Moreover, a limit of determination of 1.9×10^{-6} mol l^{-1} was also calculated according to the $10s_b$/m criterion.

3.2. Determination of methionine in real samples

Prior to the determination of methionine in real samples, an interference study from other compounds which may be present together with methionine in different types of samples was accomplished. Substances tested as potential interferences were other amino acids: His, Glu, Gly, Cys, Trp, Tyr, Leu, Lys, Arg and Val, as well as some vitamins: thiamine (Vitamin B_1), tocopherol (Vitamin E) and Vitamin D_2. The effect of the presence of these substances was checked at a concentration level of 1.0×10^{-5} mol l^{-1}. Under the experimental conditions used for the determination of methionine, the amino acids Trp, Tyr, Leu, Arg and Val did not exhibit DP voltammetric responses differentiated from the background voltammograms over the whole potential range scanned (−0.1 to +1.2 V). However, Glu, Gly, His, Cys and Lys gave rise to oxidation peaks well differentiated from the background voltammogram with E_p values close to that of methionine. The small difference between these E_p values produced an overall oxidation signal when some of these amino acids is present together with methionine in solution. Relative errors higher than 10% were found for the methionine concentration for methionine-to-interferent concentration ratios of 1:25 (Glu), 1:10 (His) and 10:1 (Gly, Lys, Cys).

Regarding vitamins, tocopherol (Vitamin E) and Vitamin D_2, which are usually present together with methionine in many drugs, did not show any interference due to their low solubility in the aqueous phosphate buffer used as working medium. Thiamine showed an oxidation peak at a potential value 100 mV more positive than that of methionine, and no interference was observed up to a methionine:thiamine concentration ratio of 1:5.

3.3. Determination of methionine peptides

The detection of small peptides containing methionine residues is of high interest since they are involved in important neuronal processes [29]. The oxidation of methionine in peptides represents a modification under conditions of oxidative stress, aging and during the pathogenesis of Alzheimer's disease. Thus, the formation of $Met^{\bullet+}$ radical plays a role in the neurotoxicity of β-amyloid peptides involved in the pathogenesis of this disease [30]. Moreover, the use of peptides such as methionine-enkephalin has been proposed therapeutic drugs [31].

Therefore, analytical methods to detect the intact peptide as well as its metabolites are needed in pharmacokinetic studies. The electrochemical behaviour of these kind of molecules at the nAu-Cyst-CPE was tested using the commercial peptide Gly-Met as a probe. DP voltammograms from peptide solutions in 0.1 mol l^{-1} $H_2PO_4^-/HPO_4^{2-}$ buffer of pH 7.0 showed an anodic peak corresponding to methionine oxidation (Fig. 4). A linear calibration graph over the 1.0×10^{-6} to 1.0×10^{-5} mol l^{-1} concentration range was obtained with a slope of (9.3 ± 0.3) $\times 10^4$ μA mol^{-1} l ($r = 0.996$), thus demonstrating the useful-

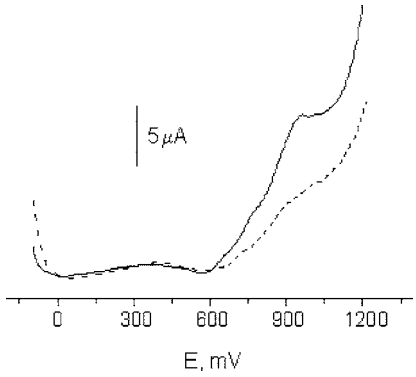

Fig. 4. Differential pulse voltammogram from a 1.0×10^{-5} mol l^{-1} solutions of a Gly-Met peptide obtained at a nAu-Cyst-CPE. (- - -) Background voltammogram in phosphate buffer of pH 7.0; $\Delta E = 50$ mV; $v = 20$ mV s^{-1}.

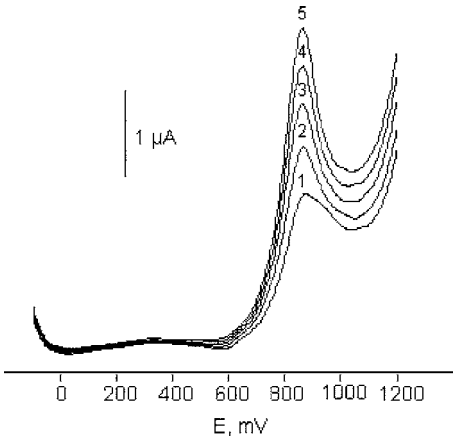

Fig. 5. (1) Differential pulse voltammograms from an "Aminoveinte" sample solution obtained at a nAu-Cyst-CPE. (2–5) Voltammograms recorded after additions of methionine in the (1.0–10.0) $\times$ 10^{-5} mol l^{-1} range (standard additions method); $v = 50$ mV s^{-1}.

ness of the colloidal-gold electrode to detect methionine peptides.

3.4. Determination of methionine in a pharmaceutical product

Methionine was determined by DPV at the nAu-Cyst-CPE in the pharmaceutical product "Aminoveinte" whose composition is described in Section 2. This product contains several vitamins, amino acids and other compounds. Clean-up of excipients from the sample solution was carried out by passing the filtered sample through a C_{18} cartridge. Following the procedure described in Section 2, a DP voltammogram for methionine was obtained (Fig. 5). No signals appeared from other components of the sample, since their concentration in the analytical solution were low enough to prevent some interfering effect. The determination of methionine was performed by using the standard additions method in order to minimize tha matrix effect. Some of the voltammograms recorded are also shown in Fig. 5. The results obtained from 10 replicates yielded a mean methionine concentration of 67 $\pm$ 2 mg for 100 ml of sample, which agrees very well with the labelled value (66.6 mg/100 ml). The R.S.D. obtained for this determination, 4.2%, indicated a good precision.

3.5. Determination of methionine in meat peptone

Meat peptone is a complex undefined sample containing enzymatically digested protein commonly used for growing yeast cultures. Therefore, this is a very complex sample in which free methionine can be present as a result of the methionine-containing peptides complete hydrolysis, at a maximum concentration level (specified for this product) of 15 μg mg^{-1}. DP voltammograms from a peptone solution (Section 2.4) showed a similar shape than that of the background supporting electrolyte solution, thus indicating the absence of free methionine in the sample. Then, samples were spiked with methionine at the mentioned 15 μg mg^{-1} concentration level, and the simple experimental procedure described in the Section 2 was followed. Application of the standard additions method gave recoveries for methionine ranging between 93 and 106%, with a mean value of 14.7 $\pm$ 0.6 μg mg^{-1}, which confirmed the validity of the analytical methodology used for the detection of this amino acid in a so complex matrix.

4. Conclusions

The results described above demonstrate fairly well that colloidal-gold cysteamine-modified carbon paste electrodes constitute excellent electrode materials for the voltammetric determination of thiocompounds such as methionine, as well as of methionine-based peptides. The electroanalytical measurements performed with these electrodes exhibit a good reproducibility, both with the same electrode and different colloidal gold-modified electrodes fabricated in the same manner, and they compare advantegeously with similar measurements carried out at bare Au electrodes and at nAu-Cyst-AuE. Finally, the suitability of nAu-Cyst-CPEs for the analysis of methionine in real samples has been proved.

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