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A Formaldehyde Biosensor Based on Potentiometric pH Transducer and Immobilised Enzyme Alcohol Oxidase

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Abstract : A potentiometric biosensor for the determination of formaldehyde was developed based on the use of a pH transducer made from methacrylic-acrylic polymer membrane and the enzyme alcohol oxidase (AOX). The biosensor was designed based on the enzymic oxidation of formaldehyde by AOX immobilized in poly(2-hydroxylethyl methacrylate) (pHEMA) hydrogel that was deposited on the pH transducer. A further layer of sol-gel was deposited on top of the pHEMA hydrogel to prevent leaching of AOX and detachment of pHEMA membrane from the pH tranducer. The biosensor gave a linear dynamic response to formaldehyde concentrations from 1-100 mM (R^2 = 0.99) with a sensitivity of 43.9 mV/decade. The sensitivity increased to near theoretical value (Nernstian slope) of 52.40 mV/decade when the formaldehye concentrations were 1-10 mM.

Key words: potentiometric biosensor, formaldehyde, sol-gel, methacrylate polymer, alcohol oxidase

Abstrak : Biopenderia potensiometrik untuk penentuan formaldehid telah dibangunkan berdasarkan transduser pH jenis membran polimer metakrilat-akrilat dan enzim alkohol oksidase (AOX). Biopenderia direkabentuk berasaskan tindak balas pengoksidaan formaldehid yang dimangkinkan oleh AOX terpegun dalam membran hidrogel poli(2-hidroksietil metakrilat) (pHEMA) yang telah disalutkan di atas transduser pH. Satu lapisan sol-gel juga disalutkan ke atas hidrogel pHEMA untuk mengelakkan larutresap enzim AOX dan juga penanggalan membran pHEMA daripada transduser pH. Biopenderia formaldehid yang dihasilkan memberi julat rangsangan linear antara 1-100 mM (R^2 = 0.99) formaldehid dengan kepekaan 43.9 mV/dekad. Kepekaannya meningkat serta menghampiri nilai teori (kecerunan Nernstian), iaitu 52.40 mV/dekad apabila julat formaldehid ialah 1-10 mM.

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Introduction

Formaldehyde is a common industrial chemical and is used in many manufacturing processes. Formaldehyde is classified as a mutagen and possible human carcinogen [1] and confirmed to cause mutagenic effect on microorganisms, mice and rats [2]. Recently, formaldehyde has been described as one of the chemical mediators of apoptosis i.e. it programmed cell death. An example is the increase in the level of formaldehyde before leaf fall in autumn [3]. Moreover, the formaldehyde generated *in situ* from some N-methylated compounds can induce apoptosis or retardation of cell proliferation of tumor cells [4].

A number of approaches to the construction of biosensors for the detection of formaldehyde have been published including the use of amperometric [5, 6], potentiometric [7] and optical [8] transducers. An important aspect of biosensor construction is the immobilization of the bioreceptor such as enyzme, which can be achieved through physical adsorption on solid supports, micro-encapsulation, covalent bonding or matrix entrapment. Among these, matrix entrapment seems to have advantages over the other methods because of its simplicity and reduced risk of inactivation of the bioreceptor during immobilization procedure. Among the immobilization matrices used as biosensor membrane, HEMA based polymer as a matrix for immobilization is becoming more popular because of its adaptability to various electrode designs and better reproducibility than the slab gels [9].

In this study, a biosensor for formaldehyde detection based on a potentiometric transducer was developed. The uniqueness of the biosensor is in the use of methacrylic-acrylic polymers in the fabrication

of the plasticiser-free pH transducer membrane apart from as a matrix for the immobilization of AOX. A sol-gel membrane was also used together with the methacrylic-acyrlic membranes as a blocking membrane to prevent AOX leaching.

Materials and Methods

Materials

Various chemicals used in the study were methyl methacrylate (MMA), n-butyl acrylate (nBA), sodium tetrakis [3,5-bis (trifluoro-triethyl) phenyl] borate (NaTFPB), poly (2-hydroxyl ethyl methacrylate) (pHEMA) from Sigma; lithium acetate, tris (hydroxymethyl)aminomethane (TrisHCl), hydrogen ionophore I (tridodecylamine), formaldehyde solution, tetraetoksilane were from Fluka; benzoyl peroxide was from Merck and dichloromethane from Baker. Alcohol oxidase (AOX) (EC 1.1.3.13) from *Hansenula polymorpha* by the method of Gibson [10] was from Sigma.. AOX was stored at 0°C before used. Standard solutions were prepared with deionised water. All chemicals used were of analytical grade.

Fabrication of pH transducer and evaluation of response

Procedure for the synthesis of the copolymer (MB28) from methyl methacrylate (MMA) and n-butyl acrylate (nBA) was as reported before [11]. The copolymer MB28 was used as a plasticiser-free membrane for the construction of a pH transducer. The pH selective membrane was fabricated by mixing 0.05g of the MB28 copolymer with 3.5mg of hydrogen ionophore and 0.9 mg of NaTFPB. The mixture was then dissolved in 250 ml dichloromethane and 20µl of this mixture was drop-coated onto a Ag/AgCl electrode (Warner, USA) and dried overnight before potentiometric measurements were carried out.

The response of the pH transducer was tested with a double junction Ag/AgCl electrode with a 1 M lithium acetate gel bridge as a reference electrode. The electrode and sensor was connected to an Orion ion meter where the difference in the potential of the cell (electromotive force, EMF (mV)) was recorded when a stable value was reached. The pH sensor was evaluated with 0.1mM TrisHCl buffer from pHs 2 -9. The pH of each buffer solution was measured with an Ecomet pH electrode before use. The EMF response of the test cell was plotted against the logarithmic concentration of the test solutions according to the Nernst Equation.

Formaldehyde biosensor fabrication and performance assessment

Poly (hydroxyl ethyl methacrylate) (pHEMA) was used to immobilize AOX. An amount of 0.06 g pHEMA was dissolved in 1 mL of water-dioxan solution followed by 1.5 mg AOX. A portion of 20 µL of the enzyme containing pHEMA solution was drop-coated onto the pH transducer that showed Nernstian response. This enzyme-pHEMA solution was then dried at 4 °C overnight before 1 µL of sol-gel solution was further coated on the enzyme-pHEMA membrane to prevent AOX leaching and detachment of the enzyme membrane from the pH transducer surface.

Formaldehyde solutions with concentrations of 0.001 to 100 mM were prepared in TrisHCl buffer (0.1mM, pH 7). The response of the formaldehyde biosensor in mV were measured at room temperature (30 ± 2 °C) using the potentiometric cell set up as described in the pH transducer section. The biosensor was immersed in the formaldehyde solution and the emf of the cell in mV was recorded after 1 min. The cell responses (mV) were plotted against the logarithmic concentrations of the formaldehyde to establish the calibration curve for the biosensor. The reproducibility of a formaldehyde biosensor was also measured by repeatedly exposed the biosensor to the same concentration of formaldehyde for three times. Possible interference substances to AOX reaction with formaldehyde are methanol, ethanol, acetaldehyde, glucose and glycerol. These substances were therefore chosen for interfering studies under the same pH and conditions as formaldehyde and the response of the biosensor in the presence of different concentrations (1 - 10 mM) of these substances was evaluated.

Results and discussion

The response of the pH transducer

Figure 1 shows the calibration curve and also the dynamic linear range of the pH transducer. The pH sensor yielded a response of 55.98 ± 1.14 mV/decade (R^2 = 0.995) with a working linear range between pH 4 – 8. The response of the transducer to H^+ ion is close to Nernstian (59.16 mV/decade at 25^{o}C) and such response is suitable for analytical purposes [13-14]. Potentiometric sensor with a response greater than 55 mV pH^{-1} at 25 ^{o}C is considered as Nernstian response [15]. Thus, the plasticiser-free H^+ ion-selective membrane from MB28 can be used as a transducer for the construction of a formaldehyde biosensor.

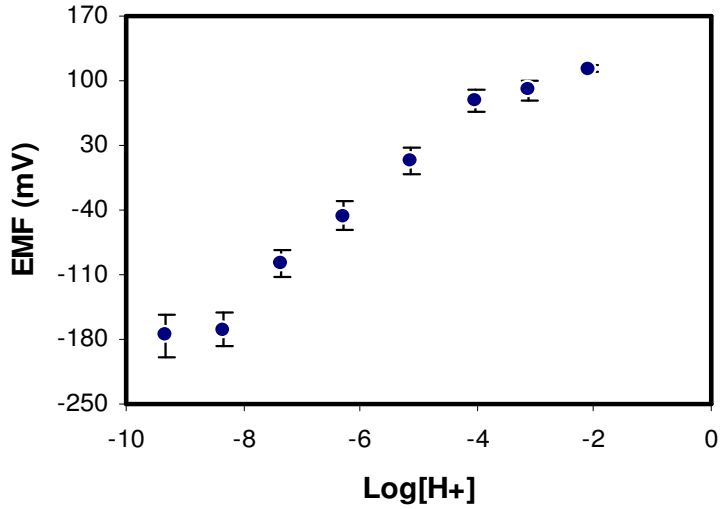

Figure 1 : The response of the pH transducer constructed from plasticiser-free methacrylic-acrylic membrane to changes in pH of 0.1 mM TrisHCl buffer.

The formadehyde biosensor response

The enzyme AOX from *Hansenula polymorpha* was selected for the potentiometric biosensor because the catalytic activity of the enzyme does not depend on the pH over a reasonably large range of 6-10 [16]. For a double layer membrane containing sol-gel and pHEMA, formaldehyde molecules first diffuse through the sol-gel membrane before transport into the pHEMA membrane and oxidize to form formic acid and hydrogen peroxide catalysed by the enzyme AOX:

$$CH_2HO + O_2 + H_2O \xrightarrow{AOX} HCOOH + H_2O_2$$

$$HCOOH \rightarrow HCOO^- + H^+$$

As a result, the changes in pH due to the dissociation of the formic acid from the oxidation of formaldehyde are detected by the plasticiser-free H^+ selective membrane (the pH transducer).

In Figure 2, the response curve of the formaldehyde biosensor with a linear response to formaldehyde concentrations sol-gel enzyme blocking membrane demonstrated from 1 – 100 mM with a slope of 43.9±2.1 mV/decade (R^2 = 0.99). However, the biosensor showed higher sensitivity to formaldehyde in the concentration range of 1 – 10 mM because the response slope increased to 52.4±4.2 mV/decade. From Figure 2, the reproducibility of the biosensor based on three different biosensors is approximately 15% relative standard deviation (RSD). On the other hand, biosensor with only pHEMA did not yield a linear response range and demonstrated much lower sensitivity to formaldehyde..During physical entrapment of an enzyme in a polymer matrix, leaching is the main problem and this will lead to poor biosensor response Clearly, the use of a layer of sol-gel membrane has stabilized the pHEMA-enzyme layer by preventing enzyme leaching from the pHEMA membrane underneath.

Several primary alcohols such as methanol, ethanol and glycerol and other substances, e.g. acetaldehyde and glucose were found to have little interference on the formaldehyde response of the potentiometric biosensor. This is demonstrated by

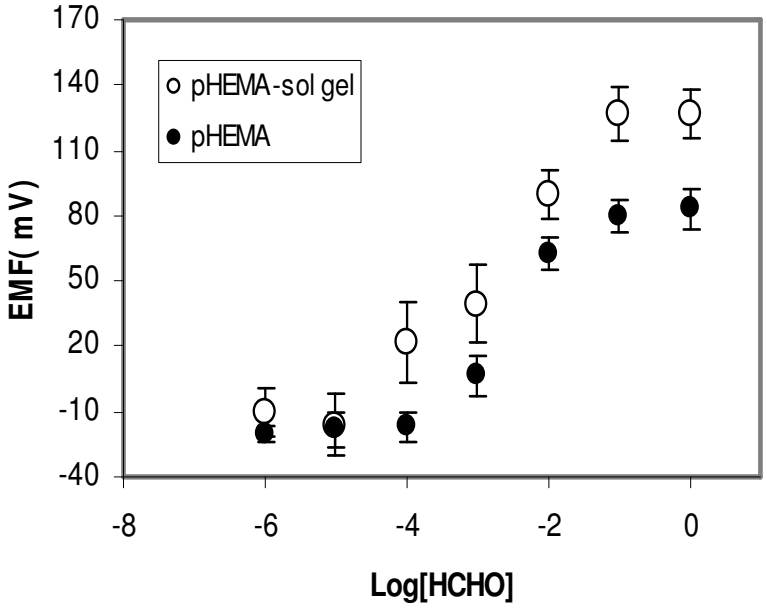

Figure 2 : A comparison of the response of two formaldehyde biosensors with (pHEMA-sol gel) and without sol-gel (pHEMA) blocking membrane. All formaldehyde standard solutions are of pH=7 at 0.1 mM TrisHCl buffer.

by Figure 3 where the response of the biosensor to various concentrations of formaldehyde is generally 2-6 times higher than all the possible interference substances evaluated at the concentration range of 1-10 mM. Hence, this shows that the biosensor designed is specific to formaldehyde especially at higher concentrations of formaldehyde. Many formaldehyde sensors reported in the literatures are based on chemical reagents as sensing elements.

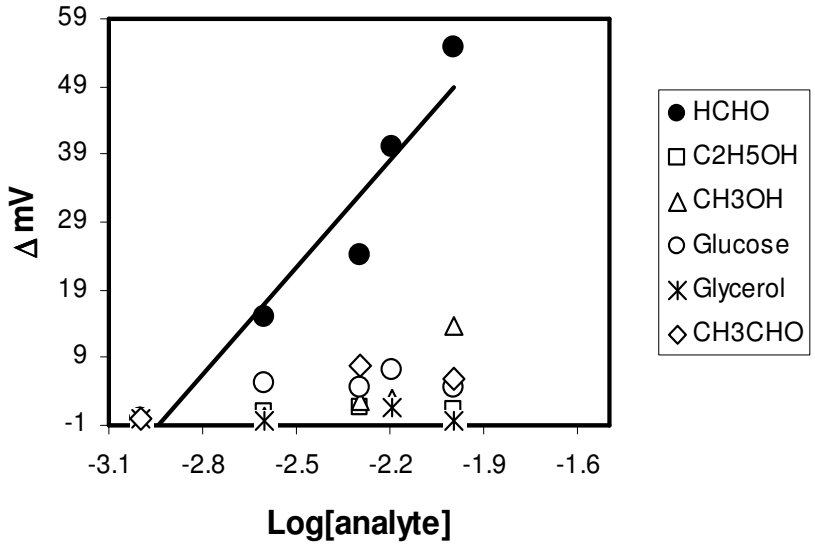

Figure 3 : The response of the formaldehyde biosensor when exposed to various concentrations of potential interference substances such as ethanol (C_2H_5OH), methanol (CH_3OH), glucose, glycerol and acetaldehyde (CH_3CHO).

Some examples are an optical sensor using paraosaniline immobilized on Amberlite resins [17], polyoxyethylene bis[amine] coated on a quartz crystal microbalance [18] and amperometric sensor using Nafion coated gold-film [19]. Recently, several biosensors for formaldehyde have also been reported using enzyme alcohol oxidase or formaldehyde dehydrogenase as the main sensing element [20-22].

Hammerle and Hall [20] reported the use of formaldehyde dehydrogenase that was confined within a dialysis membrane and an amperometric transduction method was used to measure formaldehyde vapour with linear response up to 6 vppm. Another amperometric biosensor for folmaldehyde reported also was using the same enzyme but it was immobilised in plasticised polyurethane membrane containing tetrathiafulvalene-tetracyanoquinodimethane salt [21]. This biosensor was reported to yield a linear response in formaldehyde concentrations of 0.1-1 mM and was employed to measure formaldehyde in ambient air.

A potentiometric biosensor for the detection of formaldehyde that was based on pH-sensitive FET was also reported [22]. The biosensor was constructed by direct immobilisation of AOX on the ISFET surface via cross-linking with glutaldehyde. Before cross-linking, the enzyme was stabilized by a mixture of bovine serum albumin and dextran. The linear dynamic range of this ISFET based biosensor was 10-300 mM of formaldehyde and with reproducibility of 1-3% RSD. No interference from primary alcohols was reported for this biosensor.

The potentiometric formaldehyde biosensor developed here has a linear dynamic range comparable to that of ISFET potetiometric biosensor [22] but a much larger RSD in the reproducibility of the biosensor. The concept of using sol-gel as a blocking membrane during the physical entrapment of an enzyme proved that it can yield a biosensor performance that is similar to biosensors based on chemical immobilisation of enzymes.

Conclusions

The study confirmed that alcohol oxidase from *Hansenula polymorpha* can be used as a biological active material for the fabrication of formaldehyde biosensor with methacrylate and sol gel membranes as the immobilisation matrix. The sol-gel membrane was successfully used as a blocking membrane to prevent enzyme leaching and the detachment of pHEMA layer from the pH transducer. This has contributed to the improved performance of the formaldehyde biosensor.

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References

1. Feron V.J, Til H.P, de Vrijer F, Woutersen R.A, Cassee F.R and van Bladeren P.J.(1991) *Mutat. Res.* **259**: 363.
2. Squire R.A and Cameron L.L. (1993) *Regular Toxicology Pharmacology*, **4** 107.
3. Tyihák E., Trézl L. and Szende B (1998) Formaldehyde cycle and the phases of stress syndrome. In: P. Csermely (Ed), Stress of life. From molecules to man, *Ann. NY Acad. Sci*., **851**, 259.
4. Szende B., Tyihák E., Szókán G. and Kátay G.. (1995) *Pathol. Oncol. Res*., **1**, 38.
5. Winter B. and Cammann.K. (1989) *Analytical Chemistry*, **334**, 720.
6. Hall E.A.H, Preuss M., Gooding J.J and Hammerle M. (1998) Exploring sensors to monitor some environmental discharges. In Nikolelis,D.P, Krull, U.J., Wang, J Mascini, M. (Eds). Biosensors for direct monitoring of Environmental Pollutants in field. NATO ASI Series, 2-Environment,. Kluwer, Dordreht, **38**, 227.
7. Korpan Y.I, Gonchar M.V, Starodub N.F, Shul'ga A.A, Sibirny A.A., and El'skaya A.V. (1993) *Analytical Biochemistry,* **215**, 216.
8. Rindt K.P and Scholtissek S. (1989) *Biosensors*, **13**, 405.
9. Arica Y,. and Hasirci, V. N. (1987) *Biomaterials,* **8** : 489.
10. Gibson T.D, Higgins I.J and Woodward J.R. (1992) *Analyst*, **117**, 1293.
11. Heng, L.Y. and Hall E.A.H. (2000) *Analytical Chemistry,* **72**, 41.
12. Cosofret V.V., Buck R.P. and Erdosy M. (1994) *Anal. Chem*., **66** : 3592.
13. Espadas-Torre C.and Meyerhoff. M.E. (1995) *Anal. Chem.*, **67** : 3108.
14. Cho D.H., Chung K.C. and Park M.Y. (1998) *Talanta*, **47**, 815.
15. Demirel A., Doan A., Canel E, Memon S., Yilmaz M. and Kilic E. (2004) *Talanta,* **62 :** 123.
16. Gibson, T.D and Woodward, J.R. (1992) Protein stabilization in biosensors systems. In : Edelman, P.G Wang, J. (Eds), *Biosensor and Chemical Sensors*, **40-55**.
17. Baker, J. and Narayanaswamy, R. (1994) *Analyst*, **119**, 959
18. Garcia-Verdugo-Caso C., Hidalgo-Hidalgo-de-Cisneros, Naranjo-Rodriguez, I., Bellido-Milla, D. (2001) *Fresenius J. Anal Chem*., **370**, 393.

19. Knake R., Jacquinot P. and Hauser P.C. (2001) *Electroanalysis*, **13**, 631.
20. Hammerle M, Hall E.A.H., Cade N and Hodgins, D. (1996) *Biosensors & Bioelectronics*, **11**, 239.
21. Kataky R., Bryce M.R., Goldenberg L., Hayes S., Nowak A. (2002) *Talanta*, **56**, 451.
22. Korpan Y.I, Soldatkin A.P, Gonchar M.V, Sibirny A.A, Gibson T.D and El'skaya. A.V (1997) *Chemical Technology Biotechnology*, **68** : 209.