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Immobilization of Glucose Oxidase on Cellulose/Cellulose Acetate Membrane and its Detection by Scanning Electrochemical Microscope (SECM)

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Abstracts: Cellulose/cellulose acetate membranes were prepared and functionalized by introducing amino group on it, and then immobilized the glucose oxidase (Gox) on the functionalizd membrane. SECM was applied for the detection of enzyme activity immobilized on the membrane. Immobilized biomolecules on such membranes was combined with analysis apparatus and can be used in bioassays.

Keywords: SECM, glucose oxidase, immobilization, cellulose, membrane.

The combination of the immobilized enzyme with analysis apparatus such as spectrophotometer[1], flow injection system[2], fluorescence chromatogram[3] *etc*., can be used in bioassays. This technique has received great attention, since the combination of immobilized enzyme and analysis apparatus can greatly improve the detection sensitivity of the analyte. In recent years immobilized enzyme combined with scanning electrochemical microscopy (SECM) has been used for mapping the enzyme activity or enzyme-linked immunoassay assay[4,5]. Although the immobilization of enzyme on polymer membrane has been studied extensively, the combination of immobilized enzyme on polymer membrane and SECM detection had been rare reported by now. SECM is a useful tool to map electrochemical or enzymatic reactivity of different surfaces and is therefore helpful to optimize immobilization procedures of enzymes. It even can be used to analyze the kinetics of immobilized enzymes[6,7]. We prepared the membranes from cellulose and its derivative-cellulose acetate, as natural macromolecular materials, which are compatible with biomolecules.

Cellulose solution (solution A) was made following the method proposed by C. L. Mccormick[8], 15 grams of swollen cellulose sample was solved in 100 mL 9% lithium chloride/N.N-dimethylacetamide (LiCl/DMAc) solutions. Cellulose acetate solution (solution B): 15 grams of cellulose acetate powder was dissolved in 100 mL DMAc and stirred for 2 h at room temperature. 10 mL solution A, 10 mL solution B were mixed and stirred and then placed in vacuum oven to remove air bubble in the solution, the addition of

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cellulose acetate increased the hydrophobic and mechanical property of cellulose membrane.

The cellulose/cellulose acetate membranes were prepared by phase inversion method. The above cellulose /cellulose acetate mixture solution was cast onto a glass plate, which was then immersed in a bath of distilled water after the polymer solution had been subjected for a brief period of evaporation in air, the membrane was left in water for about 5 min after separation from the glass plate, then dried at room temperature.

The above membrane (disc with 1cm diameter) was placed in 50 mL 0.05 mol/L sodium periodate solution (pH 5.5) and incubated 30 min at 30ºC. The obtained membrane was washed with double-distilled water and then further modified with 5%(w/w) 1, 6–hexanediamine solution (pH 7.0), thereafter, the membrane was washed consecutively with double-distilled water and dried at room temperature, the treatment procedure are shown in **Scheme 1**[9].

A very small droplet of a glucose oxidase (Catalog No.G 7141, EC 1.1.3.4., from *Aspergillus niger*, 105 Umg^{-1}) solution (5 mg/mL) containing 1% (v/v) glutaraldehyde and 5 mg/mL bovine serum albumin/water solution was spotted on the cellulose/cellulose acetate membrane with a self-made glass capillary (spot diameter 300 μm). Coupling was carried out by incubation of the glucose oxidase solution with the modified cellulose / cellulose acetate membrane at 4ºC overnight, after incubation, the substrate was soaked in phosphate buffer (pH 7.0).

GOX oxidizes glucose by reducing its natural cofactor, O_2, producing H_2O_2 exclusively at the site of the immobilized enzyme activity. The immobilized enzymatic activity was imaged using the generation-collection mode with an ultromicroelectrode, which act as an amperometric probe and detected the oxidation of hydrogen peroxide.

A pt-disk microelectrode for the SECM measurements was fabricated by the method reported previously[10,11]. The radius of pt disk was 5 μm and the tip radius including glasssheath was approximately 25 μm. The SECM measurements were carried out in a two-electrode configuration with an Ag/AgCl counter/reference electrode using a CHI 900 SECM system (CHI Instruments, USA). The microelectrode is first positioned in close proximity to sample surface and adjacent to the membrane surface using the feedback current overlaying the diffusion controlled O_2 reduction at the microelectrode (poised to –350 mV *vs* Ag / AgCl). To invoke the generation-collector mode, the microelectrode is biased at 0.70 V *vs.* Ag/AgCl for amperometric detection of H_2O_2 produced at the site of the immobilized enzyme activity. SECM images were acquired at a constant-height mode (approximately 20 μm from the substrate) at the scan rate of 10 μm/sec[12].

The above treated cellulose/cellulose acetate membrane was immobilized on the flat bottom of a self-made teflon well by waterproof double side adhesive tap, the substrate was then immersed in a 0.1 mol/L KCl / 0.1 mol/L Na_2HPO_4 buffer solution (pH 7.0), the buffer solution also contained 10 mmol/L glucose. **Figure 2** shows the variation of the tip current (amperometric detection of H_2O_2) when the tip moved vertically from bulk solution (1020 μm above the glucose oxidase spot) at a speed of 4.0 μm/s. The current increased rapidly, when the tip approached the surface of the membrane. The currents reached maximum at about 20 μm above the immobilization zone. While scanning the microelectrode over the surface of the immobilized enzyme zone in the x and y directions,

Scheme 1

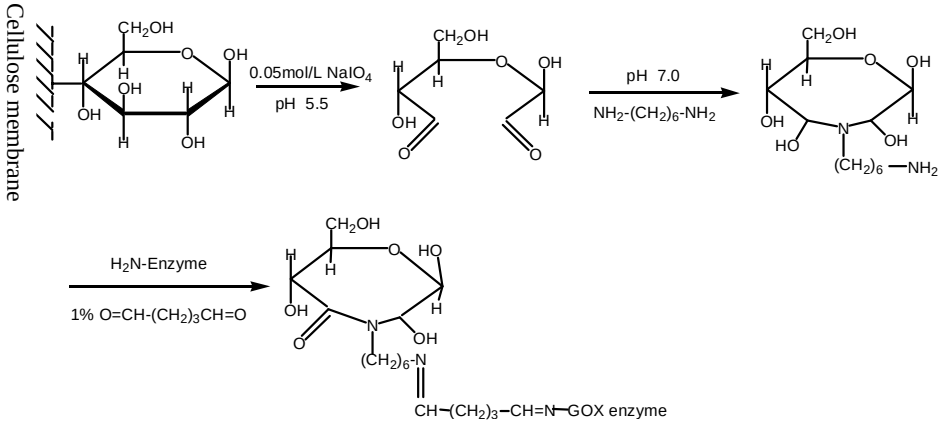


Figure 2 Vertical scan over immobilized glucose oxidase on cellulose /cellulose acetate membrane

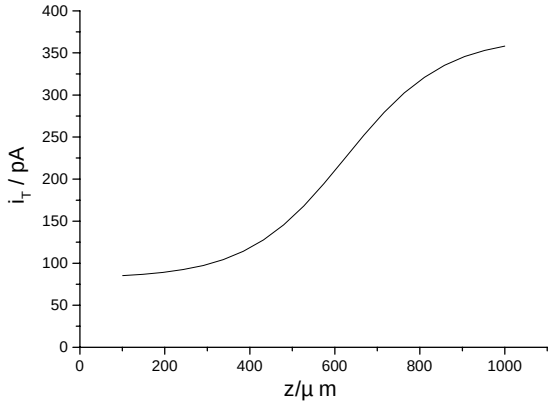
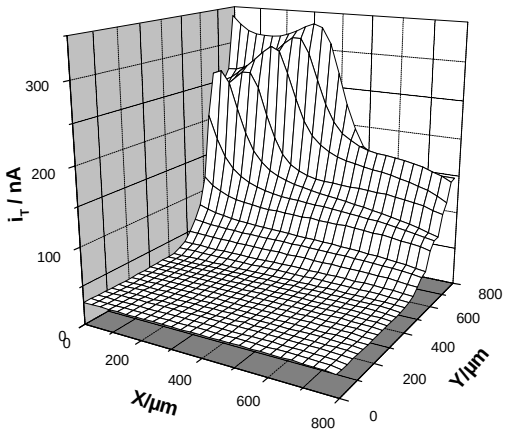


Figure 3 Two-dimensional scan over the membrane zone on which Gox was immobilized



the currents increases significantly only over the immobilized GOX structures, due to the high local concentration of enzymatically produced H_2O_2. The results of the above two-dimensional scan were shown in **Figure 3.** The transition is not sharp because the diffusion layer of the produced H_2O_2 was stirred by the presence of the microelectrode.

The oxidation currents at the summits of the images response clearly increase with the glucose concentration in the range of 0.2 mmol/L~4 mmol/L, however, the response was saturated in the range greater than 4 mmol/L.

In this letter, glutaraldehyde was used as the coupling linker which linked GOX and the amino groups of 1, 6 – hexanediamine introduced to the cellulose membrane matrix. The "arms" were longer enough to avoid changing the configuration of the certain protein segment of GOX, which mainly performs the catalytic function in the oxidation of glucose to gluconic acid. After storring the GOX immobilized on the membrane in phosphate buffer at 4ºC, the enzyme activity of the GOX remained 70% of the original level over one month. Enzymes immobilized on such membranes can be combined with different analysis apparatus for bioassays purpose.

The present study demonstrates that SECM can be a convenient tool for mapping the enzyme activities immobilized on different surfaces, with the test results obtained by SECM, we can optimize the immobilization procedure to obtain satisfactory enzyme activity.

Acknowledgments

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