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A novel conductometric creatinine biosensor based on solid-state contact ammonium sensitive PVC–NH_2 membrane

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ABSTRACT

A novel, highly sensitive and stable conductometric biosensor for creatinine determination is developed. The biosensor is based on solid-state contact ammonium-sensitive sensor. Creatininase is chemically immobilized on the surface of the solid-state contact ammonium-sensitive membrane via glutaraldehyde covalent attachment method. The developed conductometric creatinine biosensors demonstrate high sensitivity and short response time toward creatinine. The detection limit of the biosensor was about 2×10^{-6} M and the response time was shorter than 10 s in phophate buffer solution at pH 7.20. The linear dynamic range of the biosensor was between 1×10^{-1} and 9×10^{-6} M creatinine concentration in phosphate buffer solution at pH 7.20. The biosensor exhibited good operational and storage stability for at least 4 weeks kept in dry at 4–6 °C. It had a reproducible and stable response during continuous work at least for 10 h with the relative standard deviation of 0.5% ($n = 48$) for creatinine of 1×10^{-3} M in phosphate buffer solution.

1. Introduction

Creatinine is the end product of creatinine metabolism in mammalian cells. Therefore, it is an important diagnostic substance in biological fluid. Creatinine can be used for the diagnosis of renal, thyroid and muscle function [1]. The creatinine level in blood serum and urine is clinically used as a parameter of muscle damage. The physiological concentration of creatinine ranges between 40 and 150 μmol/L in serum, but pathological values due to muscle disorder or kidney dysfunction may rise to concentration higher than 1000 μmol/L [2–4].

For routine creatinine determinations in clinical laboratory, the most frequently used methods are the HPLC [3,5] and spectrophotometric one based on the Jaffe reaction [4,6,7]. However, these methods are time consuming and not specific for creatinine [6,8], and usually require complicated sample pretreatment of collected samples, relatively large amounts of biological samples, delicate and bulky equipment and sometimes a preconcentration step in order to improve detection sensitivity [5]. Therefore, several enzymatic methods have been reported [9–11] to increase specificity. For this reason, the methods based on a combination of enzyme with specific sensor, such as ion-selective electrodes or other probes have been shown to be rapid, simple and promising for reduction of time and cost for the creatinine analysis [12]. Various potentiometric and amperometric enzyme electrodes for creatinine determination have been reported in the literature and their principles and designs have been reviewed [4,13–26]. In these studies, a creatinine biosensor in a flow injection analysis system [27,28], and the application of a creatinine sensitive ion-selective field-effect transistor (ISFET) were described [29,30]. However, the shortcomings of the creatinine biosensors, including the high cost and the complexity of the fabrication process, are not commonly addressed [31].

Conductometric sensors for biosensing devices have first been introduced by Watson et al. [32]. The device consisted of a planar glass support with interdigitated gold electrode pairs on one surface in a planar configuration. The operation of the biosensor device was based on measurement of the bulk conductance of the sensitive membrane due to biochemical reaction in solution. Conductometric transducers are considerably beneficial since construction in a single way, high compatibility, rugged and relatively cheap, no need of any reference electrode [33,34]. They were also described in the detection of glucose [35,36], urea [37,38], uric acid [39], sucrose [33] and trypsin [40].

However, to our best knowledge, as alternatives to the present methods for creatinine determination, no previous work has been done on the conductometric creatinine biosensors to date. The present study reports the development of an alternative conductometric creatinine biosensor based on solid-state contact ammonium-sensitive sensor chip membrane. The main analytical

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characteristics of the biosensor, such as pH behaviour, time of immobilization and the enzyme loading were investigated with respect to the influence on sensitivity, limit of detection, dynamic range, response time, operation and storage stability.

2. Experimental

2.1. Materials

Creatinine and creatininase (*Pseudomonas* sp., 84.5 U/mg) were obtained from Fluka and used as received. High molecular mass poly(vinylchloride) (PVC), o-nitrophenyloctylether (o-NPOE), ammonium ionophore I (nonaction), potassium tetrakis (p-chlorophenyl) borate (KTpClPB), tetrahydrofuran (THF) and graphite were obtained from Fluka. Epoxy resin (Macroplast Su 2227) and hardener (Desmodur RFE) were obtained from Henkel and Bayer, respectively. Di-cyclo-hexyl 18-crown-6 was obtained from Aldrich. Glutaraldehyde and lysine were obtained from Merck. PVC–NH_2 was synthesized according to our previously described method [41].

A stock creatinine solution (1.0×10^{-1} mol/L) was prepared. The dilute solutions (1×10^{-2} to 1×10^{-6} mol/L) of creatinine were prepared by an appropriate dilution of the stock solution with 5 mM phosphate buffer solution. All of the other reagents used were of analytical reagent grade. Doubly distilled deionized water was used throughout the study.

A 2.5% (w/w) glutaraldehyde solution was prepared with 5 mM phosphate buffer at pH 7.20. 20 ml of creatininase solutions were prepared by dissolving 0.5–10.0 mg of creatininase in 20 ml of 5 mM phosphate buffer at pH 7.20.

A 10 mM lysine solution was prepared in 5 mM phosphate buffer at pH 7.20.

Sodium borohyride solution was prepared by dissolving 20 mg of sodium borohyride in 20 ml of 5 mM phosphate buffer at pH 7.20.

2.2. Apparatus

Conductometric measurements were performed by CMD 750 model conductometer (WPA Linton Cambridge, UK) at room temperature (20 ± 1 °C). A SE 120 BBC chart recorder was directly connected to the conductometer to obtain calibration graphs when required.

pH measurements were performed by using Jenway 3040 Ion Analyser.

2.3. Design of conductometric sensor

The conductometric sensor was designed as a chip using glass–fiber substrate with interdigitated copper pads. Copper pads of the glass–fiber substrate were used for the construction of sensor chip. The epoxy resin mixture used to bind the graphite in preparing the all solid-state contacts of sensor chip was made from epoxy and hardener in THF solvent in the proportions 1.0:0.5 (w/w). The powdered graphite was mixed with the epoxy–resin mixture in the proportions 1.0:10 (w/w). After mixing, the solution was allowed to stand for 20–30 min in air. When the appropriate viscosity was attained, the mixture was deposited on the central part of the copper contact pads and then allowed to stand overnight in an oven at 40 °C.

PVC–NH_2 membrane solution which defines the sensor sensitive area was layered over the central part of the solid-state contacts deposited on the copper contact pads using drop method and then allowed to dry in air for at least 3 h. The membrane solution comprised ammonium ionophore-I (1.0%, w/w), di-cyclo-hexyl 18-crown-6 (1.0%, w/w), o-NPOE (67.0%, w/w), KTpClPB (1.0%, w/w) and PVC–NH_2 (30.0%, w/w) dissolved in 4 ml of THF. The sensitive part of the sensor membrane was isolated from the copper contact pads and solid-state contacts by using PVC solution. The radius of the sensitive area of the membrane left after isolation was about 1.0 mm and thickness of the membrane was about 0.1 mm. As a result, the sensitive area of each electrode was about 1.0 mm^2. The resulted conductometric solid-state ammonium-sensitive sensor chip was soaked in a 0.1 M ammonium nitrate solution for at least 5 h before use. The conductometric performance characteristics of the ammonium-sensitive sensor were tested in steady-state conditions.

2.4. Enzyme immobilization

The enzymatic membrane was immobilized on the surface of the ammonium-sensitive sensor membrane by using a simple two step glutaraldehyde covalent attachment method as described in our previous work [41]. Briefly, the ammonium sensitive sensor membrane surface was immersed in 2.5% (w/w) glutaraldehyde solution for 2.5 h. After brief washing of the sensor membrane surface with phosphate buffer at pH 7.20, the sensor chip membrane surface was immersed in creatininase enzyme solution and left for 15 h at 4 °C in a dark place. After that the surface of the biosensor chip constructed was washed with 10 mM lysine solution to remove the excess of the unbounded enzyme. Then the biosensor was immersed in sodium borohyride solution and left for 90 min to activate the covalent bondings on the membrane surface. Before measurements, the biosensor was thoroughly rinsed with water and phosphate buffer to wash out the sodium borohyride. A schematic diagram of conductometric creatinine biosensor based on solid-state contact ammonium-sensitive sensor is shown in Fig. 1.

2.5. Measurements

Measurements were conducted at room temperature (25 °C) in a glass beaker. Ammonium-sensitive sensor and creatinine-sensitive biosensor were immersed in a measuring glass filled with appropriate standard solution. The conductivity values as steady-state responses of the ammonium sensor and creatinine biosensor were measured for different concentrations of standard solutions of ammonium and creatinine in phosphate buffer solution, respectively.

3. Results and discussion

3.1. Conductometric performance of the ammonium-sensitive sensor

Before the construction of conductometric creatinine biosensor, as preliminary studies, solid-state conductometric ammonium-sensitive sensor was designed. Conductometric performance of the ammonium-sensitive sensor was evaluated to optimize membrane composition. The conductometric results obtained by one of the evaluated ammonium-sensitive membrane compositions are summarized in Table 1. The all solid-state ammonium sensitive membrane based on 1.0% ammonium ionophore-I and 1.0% di-cyclo-hexyl 18-crown-6 resulted good selectivity toward ammonium over other cations tested. Our intense studies uncovered that the addition of di-cyclo-hexyl 18-crown-6 compound into the membrane did not only increased the ionic mobility, it also enhanced the selectivity of the membrane toward ammonium ion. Based on the determination [42], conductometric selectivity of the all solid-state ammonium sensitive sensor in respect to Na^+, K^+, Ca^{2+} and H_3O^+ were evaluated. As can be seen from the results presented in Table 1, the prepared conductometric sensor showed