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ScienceDirect
Food Chemistry 105 (2007) 1106–1111
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Catalytic determination of traces of oxalic acid in vegetables and water samples using a novel optode

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Received 9 December 2006; received in revised form 29 April 2007; accepted 2 May 2007

Abstract

A new optode has been introduced for determination of oxalic acid. The optode sensing reagent is Victoria blue 4R which is immobilized on triacetylcellulose membrane. This reagent could be oxidized by dichromate in acidic media resulting in decoloration of the membrane. Oxalic acid has a strong catalytic effect on this reaction. The difference in absorbance of the immobilized form of Victoria blue 4R at 615 nm between uncatalyzed and catalyzed reactions (ΔA) is directly proportional to the concentration of oxalic acid. Oxalic acid can be determined in the concentration range of 2–180 μg ml^{-1}. The effect of different possible interfering species has been examined and was shown that the optode has a very good selectivity. The optode has been applied for the determination of oxalic acid in different real samples such as spinach, beet root, mushroom and river water with excellent recoveries.

Keywords: Oxalate determination; Vegetables; Water; Optode; Catalytic

1. Introduction

Oxalic acid and oxalates are abundantly present in many plants. The root and/or leaves of rhubarb and buckwheat are listed being high in oxalic acid. Foods that are edible, but that still contain significant concentrations of oxalic acid include star fruit, black pepper, parsley, spinach, mushroom, beets, cocoa, chocolate, most nuts, most berries, and beans. Oxalate acts as a chelator of various positively charged metal ions, such as Fe^{2+}, Fe^{3+}, Cu^{2+}, Al^{3+} and Cr^{3+}, among others. It also forms strong chelates with dietary calcium, thus rendering the complex unavailable for absorption and assimilation. In humans, precipitation of calcium oxalate may lead to the formation of kidney stone. Also, oxalic acid irritates the lining of the gut when consumed, and can prove fatal in large doses. Therefore, sensitive, selective and precise methods for the determination of oxalic acid are demanding. Methods mostly reported for oxalate or oxalic acid determination are based on spectrophotometry (Parkinson, Sheldon, Laker, & Smith, 1987), polarography (Koolstra, Wolters, Hayer, & Elzerga, 1987), isotope dilution mass spectrometry (Zara & Bulhoes, 1987), liquid chromatography (Koolstra, Wolters, Hayer, & Rutgers, 1987; Murray, Nolen, Godon, & Peters, 1982), gas chromatography (Gelot, Lover, Belleville, & Nabet, 1980), high performance liquid chromatography (HPLC) (Utzman, 1993), and enzymatic analysis (Buttery, Ludvigson, Braiotta, & Pannall, 1983).These methods are either not so sensitive or selective or are time-consuming.

The catalytic method is one of the most attractive procedures in terms of sensitivity (Zhang, Gao, Zhan, & Liu, 1998), and the fact that no expensive or special equipment is required. However, only few methods have been described for determination of oxalic acid based on kinetic procedures (Jiang, Zhao, & Liao, 1996; Perez-Ruiz, Martinez-Lozano, Tomas, & Casajus, 1995). Zhang and Xu (2000), reported a catalytic spectrophotometric method, based on the catalytic effect of oxalic acid on the oxidation of Victoria blue B by potassium dichromate. One of the most serious limitations of the above mentioned catalytic methods is the lack of sensitivity for the analyte

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0308-8146/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2007.05.007

determination at room temperature, so that working temperatures of 50 °C or higher has been reported for this type of analysis. The need for high temperatures not only is inconvenient but also may cause the formation of air bubbles in the reaction mixture which results in poor reproducibility and some kind of alteration in sample texture. Therefore, the need for a sensitive and selective as well as simple method for oxalic acid determination at room temperature is still highly desired.

Because of its advantages of easy fabrication, good sensitivity and selectivity and low cost, optical chemical sensors have drawn much attention in analytical chemistry. In optochemical sensors (so called optrodes) (Seitz, 1984), or optodes (Simon, 1990), the sensing element consists of reagent dyes immobilized in organic or inorganic matrices. Reaction with the analyte changes the absorbance or fluorescence behaviour of the sensitive layer.

Victoria blue 4R is a dye, which has been introduced as an acid–basic indicator. This reagent has a very limited solubility in aqueous media but retained strongly on the triacetylcellulose membrane. The uses of triacetylcellulose membrane as robust and very useful membranes in optode technology have been reported for the determination of different analytes (Safavi & Bagheri, 2003, 2004, 2005a, 2005b; Safavi, Rostamzadeh, & Maesum, 2006). In the present work, the fabrication of an optode for catalytic determination of low levels of oxalic acid is described in which the sensing reagent is Victoria blue 4R (VB.4R) immobilized on triacetylcellulose membrane. It was found that in acidic media dichromate could oxidize VB.4R resulting in decoloration of the membrane. Oxalic acid has a strong catalytic effect on this reaction. The difference in absorbance of the immobilized form of VB.4R at 615 nm between uncatalyzed and catalyzed reactions (ΔA) is directly proportional to the concentration of oxalic acid. The measurements are carried out at room temperature with good selectivity and precision. The proposed method was successfully applied to the determination of oxalic acid in some vegetable and water samples.

2. Materials and methods

2.1. Reagents

All chemicals were of analytical reagent grade. Solutions were made in deionized water. Stock standard solution of oxalic acid (1.0 mg ml^{-1}) was prepared by dissolving 0.1 g oxalic acid in water and diluting to 100 ml in a volumetric flask. Working standard solutions were obtained by appropriately diluting the stock solution before use. Dichromate stock solution (0.01 mol l^{-1}) was prepared by dissolving the required amount of potassium dichromate in water. VB.4R solution (1.2×10^{-3} g ml^{-1}) was prepared by dissolving 12 mg of VB.4R (BDH, Liverpool, England) in 10 ml of ethylenediamine. A sulphuric acid solution (1.0 mol l^{-1}) was prepared from 98% purity reagent.

2.2. Apparatus

A Shimadzu 1601PC UV–Vis spectrophotometer (Kyoto, Japan) was used for recording the visible spectra and absorbance measurements. The sensing membrane was placed in a glass cell and all measurements were performed in a batch mode. A pH meter (Metrohm 780, Herisau, Switzerland) with a Metrohm glass electrode was used for monitoring pH adjustments.

2.3. Preparation of the sensor membrane

The immobilized indicator on triacetylcellulose was prepared according to the following procedure. The transparent triacetylcellulose membranes were produced from waste photographic film tapes that were previously treated with commercial sodium hypochlorite for several seconds in order to remove coloured gelatinous layers. The films were treated with a clear solution of VB.4R in ethylenediamine for 5 min at ambient temperature. Then they were washed with water for removing ethylenediamine and loosely trapped indicator. Prepared membranes were kept under water when not in use. The membranes prepared by this method were stable over several weeks of storage under pure water.

2.4. Procedure

The prepared membrane was placed in a sulphuric acid solution of pH 4 for several seconds to reach equilibrium. The membrane was placed vertically inside the cell containing 2.5 ml of dichromate solution (1.0×10^{-3} mol l^{-1}). Then oxalic acid solution with specific concentration was added and the difference in absorbance of the immobilized form of VB.4R at 615 nm between uncatalyzed and catalyzed reactions (ΔA) was measured after 400 s.

2.5. Procedure for real sample analysis

A 50.0 g of each real samples (spinach, beet root and mashroom) was cut into small pieces with a razor blade and boiled with water under reflux for 50 min. The mixture was cooled and filtered through a membrane filter. A suitable aliquot of the filtrate was used to determine oxalic acid.

3. Results and discussion

3.1. Oxidation of optode membrane by dichromate in the presence of oxalic acid

Victoria blue 4R is a dye, which has been used as acid–basic indicator. This reagent is slowly oxidized to a colourless compound by potassium dichromate; however, in the presence of oxalic acid the rate of oxidation strongly increases. Victoria blue 4R has a limited solubility in aqueous media but is retained strongly on the triacetylcellulose

membrane. This work is based on the fabrication of an optode for catalytic determination of low levels of oxalic acid using Victoria blue 4R (VB.4R) immobilized on triacetylcellulose membrane.

The catalytic effect of the oxalic acid on the oxidation of aromatic azo compounds with chromic acid has been plausibly explained by Subba Rao and Murty (1976), considering the formation of a complex between oxalic acid and chromium (VI), which decrease the redox potential of the system. Fig. 1 shows the absorption spectra of the immobilized form of VB.4R before and after catalytic oxidation.

3.2. Influence of reaction temperature

The influence of reaction temperature on the catalytic determination of oxalate was studied in the range of 15–50 °C. As can be seen from Fig. 2, (ΔA) increased rapidly with increasing reaction temperature up to 25 °C and decreased at higher temperatures. This trend is due to the fact that upon increasing temperature, the rate of uncatalyzed reaction was increased, and therefore the difference of absorbance value between catalyzed and uncatalyzed reaction (ΔA) was decreased. Thus, 25 °C was chosen for further experiments.

3.3. Optimization of reagent concentrations

For immobilization of dye on the membrane, the triacetylcellulose membrane was immersed in a solution of VB.4R in ethylenediamine. The effects of varying concentrations of VB.4R were studied in the range of 5.0×10^{-4}–2.0×10^{-3} g ml^{-1}. The ΔA (defined as the difference in absorbance value between catalyzed and uncatalyzed reaction) increased with increasing VB.4R concentrations up to 1.2×10^{-3} g ml^{-1}, above which no improvement in (ΔA) was observed. Thus, a 1.2×10^{-3} g ml^{-1} of VB.4R concentration was chosen for immobilization of VB.4R on the membrane.

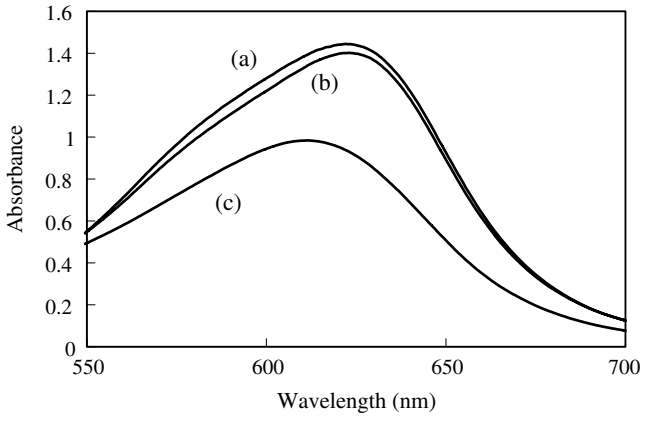

Fig. 1. Effect of oxalic acid on the oxidation of immobilized form of VB.4R by dichromate. VB.4R, 12 mg ml^{-1}; sulphuric acid, 0.4 mol l^{-1}; potassium dichromate, 0.001 mol l^{-1}; reaction time and temperature, 7 min at 25 °C. (a) membrane containing VB.4R in the absence of dichromate and oxalic acids; (b) with dichromate and without oxalic acid; (c) with dichromate and 80 μg ml^{-1} of oxalic acid.

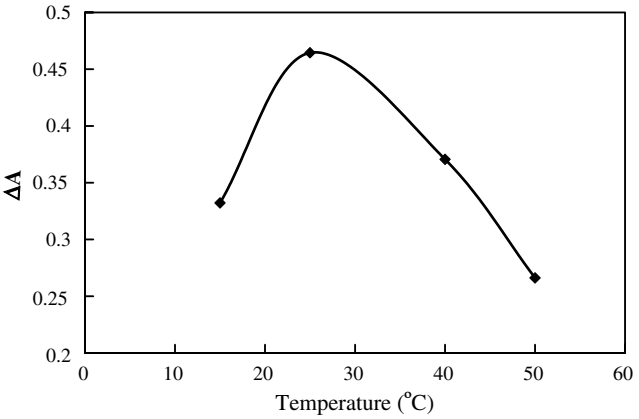

Fig. 2. Effect of reaction temperature on the determination of 80 μg ml^{-1} oxalic acid. Other conditions as in Fig. 1.

The effect of sulphuric acid concentration was studied in the range of 0.2–1.0 mol l^{-1}. The (ΔA) increased with increasing sulphuric acid concentrations up to 0.5 mol l^{-1}. A sulphuric acid concentration of 0.5 mol l^{-1} was therefore selected.

The effect of dichromate concentration was tested in the range of 1×10^{-4}–1×10^{-2} mol l^{-1}. The ΔA increased with increasing dichromate concentrations up to 1×10^{-3} mol l^{-1}, above which a decrease in ΔA was observed. This trend is due to the fact that upon increasing dichromate concentration, the rate of uncatalyzed reaction is increased, and therefore the difference of absorbance values between catalyzed and uncatalyzed reaction (ΔA) is decreased. Thus, a 1×10^{-3} mol l^{-1} of dichromate concentration was chosen for further experiments.

3.4. Response time

In this work the optode film was found to reach 95% of the final signal at 5–7 min depending on the concentration of oxalic acid (Fig. 3). At high concentrations of oxalic acid, a rapid response was achieved, while at low concentrations of oxalic acid a longer response time was observed.

3.5. Calibration graph, detection limit and precision

Under the chosen experimental conditions, the difference in absorbance between blank and sample varied linearly with the concentration of oxalic acid in the range of 2.0–180 μg ml^{-1} and fitted the equation:

$$\Delta A = 0.004\,C + 0.0946$$

with a regression coefficient of 0.9977, where C is the oxalic acid concentration expressed in μg ml^{-1}. The detection limit calculated from three times the standard deviation of the blank was 0.7 μg ml^{-1}. The relative standard deviation was 0.7% for the determinations of 80.0 μg ml^{-1} of oxalic acid.

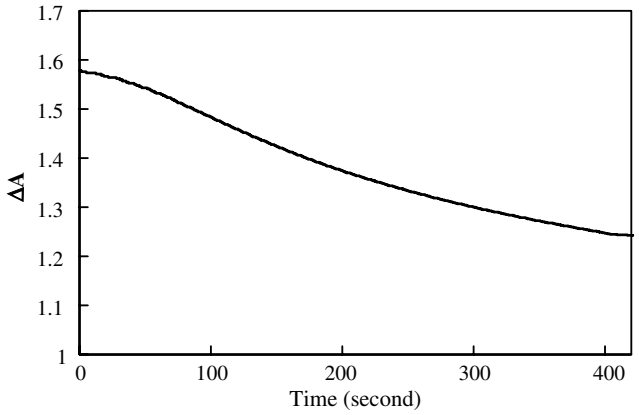

Fig. 3. Typical response curve of the optode film at 615 nm as a function of time when the film was exposed to 80 μg ml^{-1} oxalic acid.

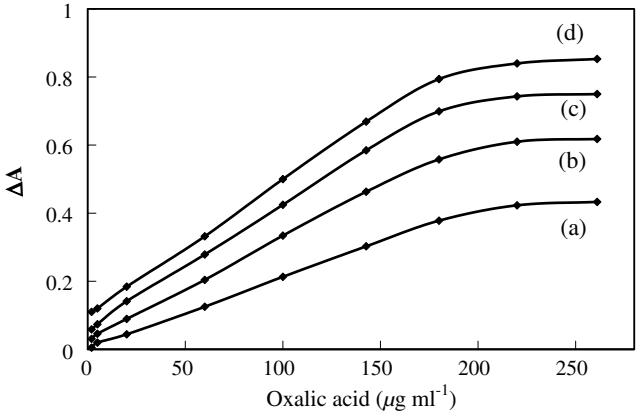

Fig. 4. Calibration graph for the determination of oxalic acid at 100, 200, 300, 400 s respectively.

Table 1 shows a comparison between the analytical performance of the proposed optode and those of previously reported methods.

3.6. Calibration graph for determination of oxalic acid at different times

Conventional measurements with optode involve obtaining the response at steady state. The results discussed so far were also based on this type of measurement. However, it was attempted to evaluate the effect of measuring the response before the optode signal reached a steady state value. For this study, it was not needed to wait for the optode to reach a steady state response as it is common in traditional optodes. Instead, the difference between the absorbance of the optode in the absence and presence of oxalic acid was measured at a fixed time which was in the dynamic region of the optode response. The advantage of this measurement method is that reliable data could be achieved in a shorter analysis time. Thus, the determination of the absorbance difference was performed before steady state was reached. Fig. 4 shows the ΔA measurements at different time values. According to this figure, the determination in dynamic range (at shorter times) has negligible effect on linearity range but slightly compromises the sensitivity of measurements.

3.7. Interference study

To study the selectivity of the proposed method, the effect of foreign species on the determination of 80.0 μg ml^{-1} of oxalic acid was tested. The tolerance limit was defined as the concentration at which the species caused an error less than 5%. The results are shown in Table 2 which demonstrate that this catalytic method has a very good selectivity. According to these results ascorbic acid is considered as the only important interference. To remove this interference, the sample should be refluxed for few minutes before analysis. Under this condition ascorbic acid is decomposed.

3.8. Application

To investigate the applicability of the proposed method to real samples, oxalic acid was determined in different vegetable and water samples, using standard addition method. The results obtained for the analysis of the samples of vegetables using the proposed method and the potassium permanganate titrimetric method (Jacobs, 1962), are summarized in Table 3. Student *t*-test was applied which showed that there is no significant difference between the results obtained by the two methods. As shown in Table 3, the results of both methods are in very good agreement.

Table 1
Comparison of analytical performance of the proposed optode with those of previously reported methods

Regent	LOD (μg ml^{-1})	RSD%	Linear range (μg ml^{-1})	Temperature work (°C)	Major interference	References
Dichromate and Rhodamine B	0.02	2.3	0.06–4	90	Ascorbic acid,uric acid	Jiang et al. (1996)
Dichromate and Bromophenol blue	0.04	2.7	0.1–8	60	Lactic acid, Cu^{2+}, Fe^{3+}	Xu and Zhang (2000)
Dichromate and Victoria blue B	0.8	1.5	1–80	50	Mn^{2+}, Cu^{2+}, Fe^{3+}, Ascorbic acid	Zhang and Xu (2000)
Dichromate and safranine	0.08	2.5	1–10	60	Lactic acid, Cu^{2+}, Fe^{3+}	Ensafi et al. (2001)
Dichromate and Victoria blue 4R	0.7	0.7	2–180	Room temperature	Ascorbic acid	This work

Table 2
Tolerance limit of diverse species in the determination of 80 μg ml^{-1} oxalic acid

Species	Max. tolerable concentration ratio
CN^-, NO_3^-, ClO_3^-, SO_4^{2-}, F^-, K^+, Mn^{2+}, Ca^{2+}, NH_4^+, glucose, salicylate, glutamic acid	100
Fe^{3+}, urea, tartaric acid, Cu^{2+}	50
Ascorbic acid	1

Table 3
Determination of oxalic acid in real samples (for $n = 5$)

Sample	Proposed method (g kg^{-1})	Reference method (g kg^{-1})	Student t-values*
Beet root	14.31 ± 0.40	14.75 ± 0.36	1.82
Spinach	4.15 ± 0.16	4.21 ± 0.16	0.59
Mushroom	0.36 ± 0.01	0.35 ± 0.01	1.58

* $t_{0.05} = 2.31$.

Table 4
Recovery for the determination of oxalic acid in real samples

Sample	Added (μg ml^{-1})	Found (μg ml^{-1})	Recovery (%)
Spinach	None	18.8	
	15	33.9	100.6
	25	45.1	106.9
	35	53.4	97.9
	45	63.7	99.5
Mushroom	None	5.6	
	15	21.0	107.1
	25	30.5	98.2
	35	40.7	101.8
	45	50.5	98.2
Beet	None	6.3	
	15	21.6	104.8
	25	31.1	96.8
	35	41.5	103.2
	45	51.4	101.6
River water	None	5.1	
	10	15.2	101.9
	20	25.0	98.0
	30	34.9	96.1
	40	45.3	103.9

The recovery results for the analysis of some real samples are shown in Table 4.

4. Conclusions

The optode described in this work provides a simple and easy-to-use means for the determination of oxalic acid. In the presence of oxalic acid, the coloured membrane becomes colourless. Although VB.4R is a very suitable reagent for oxalic acid determination at room temperature, because of its very limited solubility, it cannot be used directly in solution. Therefore, immobilization of this indicator in an optode membrane is a good alternative for oxalic acid determination. The use of membrane can enhance the selectivity of the method, because diffusion of interfering ions into the membrane may not take place in the time scale of oxalic acid measurement. Some of the advantages of this method compared to other catalytic spectrophotometric methods can be summarized as follows: (a) a wider linear range, (b) good selectivity and fewer interferences, (c) applicability at room temperature and (d) good precision.

Acknowledgements

The authors wish to express their gratitude to the Third World Academy of Sciences Iran Chapter (TWASIC) and Shiraz University Research Council for the support of this work.

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