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Amperometric needle-type glucose sensor based on a modified platinum electrode with diminished response to interfering materials

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Abstract

A needle-type glucose sensor that can be used to determine glucose in serum and whole blood samples was developed Platinum wire was used as the working electrode and a disposable hypodermic stainless-steel needle electroplated with platinum was used as the counter and reference electrode A method involving both photocross-linking of PVA–SbQ and cross-linking with glutaraldehyde was used to immobilize the enzyme [PVA–SbQ is a poly(vinyl alcohol) bearing stilbazolium groups] Nafion and cellulose triacetate membranes were used to prevent inaccuracy from interfering materials and to increase the dynamic range of the sensor, respectively The response, reproducibility and long-term stability of the sensor and the effects of temperature, pH and metal ions on the response were investigated Owing to the effective method for enzyme immobilization, the large surface area of the counter electrode and the relative inactivity of the counter electrode to chemical reactions, the sensor showed good response, stability and reproducibility The sensor did not respond to ascorbate and urate at the concentrations normally found in blood Data obtained from the sensor for glucose in serum and whole blood samples showed a good correlation ($r > 0\ 95$) with a clinical laboratory automated analyser

Keywords Amperometry, Biosensors, Enzymatic methods, Blood, Glucose, Serum

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Biosensors for glucose have been studied for a long time and many kinds of materials, including enzymes [1,2] and microbial cells [3] have been used to construct them Enzymes have been used most frequently because of their specificity to the substrate [4] Many methods for enzyme immobilization [5–11] and monitoring of enzymatic reactions [12–14] have been developed for constructing such sensors

Needle-type glucose sensors seem attractive because of their small size, simple construction and the possibility of inserting them directly into a fruit or the vascular tissue of a living organism Many studies concerning the construction of needle-type glucose sensors have been reported [15–17] However, most of them suffer from instability, a low response and the inaccuracy induced by interfering materials [18]

This paper describes the construction of a needle-type glucose sensor suitable for determining glucose in blood The method combines photocross-linking of PVA–SbQ [19] and cross-linking with glutaraldehyde to immobilize the enzyme PVA–SbQ is a poly(vinyl alcohol) bearing stilbazolium groups [20] This polymer is photo-cross-linkable with light of wavelength shorter than 460 nm Biomaterials including enzymes [21,22] and organelles [23] have been immobilized in this polymer

Nafion was used to diminish the responses to ascorbate and urate [23], which are the major interfering materials in blood [18] The responses to ascorbate and urate at their normal concentrations in blood were eliminated after coating a Nafion membrane on the working electrode Nafion is a fluorine-containing material and therefore it is difficult ot make another membrane adhere to it The high viscosity of PVA–SbQ, however, made it possible to encapsulate the tip of the electrode with an immobilized enzyme membrane

Silver is most popularly used as a counter electrode [24] However when lengths of silver and platinum wires were immersed in phosphate-buffered saline (PBS) solution at room temperature for 3 days, a dark layer formed on the surface of silver but not platinum Electroplated platinum was used as the counter electrode in this study because of its large surface area and comparative inactivity to chemical reactions Both of these properties contributed towards a stable response and good stability of the sensor

The sensor was used to determine glucose in serum and whole blood A good correlation between these results from the sensor and those obtained with an automated analyser confirms the possibility of applying this sensor in clinical analysis

EXPERIMENTAL

Chemicals

Glucose oxidase (GOD) (E C 1 1 3 4) from *Aspergillus niger* and Bis–Tris propane {1,3-bis[tris(hydroxymethyl)methylamino]propane} buffer were obtained from Sigma (St Louis, MO) PVA–SbQ was purchased from Toyo Chemical (Tokyo) Nafion perfluorinated ion-exchange powder (5% solution in a mixture of lower aliphatic alcohols and 10% water) was obtained from Aldrich (Milwaukee, WI) and used as supplied Bovine serum albumin (BSA) was obtained from Wako (Tokyo), cellulose triacetate from Eastman Kodak (Rochester, NY), glutaraldehyde (50% aqueous solution) from Tokyo Kasei (Tokyo) and the electrolyte solution for platinum electroplating (Platanex 3LS) from Japan Electroplating Engineers (Tokyo) A glucose analysis kit based on hexokinase–glucose-6-phosphate dehydrogenase was supplied by Boehringer (Mannheim) and a glucose analysis kit based on glucose oxidase by Wako

Phosphate-buffered saline (PBS) solution was prepared by dissolving 2 754 g of NaCl, 2 081 g of KH_2PO_4 and 0 477 g of NaOH in 1000 ml of distilled water and adjusting the pH to 7 4 with 0 1 M NaOH solution [25] Glucose solutions were prepared in PBS and allowed to stand for at least 24 h before use to equilibrate the α- and β-anomers All other chemicals were of the highest grade available and were used as received

Instrumentation and materials

A potentiostat (BAS LC-4B amperometric detector, Bioanalytical Systems, Lafayette, IN) was used to supply a fixed potential to the electrode

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A circulating water-bath (Thermo Minder Mini-80, Taiyo Science, Tokyo) which incorporated a water-jacketed glass reactor was used to control the temperature of the operating system Magnetic stirring was used to maintain homogeneity of the sample solutions in the reactor The response current could be read directly from the digital screen of the potentiostat and recorded simultaneously by a chart recorder (Electronic Polyrecorder EPR-100A, TOA Denpa Kogyo, Tokyo) A schematic diagram of the batch operating system is shown in Fig 1

Another potentiostat–galvanostat (HA 501, Hokuto Denko, Tokyo) was connected with a function generator (HB-107A, Hokuto Denko) to perform cyclic voltammetry and electroplating A multimeter (Digital Multimeter, TR6840, Takeda-Riken, Tokyo) and an Ag/AgCl electrode (HS-907, TOA Electric, Tokyo) were used to measure the potential drift during a determination The clinical analyser used was a Beckman Glucose Analyzer II (Beckman Instrument, Palo Alto, CA) Heat-shrink FEP (fluorinated ethylene–propylene) tubing was obtained from Junkosha (Tokyo) A stainless-steel hypodermic needle (o d 1 2 mm, i d 1 0 mm) was obtained from Terumo (Tokyo) Platinum wire of 0 3 mm diameter was obtained from Tokuriki (Tokyo)

Preparation of the working electrode

Platinum wire (50 mm × 0 3 mm diameter) was soldered to a lead wire (copper wire electroplated with tin and then insulated with Teflon) Heat-shrink FEP tubing was used to insulate the platinum wire The tip of the FEP-encapsulated platinum wire was cut at an 18° angle and successively polished with water-proof sand-papers (No 320 and 1000, Marumoto, Tokyo) and a silicon carbide Paper (No 2400, Struers, Copenhagen) After the electrode had been cleaned by sonica-

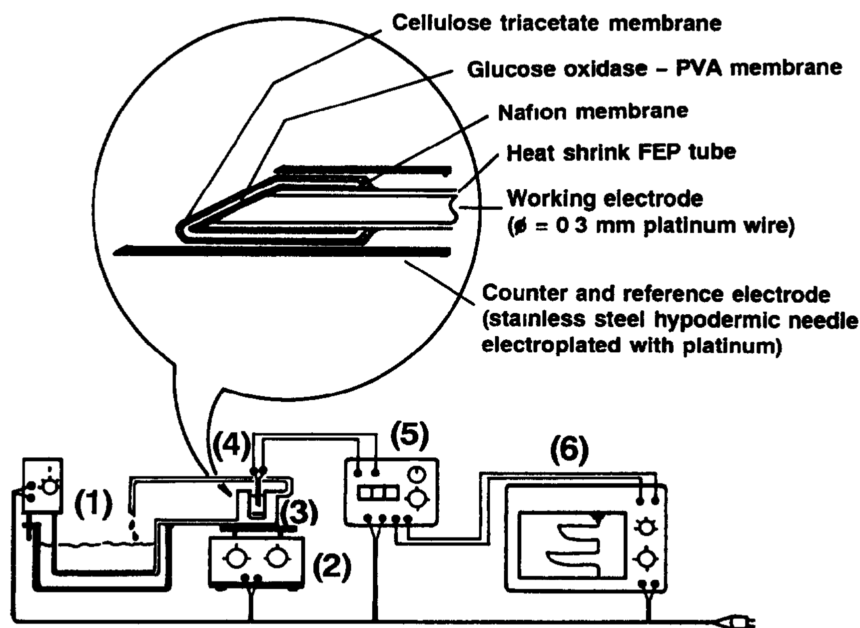

Fig 1 Schematic diagram of the batch-type operating system and the multi-layer membrane system on the tip of the working electrode (1) Thermostated circulating water-bath, (2) magnetic stirrer, (3) water-jacketed glass reactor, (4) electrode, (5) potentiostat, (6) chart recorder

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tion for 30 min, the multi-layer membrane system was coated on to the metallic surface of the working electrode

Construction of the multi-layer membrane system

Three membranes made up the multi-layer system Initially the tip of the working electrode was dipped into Nafion solution for 10 s and then dried at room temperature for 30 min The tip of the electrode was then dipped into an enzyme solution composed of 5 mg of GOD (25 U mg^{-1}), 10 mg of BSA, 100 mg of distilled water and 200 mg of PVA–SbQ for 5 s After dipping, the electrode was placed in a sealed, dark box containing glutaraldehyde vapour The box was kept at room temperature for 12 h to complete cross-linking, then the electrode was exposed to a fluorescent lamp for 10 min to induce photocross-linking of PVA–SbQ Finally, the electrode was dipped in a 0 5% (w/v) cellulose triacetate solution in dichloromethane for 3 s and dried for 5 min at room temperature The electrodes were stored dry at 4°C until used The multi-layer membrane system is shown in Fig 1

Preparation of the counter electrode

A stainless-steel hypodermic needle was cleaned with methanol and dichloromethane It was further cleaned with an oxidative acid solution [concentrated sulphuric acid–30% hydrogen peroxide (1 + 1)] and an oxidative alkaline solution [concentrated ammonia solution–30% hydrogen peroxide–water (1 + 1 + 6)] It was thoroughly washed with distilled water after each cleaning step

Nickel and platinum were electroplated successively on to the needle The processes of elec-

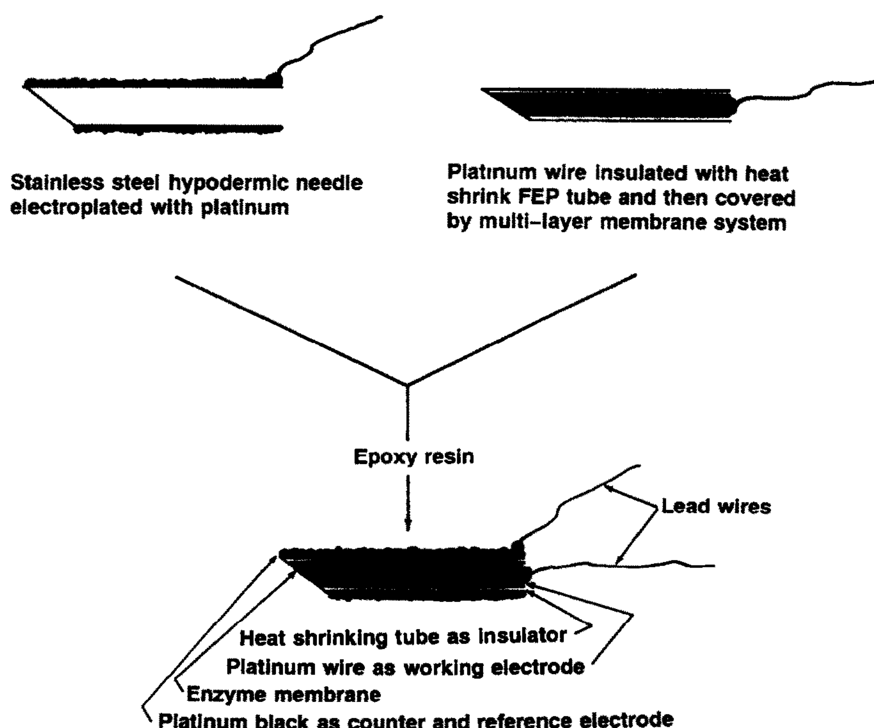

Fig 2 Procedure for constructing needle-type enzyme electrode

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troplating were conducted at 80°C The needle and a piece of nickel wire were connected to the potentiostat and immersed in an electrolyte solution prepared by dissolving 60 g of $NiCl_2$ $6H_2O$ in 30 ml of concentrated hydrochloric acid and diluting with distilled water to 250 ml A potential of −4 0 V was applied between the needle and the nickel wire for 30 s After thorough rinsing with distilled water, the needle was transferred into Platanex 3LS platinum electroplating solution A current of −20 mA was applied between the needle and a piece of platinum wire for 5 min The needle was thoroughly rinsed again with distilled water and then stored dry at room temperature until used

Fabrication of glucose sensor

The working electrode was inserted into the hollow interior of the counter electrode Epoxy resin was used to fix the two electrodes in position The procedure for constructing the glucose electrodes is shown in Fig 2

Measurement of potential drift during glucose determination

An Ag/AgCl electrode was inserted in the reaction cell with the glucose sensor During glucose determination the potential between the working electrode and the Ag/AgCl electrode was measured with a voltmeter and recorded with a chart recorder

Determination of glucose concentration

The electrode was immersed in PBS solution for 1 h to equilibrate the membrane system The copper wires were then connected to the potentiostat and a potential of +650 mV was applied between the working and the counter electrodes The baseline current was measured and then glucose solution was injected into the PBS solution using a microsyringe The response current following injection was recorded with a chart recorder until the second steady state was achieved Magnetic stirring was used during this operation to ensure homogeneity of the solution The difference between the baseline and the second steady-state currents was used to calculate the concentration of glucose in the sample according to a calibration graph

Another calibration graph was obtained by adding glucose solution to heparinized whole blood that had been incubated at 37°C for 18 h to glycolyse the glucose present [26] The concentration of glucose in serum and whole blood was determined by taking the baseline and the second steady-state currents in the glycolysed whole blood and the sample, respectively, and using them to calculate the glucose concentration according to the calibration graph obtained with glycolysed whole blood The samples were mixed in the sampling tube by gentle shaking before determination but were not stirred during determination

RESULTS AND DISCUSSION

Determination of the applied potential

The cyclic voltammograms of the electrode using platinum wire as the working electrode and platinum black as the counter and reference electrode in PBS with or without hydrogen peroxide are shown in Fig 3 The plateau of this electrode after contacting with hydrogen peroxide appeared from 450 to 750 mV The potential between the working electrode and the Ag/AgCl electrode drifted by −57 mV when determining 400 mg dl^{-1} (22 2 mM) glucose in PBS A potential of +650 mV was chosen after taking the range of the plateau region and the potential drift during glucose determination into consideration This

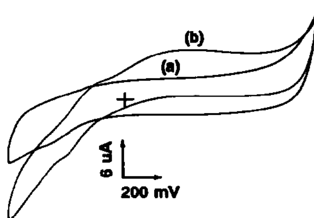

Fig 3 Cyclic voltammogram of the electrode using platinum wire as the working electrode and platinum black as the counter and reference electrode Experiments were done in (a) PBS and (b) 1 mM H_2O_2 in PBS (pH 7 4) at 37°C

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