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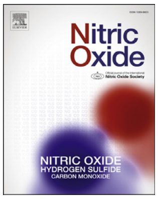

A new, fast and sensitive method for the determination of trace amounts of nitrite using differential pulse polarography

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ABSTRACT

Nitrite salts of sodium or potassium are being used for the protection of meat products. They provide color and taste of meat and they protect against clostridia. On the other hand, nitrite ions can interact with amines to form nitrosamines which are known as carcinogenic substances. They may also react with hemoglobin and reduce its oxygen carrying capacity. Thus, its concentration in food-stuff has to be controlled carefully by highly sensitive methods.

A new DP polarographic method is established for the determination of nitrite. Nitrite cannot be determined directly with any analytical methods. Long and tedious procedures are necessary for many of them. In this polarographic method arsenite, As(III), ion is used for the reduction of nitrite. The nitrite is determined from the As(III) quantity left over after the reaction with nitrite. The peak of arsenite has been used since it is sharp and responds well for the standard addition of arsenite.

The optimum conditions for the quantitative reaction between nitrite and arsenite have been studied. It was found that the pH for the reaction medium has to be 5–7, since nitrite is decomposed at lower pH values. The reaction medium has to be stirred for about 5 min with nitrogen gas in order to expel the NO gas formed and thus to shift the equilibrium to products side. The limit of detection, LOD, was found to be as 2×10^{-7} M. No interference was observed from most common ions.

1. Introduction

The food-stuff protection has taken an important place in industry. Nitrite salts of sodium or potassium are being used for the protection of meat products. They provide color and taste of meat and they protect against clostridia. On the other hand, nitrite ions can interact with amines to form nitrosamines which are known as carcinogenic substances. They also may react with hemoglobin and reduce its oxygen carrying capacity [1,2].

Thus, its concentration in food-stuff has to be controlled carefully by highly sensitive methods. The commonly used methods for nitrite determination include the spectrophotometric [3–6], chromatographic [7,8], FIA-CL flow injection-chemiluminescence [9,10] and electrochemical methods such as amperometry [11], voltammetry [12–16], and polarography [17–20].

Nitrate and nitrite in atmospheric liquids and lake waters could be determined by reverse-phase high performance liquid chromatography [7]. Nitrite and nitrate ions were determined after reduction with NaI to NO and the chemiluminescence of NO in the presence of ozone was measured [9].

The amperometric determination of both nitrite and nitrate was made using tetraruthenated porphirin-modified electrode. This method was applied for nitrite and nitrate determination in mineral water and in cured meat [11]. An anodic stripping procedure was described for nitrite using cetyltrimethylammonium bromide as a complexing agent. The oxidation of this complex on a glassy carbon electrode was used for nitrite determination [12].

Electrochemical sensors using modified electrodes based on copper complexes were used for determination of nitrite in water [13]. In one work nitrate and nitrite was determined using DPP (differential pulse polarography). The reduction peak of nitrite in the presence of Zr (IV), which acts as a catalyst, was used for the destruction of nitrite [17]. By using the selective reduction of both nitrate and nitrite into nitric oxide both ions could be determined by DPP [18]. In another work o-,m- or p-aminobenzoic acids were used for DPP-determination of nitrite from the decrease of their peak by the addition of nitrite [19]. In our former studies nitrite was determined using DP polarography by direct and indirect methods.

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Sulfite ion was used for the reduction of nitrite for the indirect determination [20].

The classical colorimetric methods widely used, are based on the formation of strongly colored azo dyes. For this purpose an amine is diazotized by means of nitrite and then the intermediate is let to couple with a selected aromatic compound. Most of the above mentioned methods are laborious, since in many of them diazotization and coupling reactions are used; some of them suffer from matrix interference by oxidizing and reducing agents.

Electrochemical methods offer useful alternatives since they allow faster and cheaper analysis. The results obtained with DPP method are very reproducible since with the use of dropping mercury electrode (DME) the behavior of the electrode is independent of its past history.

In this work, a new indirect polarographic method was developed where it was possible to determine very low concentrations of nitrite using arsenite As(III) ion for the reduction of nitrite. For this purpose as given below the electrochemical behavior of nitrite and As(III) have been studied and the optimum conditions for the quantitative reaction conditions have been determined. This method can be applied to many biological samples safely.

2. Experimental

2.1. Apparatus

A polarographic analyzer “Entek Electronics Model 2016” equipped with a PAR mercury drop timer was used. The drop time of the electrode was in the range 2–3 s (2.35 mg s^{-1}). A Kalousek electrolytic cell with a saturated calomel electrode (SCE), separated by a liquid junction, was used in the three-electrode configuration. With this three –electrode cell the danger of IR drop (where I = current, and R = resistance) is prevented. All potentials in the manuscript are given vs. SCE. The counter electrode was platinum wire. The polarograms were recorded with a Linseis (LY 1600) X–Y recorder under the conditions of a drop life of 1 s, a scan rate of 2–5 mV s^{-1}, and a pulse amplitude of 50 mV.

2.2. Reagents

All chemicals used were of analytical-reagent grade (proanalysis). Triply distilled water was used in the preparation of all solutions. In order to expel the oxygen present in polarographic cell 99.999% pure nitrogen was passed through the solution. Solutions of 1×10^{-3} M and more diluted ones were prepared before every use in order to avoid the aging process of solution.

The mercury used in the dropping mercury electrode was obtained from Merck (Darmstadt, Germany). Contaminated mercury was cleaned by passing it successively through dilute HNO_3 (3.0 M) and water columns in the form of fine droplets by using a platinum sieve. This mercury is then washed in the same way until no acidic reaction was observed. The collected mercury was stored in a closed vessel covered with water. It was dried between sheets of filter paper when it was needed. The mercury used is not lost since it was collected quantitatively, without causing any pollution. Thus, no mercury loss is possible and it can be used continuously. Before use, a DPP polarogram of this mercury was recorded in order to confirm the absence of impurities.

2.3. Preparation of reagents

2.3.1. 1.0 M AcOH/AcO^- electrolyte

It was prepared by adding 6 g of solid NaOH, which had to be washed first with distilled water to remove the carbonate formed, into 57 mL of 1.0 M AcOH and diluting to 1 L. The pH was adjusted to the desired value using a pH meter.

2.3.2. 0.1 M KNO_2 solution

Dried 0.21 g of KNO_2 was dissolved in 25 mL de-aerated distilled water. It was stored in dark.

2.3.3. 0.1 M As(III) solution

About 0.65 g $NaAsO_2$ was dissolved in distilled water and diluted into 50 mL using volumetric flask.

1.1 M Pb(II), Zn(II), and Cu(II) solutions were prepared from their standard nitrate solutions; only for Cd(II) solution the chloride salt was used.

3. Results and discussion

3.1. Electrochemical behavior of nitrite ion

In DP polarography the nature (cathodic or anodic) of a polarographic peak cannot be evaluated. For this purpose direct current (DC) polarography has to be used. In our former studies with DC, we have shown that 1×10^{-4} M nitrite ion is reduced in several electrolytes and it showed a cathodic current on DME at about −1.43 V.

The DP polarograms of nitrite were taken in varying concentrations of HCl (6, 4, 2, 1 and 0.1 M) and in acetate buffers. It was observed that in these acidic solutions nitrite was decomposing and NO gas was escaping. Thus, it is not possible to determine nitrite concentration correctly. It was found that in 4 M HCl medium while nitrogen gas was purging from 2×10^{-4} M nitrite 10% was decomposed in 8 min, in 6 M HCl on the other hand 20% of nitrite was decomposed in 4 min, Fig. 1. However, it was found that 1×10^{-4} M nitrite peak did not decrease with time at about pH = 5 HAc. Table 2.

3.2. Indirect determination of nitrite using arsenite As (III)

According to our former work it is decided that lower concentrations of nitrite cannot be determined precisely since nitrite peak is not high enough. Thus, it will be better to find a substance which is electroactive and reacts with nitrite ion quantitatively. As can be seen from the reduction potentials given below, arsenite ion possesses these feature, it can react with nitrite ion quantitatively.

$$
\begin{array}{ll}
NO_2^- + 2H^+ + e^- \leftrightarrow NO\,g + H_2O & E^0 = +1.00V \\
H_3AsO_4 + 2H^+ + 2e^- \leftrightarrow H_3AsO_3 + H_2O & E^0 = +0.56V \\
\hline
2H^+ + 2NO_2^- + H_3AsO_3 \leftrightarrow 2NO(g) + H_3AsO_4 + H_2O & \Delta E = +0.44V
\end{array}
$$