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Selective and sensitive detection of nitrite based on NO sensing on a polymer-coated rotating disc electrode

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Abstract

A new effective way of nitrite detection in complex samples is presented. It is based on chemical conversion of nitrite to nitric monoxide (NO) in acidic aqueous solution containing hexacyanoferrate(II) as a reductor. NO is then detected on a poly-eugenol coated platinum electrode. When the electrode is rotating and the reduction medium is continuously purged with nitrogen, the addition of a nitrite-containing sample produces narrowed current spikes. The peak current is proportional to nitrite content in the sample over the range of 5.0–100 μM and detection limit is 0.6 μM. The method is simple and highly reproducible. Relative standard deviation of 10 repetitions is less than 4%. Practical utility of the proposed approach is demonstrated by nitrite determination in human saliva.

Keywords: Nitrite; Nitric oxide; Poly-eugenol; Rotating disc electrode

1. Introduction

Nitrite ions appear in biological systems as the end metabolite of nitric oxide which is produced by nitric oxide synthase (NOS). Different isoforms of NOS have been identified in a variety of organisms ranging from bacteria and macrophages to humans [1,2].

In higher plants nitrite is produced by nitrate reductase (NR) especially in illuminated leaves and is the substrate for enzymatic [3–6] and non-enzymatic [7] NO production. Besides, it is involved in many physiological processes such as growth [8], seed germination [9,10], cell signaling and disease resistance [3]. Extensive nutrition of plants may lead to the accumulation of nitrite along with its precursor nitrate in fruits and vegetables and hence nitrite monitoring is important for human health. The daily intake of nitrate and nitrite with vegetables can reach 40 and 0.09 mg, while the total intake including other food was estimated to be 60 and 0.5 mg, respectively [11]. Most of the nitrate passes harmlessly out of the body through feces or urine. A small fraction of the nitrate is transformed into nitrite in the intestines by microorganisms, or under alkaline conditions in the stomachs of very young babies. Yet, nitrate is transformed in the gastrointestinal tract into nitrite, which can then be adsorbed into the blood. Although some of formed nitrite also passes harmlessly out in the feces, nitrite in the blood combines with hemoglobin to form methemoglobin, which has reduced capabilities for oxygen transfer. Excessive levels of methemoglobin result in methemoglobinemia and fall of blood pressure [12].

Nitrite monitoring in body fluids has become a powerful tool in health diagnostics. Anomalous levels of nitrite in plasma or cerebrospinal fluids were postulated to be a signal of psychiatric disorders [13]. Nitrite is normally present in human saliva due to the bacterial activity of oral bacteria living on the posterior surface of the tongue [14], but its abnormal increase may be a sign of a number of diseases. Similarly, increased levels of nitrite in urine were reported to appear during urinary track infection or kidney diseases [15]. Furthermore, nitrite content in exhaled breath condensate was identified as a symptom of lung injury or respiratory disease [16,17].

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Electrochemical nitrite sensors, described to date, do not provide required selectivity for nitrite detection in biological systems since they also respond to other species present in such a matrix. Moreover, they are often not sufficiently stabile, which can limit their use in prolonged investigations.

Transition metal complexes when immobilized on the electrode surface can serve as electrocatalysts for nitrite oxidation; however, up to now no nitrite-selective electrocatalyst has been found. For example, cobalt phthalocyanine, which is a good catalyst for nitrite electrooxidation [18], was also suggested as a promoter for electrocatalytic oxidation of hydrogen peroxide, thiols, and other compounds [19]. The selectivity problems may be partly overcome by using an outer membrane placed on the electrode working surface, which provides a selective pathway for nitrite on charge on molecular-size basis. For example 1,8-diaminonaphthalene was polymerized on a platinum electrode to give a size-selective membrane allowing selective electrochemical detection of nitrite in the presence of ascorbate [20]. The same effect could be achieved by using a cellulose acetate membrane of 100 Da molecular weight cut-off [21].

Since nitrite is also cathodically active its electrochemical detection is possible by operating in the reductive mode and many examples of chemically modified electrodes capable of reducing catalytically nitrite can be found in the literature [22,23]. Such an approach by principle should be free from interferences coming from the oxidisable substances, e.g. ascorbate. Still, the presence of a reducible compound such as nitrate and dissolved oxygen may complicate nitrite assay.

In this paper a novel method allowing for minimizing some interference problems associated with nitrite detection is described. It is based on chemical conversion of nitrite to nitric oxide and the latter is detected using rotating NO-selective electrode.

2. Experimental

2.1. Chemicals and samples

Sodium hexacyanoferrate(II) and (III), sodium nitrite and ascorbic acid (AA) were obtained from Aldrich and eugenol from Fluka. Redistilled water was used to prepare all solutions. Nitrite standard solutions were prepared just before the experiments. All electrolyte materials were reagent grade and used without further purification. Saliva sample was taken from volunteer.

2.2. Apparatus

The electrochemical experiments were carried out using an Autolab (PG-30) electrochemical analyzer and rotating disc electrode (both EcoChemie) connected to a PC for control, data acquisition and storage. A Pt electrode – 3.0 mm diameter was used as the substrate electrode for all experiments. The counter electrode was a platinum wire. All potentials reported in this paper are referenced to an Ag/AgCl electrode with no regard for the liquid junction potential.

2.3. Electrode preparation and measurements

Before its modification, the working surface was polished with alumina slurries of 1.0, 0.3, and 0.05 μm on Buehler polishing cloth, with distilled water as a lubricant, rinsed with redistilled water, and sonicated in a water bath for 3 min. Then it was activated in 0.1 M H_2SO_4 by potential cycling between −0.2 and 1.5 V (50 scans at 0.1 V s^{-1}). Electropolymerization of eugenol was then curried out by immersing the activated electrode in 10 mM eugenol in 0.1 M NaOH and scanning the potential between −0.2 and 0.6 V at 0.02 V s^{-1} and then holding the potential at +0.6 V for 20 min. The electrode was rotated at 2000 s^{-1} during the electropolymerization. Finally, the modified electrode was transferred to 0.1 M H_2SO_4 and while rotating at the same rate, constant potential of +0.9 V was applied until a steady baseline current was achieved. After addition of certain amount of ferrocyanide and after 20 min purging with nitrogen, the nitrite-containing standards or samples were added and the associated current signals were measured as explained below.

3. Results and discussion

3.1. Nitric oxide production by nitrite reduction with acidic ferrocyanide

In the acidic environment nitrite ions are protonized to form nitrous acid (p$Ka = 3.2$–3.4), a species with NO_x and NO^+ donor characteristics [24,25]. The latter might be reduced to NO by any redox system characterized by $E^{0,}$ lower than $E^{0,}$ of the NO^+/NO transition, which is ca. 1.2 V [26]. Species capable of reducing nitrite or nitrous acid to NO, reported so far, include iodide (or triiodide) [27–29] vanadium(III) [30] and ascorbic acid [31]. Their standard potentials range from −0.058 for AA [32] to +0.53 V for triiodide [33]. Thus, assuming that HNO_2 reduction proceeds via electron acceptance by transient NO^+ from a reducing molecule, ferrocyanide with its $E^{0,}$ of 0.69 V [33] should also be a good substrate for this reaction if no kinetic limitations occur.

The reduction of acidified nitrite to nitric oxide by ferrocyanide can be easily deducted from voltammetric curves recorded in an acidic electrolyte containing ferricyanide and nitrite. Fig. 1 presents cyclic voltammograms recorded in different potential frames for ferricyanide in 0.1 M sulfuric acid before and after the addition of nitrite. As can be seen, in the absence of nitrite (curve a) ferricyanide ions exhibited characteristic reversible redox behavior (peaks PI_A and PI_C) with mid-peak potential of 0.406 V assigned to ferricyanide/ferrocyanide conversion. The addition of nitrite (curve b) resulted in an increase in the cathodic peak