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An amperometric sensor based on electrochemically triggered reaction: Redox-active Ar–NO/Ar–NHOH from 4-nitrophthalonitrile-modified electrode for the low voltage cysteine detection

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Abstract

The present work describes the development of a highly sensitive amperometric sensor based on a simple and efficient carbon paste electrode modified with a new redox “mediator”, 4-nitrophthalonitrile, toward detection of cysteine (CySH) in neutral medium at an applied potential of 0.33 V vs. Ag/AgCl. The sensor showed its best performance in 0.1 mol L^{-1} phosphate buffer solution, at pH 7.0. After optimizing the operational conditions, the sensor provided a linear response range for CySH from 0.8 up to 13.2 μmol L^{-1} with sensitivity, detection and quantification limits of 37 nA L μmol^{-1}, 0.25 μmol L^{-1} and 0.80 μmol L^{-1}, respectively. The sensitivity of the proposed sensor was much higher when compared to other sensors described in the literature and showed a stable response for at least 80 successive determinations. The repeatability of the measurements with the sensor and the preparation of a series of electrodes, evaluated in terms of relative standard deviation, were 4.1 and 5.0%, respectively, for $n = 10$. The developed sensor was applied for cysteine determination in food supplement samples and the results were statistically the same with those obtained by the comparative method described in the literature at a confidence level of 95%.

Keywords: 4-Nitrophthalonitrile; Cysteine; Amperometric sensor

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1. Introduction

Cysteine (2-amino-3-mercaptopropanoic acid) is one of the most important aminoacids. It plays an important role in biological systems and has been widely used in medicine and food chemistry [1,2]; it is also present in various cosmetic and pharmaceutical preparations, for example, used in some antibiotics and treatment of skin damage [3], as well as it participates on a great number of biochemical processes [4].

Studies focusing in the cysteine (CySH) determination have attracted considerable attention in nowadays [5,6]. In this sense, several methods for its detection and quantification have been reported including, spectrofluorimetry [7], high performance liquid chromatography [8] and electrochemical methods [9–12]. However, most of them experienced difficulties with sample preparation, necessity of molecules derivatization or lack of sufficient sensitivity, which limit their practical utility [13].

On the other hand, electrochemical methods present the advantages of simplicity and high sensitivity. However, the major problem related to the direct electrochemical detection of thiols is the high overpotential required for their oxidations on the most conventional electrodes. In this

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context, the use of bare electrodes for detection has a great number of limitations, such as low selectivity, repeatability and the slow electron transfer reaction. Thus, chemically modified electrodes (CMEs) have been widely used as sensitive and selective analytical methods for detection and quantification of thiols at lower potentials [14,15], since the direct oxidation of thiols on the solid electrodes is slow and requires high overpotentials [16].

In this context, the surface modification of conventional electrodes is an important development in electroanalysis. A variety of efficient catalysts has been developed to induce the reduction or oxidation of some organic compounds [17,18], since these catalysts promote a faster electron transfer between the electrode surface and electroactive species in solution. Thus, a wide variety of compounds has been used as electron transfer mediators for electrooxidation of CySH, for example, lead ruthenate pyrochlore [19], pyrroloquinoline quinone (PQQ) [20], cobalt(II)-4-methylsalophen [21], fullerene-C60 [22], oxovanadium(IV) complex of Salen [23], Pt microelectrode modified with carbon nanotubes [24], ruthenium complex [25] and phthalocyanines [26–29]. However, these catalysts present a series of limitations as poor sensitivity, reproducibility or redox processes performed at high overpotentials.

In this sense, the present work describes the preparation and use of a highly sensitive amperometric sensor based on a simple and efficient carbon paste electrode modified with a new redox mediator, the 4-nitrophthalonitrile, towards cysteine (CySH) detection. The application of this electrode for CySH determination in food supplement samples containing L-cysteine showing its reliability is also presented.

One of the reasons to use the term "new redox mediator" refers to the versatility of the polyfunctional compound 4-nitrophthalonitrile, which presents two cyano and one nitro groups, that: (i) facilitates the electron transfer process; (ii) could be activated through the irreversible four electrons reduction of $–NO_2$ to –NHOH [30–32]. The resulting hydroxylamine can be oxidized reversibly to the corresponding nitroso compound (RNO/RNHOH couple), by a $2e^-/2H^+$ redox process. This electrogenerated system is a good candidate for catalysis, because not only two-electrons but also the released protons can be accepted by the mediator. This might be an advantage compared to some known classical mediators.

2. Experimental

2.1. Chemical and solutions

All chemicals were of analytical grade. The food supplement sample was purchased from a local drugstore. Graphite powder (99.9%) and mineral oil were purchased from Aldrich, Milwaukee–USA. Cysteine, McIlvaine (Na_2HPO_4 and citric acid), PIPES [piperazine-*N*-*N*-bis[2-ethanesulfonic acid]], HEPES[*N*-(2-hydroxyethyl)piperazine-*N*-(2-ethanesulfonic acid)] and [tris(hydroxymethyl) aminomethane] were acquired from Sigma, St. Louis, USA. Disodium and monosodium phosphate (Na_2HPO_4 and NaH_2PO_4) were acquired from Synth, São Paulo, Brazil. 4-Nitrophthalonitrile was synthesized by the methods described by Young and Onyebuago [33]. The solutions were prepared by using water purified in a Milli-Q Millipore system and the actual pH values of the buffer solutions were determined with a Corning pH/Ion Analyser model 350.

2.2. Construction of the sensors

The modified carbon paste was prepared by mixing 35 mg of graphite powder with 5 mg of 4-nitrophthalonitrile (0.029 mmol) and 20 μL of mineral oil, added to give consistence to the paste. The influence of the ratio between 4-nitrophthalonitrile and graphite powder used in the modified electrode preparation on the peak current was investigated in the 1:13, 1:7, 1:3, 1:1 and 3:1 (w/w) proportion. This paste was put into a cavity in the extremity of a glass tube containing a Pt slide, for the electrical contact with the paste. All the responses obtained with the proposed sensor were given in terms of current density. Thus, the geometric area of the work electrode (sensor) was determined as ($A = \pi r^2$) and it presented the value of 0.2 cm^2.

2.3. Electrochemical measurements

The voltammetric measurements were carried out with a potentiostat PGSTAT-30 Model from Autolab Echo Chemie (Utrecht, The Netherlands) connected to a PC (Software GPES 4.9). An electrochemical cell with three electrodes was used with a Ag/AgCl (saturated KCl) electrode as reference, a Pt wire as auxiliary and unmodified or 4-nitrophthalonitrile-modified carbon pastes as working electrodes, for all measurements. The measurements were carried out, using 5.00 ml of buffer solutions. Oxygen was removed by bubbling nitrogen through the solution.

2.4. Procedure for the sample preparation for amperometric and spectrophotometric determinations

For amperometric analysis, the food supplement samples [Dietary Supplement L-cysteine (500 mg)] were prepared by dissolving the sample (one capsule of 500 mg) in 1000.00 mL of water and then the solution was filtered through a quantitative paper filter. After this step, an aliquot of 125 μL of the filtered solution was diluted to 5.00 mL. An aliquot of 100 μL of the solution was added to the cell containing 5.00 mL of the supporting electrolyte to be measured. For the spectrophotometric measurements, the samples were diluted to 1000.00 mL with deionized water and then, the solution was filtered through a quantitative paper filter. Other steps were performed according to already described procedure [34], where an aliquot of the sample was transferred to a test tube plus 200 μL of *p*-benzoquinone adjusting the volume with acetate buffer (pH 3.0).