Selective Determination of Oxalate with a Ruthenium(II) Complex/Nafion-Modified Electrode Combined with a Carbon Dioxide Sensor

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A tris(2,2'-bipyridine)ruthenium(II)/Nafion-modified Pt gauze electrode, where CO₂ was generated by oxidation of substrates, was combined with a CO₂ sensor. The combined sensor responded most effectively to oxalate among the organic acids examined. The response to oxalate was enhanced by use of a doubly-piled, modified Pt gauze electrode. The most suitable applied potential was +0.95 V vs. Ag/AgCl; the stationary response was attained in 10 min after the potential application. The response to 0.5 mM oxalate was reproducible within 3% on 10 repeated runs and remained virtually unchanged over 10 days. The calibration curve for oxalate was linear in the concentration range of 0.1 to 5 mM, and the detection limit was 0.05 mM (*S/N*=3).

Keywords Oxalate, tris(2,2'-bipyridine)ruthenium(II), Nafion, modified electrode, carbon dioxide sensor

The determination of oxalate is important in the fields of food chemistry and clinical chemistry. At present there are many methods for such determination, including potassium permanganate titration¹, gas chromatography², colorimetry³ and enzyme assay.⁴ These methods, however, are time-consuming and cannot be used for on-line determination. Flow-through detectors consisting of an enzyme thermistor column^{5,6} have the problem that unstable or expensive enzymes must be used.

We previously reported two types of fiber-optic electrochemiluminescence (ECL) sensors for the determination of oxalate. One of the sensors consisted of a Pt working electrode coated with tris(2,2'-bipyridine)ruthenium(II) ([Ru(bpy)₃]²⁺)/Nafion;⁷ the other was a carbon paste electrode containing bis(2,2'-bipyridine)-4,4'-dinonadecyl-2,2'-bipyridine)ruthenium(II).⁸ Performances of these sensors, however, were unsatisfactory: the former was sensitive to trialkylamines as well and the latter, in addition to similar low selectivity, needed troublesome repacking of the carbon paste before each measurement.

In the present work, we fabricated an oxalate sensor having a [Ru(bpy)₃]²⁺/anionic perfluoropolymer (Nafion)-modified electrode combined with a CO₂ sensor which responds to CO₂ generated by the following reaction sequence:⁹

and

This sensor exhibited essentially no response to trialkylamines.

Experimental

Reagents

Ru(bpy)₃Cl₂ and 5 wt% Nafion solution in aqueous lower aliphatic alcohols were from Aldrich. All other reagents were of reagent grade or better and were used without further purification.

Preparation of the Ru^{II} complex-modified electrode

A Nafion-modified electrode was prepared by dropping 60 µl of the Nafion solution on a piece of Pt gauze (80 mesh, 16 mm in diameter) and evaporating this solution to dryness at room temperature. The modified electrode was dipped in a 1 mM [Ru(bpy)₃]²⁺/aqueous solution for 3 h to incorporate the Ru^{II} complex into the Nafion film and subsequently rinsed with deionized water.

Apparatus and procedure

As shown in Fig. 1, the [Ru(bpy)₃]²⁺/Nafion electrode was doubly piled and was fixed on the bottom face of a TOA CE235 CO₂ sensor coupled with a TOA 1M.1E Ion

Fig. 1 Configuration of the end of the sensor: (a) side view, (b) bottom view: 1, CO₂ sensor; 2, Pt wire; 3, counter electrode (Pt plate); 4, Ag/AgCl electrode; 5, CO₂ sensor membrane; 6, working electrode (Pt gauze modified with [Ru(bpy)₃]²⁺/perfluoropolymer).

Meter. A Pt counter electrode (12 × 22 mm) and Ag/AgCl reference electrode (1 mmφ) were fixed on the side face. The working electrode potential was +0.95 V vs. Ag/AgCl, controlled with a Hokuto Denko HA-301 potentiostat, unless otherwise noted. The current was monitored with an Advantest TR6846 Digital Multimeter. The electrolytic solution was 10 cm³ of 0.2 M phosphate buffer of pH 6.9 thermostated at 25.0 ± 0.1 °C, except in studying the pH effect, where 0.2 M phthalate buffer was used at pH 4.4 and 0.2 M phosphate buffer at pH 6.2, 6.9 and 7.7. A freshly prepared sensor was subjected to aging until a stable response to 1 mM oxalate was obtained.

Results and Discussion

Figure 2 shows a typical response when the sensor was immersed into a 0.5 mM oxalate solution under stirring. The signal gradually increased until it reached a stationary value 10 min after application of the potential.

As shown in Fig. 3, the response to a 1 mM oxalate solution with the [Ru(bpy)₃]²⁺-modified electrode was detectable at +0.6 V; it increased with a rise in potential, reaching a constant value at +1.0 V. A preferable potential was determined to be +0.95 V, in view of the accelerated decay of the response at higher potentials. At +0.95 V, a very weak response to 0.2 M phosphate buffer was also observed and therefore was subtracted from the response intensity with the substrate solution. A response to oxalate with an unmodified electrode was detectable only at potentials over +1.0 V in the presence of 0.1 mM [Ru(bpy)₃]²⁺, showing the high electroactivity of the modified electrode because of a high concentration of [Ru(bpy)₃]²⁺ in the film. The response in the absence of the Ru^{II} complex with the bare electrode, probably due

Fig. 2 Response profile of the sensor to 0.5 mM oxalate in 0.2 M phosphate buffer of pH 6.9. Applied potential: +0.95 V vs. Ag/AgCl.

Fig. 3 Effects of the applied potential on responses of the [Ru(bpy)₃]²⁺-modified electrode to 0.2 M phosphate buffer of pH 6.9 containing 1 mM oxalate (○) and to the phosphate buffer containing no oxalate (●) and on responses of a bare Pt electrode to the buffer containing 1 mM oxalate (△) and to the buffer containing 1 mM oxalate and 0.1 mM [Ru(bpy)₃]²⁺ (▲).

to the Kolbe reaction, was negligible below +1.0 V.

Piling two or three of the [Ru(bpy)₃]²⁺/Nafion-modified electrodes was examined. The doubly piled electrode gave a signal intensity 1.2 times as large as that of a singly-piled electrode and, in addition, the latter showed a slower response rate. It thus seems that the outside Pt gauze of the doubly-piled electrode kept the generated CO₂ from diffusing into the bulk solution. The triple pile did not enhance the signal intensity further, and consequently the doubly-piled electrode was chosen.

Figure 4 shows the effect of solution pH on the response to oxalate. The largest net response was observed at pH 6.2, while the net response at around pH 7 was not so much reduced and thus was sufficient to apply to *in vivo* measurements.

The signal intensity for 0.5 mM oxalate was reproducible within 3% on 10 repeated runs. The response was so stable as to give almost the same intensity even after repeated use of 10 days, suggesting that the present sensor is applicable to practical use. Figure 5

Fig. 4 pH-Dependence of the response to 0.5 mM oxalate (O) and a buffer solution without oxalate (□). Applied potential, +0.95 V vs. Ag/Ag Cl.

Fig. 5 Calibration curve for oxalate in 0.2 M phosphate buffer of pH 6.9. Applied potential, +0.95 V vs. Ag/AgCl.

shows a linear calibration curve for oxalate in the concentration range of 0.1 to 5 mM (correlation coefficient, 0.971). The detection limit was 0.05 mM ($S/N=3$), sufficient to determine oxalate in vegetables and urine ranging in concentration from 0.1 to 3 mM. The performance of the present sensor was comparable with those of the flow-through detectors mentioned above.^{5,6}

The response intensities for several organic substrates are summarized in Table 1. No response was observed with tripropylamine as expected. On the other hand, the sensor responded to some other carboxylic acids and amino acids, albeit more weakly than to oxalate. Accordingly, care must be taken in handling a sample solution where the responsive acids coexist. In the case of urine, particularly, uric acid coexisting in the concentration of a few mM should be previously removed by electrooxidation at a proper potential.

A substrate to which the sensor strongly responds must have an oxidation potential low enough to reduce the electrochemically generated $[\text{Ru}(\text{bpy})_3]^{3+}$, and the oxidized intermediate must undergo an efficient decarboxylation or degradation leading to CO_2 generation if

Table 1 Relative intensities for organic substrates in 0.2 M phosphate buffer of pH 6.9

Substrate ^a	Relative intensity ^b	E_p/V vs. SCE ^c
Oxalic acid	100	1.38
Formic acid	14	— ^d
Pyruvic acid	0	1.52
3-Methyl-2-oxobutanoic acid	4	
4-Methyl-2-oxopentanoic acid	0	1.45
Oxalacetic acid	59	1.55
2-Oxoglutaric acid	14	—
Lactic acid	6	—
Malic acid	6	—
Tartaric acid	7	—
Glycine	0	
Phenylglycine	0	
Leucine	0	
Phenylalanine	0	
Proline	0	
Aspartic acid	7	
Glutamic acid	9	
Serine	8	
Homoserine	8	
Threonine	0	
Cysteine	10	
Methionine	0	
Histidine	17	
Tryptophan	5	
Asparagine	13	
Arginine	0	
Uric acid	26	0.42
Tripropylamine	0	

a. 5×10^{-4} M.

b. Applied potential, +0.95 V vs. Ag/AgCl.

c. Anodic peak potential E_p measured in 0.2 M phosphate buffer of pH 6.8 by cyclic voltammetry on a glassy carbon electrode.

d. Not observed.

CO_2 is generated only through a series of reactions starting with a one-electron oxidation of the substrate with the $[\text{Ru}(\text{bpy})_3]^{3+}$ complex, as illustrated by equations (1)–(3) for oxalate. Oxalacetic acid exhibited a strong response, though it has a higher oxidation potential than those of other α -keto acids, 4-methyl-2-oxopentanoic acid, 2-oxoglutaric acid and pyruvic acid. Uric acid with a very low oxidation potential showed a weaker response than those of oxalate and oxalacetic acid. Thus, the response intensity appears to depend not only on the oxidation potential but also on the efficiency of the decarboxylation or degradation process, though this point should be substantiated by further studies. The highest response of oxalic acid may be attributed partly to generation of two CO_2 molecules through a series of reactions (2)–(4).

In conclusion, the present sensor is applicable to the selective oxalate determination and also to the determination of trialkylamines, when combined with

the ECL sensor which can respond to both oxalate and trialkylamines.⁷

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