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Application of Rodamine B Optical Detector in Environmental and Food Analysis

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An optical oxalic acid detector was developed by immobilizing a direct indicator dye in an optical detecting film for food and environmental monitoring by FIA. This detector was fabricated by binding rhodamine B to a cellulose acetate film that had previously been subjected to an exhaustive base hydrolysis. The membrane has good durability (> 5 months) and a short response time (< 9 s). Oxalic acid can be determined for the range 0.07–3.50 μg mL^{-1} with 36 detection limits of 12 ng mL^{-1}. The method is easy to perform and uses acetyl cellulose as a carrier. The reagents used for activating the cellulose support are inexpensive, non-toxic and widely available.

Key Words: Rodamine B, Optical detector, Oxalic acid, Food, Environmental analysis, FIA.

INTRODUCTION

The development of optical oxalic acid detectors is of great interest because of their possible application in biological and non-biological materials[1] and they are used in a variety of manufacturing[2] and analytical procedures[3].

It has been shown that increased urinary oxalic acid excretion may lead to the development and formation of renal and urinary tract stones[4], so that sensitive, selective and precise detectors are necessary for the determination of oxalic acid content in food stuffs. The most important problem with this type of detector is related to the stability of the bond between the reagents and the carrier. This can be improved by using an efficient procedure for immobilization of the indicator on an appropriate polymer matrix[5, 6].

The purpose of this work was to modify the above methods for the covalent immobilization of new indicators on an optically transparent acetyl cellulose 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 oxalic acid detectors were evaluated. According to our knowledge, only dye molecules with amino, carboxy acid and hydroxyl groups on the ring have been used to construct optical oxalic acid detectors based on chemical modification of polymer films. In this paper, thiourea in linking a dye (with carboxyl acid groups on the ring) to a cellulose acetate film with satisfactory results have been used. The dye used is rhodamine B detector, which can be used for direct determination of oxalic acid ion as a catalyst for dye oxidation by dichromate in acidic media.

The determination of oxalic acid ion is an important factor in the analysis of food and natural waters. Various methods such as polarography[7], liquid chromatogra-

phy[8, 9], ion chromatography[10], spectrophotometry[11] and enzymatic procedures[12] have been used to quantify oxalic acid. These methods are less sensitive than the proposed method[7–9] and have many interferences[5, 11, 12], or use complicated procedures. Jiang *et al.*[13] determined oxalic acid ion by its catalytic effect on the oxidation of rhodamine B, but this method is a conventional spectrophotometic method and thus needs a rigid control of reaction conditions. Narayanan *et al.*[14] determined dichloroacetic acid and its metabolites in blood and urine by using a sensitive high performance liquid chromatography. Shaidarova *et al.*[15] determined oxalic acid by using chemically modified electrodes. Matsumoto *et al.*[16] determined oxalic acid ion by amperometric flow injection analysis. In this paper, the catalytic effect on the redox reaction between oxalic acid ion and rhodamine B by potassium dichromate was used for determination of ultra trace amounts of oxalic acid with optical detection. The change in the colour of rhodamine B was monitored at 543 nm. The method is fast or simpler and more sensitive than the present methods for the determination of oxalic acid.

EXPERIMENTAL

All chemicals used in this work were analytical-reagent grade (Merck). Distilled water was used throughout. A standard solution of oxalic acid (1000 μg mL^{-1}) was prepared by dissolving 0.1 g of the reagent ($H_2C_2O_4$, Merck) in distilled water and diluted to 100 mL in a standard flask. The working standard solutions were freshly prepared before use. A 0.050 mol L^{-1} potassium dichromate solution was prepared by dissolving 0.2450 g of $K_2Cr_2O_7$ (Merck) in water and diluting to 100 mL in a volumetric flask. Rhodamine B (Merck) 0.030% solution was prepared by dissolving the dye in water and diluting with water in a 100 mL volumetric flask. Poly(vinyl alcohol) solution was prepared by dissolving 0.60 g of the reagent in 100 mL of water. Thiourea solution was prepared by dissolving 0.45 g of the reagent in 100 mL of water.

For optical measurements, Shimadzu UV-Vis 2100 double-beam spectrophotometer and a thermostated cell at 80 ± 010C controlled temperature were used. Experiments were carried out using conventional system for FIA with a transmission flow through cell having one of the windows covered with sensing film. Simple single line manifold consists of 12 channel peristaltic pump (Desaga, PLG, 70 W) was fitted with three silicon rubber tubes (10 mm i.d.), rotary injection valve and flow-through optical cell.

Preparation of detectors: 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 0.45% (w/v) thiourea and 0.60% (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 rhodamine B 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 the washings showed no absorbance at the wavelength of the dye during rinsing. Finallythe film was dried at 45°C for 20 min

Effect of reagent concentration and temperature: The effects of potassium dichromate and sulfuric acid on the catalyzed reaction were studied with 0.030%

(w/v) rhodamine B solutions, with various potassium dichromate and sulfuric acid solutions with oxalic acid concentration of 0.080 μg mL^{-1} and a temperature of 25°C. From the results, 0.060 and 0.050 M were selected as the optimum potassium dichromate and sulfuric acid for the study.

The effect of rhodamine B concentration in the presence of potassium dichromate and sulfuric acid 0.060 and 0.050 M at temperature 25°C was studied. The results show that by increasing rhodamine B concentration to greater than 0.030% (w/v), the change in absorbance was diminished. Thus, 0.030% (w/v) rhodamine B solution was selected for the best sensitivity.

The effect of temperature on the peak height was studied in the temperature range 5–50°C at the optimum condition. From the results, 25°C was selected for the study.

The sensitivity of the oxalic acid determination largely depends on the flow rate of the reagents. The peak heights increased as the flow rate decreased. Thus, a flow rate of 0.40 mL/min was selected for all reagents.

By increasing the length of the reaction coil from 50–250 cm the peak heights increased, because the longer residence time of the sample zone allowed the reaction to proceed further. However, peak broadening and tailing were observed with longer reaction coils (200 cm) due to dispersion. Thus, 200 cm was selected for the length of the reaction coil.

The effect of sample volume was investigated for the best sensitivity. The sensitivity increased by increasing the sample volume from 50–200 μL. However, injection of the larger sample volume into the water stream results in peak broadening and tailing. Therefore, 100 μL sample volume was selected.

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 measurements were 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 rhodamine B were measured by FIA with oxalic acid in (sulfuric acid 0.050 M, potassium dichromate 0.060 M, Fig. 2).

RESULTS AND DISCUSSION

Rodamine B has amino and carboxy acid groups in the ring. These dye molecules can be linked to the cellulose acetate film by special treatment. Kostov *et al.*[6] showed that only the dye with amino groups could be linked with cellulose acetate. It is found that using thiourea, dyes with or without amino group can be linked to cellulose acetate film. The optical properties of immobilized rhodamine B on a hydrolyzed cellulose membrane as a function of oxalic acid concentration is shown in Fig. 2. The absorbance change is linear only for 0.07–3.50 μg mL^{-1} oxalic acid. The absorbance maximum of the immobilized rhodanmie B is located at 543 nm. The above result can be interpreted as the influence of the immobilization procedure on the behaviour of the indicator. Koolstra *et al.*[7] have shown that the condensation between the hydroxymethyl 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

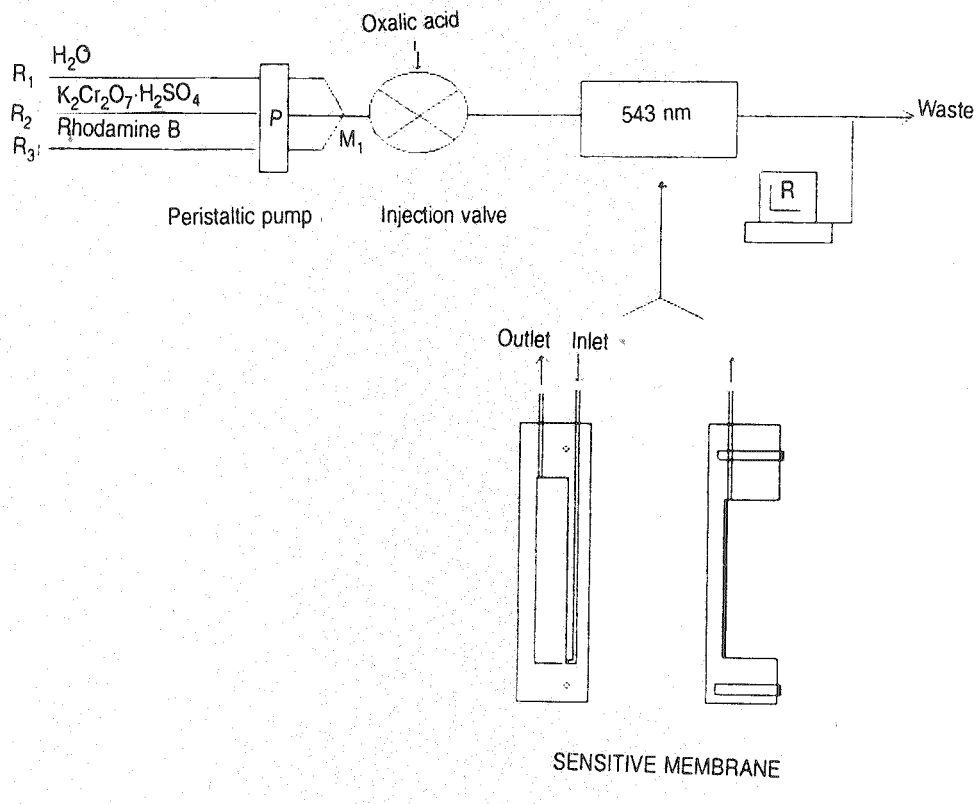

Fig. 1. Schematic diagram of the frame on which the membranes are stretched, inside the cuvette

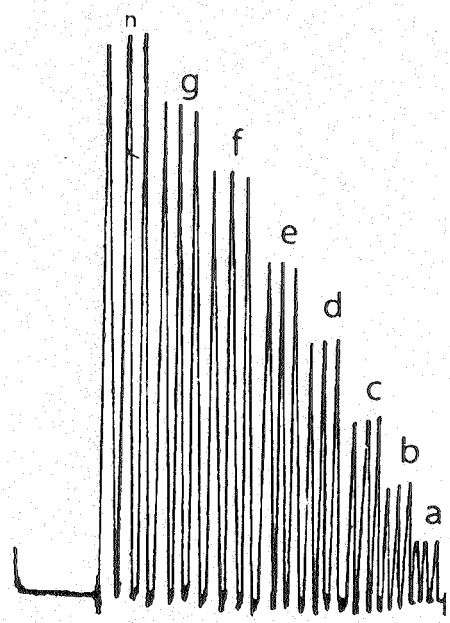

Fig. 2. Absorption spectra for rhodamine B in membrane at oxalic acid concentration: (a) 30×10^{-9}, (b) 60×10^{-9}, (c) 90×10^{-9}, (d) 12×10^{-9}, (e) 150×10^{-9}, (f) 180×10^{-9}, (g) 210×10^{-9}, (h) 240×10^{-9} μg mL^{-1}

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 and carboxy acid groups or free *para*-positions in their structure can be used. For this reason, thiourea was used as a bridge to connect the dye molecules

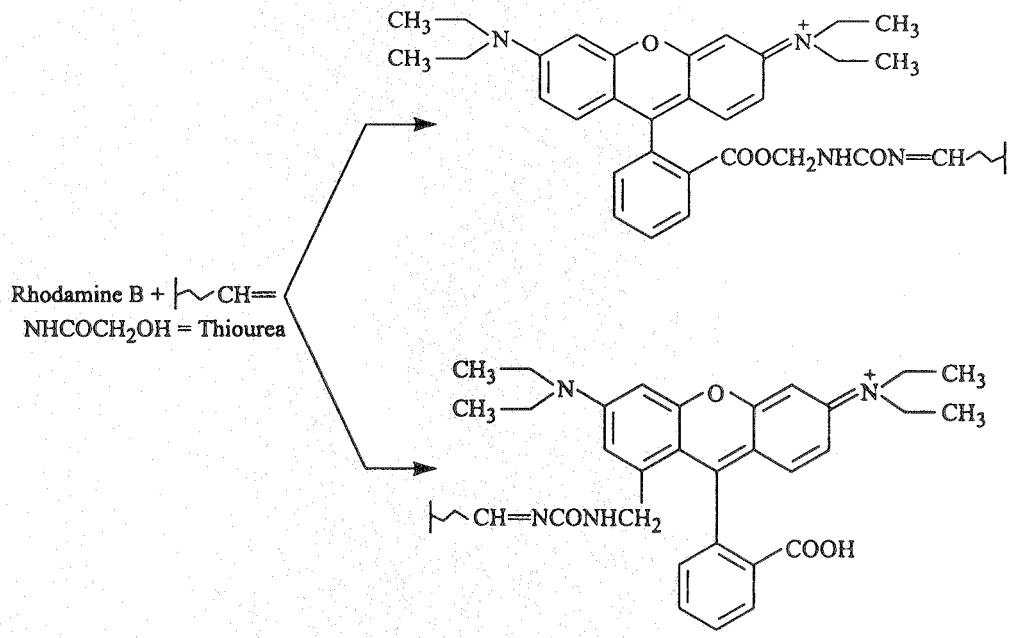

Fig. 3. Possible scheme of reaction between activated membrane and rhodamine B

to the membrane cellulose acetate film. A possible scheme for the reaction is shown in Fig. 3. This makes it possible to achieve covalent binding to the activated matrix. The fact that the immobilization changes the heights and positions of the absorption maxima to that of free indicators shows that the carboxy acid groups are auxochromic elements of the molecule and the loss of the proton after covalent binding influences the charge distribution during the dissociation of the immobilized dye. Rhodamine B is a dye that can be oxidized with potassium dichromate in an acidic solution in the presence of oxalic acid.

Detector stability and response time: It was shown that the changes in the absorbance on making repeated measurements in aqueous solutions for a month were < 5%. The stability of the membrane detectors based on a recycled support is higher than with other methods in which acetyl cellulose is also used for producing optical detectors[5]. This is due to the higher mechanical strength of the carrier as well as the covalent binding with the indicator. The change in optical properties of membranes with immobilized rhodamine B is measured at 543 nm. Fig. 4 shows a typical curve for the transition process. It can be seen that the output signal reaches 98% of the steady state response of the membrane in 9 s.

Sample treatment and digestion: Some analytical performance characteristics are given in Table-1.

Determination of oxalic acid in food samples: To check the applicability of the method, the determination of oxalic acid was carried out on various spinach samples. The results are shown in Table-2. The results showed good reproduc-ibility and accuracy in comparison to the standard method[17].

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

Characteristics	Oxalic acid
Linear range ($\mu g\ mL^{-1}$)	0.07–3.50
Detection limit ($\mu g\ mL^{-1}$)	0.012
Precision (RSD)*:	
(1) 0.10 $\mu g\ mL^{-1}$ $C_2O_4^{2-}$	1.55%
(2) 0.50 $\mu g\ mL^{-1}$ $C_2O_4^{2-}$	1.30%
(3) 1.50 $\mu g\ mL^{-1}$ $C_2O_4^{2-}$	1.15%

*For 10 replicate measurments.

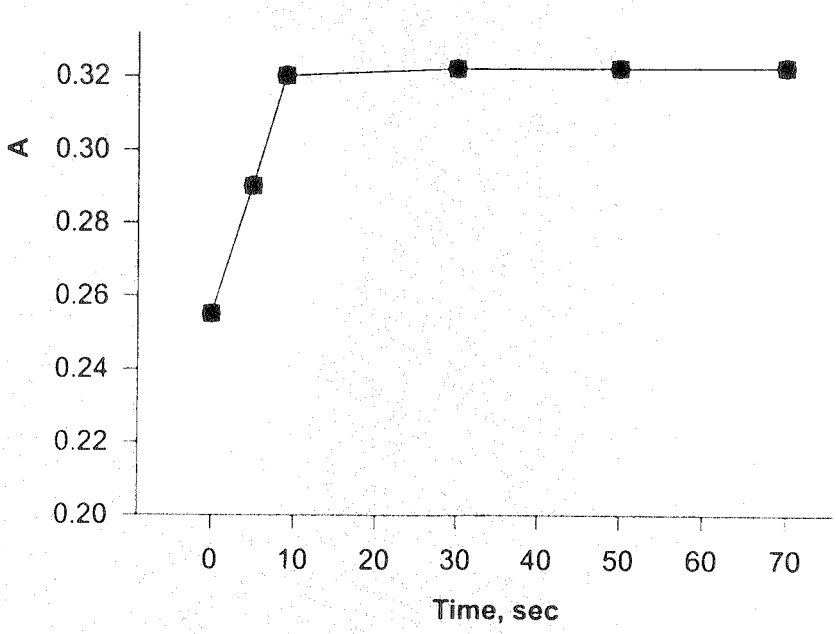

Fig. 4. Transition process of the oxalic acid detector constructed by covalent binding of rhodamine B to cellulose in acidic media (Condition: the initial oxalic acid was 24×10^{-7} $\mu g\ mL^{-1}$ at t = 0; the oxalic acid was changed to 8×10^{-7} $\mu g\ mL^{-1}$)

TABLE-2
DETERMINATION OF OXALIC ACID IN SPINACH SAMPLES

Samples	Oxalic acid ($\mu g\ mL^{-1}$)		Concentration of oxalic acid* (mg/g†)	
	Added	Found	Proposed method	Standard method
Spinach leaf (1)	—	1.18 ± 0.003	4.27 ± 0.002	4.18 ± 0.003
	1.0	2.12 ± 0.002		
	2.0	3.15 ± 0.002		
Spinach leaf (2)	—	1.25 ± 0.002	3.65 ± 0.004	3.72 ± 0.006
	1.0	2.51 ± 0.003		
	2.0	3.58 ± 0.002		

*Mean for five determinations. †For spinach samples.

Preparation of water samples: The proposed method was applied to the analysis of some water samples under optimum conditions. The water sample was initially filtered over Whatmann No. 1 paper. The results are shown in Table-3. Significant differences between the proposed method and the standard method[17] were found.

TABLE-3
DETERMINATION OF OXALIC ACID IN ENVIRONMENTAL WATER SAMPLES (KARAJ WATER)

Samples	Oxalic acid ($\mu g\ mL^{-1}$)		Concentration of oxalic acid* ($\mu g\ mL^{-1}$)	
	Added	Found	Proposed method	Standard method
Sample 1	—	1.44 ± 0.002	1.48 ± 0.003	1.55 ± 0.003
	1.0	2.36 ± 0.002		
	2.0	3.48 ± 0.002		
Sample 2	—	1.70 ± 0.004	1.75 ± 0.006	1.68 ± 0.007
	1.0	2.36 ± 0.003		
	2.0	3.48 ± 0.004		

*Mean for five determinations.

Interference study: In order to assess the application of the proposed method to real samples, the influences of some other substances were studied in the presence of 0.1 $\mu g\ mL^{-1}$ of oxalic acid ion. The tolerance limit of a foreign species was taken as a relative error not greater than 5% (three times standard deviation for 0.10 $\mu g\ mL^{-1}$); the results are shown in Table-4.

TABLE-4
EFFECT OF INTERFERENCE FOR THE DETERMINATION OF 0.10 $\mu g\ mL^{-1}$ OF OXALIC ACID

Species	Tolerance limit ($\mu g\ mL^{-1}$)
Li^+, Na^+, NH_4^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Hg^{2+}, Sn^{2+}, Co^{2+}, Ni^{2+}, Ag^+, F^-, Cl^-, NO_3^-	10000*
Acetate	500
Cu^{2+}, Fe^{2+}	200
Al^{3+}	150
Glucose, ascorbic acid	100
Formic acid, Br^-	80
Tartaric acid, citric acid, uric acid	30

*Maximum concentration tasted.

Conclusion

The detector exhibits a dynamic range of 0.07–3.50 $\mu g\ mL^{-1}$ oxalic acid. The described method for producing oxalic acid-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 detectors to be produced with a short response time and (c) its activation is performed by using inexpensive and easily available reagents with suitable time stability for large number of measurements.

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