

Development of potentiometric urea biosensor based on urease immobilized in PVA–PAA composite matrix for estimation of blood urea nitrogen (BUN)

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Received 7 June 2006; received in revised form 6 September 2007; accepted 31 December 2007

Abstract

A urea biosensor was developed using the urease entrapped in polyvinyl alcohol (PVA) and polyacrylamide (PAA) composite polymer membrane. The membrane was prepared on the cheesecloth support by gamma-irradiation induced free radical polymerization. The performance of the biosensor was monitored using a flow-through cell, where the membrane was kept in conjugation with the ammonia selective electrode and urea was added as substrate in phosphate buffer medium. The ammonia produced as a result of enzymatic reaction was monitored potentiometrically. The potential of the system was amplified using an electronic circuit incorporating operational amplifiers. Automated data acquisition was carried by connecting the output to a 12-bit analog to digital converter card. The sensor working range was 1–1000 mM urea with a response time of 120 s. The enzyme membranes could be reused 8 times with more than 90% accuracy. The biosensor was tested for blood urea nitrogen (BUN) estimation in clinical serum samples. The biosensor showed good correlation with commercial Infinity™ BUN reagent method using a clinical chemistry autoanalyzer. The membranes could be preserved in phosphate buffer containing dithiothreitol, β -mercaptoethanol and glycerol for a period of two months without significant loss of enzyme activity.

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Keywords: Urea biosensor; Polyvinyl alcohol; Potentiometric; Ammonia selective electrode; Blood urea nitrogen (BUN); Gamma-irradiation

1. Introduction

Determination of blood urea nitrogen is an important routine test widely used in clinical laboratories, as elevated urea level in blood sera deciphers kidney disease, stone in urinary tract or even bladder tumour. Whereas, its decreased level indicates severe liver malfunction. The normal range of urea in human serum is between 1.7 and 8.3 mM and level increases up to 100 mM under patho-physiological conditions [1]. Although colorimetric and spectrometric methods are most commonly used [2,3], simple and fast method for determination of urea is in demand.

More recently, biosensors have emerged as a promising technology, especially for applications requiring rapid and continuous monitoring. Biosensors are being applied to a wide variety of analytical problems such as in medicine [4–6], environment [7–9], food and process industries [10,11], security and defence [12]. Although urea biosensors emphasising on better sensitivity [13–20] or higher response range [21] are reported, not much effort has been made in resolving the drawbacks of enzyme instability, difficulty in storage and handling, and fragility of the immobilization matrix.

Some of these drawbacks were obviated in our recent studies, wherein we developed a mechanically stable conjugate membrane of polyvinyl alcohol (PVA) and polyacrylamide (PAA) prepared on cheesecloth support by gamma-irradiation [22]. PVA provided flexibility to the membrane and cryo-polymerization generated porosity in the structure. The mixed properties of the individual polymers and use of cheesecloth as the impregnating support provided sufficient tensile strength to the membrane to

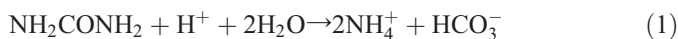
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withstand rough treatments such as repeated washings. The membranes entrapped sufficient amount of the enzyme, however a slow leakage of the enzyme was seen during reuse. Cross-linking the enzyme with 0.2–0.4% glutaraldehyde in cold minimized the leaching.

We used this membrane in our present study to develop a potentiometric biosensor for urea. The sensor was based on the enzymatic reaction of urea hydrolysis catalysed by urease, which is described in Eq. (1).



The electronic circuit was designed for automated measurements and the biosensor was developed as standalone equipment to suite clinical needs.

2. Materials and methods

2.1. Materials

Polyvinyl alcohol (PVA) of degree of polymerization [1700–1800] and degree of hydrolysis [98–99 mol%] was procured from Loba Chemie, India. Urease (E.C. 3.5.1.5.) powder from Jack Bean was purchased from Sigma–Aldrich (catalogue no. U1500). Infinity™ Urea (Nitrogen) (Powder) reagent and ammonia ion selective electrode (ISE) (model no. 9512 BNWP) were from Thermo Scientific Inc. All other chemicals were of analytical reagent grade. A 12-bit analog to digital converter card (ADC) (model PCL 207) was procured from Dynalog India Ltd. Other electronic components were purchased from the local market.

2.2. Preparation of enzyme membrane

The enzyme membranes were prepared as per the procedure described earlier [22]. Polymerization mixture contained PVA (5%) (w/v), acrylamide (10%) (w/v) and bis-acrylamide (3%) (w/v), dissolved in 10 mM sodium phosphate buffer of pH 7.4 by heating under boiling water bath for 10 min, followed by cooling down to room temperature. Urease (2.5 g) (w/v), dithiothreitol (DTT) (1 mM), β -mercaptoethanol (β -ME) (1 mM) and 125 μ l

glycerol were mixed with 50 ml of this viscous slurry and homogenized by stirring with a glass rod. Ten 100 cm² cheesecloth pieces were soaked in the slurry and frozen in toluene-dry ice bath (–78 °C). These were then exposed to 1.2 kGy of Co⁶⁰ γ -rays in a gamma-cell (model: 220, Atomic Energy of Canada Ltd, Ottawa, Canada) at a dose rate of 10 Gy/min using air as the gas phase. After irradiation, membranes were soaked in phosphate buffer (pH 7.4) containing 0.2% glutaraldehyde (v/v) for 20 min and were finally washed with phosphate buffer. The membranes were partially dried at room temperature and stored at 4 °C.

2.3. Analytical methods

Urease activity in the membrane was determined as previously reported [22]. Membranes of size 5 cm² (2 cm \times 2.5 cm) were soaked in phosphate buffer containing 50 mM urea for 1 min. 20 μ l aliquot of the sample was removed and the ammonia liberated was estimated colorimetrically by the phenol-hypochloride method [23].

Urea was estimated by Infinity™ blood urea nitrogen (BUN) reagent method [24]. Appropriate amount of serum sample was mixed with 300–1000 μ l of reagent and analyzed by a chemistry autoanalyzer (model-express plus, Ciba–Corning Ltd.). The urea concentration was proportional to the absorbance change at 340 nm over a fixed time interval.

2.4. Reaction vessel and electronic circuit designs

The reaction vessel and electronic circuit used for biosensor studies were developed in our laboratory. The schematic diagram of the vessel is shown in Fig. 1. An ammonia ion selective electrode (ISE) was placed in a measuring cylinder with provision of buffer flow through tubes. Samples were injected into the vessel through a syringe. A buffer tank was placed at a height to maintain the flow of buffer to the vessel by siphon action. The flow was controlled with the help of stopper cocks. The working volume of the vessel was 10 ml.

An electronic circuit was designed to acquire sensor signal. This comprised of operational amplifiers (op-amp) to amplify the output potential of the ammonia selective electrode. Due to

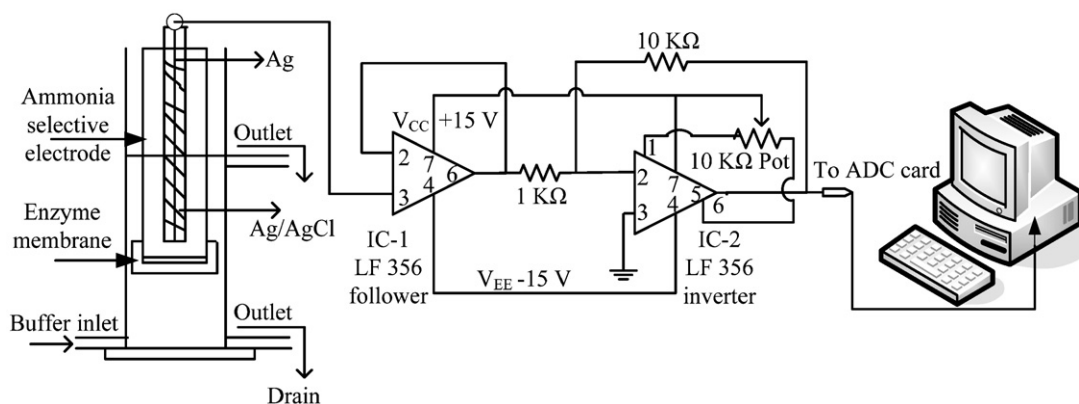


Fig. 1. Schematic of flow-through reaction vessel and circuit diagram of electronic assembly used for urea biosensor.

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