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Development of New Optical Nitrite Detector

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A new, low-cost nitrite detector was developed by immobilizing a direct indicator dye in an optical sensing film for food, environmental and clinical monitoring. This detector was fabricated by binding pyrogallol red to a cellulose acetate film that had previously been subjected to an exhaustive base hydrolysis. The membrane has good durability (> 12 months) and a short response time (< 9 s). Nitrite can be determined for the range 0.006–1.50 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.

Key words: Optical detector, Nitrite, Environmental and clinical analysis.

INTRODUCTION

The development of optical nitrite detectors is of great interest because of their possible application in biotechnology, ecology, medicine, food, environmental and clinical 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 detector 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[2, 3].

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, catalase and 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(vinyl alcohol). The characteristics of the membrane produced were investigated and the possibilities for its use in the design of optical nitrite detectors were

evaluated. According to our best knowledge, only dye molecules with amino and hydroxyl groups on the ring cycle have been used to construct optical nitrite detectors based on chemical modification of polymer films. In this paper, thiourea has been used in linking of dye (with hydroxyl groups on the ring) to cellulose acetate film with satisfactory results. The pyrogallol red detector can be used for direct determination of nitrite ion by bromate in acidic media.

The determination of nitrite 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 plant[7]. A limit of 45 μg/mL has been proposed for the nitrite present in drinking water, since excessive amounts lead to methaemoglobinaemia in infants[8]. Nitrite 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 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 liquid-liquid extraction method[11–16]. According to literature surveyed, only one paper is based on the diazonium salt system for the simultaneous determination of nitrite and nitrate[17]. The proposed method is based on catalytic effect of nitrite on the oxidation of pyrogallol red 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.

EXPERIMENTAL

UV-visible spectra were measured with a Shimadzu UV-Vis 2100 double-beam spectrophotometer controlled by a thermostated cell. All chemicals used 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. Pyrogallol red (Aldrich) 0.020% solution was prepared by dissolving the dye in 20 mL of ethanol solution and diluting to 100 mL with distilled water in a 100 mL volumetric flask. Poly(vinyl alcohol) solution was prepared by dissolving 0.80 g of the reagent in 100 mL water. Thiourea solution was prepared by dissolving 0.55 g of the reagent in 100 mL water.

Preparation of Detectors: The triacetyl cellulose was previously hydrolyzed in order to de-esterify the acetyl groups and to increase the porosity of the membrane. Separate pieces of transparent film (34 × 8 × 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 0.55 per cent (w/v) thiourea and 0.80 per cent (w/v)

polyvinyl alcohol solution for 48 h at 25°C. The cellulose membranes were separately treated with 0.020% (w/v) solution of pyrogallol red 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 absorption at the wavelength of the dye during rinsing. Finally the film was dried at 45°C for 20 min.

Spectrophotometric measurements: The measurements were made on the membrane, which was stretched on a special frame. The size of the aperture was 8.5 × 35 mm (Fig. 1). The control sample against which the measurement was performed consisted of a film treated in the same way but without indicator. The control sample was stretched in the same way inside the cuvette using a frame of the same size. The spectral characteristics of pyrogallol red were measured with nitrite in sulfuric acid (0.14 M) and bromate solution (0.060M) (Fig. 2).

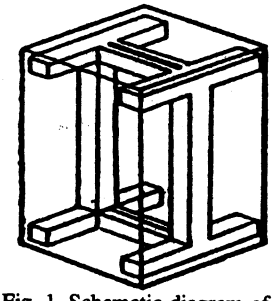

Fig. 1. Schematic diagram of the frame on which the membranes are stretched, inside the cuvette

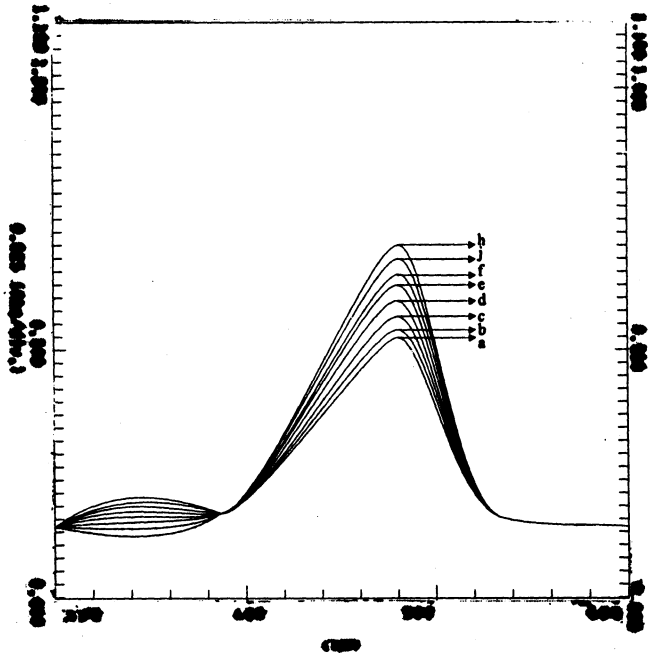

Fig. 2. Absorption spectra for pyrogallol red in membrane at nitrite concentration: (a) 80×10^{-9} (b) 120×10^{-9} (c) 160×10^{-9} (d) 200×10^{-9} (e) 240×10^{-9} (f) 280×10^{-9} (j) 320×10^{-9} and (h) 350×10^{-9} μg/mL

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 then taken in a 100 mL beaker and digested carefully following the

method recommended by the AOAC[18]. For the flour samples, 2.0 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[18].

RESULTS AND DISCUSS ION

It was found that a considerable amount of indicator with amino and hydroxyl groups is linked to the activated membrane *via* covalent binding, whereas the indicators without amino groups such as phenol red[3] cannot be linked with cellulose membrane. The optical properties of immobilized pyrogallol red at hydrolyzed cellulose membrane as a function of nitrite concentration are shown in Fig. 2. The absorbance change is linear only for nitrite ranges from 0.006–1.50 μg/mL. The absorption maxima of the immobilized pyrogallol red are located at 475 nm. The ratio of the peak-height absorption maxima of immobilized pyrogallol red (PGR) is 0.475. The above result can be interpreted as the influence of the immobilization procedure on the behaviour of the indicator. Kresteva and co-workers[6] have shown that the condensation between the hydroxylmethyl groups of the carrier and the protein is accomplished through the transformation of the amino groups in the acidic range. In addition, the reactivity of the activated carrier is so high that it was considered that interaction is possible with low molecular weight compounds that have a free para position in the molecules. For this reason, indicators having hydroxyl groups or free para positions in their structure can be used. Therefore, thiourea was used as a bridge to connect the dye molecules (with hydroxyl groups) to the membrane cellulose acetate film. A possible scheme for the reaction is shown in Fig. 3. This makes it possible to

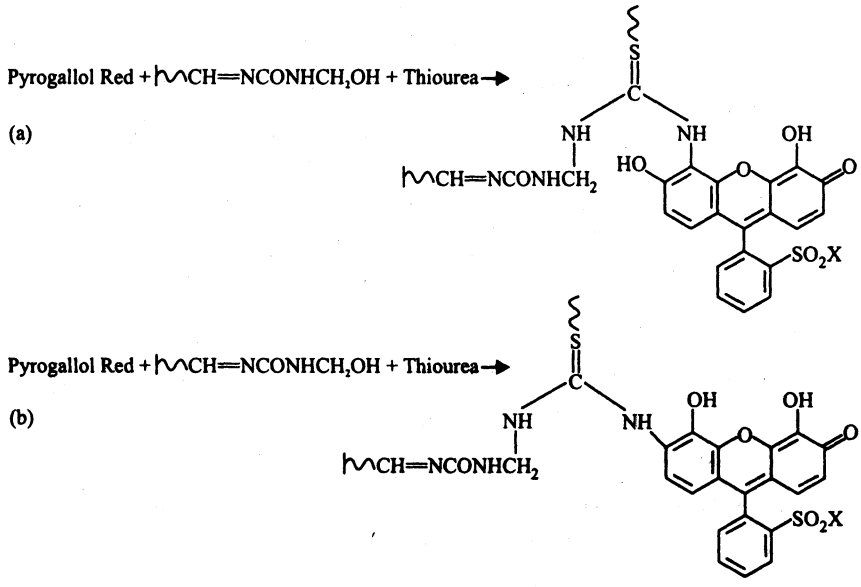

Fig. 3. Possible scheme of reaction between activated membrane and pyrogallol red

achieve covalent binding to the activated matrix. In the experiments, it was found that a considerable amount of indicator was linked to the activated membrane *via* covalent binding. The fact that the immobilization changes the ratio of the heights and positions of the absorption maxima of the indicators shows that the hydroxyl groups are auxochrome elements of the molecule and the loss of the proton after the covalent binding influences the charge distribution during the dissociation of the immobilized dye. The reasons for the transition interval shift are possibly the new covalent binding and the influence of the nearby acetylcellulose carrier. Pyrogallol red is a dye that can be reacted with nitrite by bromate solution in acidic media. This is a fast reaction in the presence of nitrite. The reaction is monitored spectrophotometrically by measuring the decrease in absorbance of the dye at 475 nm.

Detector Stability and Response Time: Repeated measurements showed that the changes in the absorption coefficient after keeping the measurements in aqueous solutions for a month were less than 5%. The stability of the membrane sensors based on a recycled support made from waste films is higher in comparison with other methods in which acetylcellulose is also used for producing optical detectors[2]. This is due to the higher mechanical strength of the carrier and to the covalent binding between the indicator and the change in optical properties of membranes with immobilized pyrogallol red was measured at 475 nm. Fig. 4 shows a typical curve for the transition process. As shown in the figure, after less than 9 s, the output signal reaches 98% of the steady-state response for membrane for about 8 s.

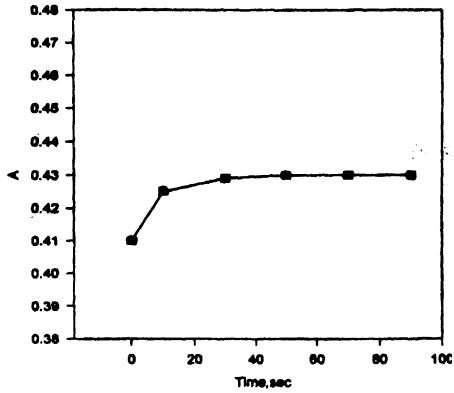

Fig. 4. Transition process of the nitrite sensor constructed by covalent binding of pyrogallol red to acetylcellulose in acidic media: (Condition: the initial nitrite was 320×10^{-9} μg/mL at t = 0, the nitrite was changed to 1320×10^{-9} μg/mL)

Determination of nitrite: The calibration curve for several nitrite standards assayed with the proposed detecting film, while the performance of both methods is compared in Table-1. In this table, the detection limit was calculated as three-fold of the standard deviation of the peak height measured for 10 samples and the precision (RSD %) was obtained from 3 samples; for each ten replicates determination for each sample was done.

TABLE-1
CALIBRATION RANGE, DETECTION LIMIT AND REPRODUCIBILITY FOR THE DETERMINATION OF NITRITE

Characteristics	Nitrite
Linear range (μg/mL)	0.006–1.50
Detection limit (μg/mL)	0.001
Precision (RSD)[a]	
(1) 0.010 μg/mL NO_2^-	1.50%
(2) 0.050 μg/mL NO_2^-	1.15%
(3) 0.50 μg/mL NO_2^-	0.95%

[a]For 10 replicate measurements.

Effect of interfering ions: The effect of potential interfering ions on the catalytic effect of nitrite on the oxidation reaction between pyrogallol red and bromate was studied with 0.1 μg mL^{-1} nitrite. The results showed that at least 10000-fold weight ratios of the alkali and alkaline earth metals and Al(III), F^-, CN^-, $B_4O_7^{2-}$, Zn(II), Ni(II) and 5000-fold ClO_3^-, NO_3^- did not interfere. However 10-fold CH_3COO^-, $S_2O_3^{2-}$, Ag(I), Cr(IV), Hg(II), Hg(I), As(III) and Fe(II) interfered. Use of a cation exchanger resin (Na^+-form) can decrease the interference effect of the cation.

Determination of Nitrite in Real Samples: To check the applicability of the method, the determination of nitrite in various samples such as meat product (beef), flour sample and environmental samples was carried out. The results are shown in Tables 2 and 3. The results showed good reproducibility and accuracy in comparison to the standard method[18].

TABLE-2
DETERMINATION OF NITRITE IN FOOD SAMPLES

Samples	Concentration of nitrite found* (μg/mL)	
	Proposed method	Standard method
Beef sausage	0.148 ± 0.003	0.150 ± 0.002
Flour	0.235 ± 0.005	0.242 ± 0.004

*Mean for five determinations.

TABLE-3
DETERMINATION OF NITRITE IN ENVIRONMENTAL WATER SAMPLES

Samples	Concentration of nitrite* (μg/mL)	
	Proposed method	Standard method
Karaj water:		
Sample 1	0.03 ± 0.002	0.035 ± 0.002
Sample 2	0.05 ± 0.003	0.052 ± 0.003

*Mean for five determinations.

Conclusion

The spectra for the pyrogallol red indicate that this detector exhibits a dynamic range of more than 0.01–1.50 μg/mL nitrite units. The described method for producing nitrite-sensitive optical membranes has the following advantages in comparison with other methods: (a) a waste cellulose material with good optical and mechanical properties is used as a matrix for immobilization; (b) immobilization of the indicators on the membrane surface reduces the diffusion limitations and allows sensors with short response time; (c) its activation is performed by using non-expensive and available reagents with suitable time stability for large numbers of measurements.

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