Flow-injection Analysis Technique Used to Electrochemically Measure Nitrite through a Gold Working Electrode Modified with 1-2 Diaminobenzene (DAB)

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ABSTRACT
In this paper, it is presented nitrite analysis, electrochemically measured, using a Flow-injection Analysis (FIA) system. The importance of determining nitrite from water, food and blood is based on its toxicity for human health, even at low concentrations (<4 mM). The presented electrochemical planar sensor uses silk-screen technology and has a working electrode covered with 1-2 Diaminobenzene (DAB) that allowed good selectivity, reproducibility, stability and sensitivity at 0.75 VAg/AgCl Nafion® 117, 0.5 VAg/AgCl Nafion® 117 and 0.3 VAg/AgCl Nafion® 117. It was noteworthy that nitrite response adds to the response of the studied interfering elements (paracetamol, ascorbic acid and uric acid) and it is predominant for concentrations lower than 175 µmol L⁻¹. Finally, the nitrite sensitivity was 11.2 mA mol⁻¹ L mm⁻² using the working electrode recovered with DAB at 0.5 VAg/AgCl Nafion® 117.

Index Terms: nitrite, electrochemical sensor, 1-2 Diaminobenzene (DAB) and silk-screen.

1. INTRODUCTION

The need for quantitative determination of nitrite ions (NO₂⁻) has increased due to its potential toxicity to human health and environmental security. Nitrites can react with amines in stomach forming nitrosamines, which are known to be carcinogenic [1-3]. If present at high concentrations in blood, nitrite can react with hemoglobin, forming methemoglobin, which has no oxygen carrying ability [4,5].

Nitrite, as a key specie in the global nitrogen cycle [6,7], is present in soils, waters, foods and physiological systems. Also, it is employed as food preservative against poisoning by microorganisms such as clostridium botulinum and, it appears in water during chloramination. In addition, nitrite ion is of interest as one of the NO metabolites in biological systems and has recently been investigated as a substitute for indirect in vivo NO detection since it is an important element associated to some physiological events like neurotransmission, vasodilatation [8], inflammation and cell proliferation. Reaction 1, as follows, illustrates the formation of Nitric Oxide (NO), which may occur in the human body [3].

\[ 2NO_2^- + 2O_2 + 6H^+ \rightarrow 2NO + H_2O_2 + 2H_2O + O_2 \] (1)

In Intensive Therapy Units, it is of importance to control the nitrite concentration to avoid patient intoxication due to diet or medicaments. For this, both must be balanced in order to control the nitrite in the organism at low concentrations (<4 µM) [3].

The review by Moorcroft et al. [3] describes the analytical techniques used for determination of nitrate and nitrite. These include spectroscopic, chromatographic, electrochemical and capillary electrophoresis. Electrochemical methods are cheaper and, in general, involve either oxidation or reduction of nitrite. Oxidation is preferred since interferences from reduction of nitrate and molecular oxygen are avoided. The oxidation reaction of nitrite ions in water is given as follows [3]:

\[ NO_2^- + 2OH^- \rightarrow NO_3^- + 2H_2O + 2e^- \quad E^0 = 0.01 \text{ V}_\text{H} \] (2)

Nitrite is electroactive at glassy carbon, platinum, diamond, gold, copper and transition metal oxide electrodes. However, several species can poison the electrode surface and decrease the sensitivity and accuracy [3].
The interfering elements that can poison the electrode surface include paracetamol, ascorbic acid and uric acid, which may be present in plasma extracted from blood of patients of Intensive Therapy Units due to medicaments. Therefore, quantitative determination of nitrite in plasma may be overshadowed.

On the other hand, 1-2 Diaminobenzene (DAB) was successfully used as coating of Pt, Au and graphite electrodes, which is sensitive to nitrite and not to nitrate [9]. The activity of the DAB coated electrodes for the oxidation of paracetamol, ascorbic and uric acid was reduced by approximately 90% as compared to the bare electrodes during evaluation of dehydrogenase enzyme electrodes [10]. In addition, we have recently reported the influence of uric acid, ascorbic acid and paracetamol as interfering elements on the nitrite electrochemical detection in the amperometric determination of nitrite [11].

This article shows an electrochemical planar sensor to determine nitrite concentration using Au working electrodes covered with 1-2 Diaminobenzene (DAB). At first, the minimum ranges of nitrite and interfering elements are established and are compared to the therapeutic maximum concentrations of uric acid, ascorbic acid and paracetamol as interfering elements. At second, it is presented nitrite detection based on flow injection analysis in order to improve selectivity, repeatability, stability and sensitivity.

1. EXPERIMENTAL METHODS

A. Electrochemical sensors

Microfabrication, microelectronic and silk-screen are the main fabrication technologies employed in development of electrochemical sensors and biosensors. Silicon, glass, alumina and catheter substrate are the usual materials utilized. In the 70’s and 80’s, Hiroaki et al [12] started working with microfabrication and microelectronic technologies, but only in 90’s, they have gained expressive summit [12]. That moment was also marked by technologies based on silk-screen, mainly in fabrication processing of hybrid devices.

The amperometric electrochemical sensors are devices that record electric current of a given electro-chemical reaction at a constant potential. It allows one to qualify and to quantify substances through the relation between current response and their concentration [1,13]. The electrochemical planar sensors were fabricated with three electrodes: working, reference and auxiliary, is described below.

B. Sensor project

The fabricated sensor is 17.0 mm width and 43.0 mm length with a working electrode of 2.5 mm in diameter, having alumina as substrate (Figure 1). The geometric configuration of the planar sensor based on a set of three electrodes was chosen to reduce the reference electrode area in order to permit a stable and fixed reference.

![Figure 1. Scheme of the planar sensor, with respective electrodes.](image)

The sensor was defined by silk-screen technique: I) DuPont® 5142 gold paste for working electrode, where the electrochemical detection takes place; II) Modified DuPont® 6146 silver/palladium paste to obtain silver-chlorine-film reference electrode (Ag/AgCl), a stable and reference potential inside of the sensor’s environment and finally III) ESL 5545-LS platinum paste for auxiliary or counter electrode. Contacts and interconnections were manufactured with the same gold paste. DuPont® 9615 dielectric paste was used as the passivation layer.

C. Cleaning electrodes and characterization

After the fabrication, it was necessary to activate the electrodes surface to guarantee increasing sensitivity. It was performed a physical, chemical and electrochemical standard cleaning process followed by the working and reference electrodes characterization to attain maximum repeatability and reproducibility as described in [14]. Black spots on the electrode surface were observed under optical microscopy, before cleaning. They were removed by cleaning, promoting homogeneity of the gold surfaces, as shown in the figure 2.

Normally, during fabrication process of electrochemical sensors or biosensors, it is convenient to deposit a conductor film above the working electrode surface to eliminate interfering elements and increase the measured signal.

In our work, 1-2 Diaminobenzene (DAB) was chosen due to its previous use [9,10]. In the present work, gold electrode surface was polished with 0.05-mm colloidal alumina and DAB was electro deposited without occurrence of black sports (see figure 3). In addition, we have studied this membrane with Flow-injection Analysis (FIA) Technique in order to improve selectivity, repeatability, stability and sensitivity.
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D. 1-2 Diaminobenzene (DAB) electrodeposition

The DAB film was electrodeposited over the gold electrode at a constant voltage (0.65 V $\text{Ag/AgCl}_{\text{3M KCl}}$) during 10 min using the Bionalytical System CV-50W.

E. Nitrite solution

The experimental was performed using three nitrite concentrations of 50 $\mu$mol L$^{-1}$, 250 $\mu$mol L$^{-1}$ and 500 $\mu$mol L$^{-1}$ in physiologic serum (NaCl 0.9 % and pH = 7.3).

F. Analysis system

The following assumptions were taken into account in order to design the experimental set-up utilized to measure nitrite and interfering elements:

a) easiness to clean the sensor and the system;
b) reduced dead volume of the analysis cell;
c) minimum inductance and complacence in analysis cell, connectors and pipes;
d) materials extremely clean to eliminate impurities;
e) versatile system to allow one easy exchange of sensors (discartable) and

f) system highly integrated (sensor, analysis cell, fluid system, voltammetric analyzer and date acquisition).

The experimental set-up is shown in figure 4. The system was arranged for the items injector/commutator, discard, analysis cell, peristaltic-pump and analyte. It is also shown the analysis cell and its fluidic and electrical connections.

This analysis system was designed to simulate bloody flow utilizing physiologic serum (NaCl 0.9 % and pH = 7.3) as carrier and cleaning solution in the FIA system.

3. RESULTS AND DISCUSSIONS

The nitrite measuring results using FIA technique in three different concentrations (50 $\mu$mol L$^{-1}$, 250 $\mu$mol L$^{-1}$ and 500 $\mu$mol L$^{-1}$) are presented in figure 5. The obtained results show good stability, sensitivity and selectivity of the nitrite sensor in the studied concentration range. One observes signals with excellent repeatability and reproducibility.

On the other hand, it was possible to observe that the nitrite monitored experiment is free of interfering-elements if Flow-injection Analysis is used together the analysis system. All signals were registered without presence of interfering-elements as observed in figure 5.
The sensitivity curve (Figure 6) was recorded (current response vs. concentration); as a result, we obtained the sensitivity equation of nitrite with an excellent linear regression coefficient ($r^2 = 0.999$).

$$I = 0.89 \text{ [nitrite]} + 10.16 \text{ (nA)}$$

Following, we also present two set of experiments using nitrite solution with upper limits of therapeutic concentrations of the interfering elements, this is to say, uric acid (0.5 mmol L$^{-1}$), ascorbic acid (0.1 mmol L$^{-1}$) and paracetamol (0.15 mmol L$^{-1}$).

These are the best conditions which allow one to analyze interfering elements without need to perform additional experiments for different concentrations of them since, as observed, the nitrite signal adds to the interfering elements signal for different potentials (see Figures 7 and 8).

Figure 9 shows the calibration curves and linear fits for upper limits of interfering elements at two different potentials. At 0.3 V$_{Ag/AgCl}$ Nafion$^\circledR$ 117 potential, the nitrite response is negligible, practically null. On the other hand, at 0.5 V$_{Ag/AgCl}$ Nafion$^\circledR$ 117 potential, the
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nitrite signal is added to the interfering-elements signal. As a result:

\[ I = 6.31 \times 10^{-4} \text{[nitrite/interfering-elements]} + 1.43 \text{ (nA)} \]  \hspace{1cm} (5)

\[ I = 2.29 \text{[nitrite/interfering-elements]} + 7.67 \times 10^{2} \text{ (nA)} \]  \hspace{1cm} (6)

Finally, to shed further light on the influence of the interfering elements, figure 10 shows individual signals for nitrite, uric acid, paracetamol and ascorbic acid for concentrations ranging from 0 to 0.25 mmol L\(^{-1}\). It is noteworthy that the nitrite response was higher than the interfering-elements ones for concentrations lower than 0.175 mmol L\(^{-1}\). As an analytical criterion, we have adopted nitrite signal lower or equal to each one of the interfering elements in order to analyze nitrite which adds to these signals. On the other hand, it is important to point out that the nitrite curve in figure 10 is influenced by the interfering elements since the straight line for nitrite has a lower angular coefficient compared to figure 6.

Therefore, a systematic study of the influence of the interfering elements on the nitrite detection was performed, which is an important contribution for the development of nitrite sensors utilizing DAB as a selective polymer.

4. CONCLUSIONS

In this work, we have shown the activation and characterization of an electrochemical planar sensor developed by silk-screen technique and using Flow-injection Analysis system to achieve nitrite detection using concentrations of 50 µmol L\(^{-1}\), 250 µmol L\(^{-1}\) and 500 µmol L\(^{-1}\) which are compatible with the nitrite concentration in the human body. It is important to point out that is essential to monitor the nitrite specimen concentrations of patients submitted to Intensive Therapy Unity.

We performed experimental tests with uric acid, ascorbic acid and paracetamol as interfering elements to nitrite detection. We have obtained planar electrochemical sensor recovered with electrodeposited 1-2 Diaminobenzene (DAB) films with good response to nitrite until 0.175 mmol L\(^{-1}\).

The presented results indicated excellent linearity, repeatability, reproducibility, sensitivity and selectivity for nitrite detection. The calculated sensitivity for nitrite specimens was on order of 0.217 mA mol\(^{-1}\) L mm\(^{-2}\), 11.243 mA mol\(^{-1}\) L mm\(^{-2}\) and 0.003 mA mol\(^{-1}\) L mm\(^{-2}\) with a working electrode of 2.5 mm in diameter and 0.75 V\(_{\text{Ag/AgCl Nafion® 117}}\), 0.5 V\(_{\text{Ag/AgCl Nafion® 117}}\) and 0.3 V\(_{\text{Ag/AgCl Nafion® 117}}\) potentials, respectively.

We observed that the cleaning process in flow-injection and DAB over working electrode allows one an increasing of the sensor lifetime and better sensitivity for nitrite utilizing the 0.5 V\(_{\text{Ag/AgCl Nafion® 117}}\) potential. We also obtained a versatile system, which allows one changing sensors in a quick and easyway.

Electrochemical planar sensors based on silk-screen process are largely employed for measuring systems. They are attractive due to high yield (around 100 %). Finally, we have shown results for a low cost fabrication electrochemical sensor to electrochemically detect nitrite.

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REFERENCES


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