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Spectrochimica Acta Part A 61 (2005) 1871–1875
SPECTROCHIMICA ACTA PART A
www.elsevier.com/locate/saa

Optical nitrite sensor based on chemical modification of a polymer film

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Received 1 December 2003; accepted 10 June 2004

Abstract

A new, low-cost nitrite sensor was developed by immobilizing a direct indicator dye in an optical sensing film for food and environmental monitoring. This sensor was fabricated by binding gallocyanine to a cellulose acetate film that had previously been subjected to an exhaustive base hydrolysis. The membrane has good durability (>6 months) and a short response time (<7 s). Nitrite can be determined for the range 0.008–1.50 μg/ml with 3δ detection limits of 1 ng/ml. The method is easy to perform and uses acetylcellulose as a carrier. The reagents used for activating the cellulose support are inexpensive, non-toxic and widely available.

Keywords: Optical sensor; Nitrite; Environmental analysis; Membrane

1. Introduction

The development of optical nitrite sensors is of great interest because of their possible application in biotechnology, ecology, medicine, food and environmental studies [1]. They are suitable for application where conventional electrodes cannot be used because of their large size or because of the risk of electrode shock during the in vivo measurements. The most important problem with this type of sensor is related to the stability of the binding of the reagents to the carrier.

This can be improved by using an efficient procedure for immobilization of the indicator on an appropriate polymer matrix. A method for the covalent binding of an enzyme to a cellulose carrier was described previously [4], which includes activation of the cellulose by using urea and formaldehyde and binding the enzyme to it. This method has also been used successfully to prepare multi enzyme membranes for biosensors [5]. Double enzyme complexes of glucose oxidase and catalase invertase were produced on a cellulose matrix. A modification of this method was used for the covalent binding of enzyme to a synthetic membrane [6].

The purpose of this work was to modify the above methods for the covalent immobilization of new indicators on an optically transparent acetylcellulose membrane that had previously been hydrolyzed and activated using thiourea and poly(viny alcohol). The characteristics of the membrane produced were investigated and the possibilities for its use in the design of optical nitrite sensors were evaluated. According to our knowledge, up to now, only dye molecules with amino and hydroxyl groups on the ring cycle have been used to construct optical nitrite sensors based on chemical modification of polymer films. In this paper, we have used thiourea in linking a dye (with hydroxyl groups on the ring) to a cellulose acetate film with satisfactory results. The dye used is GC sensor, which can be used for direct determination of nitrite ion as a catalyst for dye oxidation by bromate in acidic media.

The determination of nitrate ion is an important factor in the analysis of soils, food and natural waters. Nitrite is intimately involved in the overall nitrogen cycle in the soil and higher plants [7]. A limit of 45 μg ml^{-1} has been proposed for the nitrite present in drinking water. Since excessive amounts lead to methaemoglobinalmia in infants [8]. Nitrite

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doi:10.1016/j.saa.2004.06.057

is formed during the biodegradation of nitrate and ammoniacal nitrogen or nitrogenous organic matter is an important indicator of fecal pollution of natural water. The determination of nitrite is of general importance because of its harmful impact on human health. The toxicity of nitrite is primarily due to its interaction with blood pigment to produce methaemoglobinaemia. The reaction between nitrite and secondary or tertiary amines leads to the formation of *N*-nitroso compounds, some of which are known to be carcinogenic and mutagenic [9,10]. Most of the flow-injection methods for the simultaneous determination of nitrite and nitrate are based on the diazo-coupling reaction (Griess method) or a liquid–liquid extraction method [11–16]. According to our knowledge, only one paper is based on the diazonium salt system for the simultaneous determination of nitrite and nitrate [17,18]. Our proposed method is based on catalytic effect of nitrite on the oxidation of gallocyanine by bromate in an acidic solution. The method is fast, simple and sensitive compared to all of the present methods for the determination of nitrite.

2. Experimental

Reagents all of the chemicals used in this work were analytical-reagent grade (Merck). Distilled water was used throughout.

Standard nitrite solution (1000 μg/ml) was prepared by dissolving 0.15 g of dried (for 4 h at 105–110 °C) sodium nitrite in distilled water and diluted to 100 ml in a standard flask. A pellet of sodium hydroxide was added to prevent the liberation of nitrous acid and 1 ml of chloroform was added to inhibit bacterial growth. The working standard solutions were freshly prepared by diluting the stock solution with distilled water (each day).

Sodium bromate solution (0.060 M) was prepared by dissolving 2.2635 g of $NaBrO_3$ (Merck) in water and diluting to 250 ml in a volumetric flask.

Gallocyanine (GC) (Aldrich) 0.030% solution was prepared by dissolving the dye in 1×10^{-5} M NaOH and diluting with water in a 100 ml volumetric flask.

Poly(vinyl alcohol) solution was prepared by dissolving 0.70 g of the reagent in 100 ml of water.

Thiourea solution was prepared by dissolving 0.65 g of the reagent in 100 ml of water.

2.1. Preparation of food samples

For the preparation of meat sample, 2.00 g of beef was mixed with sand and homogenized in a mortar. The thoroughly mixed sample was taken in a 100 ml beaker and digested carefully following the method recommended by the AOAC [19].

For the flour samples, 2.00 g of the sample was taken in a 150 ml beaker and mixed with 80 ml of doubly distilled water. The beaker was placed in a water bath at 40 °C and the contents digested for 15 min following the method recommended by the AOAC [19].

2.2. Apparatus

UV–visible spectra were measured with a Shimadzu UV–vis 2100 double-beam spectrophotometer controlled by a thermostated cell.

2.3. Preparation of sensors

Triacetyl cellulose was previously hydrolysed in order to de-esterify the acetyl groups and to increase the porosity of the membrane. Separate pieces of transparent film (34 mm × 8 mm × 0.1 mm) were treated in 0.10 mol l^{-1} KOH for 24 h. The films were washed with water and immediately treated with a mixture of 0.65% (W/V) thiourea and 0.70% (W/V) poly(vinyl alcohol) solution for 48 h at 25 °C. The cellulose membranes were separately treated with a 0.030% (W/V) solution of GC at 25 °C with magnetic stirring of the solution for 14 h. After washing, the film was dried at 45 °C for 20 min. Next the membrane was washed with distilled water until there was no absorbance of the washings at the wavelength of the dye during rinsing. Finally film was dried at 45 °C for 20 min.

2.4. Spectrophotometric measurements

The measurements were made on the membrane, which was stretched on a special frame. The size of the aperture was 8.5 mm × 35 mm, as shown in (Fig. 1). The known amount of nitrite sample consisting film is placed in the cell and measurements are performed against the film without indicator in other compartment of cell. The optimum condition was set and the calibration curve was obtained in the appropriate detection limit and dynamic range. The spectral character-

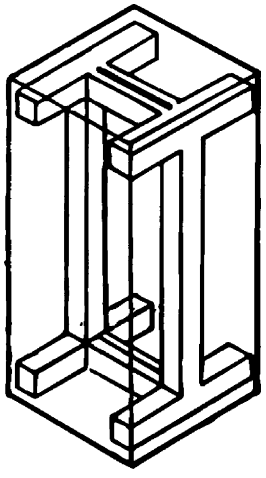

Fig. 1. Schematic diagram of the frame on which the membranes are stretched, inside the cuvette.