Preparation of Glucose Oxidase Membrane for the Application of Glucose Sensor by Plasma Activation

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Synopsis

Plasma activation is a method that takes the advantage of low temperature plasma to immobilize the bioactive materials. An active immobilized glucose oxidase membrane was obtained via plasma-initiated polymerization of acrylic acid. The obtained immobilized enzyme is very active and stable. After 100 tests, the response error of the glucose sensor is less than 5% and its linear detecting range is 0–300 ppm. A hydrophilic PP film treated by Ar or NH_3 plasma was used for the preparation of the immobilized glucose oxidase membrane. The obtained immobilized enzyme is also very active and stable. After 180 tests, the response error of the glucose sensor is less than 4% and its linear detecting range is 0–300 ppm. Furthermore, SEM was used to study the mophology of the glucose oxidase membranes obtained via both methods. And ESCA was used to analyze the plasm-initiated polymerization products and the plasma-treated PP films so as to obtain the optimum conditions for the immobilization of bioactive materials.

INTRODUCTION

Enzymes, a kind of biocatalysts, can be used to increase the rate of a specific reaction in aqueous solution at ambient temperature and pressure. A disadvantage of this kind of water-soluble enzymes is that it is difficult to reuse it. However, when an enzyme is immobilized, not only can it be recycled, but its thermal stability and applicable pH range can also be improved.[1] Furthermore, various kinds of biosensors can be prepared, by modifying the electrode surfaces with the immobilized enzyme, and the characteristic reaction properties, sensitivity, and application range of the electrodes can be improved.[2]

Basically, there are three kinds of immobilization methods, i.e., the entrapped method, the adsorption method, and the chemical method. The applications of these traditional immobilization methods are restricted by the limited selection of insoluble support materials and reagents and by their complex preparation procedures.[3,4] Recently, the application of low temperature plasma to treat the polymer material has gotten more and more attention.(2) The plasma activation method fully utilized the advantage of low temperature plasma to immobilize the bioactive materials.[5]

For the applications of organic synthesis and surface modification, the operation pressure is kept at 0.1–10 torr and the radio frequency for the illumination discharge is 13.56 MHz. The electrons, ions, free radicals, and excited molecules in the plasma produced by low pressure illumination dis-

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charge can be used for the application of plasma deposition polymerization, plasma-initiated polymerization and plasma surface modification of a polymer.[6-12] Recently there were some studies based on the utilization of low energy plasma to immobilize the bioactive materials.[13,14] This paper presents the results of using plasma-initiated polymerization of acrylic acid and plasma surface treatment of hydrophilic polypropylene (PP) film to immobilize an enzyme. The obtained enzyme immobilized membranes were assembled together with electrodes to form biosensors and the optimum conditions for the enzyme immobilization were investigated.

EXPERIMENTAL

Materials

Glucose oxidase (E.C.1.1.3.4., from *Aspergillus niger*, type X) was a product of the Sigma Chemical Co. Hydrophilic polypropylene (PP) film was obtained from Celgrad 3501 microporous film. All of the other chemicals used in this study are reagent grade.

Apparatus

Plasma-initiated polymerizations and plasma surface treatments were carried out on a Samco PD-2 plasma deposition system. The ESCA (Perkin-Elmer, PHI-560 AM SAM/1605) was used to analyze the plasma-treated PP film. The scanning electron micrographes were taken on a Joel JAX-50A scanning electron microscope. The sensor system was made of a dissolved oxygen electrode (YSI Model 5750, BOD bottle probe), D. O. Meter (Suntex Model SD-60), and a recorder (Eyela Model TR-250).

EXPERIMENTAL PROCEDURES

Preparation of Immobilized Glucose Oxidase Membrane via the Plasma-Initiated Polymerization Method

A hydrophilic PP film was dipped in 5% glucose oxidase aqueous solution for 5 min and then taken out. The PP film was placed over the electrode of a plasma reactor at $-20°C$. After the glucose aqueous solution on the PP film was frozen, the N_2 gas was introduced and then evacuated. This process was repeated three times. When the vacuum of the reactor decreased to 0.1 torr, the acrylic acid monomer was introduced until the pressure of the reactor was stable. Several different power levels and reaction time periods were used for the plasma polymerization. The obtained products were put in air for 24 h and then soaked in a buffer solution and ultrasonicatred for 15 min. The activity of glucose oxidase was determined.

Preparation of Immobilized Glucose Oxidase Membrane via Plasma Surface Treatment Method

The hydrophilic PP films and LDPE films (diameter, 5/8 in.) were put over the electrode of plasma reactor. Two kinds of gases (Ar and NH_3) were introduced, respectively, and then evacuated by rotary pump for 15 min. A needle value was used to control the flow rate of the gas and the pressure of the reactor was kept constant. Different power levels were used to generate

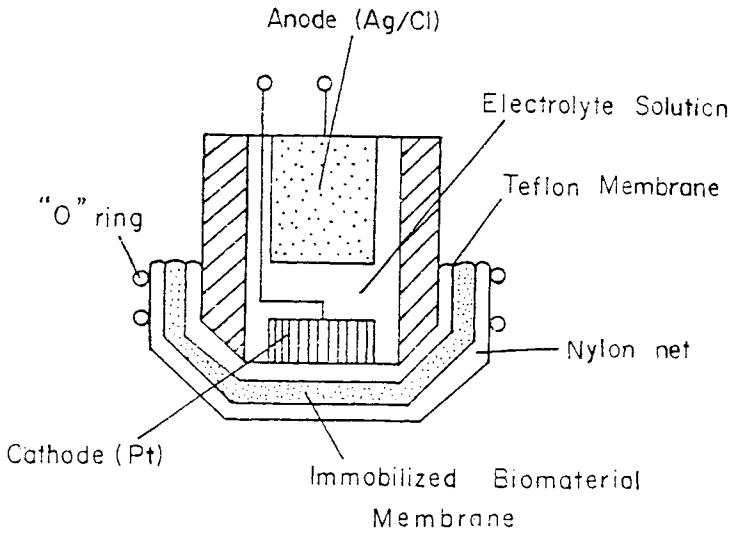


Fig. 1. Schematic drawing of bioelectrode.

the plasma which was used to treat the PP film. The treated films were taken out under N_2 atmosphere, and then washed with deionized water. The washed films were dipped in 25% glutaraldehyde for 12 h, washed again with deionized water, and soaked in GOD solution. The obtained films were used for the preparation of biosensors.

Preparation of Biosensors

The biosensors were prepared by combining the enzyme immobilized membranes with the electrode (Fig. 1).

RESULTS AND DISCUSSIONS

Preparation of Immobilized Glucose Oxidase Membrane via the Plasma-Initiated Polymerization Method

The effect of plasma power on the activity of immobilized glucose oxidase is shown in Figure 2. The maximum activity was obtained when the plasma power was 40 W. When the plasma power was higher than 40 W, the activity of immobilized glucose oxidase decreased as the power increased. This might be due to the destruction of the glucose oxidase by the high energy particles. When the plasma power was 20 W, no activity of immobilized glucose oxidase was detected. Figure 3 shows the effect of dipping time of PP film in the enzyme solution on the activity of immobilized enzyme. Higher activities were obtained when the dipping time was 10–40 min; longer or shorter dipping times were not as satisfactory. The effect of plasma-initiated polymerization time on the activity of immobilized glucose oxidase is presented in Figure 4. The optimum polymerization time was 6 min.

The effect of acrylic acid monomer concentration on the activity of immobilized enzyme is presented in Figure 5. Higher acrylic acid concentration

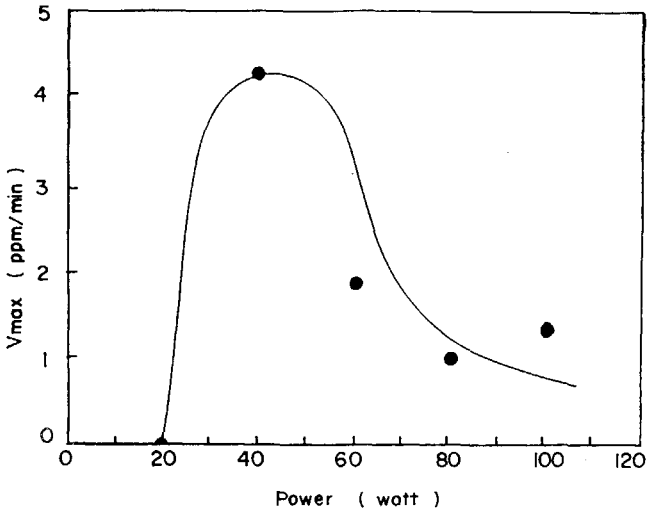


Fig. 2. Power effect of plasma deposition on immobilized enzyme's activity.

resulted in the decrease of the activity of glucose oxidase. This was due to the denaturization of glucose oxidase at low pH environment.

In general, the optimum conditions for the utilization of plasma-initiated polymerization to immobilize the glucose oxidase on the porous PP film were: dipping the PP film in GOD solution for 20 min, introducing 50 mm torr of acrylic acid monomer, and letting the polymerization occur at 40 W of plasma power for 6 min.

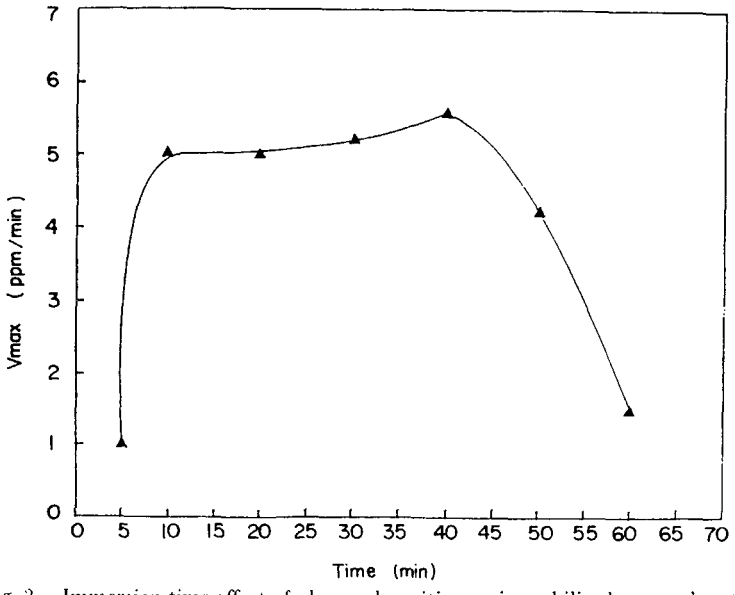


Fig. 3. Immersion time effect of plasma deposition on immobilized enzyme's activity.

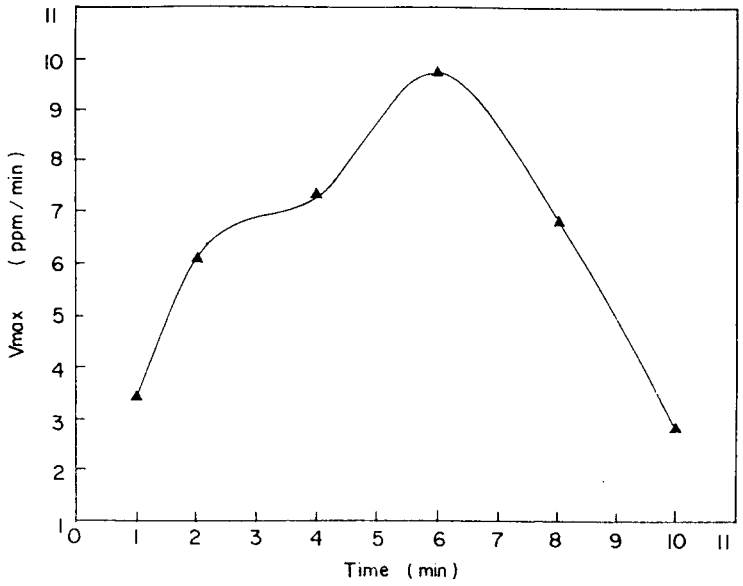


Fig. 4. Exposure time effect of plasma deposition on immobilized enzyme's activity. Plasma treatment conditions: immersion time 20 min and 0.1 torr acrylic acid.

Figure 6 shows the effect of pH values of the buffer solution on the activity of immobilized glucose oxidase. The maximum activity was obtained at pH 5.6. Therefore, the pH 5.6 buffer solution was most suitable for the storage of glucose-oxidase-immobilized membrane.

The results of using different glucose solutions to test the performance of immobilized glucose oxidase membranes were shown in Figure 7. A linear

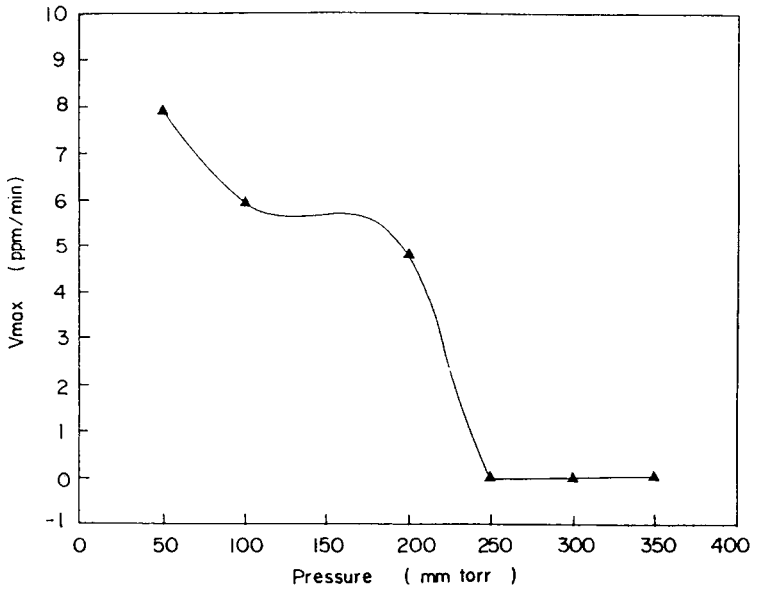


Fig. 5. The amount of acrylic acid effect of plasma deposition on immobilized enzyme's activity. Plasma treatment conditions: immersion time 20 min and exposure time 6 min.

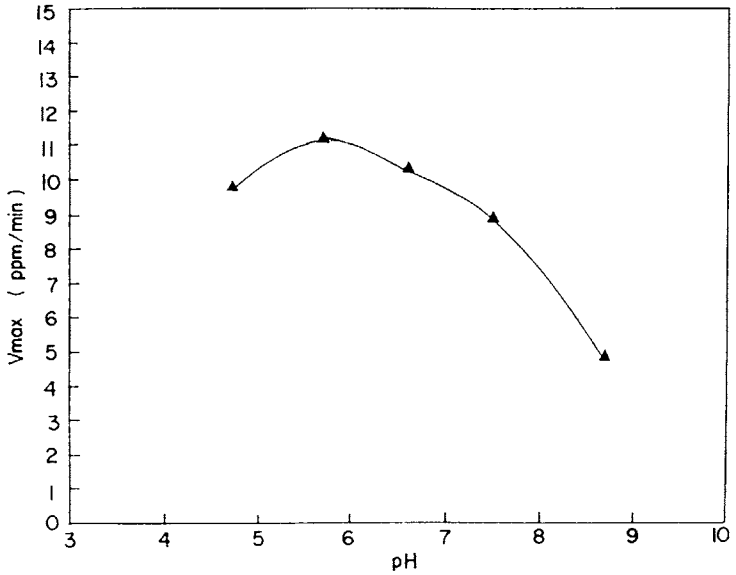


Fig. 6. The pH effect of plasma deposition on immobilized enzyme's activity. Plasma treatment conditions: immersion time 20 min, 0.05 torr acrylic acid, 40 W and exposure time 6 min.

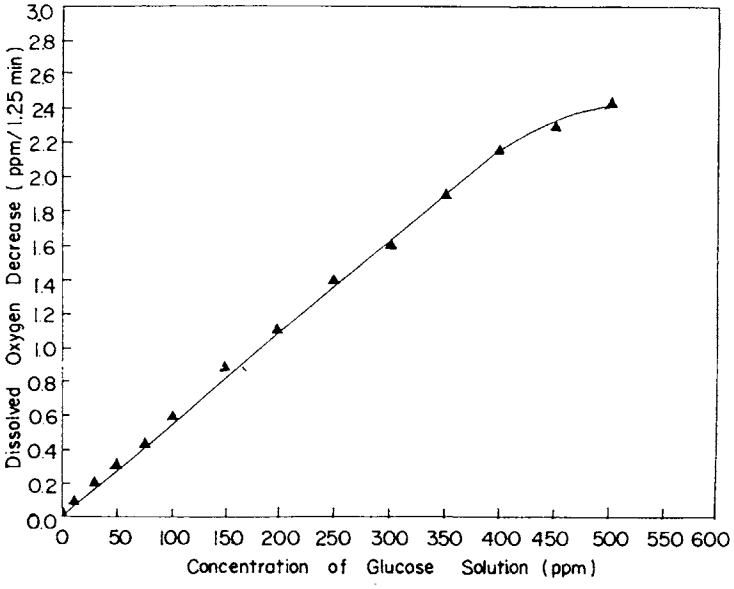


Fig. 7. The calibration curve of GOD sensor treated by plasma deposition. Plasma treatment conditions: immersion time 20 min, exposure time 6 min and 0.05 torr acrylic acid.

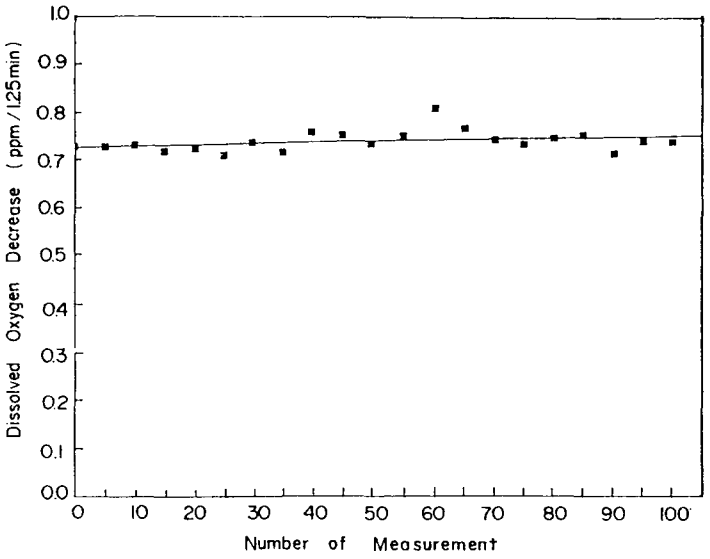


Fig. 8. Stability test of GOD sensor by plasma deposition. Plasma treatment conditions: immersion time 20 min, exposure time 6 min and 0.05 torr acrylic acid.

relationship was obtained at 0–400 ppm glucose concentration. The stability of the obtained GOD sensor was very good. The GOD sensor was continuously tested in a pH 5.6 aqueous solution containing 200 ppm glucose at 30°C. Even after 100 measurements, the response error was less than 5% (Fig. 8).

Preparation of GOD Immobilized Membrane via Plasma Surface Treatment Method

Figures 9–11 present the effects of Ar pressure, plasma power, and exposure time on the activity of GOD-immobilized membrane. The activity increased as

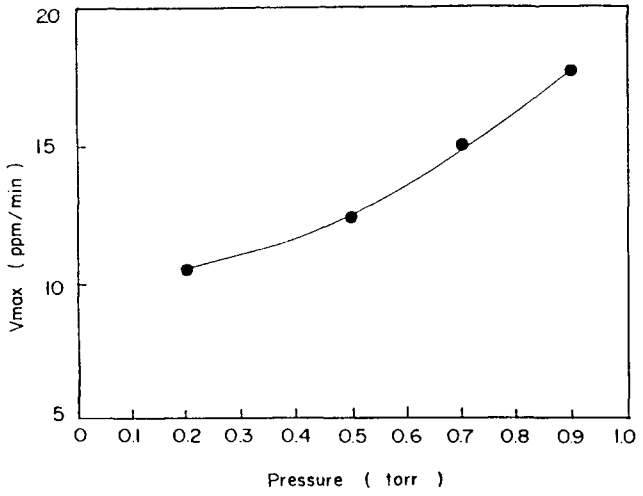


Fig. 9. Pressure effect on the activity of immobilized enzyme. Plasma treatment conditions: 100 W, 150 sec and Ar gas.

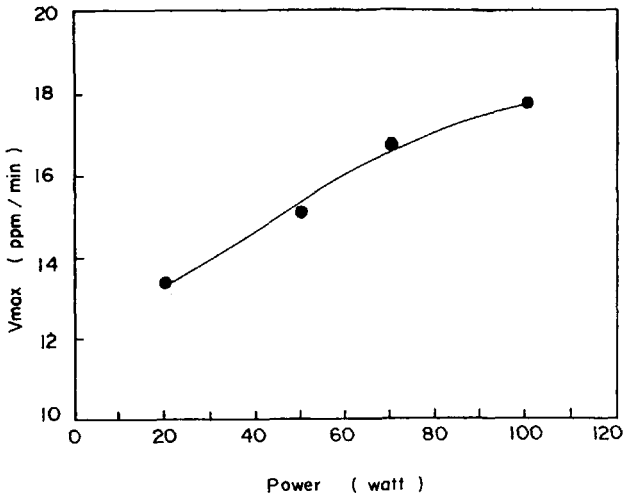


Fig. 10. Power effect on the activity of immobilized enzyme. Plasma treatment conditions: 150 sec and 0.9 torr Ar gas.

the Ar pressure and plasma power increased. The reason is that there are more high energy particles generated at high Ar pressure and plasma power, and hence more active sites were produced on the PP film. This resulted in more glucose oxidase being immobilized. The optimum exposure time was 150 s because longer exposure resulted in PP film destruction and too short exposure resulted in fewer active sites being formed on the PP surface.

The PP film, which was treated with the ammonia plasma generated at different power, underwent the same immobilization process and activity test. The results are presented in Figure 12. The best result was obtained when the

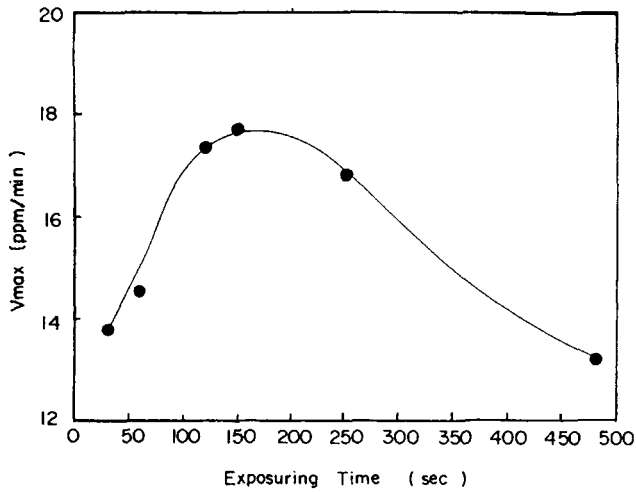


Fig. 11. Exposure time effect on the activity of immobilized enzyme. Plasma treatment conditions: 100 W and 0.9 torr Ar gas.

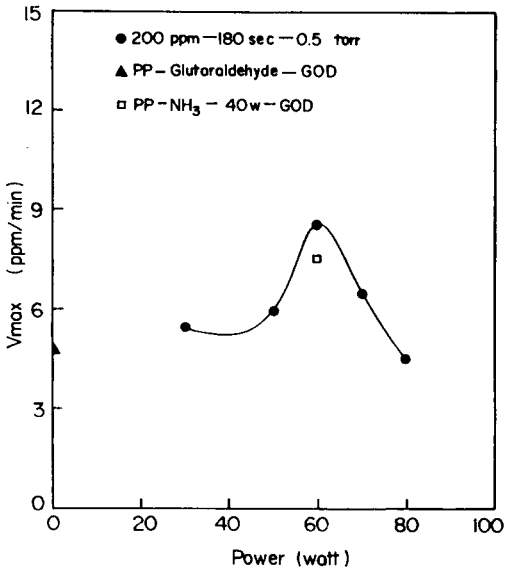


Fig. 12. Power effect on the activity of immobilized enzyme. Plasma treatment conditions: 180 sec, 0.5 torr NH_3 gas.

power was 60 W. Figure 13 shows the effect of ammonia vapor pressure on the activity of the immobilized glucose oxidase. The maximum activity was obtained at 0.5 torr ammonia. Figure 14 presents the effect of reaction time on the activity of the immobilized glucose oxidase for the PP film which was treated with the plasma generated at 60 W and 0.5 torr ammonia. When the

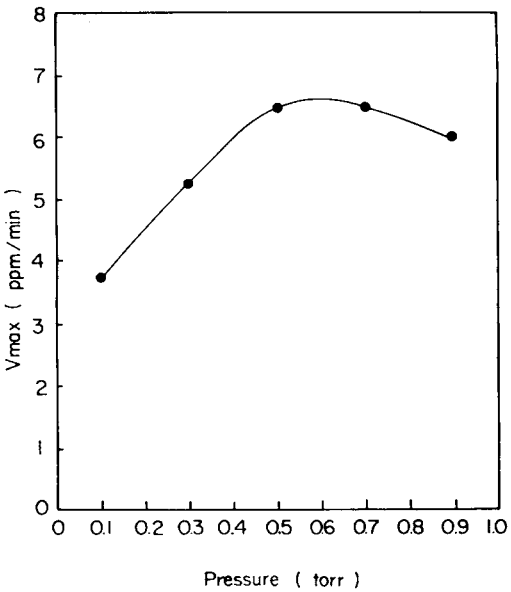


Fig. 13. Pressure effect on the activity of immobilized enzyme. Plasma treatment conditions: 180 sec, 80 W and NH_3 gas.

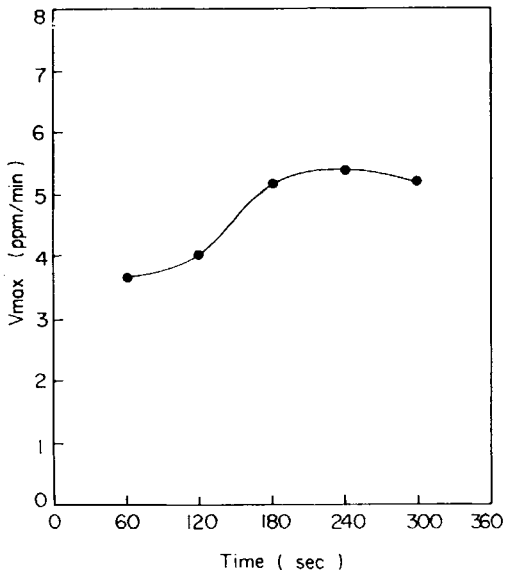


Fig. 14. Exposure time effect on the activity of immobilized enzyme. Plasma treatment conditions: 60 W and 0.5 torr NH_3 gas.

reaction time was longer than 180 s, the activity reached a stable value. The surface of the ammonia plasma treated PP film was analyzed by ESCA. From Figure 15, we found the nitrogen element present on the ammonia plasma treated PP film. This means that the amino group ($-NH_2$) was introduced onto the PP film surface by ammonia plasma. Thereafter, the immobilization reaction occurred. The whole process was shown as below:

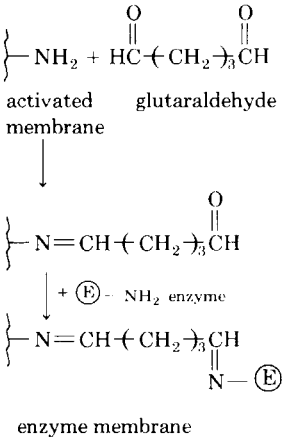


Figure 16 presents the scanning electron micrograph of untreated and treated PP films. (a) is the untreated porus PP film: (b) and (c) are the films treated, respectively, by Ar plasma or NH_3 plasma. Obviously, the hole was

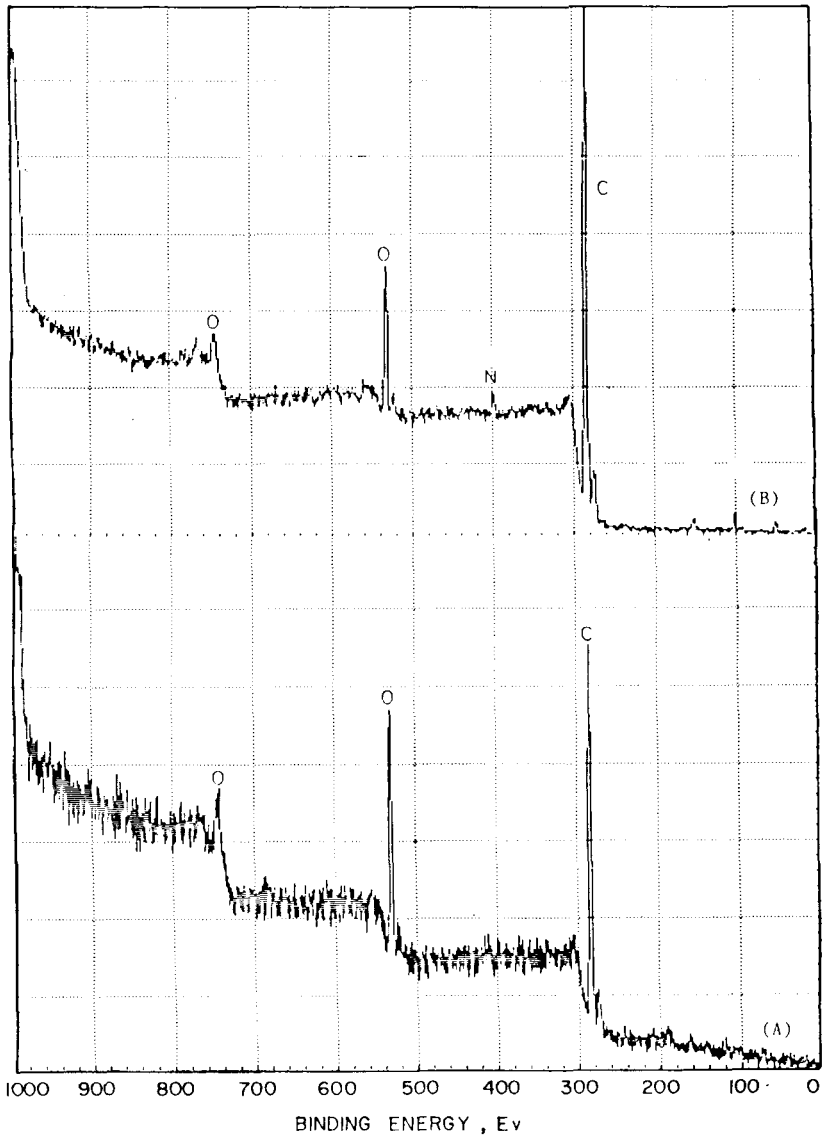
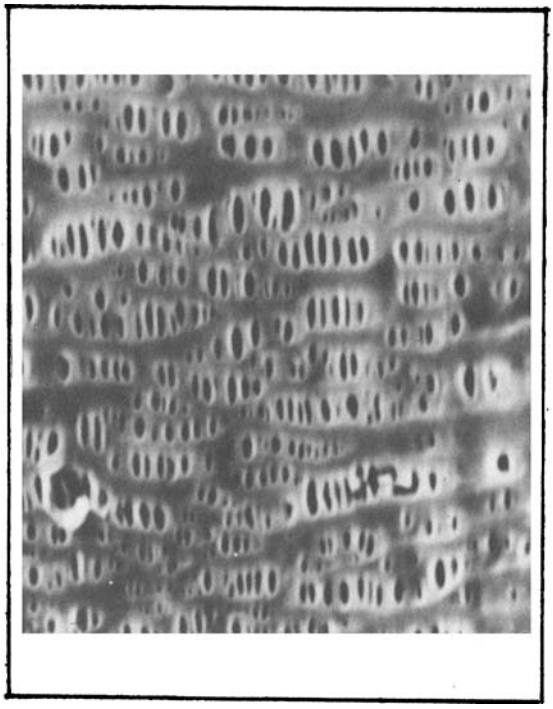


Fig. 15. ESCA of the surface of (a) untreated PP film and (b) PP film treated by plasma.

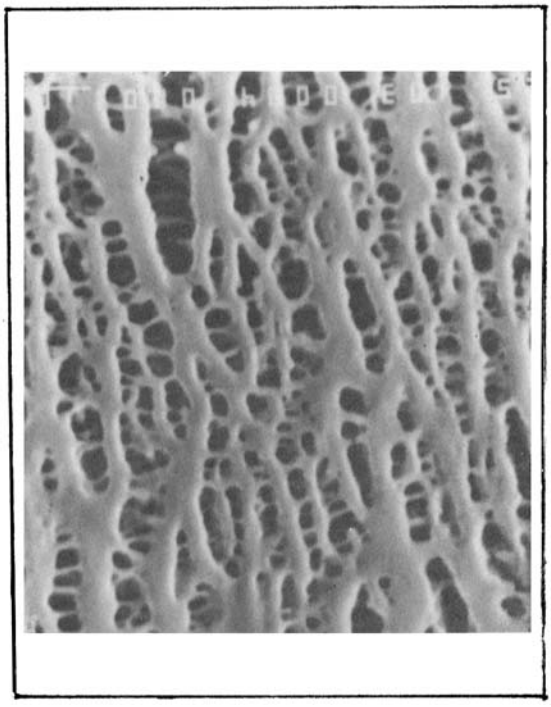
enlarged after plasma treatment, especially for the samples treated by Ar plasma.

The optimum conditions for the Ar and NH_3 plasma treatment of the porous hydrophilic PP film to prepare the GOD sensors were: introducing 0.9 torr of gas, irradiating the film with 100 W of plasma for 150 s, and dipping in GOD solution for 12 h.

Glucose solution (200 ppm, ph 5.6) was used to test the obtained GOD sensors at 30°C. The results showed that the sensor was very stable. Even after 180 measurements, the standard deviation of the response was within 3.5% (Fig. 17).

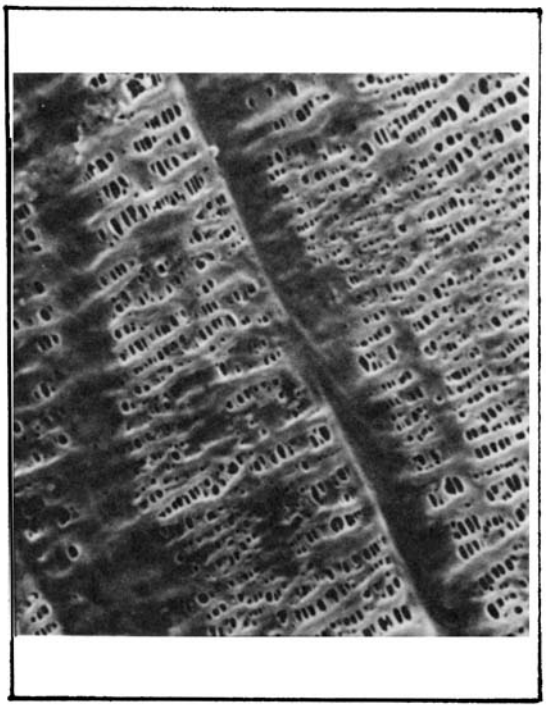


(a) non-treated X10,000



(b) Ar plasma X10,000

Fig. 16. Scanning electron micrograph of the surface of (a) the untreated PP film, (b) PP film treated by NH_3 plasma and Ar plasma.



(c) NH_3 plasma X4,000

Fig. 16. (*Continued from the previous page.*)

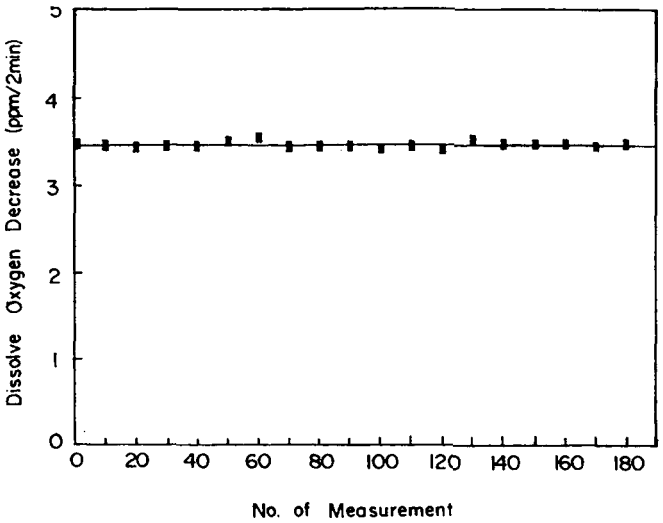


Fig. 17. Stability test of GOD sensor.

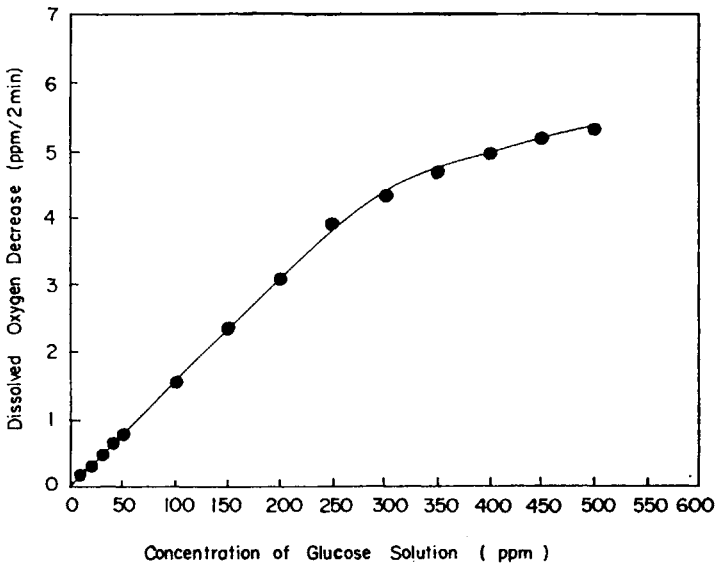


Fig. 18. Calibration curve of GOD sensor.

Further, when using different concentrations of glucose solution to test the obtained GOD sensors, a linear relationship was obtained at 0–300 ppm of glucose concentration (Fig. 18). The obtained glucose oxidase immobilized hydrophilic PP membranes were kept in pH 5.6 buffer solution at 4°C. The GOD activity of the membrane was determined at 7-day intervals. The results presented in Figure 19 show that the membranes still have 90% GOD activity even after 35 days of storage.

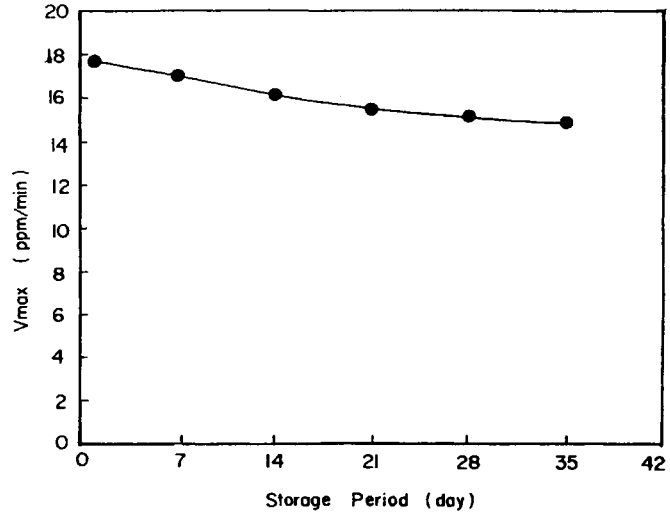


Fig. 19. Storage stability of immobilized GOD hydrophilic PP film.

From the above experimental results, several conclusions can be summarized:

1. The higher the activity of immobilized glucose oxidase the smaller the linear detecting range of a sensor is obtained.
2. Low temperature plasma can be used for the preparation of various types of immobilized membrane. Plasma-initiated polymerization of acrylic acid was proved to be able to immobilize the glucose oxidase.
3. By using plasma surface treatment, hydrophic $-NH_2$ group could be introduced on the surface of PP film. The glucose oxidase was easily immobilized on these modified PP films. The obtained enzyme immobilized membranes have good properties of high activity and high stability.
4. Both the low energy plasma-initiated polymerization method and plasma surface treatment method can be used to immobilize the enzyme. Better results were obtained by using the plasma surface treatment method. Further studies on the low energy plasma-initiated polymerization of the other monomer systems will be valuable.

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