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Optical Sensor for the Determination of Glucose Based on KIO_4 Chemiluminescence Detection

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A method to determine glucose using an optical sensor prepared by entrapping glucose oxidase into silica sol-gel column has been developed. The silica sol-gel film was coated on alumina substrate. The optical sensor is based on the chemiluminescence intensity from the reaction of periodate and hydrogen peroxide in K_2CO_3 medium. The effect of the ratio of water and alcohol for the preparation of TEOS sol on chemiluminescence intensity was investigated. The effects of pH of enzyme reactor, concentrations of potassium periodate and SDS, and flow rate on the chemiluminescence intensity were studied to find the optimum experimental conditions to determine glucose. The chemiluminescence intensity increased linearly with increasing glucose concentration from 5.0×10^{-4} M to 1.0×10^{-7} M and the detection limit was 4.0×10^{-8} M. Interference effects from some metal ions on chemiluminescence intensity were also investigated.

KEY WORDS: Glucose sensor; sol-gel; chemiluminescence; glucose oxidase.

INTRODUCTION

Glucose sensors are of great interest since glucose content is one of the most important parameters checked in routine medical analysis. These sensors also have been used to measure glucose content in other biological fluids, molasses and fermentation processes. The capacity of glucose oxidase (GOx) to catalyze the oxidation of glucose to gluconic acid has been widely promoted as a model system for the design of glucose sensor. The enzyme is highly active, relatively stable and readily available, and thus has been extensively utilized to develop electrochemical and optical biosensors. Conventional methods of enzyme immobilization include physical or chemical adsorption at a solid surface [1], covalent binding [2–4] or cross-linking [5,6] to a matrix, and entrapment within a membrane, surfactant matrix, polymer or microcapsule [7–10]. More recent work has demonstrated that sol-gel method could be a promising alternative method for the enzyme immobilization [11,12]. With the combination of the unique features of sol-gel process including high purity and uniformity, low process temperature and easy control on the reaction degree, the sol-gel encapsulation method is supposed to offer several advantages over conventional entrapment method. The principle of glucose determination by enzymatic method is to measure enzymatically produced hydrogen peroxide during the oxidation reaction of glucose in the presence of molecular oxygen [13–15]. Electrochemical biosensors for glucose play a leading role in this direction. Amperometric enzyme electrodes, based on GOx bound to electrode transducers, have thus been the targets of substantial research. Another method developed for the determination of hydrogen peroxide produced in the enzymatic reaction is to measure the difference of fluorescence or chemiluminescence (CL) signal due to the change of hydrogen peroxide content [16–19]. Recently, Lin *et al*. reported that the CL intensity was enhanced by the addition of potassium carbonate to the alkaline solution in the absence of a specific luminescent reagent [20].

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Periodate ion is known to react quantitatively with hydrogen peroxide in acid, neutral and alkaline solutions to give IO_3^- and singlet oxygen ($1O_2$) [21].

This paper describes a method to determine glucose with low interference effect by CL method. A flow injection system was employed for sample introduction. A KIO_4-K_2CO_3 system has been used for producing chemiluminescence in the present work. GOx was easily immobilized by sol-gel method and the sol-gel film was coated on alumina particle. The resulting particles were packed in a glass column. The optimum analytical conditions for calibration were obtained on the basis of the results of the effects of pH for the enzyme and CL reactions, flow rate and anionic surfactant on the CL intensity. The effects of interferences from some metal ions on CL intensity to determine glucose were also discussed.

EXPERIMENTAL

Materials

GOx (type-VII from *AspergiUus niger* EC 1.1.3.4), D-glucose, sodium dodecyl sulfonate (SDS) and tetraethyl orthosilicate (TEOS) were obtained from Sigma (St. Louis, MO, USA). Glucose solutions (freshly prepared in phosphate buffer, pH 7.5) were allowed to mutarotate at 4°C overnight before use. Potassium periodate was obtained from Kanto Chemical (Tokyo, Japan). All chemicals were of analytical-reagent grade and were used as received.

Apparatus

A schematic diagram of an automated stopped flow injection analyzer used in the chemiluminescence measurements is shown in Fig. 1. A flow system consisted of two peristaltic pumps (Ismatec Model MS-4 Reglo/6-100, Glattbrugg-Zürich, Switzerland). One (P1) delivered glucose sample solution. The other (P2) delivered KIO_4, SDS and K_2CO_3 solution in the flow system. A 100 μL aliquot of sample solution was injected by a six-way injection valve into the carrier stream of phosphate buffer solution. A mixture of P1 stream was carried through an enzyme column reactor. The P1 stream was mixed with a P2 stream in a T-shaped element connected to a flow cell. PTFE tubing (0.4 mm i.d.) was used to connect all the components of this system. A flow cell was fixed at 10 mm before the emission port of the cell compartment of a spectrofluorimeter (Model FL111, Spex, Edison, NJ, USA). A Hamamatsu Model R928 photomultiplier tube was used. The enzyme reactor was housed in a laboratory made temperature controlled chamber, in which temperature was set as 37°C. To measure chemiluminescence intensity, the Xe lamp was shut off and the luminescence emitted from the cell was fed to a photomultiplier tube (Model R928, Hamamatsu, USA). The voltage used for the photomultiplier tube was 900 V. For the chemiluminescence measurements the integration time and slit width was 1 s and 5 mm, respectively.

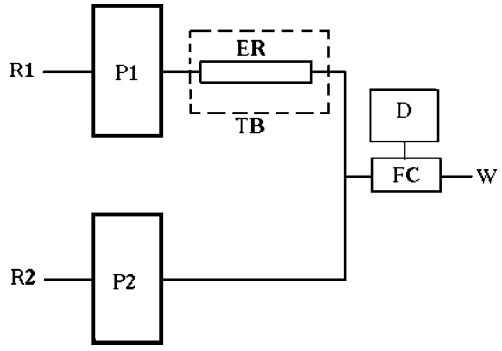

Fig. 1. Schematic diagram of a flow sensor for chemiluminescence determination of glucose in aqueous medium: R1, water; R2, KIO_4 + buffer solution; P1 and P2, peristaltic pump; ER, enzyme reactor; TB, thermal box; FC, flow cell; D, detector; W, waste.

Immobilization of GOx and Preparation of GOx Column

Silica gels derived from TEOS were synthesized using an acid-catalyzed sol, which was prepared by mixing 2 mL TEOS, 0.5 mL alcohol, 25 μL 1:20 HCl and a desired amount of water [22]. The mixed solution was sonicated for 3 hr until it became clear. A 0.4 mL of buffer solution containing 3.0 mg/mL GOx was then added to the sol solution. After thoroughly mixing, a 0.5 mL aliquot of the sol solution was transferred to a vial and 0.500 g of alumina with particle size at 80–100 mesh was added. The alumina particles doped with silica sol-gel was sonicated for 10 min, and the modified particles were kept at 4°C. The resulting alumina particles were used to prepare an enzyme column reactor using a glass tube with 2 mm of diameter and 4 cm of length. Measurement of glucose with CL-based sensor with sol-gel entrapped column.

Measurement Procedure

Flow lines R1 and R2 (Fig. 1) were inserted into KIO_4-K_2CO_3 solution and phosphate buffer solution, respectively. A 100 μL aliquot of sample was injected into the carrier stream of phosphate buffer stream which reacted with GOx in the column reactor to produce H_2O_2. All experiments were performed with 0.02 M potassium carbonate and 0.9 M potassium hydroxide, at which conditions the CL system could produce maximum CL signals.

RESULTS AND DISCUSSION

Immobilization of GOx in Silica Sol-Gel Column

Conventional sol-gel procedures generally involve extremes of pH and high concentration of alcohol, both of which affect the stability of biomolecules. The synthetic condition is to raise the pH of the sol system to biologically compatible values so as to prevent acid denaturation or aggregation of the biomolecules. The pH of the sol stock solution was adjusted to the range from 5 to 8 before GOx was added in our experiment. It has been reported that when the mol ratio of water and alcohol (R) is controlled in the range of 4–20, the gel molecules obtained from TEOS grow linearly [23]. In the course of gelation, the linear chain molecules crossly twist to form uniform network of the matrix. The higher R is also favorable for the enzyme owing to more water exited in the network. The CL intensities of the system were measured on variation of the value of R from 4–10. The maximum CL intensity of the present system was obtained when the value of R was 7.

Optimization of Analytical Parameters

A series of experiments were conducted to establish optimum analytical variables in a flow injection system. The parameters optimized include flow rates, pH of the enzyme reactor, concentrations of reagents and surfactant. As shown in Fig. 2, the CL intensity increased with increasing pH value of enzyme reactor, reaching a maximum at 7.5. When pH value higher than 7.5 by addition of 0.5 M KOH, the CL emission was gradually decreased.

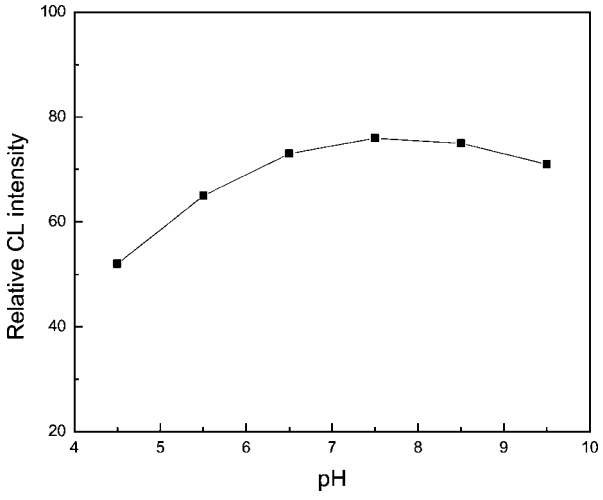

Fig. 2. Effect of pH of enzyme reaction system on CL intensity: [KIO_4], 20 mM; [SDS], 10 mM.

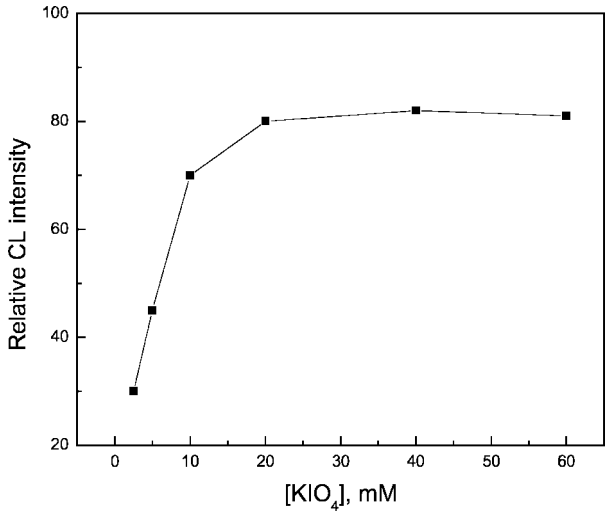

Fig. 3. Effect of KIO_4 concentration on CL intensity: [SDS], 10 mM; pH of enzyme reaction 7.5.

The effects of potassium periodate concentration on the CL intensity are shown in Fig. 3. The CL intensity increased with increasing the concentration of KIO_4 in the range of 2.5 mM–20.0 mM, and reached a plateau at 20 mM. Thus a 20 mM potassium periodate was selected for the subsequent experiments.

The effects of the concentration of SDS on the CL intensity were also studied (Fig. 4). The CL intensity increased rapidly with increasing SDS concentration up to about 10 mM and then was stayed constant after this concentration. The concentration of 10 mM is close to the reported CMC value (8 mM) [24]. The present results might

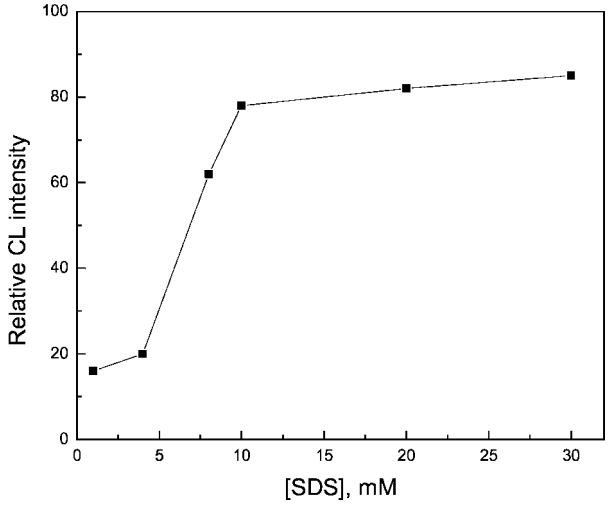

Fig. 4. Effect of SDS concentration on CL intensity: [KIO_4], 20 mM; pH of enzyme reaction 7.5.

Table I. Comparison of Analytical Characteristics for Selected Sensors to Determine Glucose

Type of sensor	Detection method	Dynamic range (M)	Detection limit (M)	[Ref.]
Polymer modified electrode	Voltammetry	1.0×10^{-3}–5.0×10^{-7}	—	[4]
Sol-gel derived composite electrode	ECL	1.0×10^{-2}–1.0×10^{-5}	8.2×10^{-6}	[12]
Solution	CL	1.0×10^{-5}–1.0×10^{-7}	8.0×10^{-8}	[13]
Sol-gel column	ECL	2.0×10^{-4}–1.0×10^{-6}	1.0×10^{-6}	[15]
Sol-gel column	CL	5.0×10^{-4}–1.0×10^{-7}	4.0×10^{-8}	Present method

be explained with the micro-cage structure of micelle that is helpful for stabilizing the excited state and prevents it from quenching.

Since the CL reaction is very fast, higher flow rates are favorable for the sensitivity of measurements. A total flow rate of 1.5 mL/min was chosen in the present studies because higher flow rates than 1.5 mL/min showed decreased signals.

Performance of the Flow Sensor

Under the optimum experimental conditions, a typical calibration curve was obtained for the determination of glucose by plotting CL signal versus glucose concentration. The calibration curve was linear in the range from 5×10^{-5} M to 1.0×10^{-7} M, and the detection limit (3σ) was 4.0×10^{-8} M. The reproducibility of this CL sensor was studied by repeating measurements with 5.0×10^{-6} M glucose solutions. The relative standard deviation (RSD) was 3.9% for nine measurements. In Table I, the detection limits and dynamic range of the present method are compared with those of selected methods reported to determine glucose using voltammetric and electrochemiluminescence (ECL) detection [4,12,13,15].

Sensor Stability

The stability of the enzyme reactor used for the determination of glucose was investigated by performing 20 successive injections of 5.0×10^{-6} M glucose standard solution once in every 3 days for 2 months. The enzyme reactor was stored in pH 7.2 phosphate buffer solution at 4°C when it is not used. For the 20 measurements, the response of CL intensity was retained 94% of its initial CL response.

Interferences

The effects of foreign ions on the determination of glucose were studied. A series of foreign ions were considered which are known to have catalytic effects on the luminol-H_2O_2 CL reaction [25]. Under the optimal experimental conditions, the tolerance of each foreign ion was taken as the largest amount yielding an error of less than 95% in the CL intensity for 1.0×10^{-6} M glucose. The following foreign substances were considered to be tolerable: a 1000-fold Cr^{3+}, Fe^{3+}, Co^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Mg^{2+} and Ag^{+}. Unlike the luminol CL method [26], the proposed method has no interference from most transition metal ions.

CONCLUSION

A low interference optical flow sensor for the determination of glucose has been prepared by entrapping glucose oxidase into silica sol-gel film coated on alumina substrate. The CL intensities increased rapidly with addition of anion surfactant to its CMC value. The chemiluminescence intensity increased linearly with increasing glucose concentration from 5.0×10^{-4} M to 1.0×10^{-7} M and the detection limit was 4.0×10^{-8} M.

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REFERENCES

1. X. D. Dong, J. T. Lu, and C. S. Cha (1997). Characteristics of the glucose oxidase at different surfaces. *Bioelectrochem. Bioenerg.* **42**(1), 63–69.
2. H. M. Wu, R. Olier, N. Jaffrezic-Renault, P. Clechet, A. Nyamsi, and C. Martelet (1994). Covalent immobilization of glucose oxidase onto graphitic electrodes. *Electrochim. Acta* **39**(3), 327–331.
3. X. H. Yang, L. Hua, H. Q. Gong, and S. N. Tan (2003). Covalent immobilization of an enzyme (glucose oxidase) onto a carbon sol-gel silicate composite surface as a biosensing platform. *Anal. Chim. Acta* **478**(1), 67–75.
4. S. Yabuki, F. Mizutani, Y. Sato, and Y. Hirata (2003). Immobilization of polyglutamate-glucose oxidase onto a cysteamine-modified gold electrode.*Sens. Actuators B* **91**(1–3), 187–190.
5. A. Guerrieri, G. E. De Benedetto, F. Palmisano, and P. G. Zambonin (1998). Electrosynthesized non-conducting polymers as permselective membranes in amperometric enzyme electrodes: a glucose

biosensor based on a co-crosslinked glucose oxidase/overoxidized polypyrrole bilayer. *Biosen. Bioelectron.* **13**(1), 103–112.
6. C. E. Hall and E. A. H. Hall (1995). Tuning of glucose oxidase modified alkylacrylate polymers employed in amperometric sensor systems. *Anal. Chim. Acta* **310**(2), 199–210.
7. S. M. Reddy and P. Vadgama (2002). Entrapment of glucose oxidase in non-porous poly(vinyl chloride). *Anal. Chim. Acta* **461**(1), 57–64.
8. H. Y. Liu, Y. C. Liu, J. H. Qian, T. Y. Yu, and J. Q. Deng (1996). Feature of entrapment of glucose oxidase in regenerated silk fibroin membranes and fabrication of a 1,1′-dimethylferrocene-mediating glucose sensor. *Microchem. J.* **53**(2), 241–252.
9. J. Wang, D. Leech, M. Ozsoz, and S. M. R. Smyth (1991). One-step fabrication of glucose sensors based on entrapment of glucose oxidase within poly(ester-sulfonic acid) coatings. *Anal. Chim. Acta* **245**, 139–143.
10. D. Trau and R. Renneberg (2003). Encapsulation of glucose oxidase microparticles within a nanoscale layer-by-layer film: Immobilization and biosensor applications. *Biosens. Bioelectron.* **18**(12), 1491–1499.
11. D. R. Shankaran, N. Ueheara, and T. Kato (2003). A metal dispersed sol–gel biocomposite amperometric glucose biosensor. *Biosens. Bioelectron.* **18**(5/6), 721–728.
12. L. D. Zhu, Y. X. Li, F. M. Tian, B. Xu, and G. Y. Zhu (2002). Electrochemiluminescent determination of glucose with a sol–gel derived ceramic–carbon composite electrode as a renewable optical fiber biosensor. *Sens. Actuators B* **84**(2/3), 265–270.
13. Y. X. Zhou, T. Nagaoka, F. Li, and G. Y. Zhu (1999). Evaluation of luminol–H_2O_2–KIO_4 chemiluminescence system and its application to hydrogen peroxide, glucose and ascorbic acid assays. *Talanta* **48**(2), 461–467.
14. A. M. Almuaibed and A. Townshend (1997). Flow injection amperometric and chemiluminescence individual and simultaneous determination of lysine and glucose with immobilized lysine oxidase and glucose oxidase. *Anal. Chim. Acta* **338**(1/2), 149–154.
15. C. Y. Wang and H. J. Huang (2003). Flow injection analysis of glucose based on its inhibition of electrochemiluminescence in a $Ru(bpy)_3^{2+}$-tripropylamine system. *Anal. Chim. Acta* **498**(1/2), 61–68.
16. O. S. Wolfbeis, I. Oehme, N. Papkovskaya, and I. Klimant (2000). Sol–gel based glucose biosensors employing optical oxygen transducers, and a method for compensating for variable oxygen background. *Biosens. Bioelectron.* **15**(1/2), 69–76.
17. Y. X. Li, L. D. Zhu, G. Y. Zhu, and C. Zhao (2002). A chemiluminescence optical fiber glucose biosensor based on co-immobilizing glucose oxidase and horseradish peroxide in a sol–gel film. *Chem. Res. Chin. Univ.* **18**(1), 12–15.
18. O. S. Wolfbeis, M. Schaeferling, and A. Duerkop (2003). Reversible optical sensor membrane for hydrogen peroxide using an immobilized fluorescent probe, and its application to a glucose biosensor. *Microchim. Acta* **143**(4), 221–227.
19. X. Z. Liu and E. H. Hansen (1996). Sequential injection determination of D-glucose by chemiluminescence using an open tubular immobilised enzyme reactor. *Anal. Chim. Acta* **326**(1–3), 1–12.
20. J. M. Lin, H. Arakawa, and M. Yamada (1998). Flow injection chemiluminescent determination of trace amounts of hydrogen peroxide in snow-water using KIO_4–K_2CO_3 system. *Anal. Chim. Acta* **371**(2/3), 171–176.
21. A. U. Khan and M. Kasha (1963). Red chemiluminescence of molecular oxygen in aqueous solution. *J. Chem. Phys.* **39**, 2105–2106.
22. B. X. Li, Z. J. Zhang, and L. X. Zhao (2001). Chemiluminescent flow-through sensor for hydrogen peroxide based on sol–gel immobilized hemoglobin as catalyst. *Anal. Chim. Acta* **445**(2), 161–167.
23. J. D. Wright and N. A. J. M. Sommerdijk (2000). *Sol–Gel Materials Chemistry and Applications*, Gordon and Breach Science Publisher, Amsterdam.
24. J. C. Jacquier and P. L. Desbène (1996). Determination of critical micelle concentration by capillary electrophoresis application to organo-saline electrolytes *J. Chromatogr. A* **743**(2), 307–314.
25. I. S. Joo, S. H. Lee, J. K. Suh, and C. J. Kim (2002). Determination of Europium ion in aqueous media by chemiluminescence method. *Anal. Sci.* **17**(Suppl.), a117–a120.
26. S. Hanaoka, J. M. Lin, and M. Yamada (2001). Chemiluminescent flow sensor for H_2O_2 based on the decomposition of H_2O_2 catalyzed by cobalt(II)-ethanolamine complex immobilized on resin. *Anal. Chim. Acta* **426**(1), 57–64.