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Entrapment of Glucose Oxidase in Silica Gel by the Sol-Gel Method and Its Application to Glucose Sensor

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The polymerization of tetraethyl silicate in the presence of glucose oxidase gave the silica gel with the enzymatic activity. As raising the aging temperature, the yield increased but the activity decreased. The flow injection-sensor constructed with the oxygen electrode covered by glucose oxidase-entrapped silica gel responded to glucose of physiological concentration.

Several techniques have been developed for the immobilization of enzymes.[1] Among them, inorganic matrices have a number of advantages over organic ones: they are not subject to microbial attack; no swelling and porosity change with pH occur; and the excellent storage stability of enzymes.[2] The sol-gel techniques is an attractive method for immobilization of bio-molecules in the silica gel because of low temperature preparation, a large amount entrapping, and simple preparation.[3] In a few reports, however, the technique has been applied for the immobilization of trypsin,[4] alkaline phosphatase,[5] and whole cell yeast.[6] Here we first report the preparation and properties of glucose oxidase-entrapped silica gel and its application to glucose sensor.

Glucose oxidase was obtained from Biozyme. The glucose oxidase-entrapped silica gel was prepared as follows. To tetraethyl silicate (40 vol% of ethanol solution, 1.67 ml), glucose oxidase (100 mg / ml of water solution, 0.33 ml) was added under stirring at 3 - 5 °C for 20 min and stored at -20 °C, 4 °C, or room temperature for prescribed periods. The resulting solidified material was centrifugally collected and washed several times with water. The gel was lyophilized and stored at 4 °C. The activity of glucose oxidase was measured by using *o*-dianisidine and peroxidase.

The color of the entrapped silica gel was cream-yellow, which is attributable to glucose oxidase. Figure 1

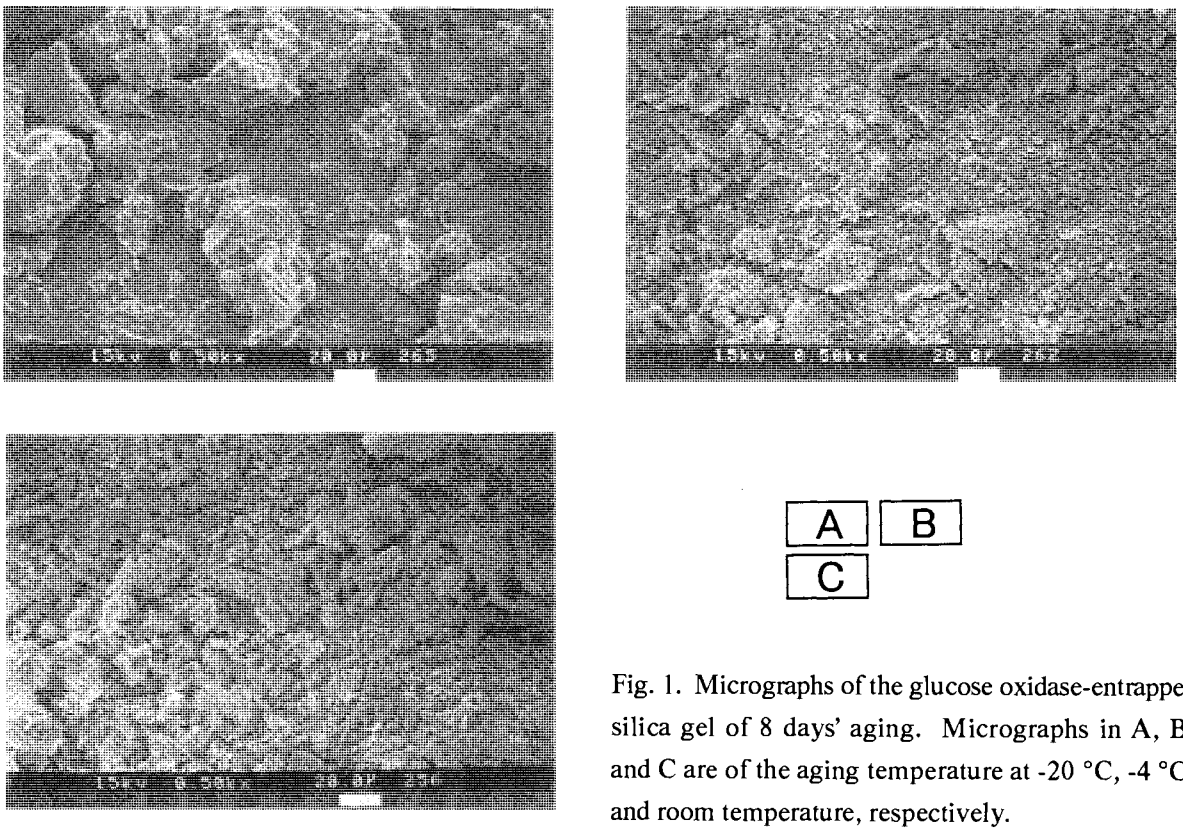

Fig. 1. Micrographs of the glucose oxidase-entrapped silica gel of 8 days' aging. Micrographs in A, B, and C are of the aging temperature at -20 °C, -4 °C, and room temperature, respectively.

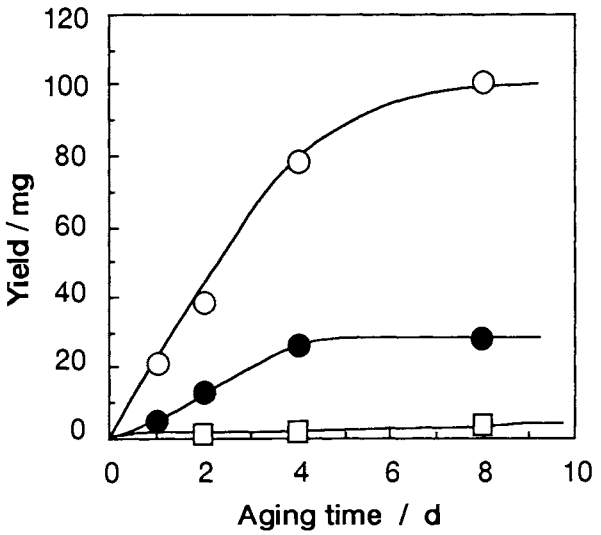

Fig. 2 (left side). The yield of glucose oxidase-entrapped silica gel. Square and closed and opened circles are of the aging temperature at -20 °C, -4 °C, and room temperature, respectively.

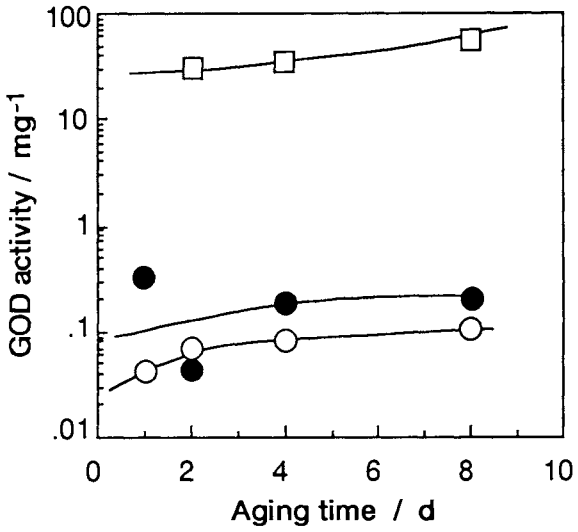

Fig. 3 (right side). The glucose oxidase-activity of the gel. The sample and symbols are the same as those in Fig. 2.

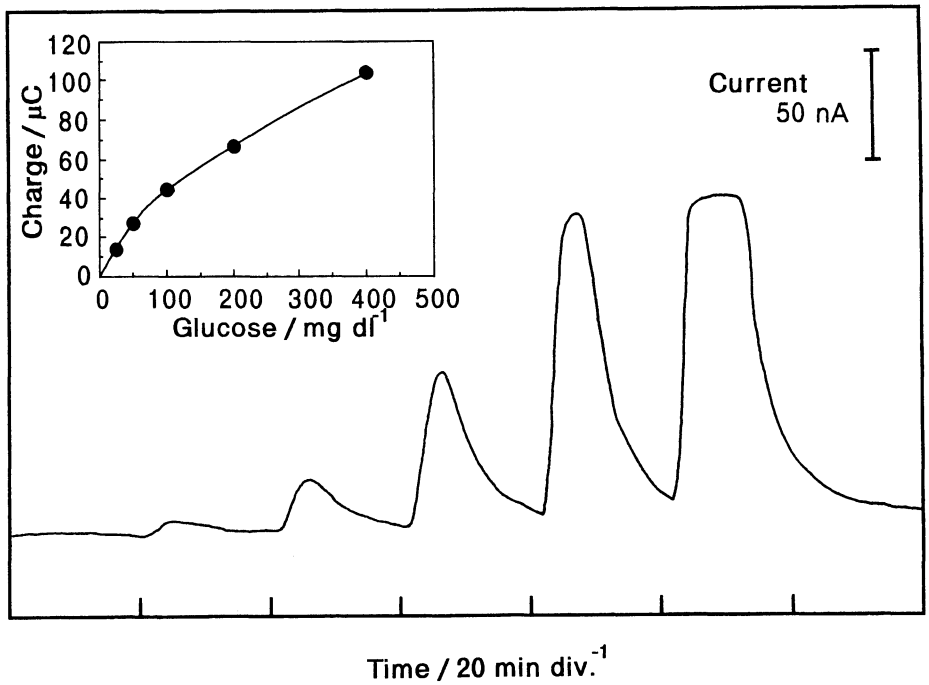

Fig. 4. The time course of the output current during injections of glucose solution. The standard solutions of 0, 25, 50, 100, 200, and 400 mg / dl of glucose was injected in this sequence with 20 min's period. The stock solution of glucose was used after one week of preservation. The carrier buffer of 0.1 mol dm^{-3} sodium phosphate (pH 5.1) was flowed at 1 ml / min. The inset is the dependence of integrated peak area, i.e. the charge, on the glucose concentration.

shows micrographs of the glucose oxidase-entrapped silica gel of 8 days' aging. The ragged grains are seen. As the aging temperature was lowered, the size of the grain increased. Figure 2 shows the dependence of the yield of the entrapped silica gel on the aging time. The yield increased with time and reached to the saturation after 4-8 days. As the aging temperature was lowered, the yield decreased. The yield also depended on the concentration of glucose oxidase. Figure 3 shows the dependence of the enzymatic activity of the gel on the aging time. Since glucose oxidase is a stable protein, the activity remains even after the treatment with such reagents. The enzymatic activity of the gel was still found after 2 months' storage desiccated at 4 °C. The enzymatic activity in a unit amount of the gel decreased with raising of aging temperature, which would be attributed to the progress of solidification or to the denaturation of glucose oxidase. Although the state of glucose oxidase in silica gel matrix is not clear yet, the oxidase will be entrapped geometrically in the pore of the silica gel.

The flow injection-sensor was constructed with the infusion pump, 6-way injector with 2.8 ml of sample loop, the oxygen electrode (YSI, type 5331), the hand-made flow cell, and the potentiostat (Yanaco, V10-PG).

On the tip of the oxygen electrode, the glucose oxidase-entrapped silica gel was covered with a nylon net and a cellulose membrane (Visking). The flow cell was immersed in water bath at 37 °C. To the electrode, -0.5 V was applied and the output current was recorded. Figure 4 shows the time course of the output current during the serial injection of glucose solution. The response peaks within 4 min. The peak current was saturated over 200 mg / dl of glucose. The integrated current, i.e. the charge, was plotted against the concentration of glucose in the inset of Fig. 4. The standard curve in the range of 0-400 mg / dl of glucose concentration is applicable for the glucose in normal human serum, which is 45-95 mg / dl.[7)]

Entrapment of glucose oxidase in silica gel could be performed by the sol-gel technique and the technique will be applicable to the other bio-molecules. Besides the application of the silica for sensors, protein-entrapped silica gels will be applicable to the enzyme reactor and the affinity chromatography.

References

1) G. G. Guilbault, "Analytical Uses of Immobilized Enzymes," Marcel Dekker, New York (1984), p.77.

2) H. H. Weetall, *Biochim. Biophys. Acta*, **212**, 1 (1970).

3) M. Yamaguchi, H. Morikawa, and I. Soma, *Chem. Express*, **2**, 333 (1987).

4) P. Johnson and T. L. Whateley, *J. Colloid Interface Sci.*, **37**, 557 (1971).

5) S. Braun, S. Rappoport, R. Zusman, D. Avnir, and M. Ottolenghi, *Mater. Lett.*, **10**, 1 (1990).

6) G. Carturan, R. Campostrini, S. Diré, V. Scardi, and E. De Alteriis, *J. Mol. Catal.*, 57, L13 (1989).

7) "Seikagaku Data Book," Tokyo Kagaku Dojin , Tokyo (1979), Vol. 1, p.1549.

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