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Amperometric biosensor for inert organic solvents based on a sol–gel hybrid material

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A unique sol–gel enzyme electrode for inert organic solvents is developed that is based on the partition equilibrium of the substrate between water–organic solvent media and the enzyme membrane.

Since the introduction of the first enzyme electrode over 30 years ago, growing interest in biosensors has resulted in increasingly widespread development. The development direction of biosensors can be divided into two aspects: First, the scope of the detection medium was expanded. In order to meet specific requirements, biosensors were developed from the aqueous phase, then to organic solvents saturated with water,[1] then to pure organic solvents,[2] and finally to a universal organic solvent.[3] Second, the scope of the determinable substance was expanded. Enzyme electrodes were first used to detect various substrates,[4,5] later inhibitors could be quantified due to their inhibition of enzyme activity.[6] Recently, Wang[7] applied biosensors to water determination based on the effect of water content on enzyme activity in organic solvents. In this study, we further expand the application of biosensors to inert organic solvent determination.

Recently the use of sol–gel glass as a biosensor encapsulation matrix has recieved much interest because of its high stability.[8] We have fabricated a sol–gel/hydrogel hybrid material, which retained the high activity of enzymes and exhibited negligible swelling.[9] In order to eliminate the influence of matrix swelling on the response, a tyrosinase enzyme electrode based on this hybrid material was chosen to demonstrate the feasibility of the biosensor for the determination of polar organic solvents. The preparations of fresh sol-gel solution and the enzyme electrode have been described elsewhere.[9]†

Steady-state amperometry was used to characterize the enzyme electrode, and Fig. 1(A) shows the typical steady-state current–time responses for successive addition of 50 μl acetonitrile in the presence of catechol. The first 'jump' in the current resulted from the response of catechol to the enzyme electrode, after the steady-state current reached a plateau, injection of acetonitrile caused a current drop. The current decrease is proportional to the content of the organic solvents added [Fig. 1(B)], therefore, electrochemically inert organic solvents can be amperometrically quantified by the enzyme electrode. Fig. 1(B) also shows the effect of enzyme loading upon the response for acetonitrile. Obviously, the response for acetonitrile increases upon increasing the enzyme loading between 266 and 400 units. Therefore, the increase in the enzyme loading can improve detection sensitivity, consequently decreasing the detection limit.

The dilution effect on the substrate in the solution can be ruled out by the control experiments because the injection of the same volume of buffer into the solution did not produce obvious current changes.[11] Thus the mechanism can be ascribed to the change of the sol–gel enzyme membrane due to the addition of organic solvent. Sol–gel encapsulation provides a sufficiently hydrophilic microenvironment that would both retain the essential hydration layer and exclude potentially denatured solvent components,[2,4,8] so it has a stabilization function on the enzyme system. Moreover, the content of organic solvent in this system is considerably lower than that in non-aqueous media,[1–3] so the addition of small amounts of organic solvent will not greatly influence the activity of the sol–gel encapsulated enzyme.[10] If the addition of organic solvent will inhibit the enzyme activity to a large degree, then after the 12th injection of polar organic solvents, the biosensor should exhibit a small current response to the same concentration of catechol. This was not the case. Hupp also found the sol–gel-encapsulated enzyme could retain its catalytic activity when alcohol and aldehyde were successively added into the enzyme monolith.[4]

To accurately demonstrate the response mechanism, we did steady-state QCM experiments at sol–gel modified gold electrodes. Fig. 2 shows the frequency changes following the injection of catechol and acetonitrile when the solution was under rapid stirring. When no enzyme was added into the sol–gel film, the injection of organic solvent did not produce noticeable changes in the frequency (curve *a*). Therefore, the nature of the sol–gel/electrode interface does not change on addition of solvent, which further proves the high stability of the sol–gel hybrid material.[8,9] The responses of the enzyme electrode are shown in curve *b*. With a constant frequency established at time T1, injection of catechol caused a frequency decrease. This can be explained by the fact that catechol diffuses

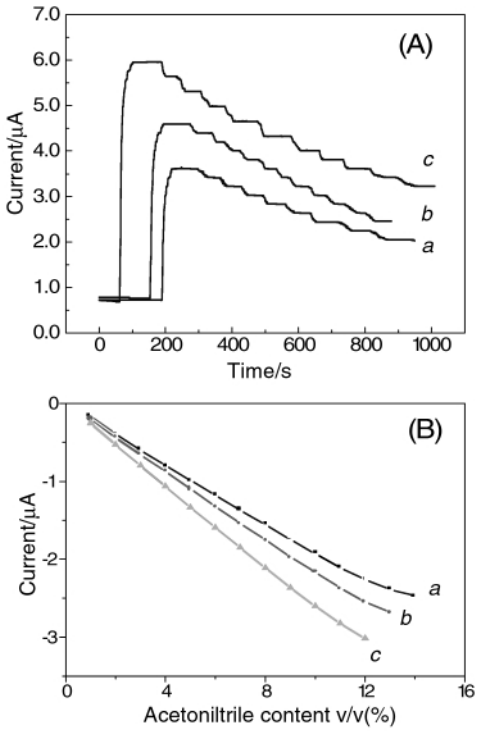

Fig. 1 (A) Amperometric responses of biosensors with different enzyme loadings to addition of 60 μM catechol, followed by successive additions of 50 μl acetonitrile to 5 ml PBS. (B) Corresponding calibration curve for acetonitrile. Enzyme loading, (a) 266 units, (b) 320 units, (c) 400 units; potential, −100 mV *vs* Ag/AgCl (sat. KCl).

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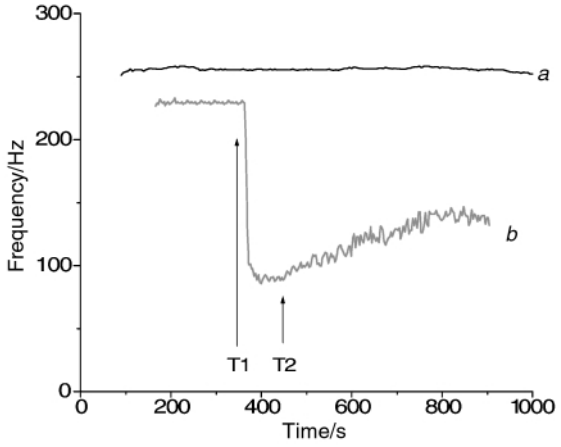

Fig. 2 Frequency–time response curves at the sol-gel-modified gold electrodes with 0 (a) and 320 units tyrosinase (b). At time T1, injections of 60 μM catechol; at time T2, injection of 1% (*v*/*v*) acetonitrile.

into the enzyme membrane and causes an increase in mass, accordingly the frequency decreases. When the response reached a steady state at time T2, injection of acetonitrile caused a frequency increase, which illustrates that the mass of the enzyme membrane does not increase but decreases. If the addition of organic solvent mainly inhibits the enzyme activity, then the mass of the enzyme membrane would not decrease. Therefore, this phenomenon further proves that organic solvent will not greatly inhibit the enzyme activity. In the enzyme membrane, only the amount of catechol may change; therefore, we attribute the current decrease to the extraction effect of organic solvent on the catechol in the enzyme membrane.[11] The solubility of catechol in organic solvents is much greater than in water, and the injected polar organic solvent quickly hydrates and concentrates catechol in the solution, which causes a decrease of the substrate concentration in the solution. Because of the dynamic distribution balance of the substrate between membrane and solution, the catechol in the membrane diffuses toward the solution; this brings about the substrate decrease in the enzyme membrane, accordingly, the response of the biosensor decreases. This mechanism is consistent with our previous report.[11] Both the working electrode and the configuration of the electrochemical cell in QCM experiments are different from those used in Fig. 1, so the response is relatively slower in Fig. 2. Moreover, because of the high sensitivity of QCM (~ng), the noise in Fig. 2 is larger than that in Fig. 1(A).

The sensitivity of the enzyme electrode to organic solvent is solvent dependent. The dielectric constant (ε) and the viscosity (λ) of organic solvents influence the response of the biosensor. The higher the $1/\varepsilon\lambda$, the lower the frictional resistance forces of the solvents on the substrate. This will bring about a higher diffusion of the substrate through the sol–gel film, therefore resulting in a greater sensitivity.[12] In addition, the hydrophobicity of the organic solvent plays an important role in the sensitivity of the biosensor. Methanol, *n*-propanol and *n*-butanol were selected to study the relationship between the sensitivity and the hydrophobicity of organic solvents, because they have similar molecular structures. The biosensor showed a sensitivity sequence as *n*-butanol > *n*-propanol > methanol. log *P* values for *n*-butanol, *n*-propanol and methanol are 0.88, −0.16 and −0.76, respectively. log *P* is a measure of the hydrophobicity of an organic solvent (*P* is the partition coefficient of a solvent in a standard octanol–water two-phase system[13]). The higher the log *P* value is, the more hydrophobic the organic solvent.[14] Obviously, the sensitivity sequence for the three organic solvents conforms to the hydrophobicity sequence. This is because the solubilities of catechol in the three solvents are in the sequence: *n*-butanol > *n*-propanol > methanol.

The biosensor proposed here determines organic solvents well. The response time for acetonitrile is about 40 s, and the detection limit of 1,4-dioxane, an explosive compound, is 0.023% *v*/*v* (*S*/*N* = 3). Moreover, the biosensor can be used to determine some organic solvents such as acetone, dimethylformamide, tetrahydrofuran *etc*., for which no specific enzyme has been found to fabricate a specific biosensor.

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Notes and references

† The enzyme electrodes were prepared as follows: a suitable amount of tyrosinase (E.C.1.14.18.1) was dissolved in 30 μl of phosphate buffer solution (PBS, pH 7.0), and then 20 μl of hydrogel and 10 μl of silica sol were added. After complete mixing, 10 μl of the mixture was dropped on a glassy carbon electrode (diameter 4 mm). The film was allowed to dry at 4 °C for 16 h and then washed thoroughly with PBS.

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