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Application of Safranine O Optical Detector in Environmental and Food Analysis

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The work presented here describes the oxalic acid detector was developed by immobilizing a direct indicator dye in an optical detecting film for food and environmental monitoring. This detector was fabricated by binding sarfranine O to a cellulose acetate film that had previously been subjected to an exhaustive base hydrolysis. The membrane has good durability (> 8 months) and a short response time (< 10 s). Oxalic acid can be determined for the range 0.15–6.50 μg mL^{-1} with 3σ detection limits of 60 ng mL^{-1}. 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; Oxalic Acid, Food, Environmental analysis.

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]. Selective and precise detectors are necessary for the determination of oxalic acid content in foodstuffs. 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 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 oxalic acid detectors were evaluated. According to our knowledge, up to now, only dye molecules with amino 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,

we have used thiourea in linking a dye to a cellulose acetate film with satisfactory results. The dye used in safranine O 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 amperometric[7], high performance liquid chromatography[8, 9], gas chromatography[10], spectrophotometry[11] and enzymatic procedures[12] have been used to quantify oxalic acid ion. These methods are less sensitive than the proposed method[7–9] and have many interferences[5, 11, 12], or use complicated procedures. Jiang and co-workers[13] determined oxalic acid ion by its catalytic effect on the oxidation of rhodamine B, but this method is a conventional spectrophotometric method and thus needs a rigid control of reaction conditions. L. Narayanan and co-workers[14] determined dichloroacetic acid and its metabolites in blood and urine by using a sensitive high performance liquid chromatography. Shaidarova and co-workers[15] determined oxalic acid by using chemically modified electrodes. Matsumoto and co-workers[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 safranine O by potassium dichromate was used for determination of ultra trace amounts of oxalic acid with FIA and optical detection. The change in the colour of safranine O was monitored at 545 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 0.1000 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 0.030 mol/L potassium dichromate solution was prepared by dissolving 0.1470 g of $K_2Cr_2O_7$ (Merck) in water and diluting to 100 mL in a volumetric flask. Safranine O solution was prepared by dissolving 0.1096 g of safranine O (Merck) in 20 mL of ethanol and diluting with water in a 100 mL volumetric flask. Poly (vinyl alcohol) solution was prepared by dissolving 0.80 g of the reagent in 100 mL of water. Thiourea solution was prepared by dissolving 0.55 g of the reagent in 100 mL of water. A sulfuric acid solution (1 M) was prepared from the reagent of 98% (Merck).

For optical measurements Shimadzu UV-Vis 2100 double-beam spectrophotometer and a thermostated cell at 80 ± 0.1°C controlled temperature. 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 consisting 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 0.55% (w/v) thiourea and 0.80% (w/v) poly (vinyl alcohol) solution for 48 h at 25°C. The cellulose membranes were separately treated with a 3.126×10^{-3} M solution of safranine O at 25°C with magnetic stirring of the solution for 1 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. 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 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

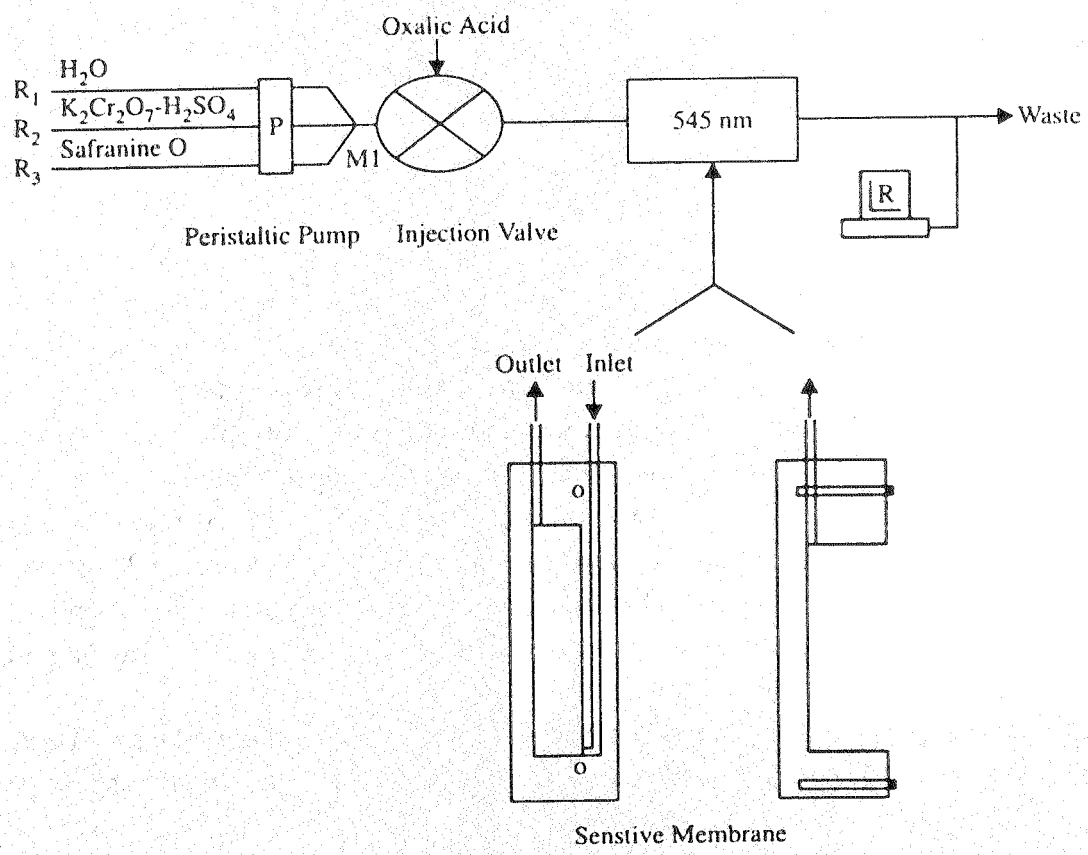
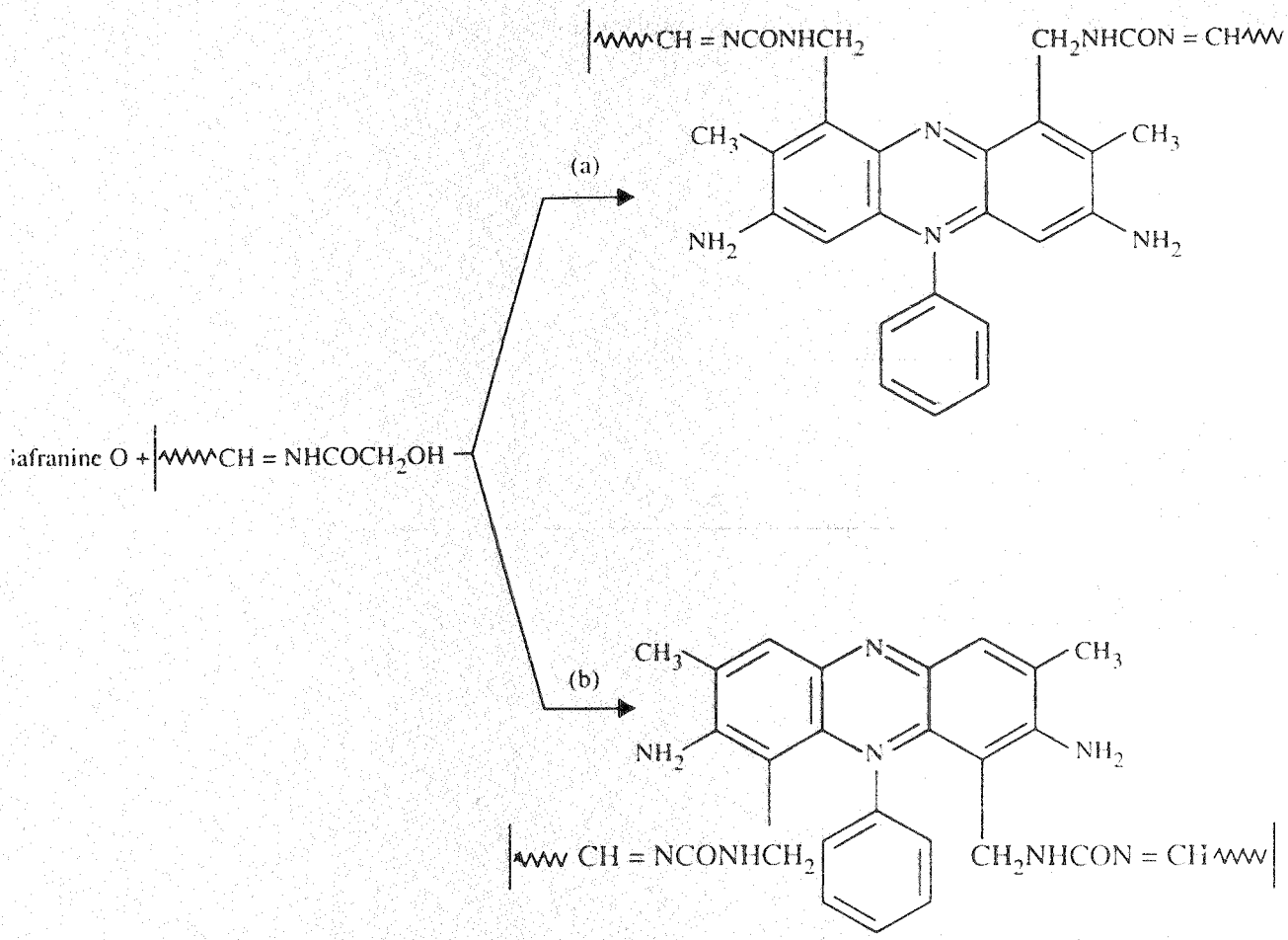


Fig. 1. Schematic diagram of FIA and the frame on which the membranes are stretched, inside the cuvette



Scheme-1. Possible scheme of reaction between activated membrane and safranine O

to achieve covalent binding to the activated matrix. The fact that the immobilization changes the heights and position of the absorption maxima to that of free indicators shows that the hydroxyl 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. Safranine O 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 sensors based on a recycled support is higher than with other methods in which acetylcellulose 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 safranine O measured at 545 nm. Fig. 3 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 10 s.

characteristics of safranine O were measured with oxalic acid in (sulfuric acid 0.15 M, potassium dichromate 0.015 M, Fig. 2).

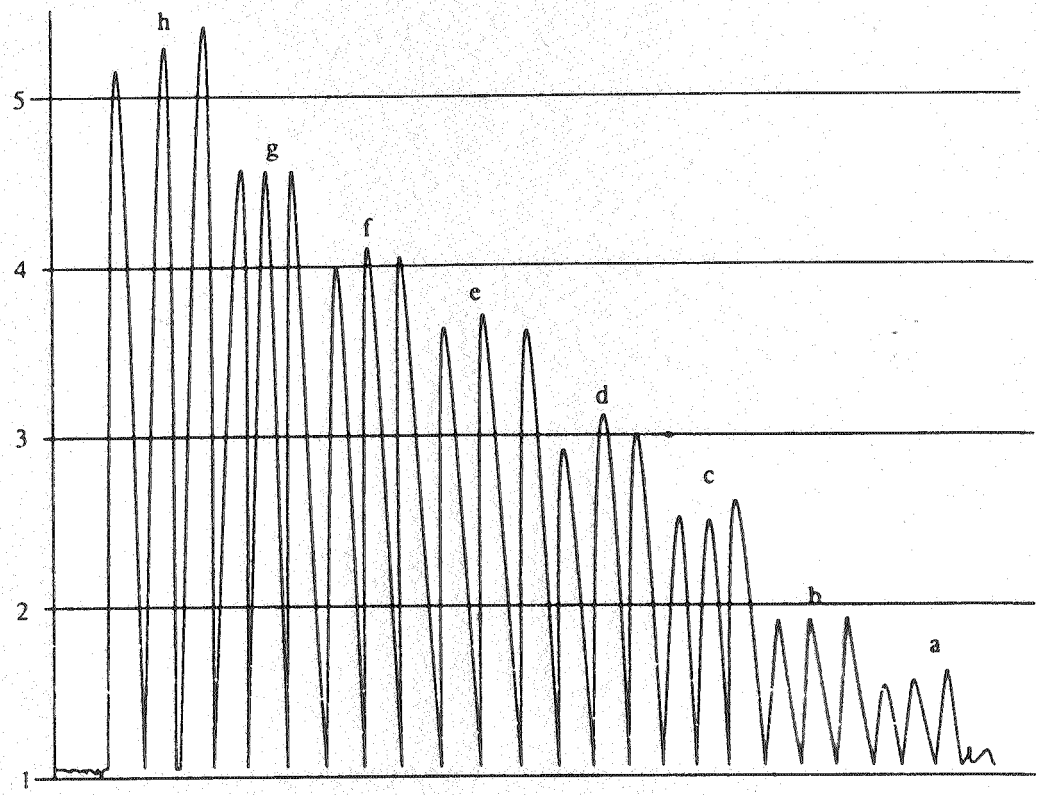


Fig. 2. Recording obtained in the flow-injection and sensitive membrane for determination of oxalic acid: concentrations: (A) 200×10^{-9}, (b) 400×10^{-19}, (c) 600×19^{-9}, (d) 800×10^{-9}, (e) 100×10^{-8}, (f) 120×10^{-8}, (h) 140×10^{-8} μg mL^{-1}

RESULTS AND DISCUSSION

Safranine O has two amino groups in the ring. These dye molecules can be linked to the cellulose acetate film by special treatment. Kostov and coworkers[6] showed that only dyes with amino groups could be linked with cellulose acetate. We find that using thiourea, dyes with or without amino group can be linked to cellulose acetate film. The optical properties of immobilized safranine O on a hydrolyzed cellulose membrane as a function of oxalic acid concentration are shown in Fig. 2. The absorbance change in linear only for 0.15–6.50 μg mL^{-1} oxalic acid. The absorbance maximum of the immobilized safranine O is located at 545 nm. At the above result can be interpreted as the influence of the immobilization procedure on the behaviur of the indicator. 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. For this reason, thiourea was used as a bridge to connect the dye molecules to the membrane cellulose acetate film. Possible scheme for the reaction is shown in **Scheme-1**. This makes it possible

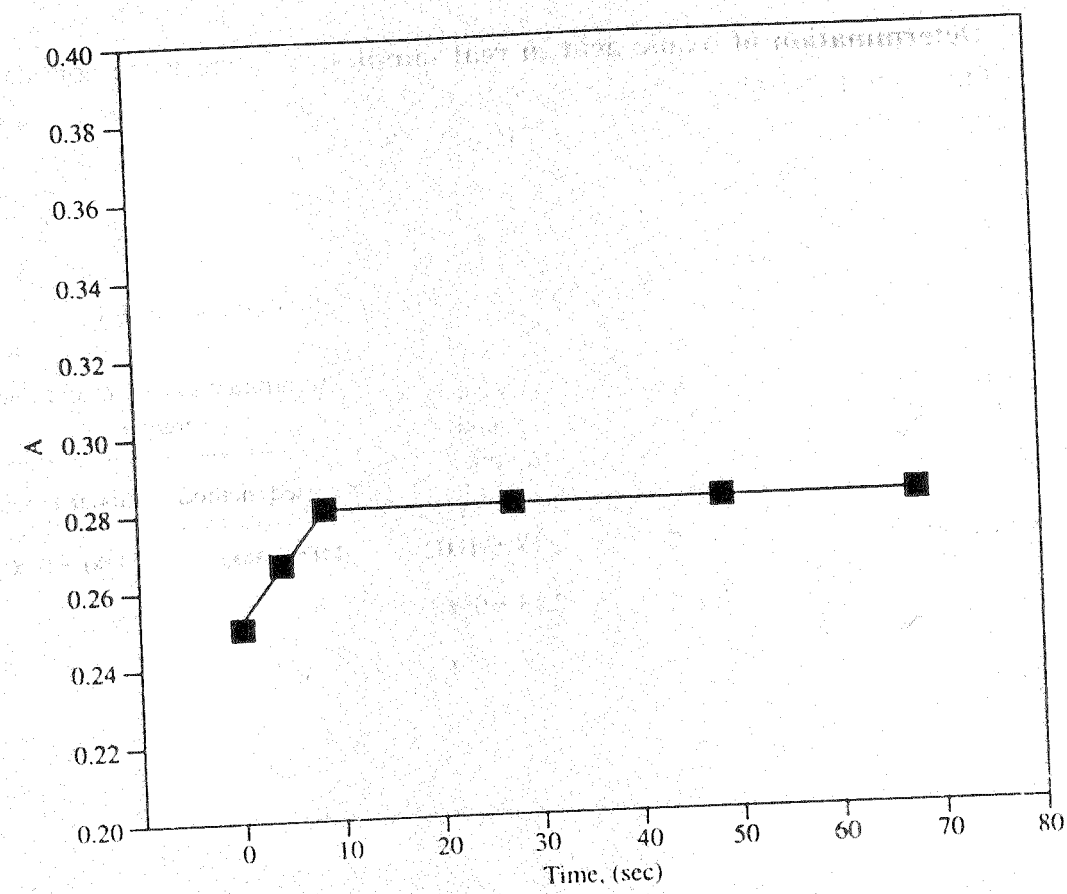


Fig. 3. Transition process of the oxalic acid detector constructed by covalent binding of safranine O to cellulose in acidic media (Condition: the initial oxalic acid was 36×10^{-7} μg mL^{-1} at t = 0, the oxalic acid was changed to 12×10^{-7} μg mL^{-1})

Sample treatment and digestion

Calibration curve: Some analytical performance characteristics are given in Table-1.

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

Characteristics	Oxalic acid
Linear range (μg mL^{-1})	0.15–6.50
Detection limit (μg mL^{-1})	0.060
Precision (RSD)[a]	
(1) 0.10 μg mL^{-1} $C_2O_4^{2-}$	1.80%
(2) 0.50 μg mL^{-1} $C_2O_4^{2-}$	1.55%
(3) 1.50 μg mL^{-1} $C_2O_4^{2-}$	1.20%

[a]For 10 replicate measurements ± S.D.

Determination of oxalic acid in real 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 reproducibility and accuracy in comparison to the standard method[17].

TABLE-2
DETERMINATION OF OXALIC ACID IN SPINACH SAMPLES

Sample	Oxalic acid added ($\mu g\ mL^{-1}$)	Oxalic acid found ($\mu g\ mL^{-1}$)	Concentration of oxalic acid found* (mg/g^b)	
			Proposed method	Standard method
Spinach leaf (1)	—	1.18 ± 0.002	4.07 ± 0.003	3.90 ± 0.002
	1.0	2.13 ± 0.001		
	2.0	3.16 ± 0.002		
Spinach leaf (2)	—	1.10 ± 0.003	3.47 ± 0.005	3.58 ± 0.008
	1.0	2.36 ± 0.002		
	2.0	3.44 ± 0.003		

*Mean for five determinations ± S.D.
[b]For spinach samples.

Preparation of food 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)

Sample	Oxalic acid added ($\mu g\ mL^{-1}$)	Oxalic acid found ($\mu g\ mL^{-1}$)	Concentration of oxalic acid* ($\mu g\ mL^{-1}$)	
			Proposed method	Standard method
Sample (1)	—	1.24 ± 0.002	1.28 ± 0.002	1.35 ± 0.004
	1.0	2.15 ± 0.003		
	2.0	3.25 ± 0.003		
Sample (2)	—	1.42 ± 0.002	1.52 ± 0.005	1.48 ± 0.005
	1.0	2.16 ± 0.003		
	2.0	3.28 ± 0.004		

*Mean for five determinations ± SD.

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 0.50 μg 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 0.50 μg mL^{-1}). The results are shown in Table-4.

TABLE-4
EFFECT OF INTERFERENCE FOR THE DETERMINATION OF 0.50 μg mL^{-1} OF OXALIC ACID

Species	Tolerance concentration Ratio ($W_{substance}/W_{oxalic\ acid}$)
Na^+, K^+, NH_4^+, Ca^{2+}, Al^{3+}, Ba^{2+}, Sn^{2+}, Cd^{2+}, Ni^{2+}, Mg^{2+}, Cl^-, NO_3^-	10000[a]
Urea, acetate, Zn^{2+}	600
Uric acid, formic acid	80
Glucose, ascorbic acid	
Formic acid, Br^-, lactic acid	
Benzoic acid, citric acid, Fe(II), Cu(II)	
Mn^{2+}	0.1

[a]Maximum concentration of the foreign ions tested ± S.D.

Conclusion

The detector exhibits a dynamic range of 0.15–6.50 μg 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 maxtrix for immobilization, (b) immobilization of the indicators on the membrane's surface reduces the diffusion limitations and allows detectors to be produced with a short response time, (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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