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New potentiometric sensors for creatinine

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Abstract

Three main types of creatinine potentiometric membrane sensors are described. They are based on the use of dibenzo-30-crown-10 (DB30C10) with potassium tetrakis(*p*-chlorophenyl)borate type (I), dibenzo-30-crown-10 alone type (II), and potassium tetrakis(*p*-chlorophenyl)borate alone type (III), incorporating in poly(vinyl chloride) matrix membrane plasticized with either *o*-nitrophenyl octyl ether or dioctylphthalate. The sensors are used for quantification of creatinine after soaking the membranes in 0.1 M creatinine solution for 2 days. The sensors show almost the same potentiometric response characteristics. Sensor type (I) exhibits Nernstian responses over a concentration range of 5.0×10^{-5} mol l^{-1}–1.0×10^{-2} mol l^{-1} creatinine with cationic slopes of 59.5 ± 0.1 and 60 ± 0.2 mV decade^{-1} and detection limits of 1.1×10^{-5} mol l^{-1} and 8×10^{-6} mol l^{-1} creatinine, over the pH range of 3.5–6.5 and 3.5–7.0, for *o*-NPOE and DOP solvent mediators, respectively. Sensor type (II) displays Nernstian responses over a concentration range of 6.0×10^{-5} mol l^{-1}–1.0×10^{-2} mol l^{-1} creatinine with cationic slopes of 60.0 ± 0.1 and 65.0 ± 0.2 mV decade^{-1} and detection limits of 1.5×10^{-5} mol l^{-1} and 1.4×10^{-5} mol l^{-1} creatinine over the pH range of 2.6–6.2 and 2.5–6.0, for *o*-NPOE and DOP solvent mediators, respectively. Sensor type (III) shows Nernstian responses over a concentration range of 7.0×10^{-5} mol l^{-1}–1.0×10^{-2} mol l^{-1} creatinine with cationic slopes of 60 ± 0.1 and 62.0 ± 0.2 mV decade^{-1} and detection limits of 2.7×10^{-5} mol l^{-1} and 2.0×10^{-5} mol l^{-1} creatinine over the pH range of 2.5–6.0, for *o*-NPOE and DOP solvent mediators, respectively. The response times of the sensors for 10^{-3} mol l^{-1} creatinine solution are instantaneous (4–10 s). The sensors show long-term stability with life span of ~6 months. The sensors are used for determination of serum creatinine of rats (*Rattus Norvigicus*) with mean R.S.D. of 2.62%, and the results agreed well with the Jaffe kinetic method.

Keywords: Neutral carrier; Crown ether; Potentiometric sensors; Lipophilic additives; Creatinine determination; Real samples

1. Introduction

Creatinine (2-amino-1,5-dihydro-1-methyl-4H-imidazol-4-one) is the internal anhydride of creatine and is the end product of creatine catabolism. Creatinine is found together with creatine in muscle tissue and blood. Creatinine is normal constituent of urine; daily output about 25 mg per kg of body weight as a breakdown product of the body tissues. Therefore, it is an important diagnostic substance in biological fluids. Creatinine analysis can be used for the diagnosis of renal, thyroid and muscle function. The normal range for serum/plasma creatinine is 35–140 μM [1] and during kidney dysfunction, it can rise to concentration higher than 1000 μM [2]. Creatinine is most widely analysed using Jaffée spectrophotometric reaction [3] or enzymatically [4,5].

Furthermore, sensors and enzymatic biosensors have many advantages over other techniques used to analyse creatinine in clinical laboratory. They reduce the time, complexity and cost of routine clinical analysis. Enzymatic biosensors include both amperometric using mono and trienzyme system [6–13] and potentiometric based on ion or gas sensitive electrodes [14–22] and ISFET [16,17]. Each system is characterized by certain advantages and disadvantages. Most of amperometric biosensors for creatinine are based on the three-enzyme method that involves the three-stage conversion of creatinine to creatine, creatine to sarcosine and sarcosine to glycine and H_2O_2 which is electrochemically detected. The three-enzyme system represents a complexity and low sensitivity for the amperometric system. Most of potentiometric biosensors are based on the catalysis of creatinine by creatinine iminohydrolase (CIH) at the surface of an NH_4^+-sensing ion-selective electrode. These systems have the advantage of relative simplicity but there is interference from endogenous NH_4^+ in blood and urine specimens.

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On the other hand, sensors based on neutral carrier ligands are well established for many cations and chiral ammonium ions [23]. The selection of suitable neutral carriers for ion sensing can be helped by structural studies on interactions between the carriers and ions. In this respect, Stoddart and co-workers [24,25] have shown interesting features concerning the interactions between macrocyclic polyethers and molecules and cations e.g., the bonding coordination through [N–H···O] hydrogen bonds as in the complexation between polyether and primary alkylammonium ions [24,25]. These studies encourage us to try dibenzo-30-crown-10 as a neutral carrier sensor for creatinine.

So far, there is no potentiometric sensor rather than the enzymatic biosensors for creatinine has been reported in the literature. In the present work we report three novel main types of potentiometric sensors for creatinine based on neutral carrier dibenzo-30-crown-10 with potassium tetrakis(*p*-chlorophenyl)borate, dibenzo-30-crown-10 alone and potassium-tetrakis(*p*-chlorophenyl)borate alone, incorporating in PVC matrix membranes and plasticized with either *o*-nitrophenyl octyl ether or dioctylphthalate. The sensors were used for monitoring creatinine after soaking the membranes in $0.1\,mol\,l^{-1}$ creatinine solution for at least 2 days. The sensors showed long-term stability, high sensitivity and selectivity for creatinine over creatine and NH_4^+ that interfere with the previously reported amperometric [7,9,11–13] and potentiometric [18,19] enzymatic biosensors. Furthermore, the developed sensors based method is simple, accurate, very low cost and far from the complexity of three-enzyme and single enzyme methods used in amperometric and potentiometric biosensors, respectively.

2. Experimental

2.1. Reagents and solutions

All of the reagents used were of analytical reagent grade unless otherwise stated and doubly distilled deionized water was used throughout. Dibenzo-30-crown-10 (DB30C10), potassium tetrakis (*p*-chlorophenyl)borate (PT*p*-ClPB) and *o*-nitrophenyl octyl ether were obtained from fluka (Buchs, Switzerland). Creatinine hydrochloride and dioctylphthalate were purchased from BDH (Poole, England). Poly(vinyl chloride) PVC (Breon S 110/OP) was obtained from BP Chemical International (Barry, UK). Tetrahydrofuran (THF) were obtained from Aldrich Chemical Co. (Milwaukee, USA).

A stock creatinine solution ($1.0\times10^{-1}\,mol\,l^{-1}$) was prepared, and dilute solutions ($1.0\times10^{-2}\,mol\,l^{-1}$–$1.0\times10^{-6}\,mol\,l^{-1}$) were prepared by serial dilutions, all the solutions were kept in fridge. A $1.0\times10^{-3}\,mol\,l^{-1}$ NaCl solution with acetate buffer of pH ≅ 5 was used as an ionic-strength adjuster, as appropriate.

2.2. Apparatus

Electrochemical measurements were made at room temperature ($25\pm1\,^{\circ}C$) with a PTI-15 digital pH meter using the developed potentiometric sensors in conjunction with an EIL-type RJ 23 calomel reference electrode. A glass Ag–AgCl combination electrode (consort, 5210 B BB5) was used for pH measurements. The ISE internal filling solution was a $0.1\,mol\,l^{-1}$ creatinine solution.

2.3. Creatinine PVC membrane sensors

The preparation and assembly of creatinine sensors were performed as described previously [26,27]. For sensor type (I), the quantities were DB30C10 (10 mg), KT*p*-ClPB (10 mg), *o*-nitrophenyl octyl ether or dioctylphthalate (360 mg) and PVC (170 mg). For sensor type (II), the quantities were DB30C10 (10 mg), *o*-nitrophenyl octyl ether or dioctylphthalate (360 mg) and PVC (170 mg). For sensor type (III), the quantities were KT*p*-ClPB (20 mg), *o*-nitrophenyl octyl ether or dioctylphthalate (360 mg) and PVC (170 mg). Table 1 summarizes the three main types of membrane compositions for creatinine sensors. The sensors were conditioned by soaking in $0.1\,mol\,l^{-1}$ creatinine solution for 2 days before use, and stored in the same solution when not in use.

2.4. Sensor calibration

The sensors were calibrated by spiking with successive aliquots of standard solution into a $10^{-6}\,mol\,l^{-1}$ solution of the calibrant. Alternatively, the calibration was carried out by immersing the sensors into 50 ml beaker containing 20 ml aliquots of standard $1.0\times10^{-6}\,mol\,l^{-1}$–$1.0\times10^{-2}\,mol\,l^{-1}$ creatinine solutions, starting from low to high concentrations. The calibration was also performed in presence of the ionic strength adjuster. The e.m.f values were recorded and plotted as a function of the logarithm of creatinine concentrations. The calibration graph was used for subsequent determinations of unknown concentrations of creatinine.

2.5. Selectivity coefficients

The potentiometric selectivity coefficients ($k^{pot}_{creat,B}$) were evaluated by the separate solution method [28] according to:

$$-\log(k^{pot}_{creat,B}) = \frac{E_{creat} - E_B}{S} \quad (1)$$

where E_{creat} and E_B are the response potentials of the sensors for the creatinine ion and interferent (B), respectively, at $10^{-2}\,mol\,l^{-1}$ and S is the sensor slope ($mV\,decade^{-1}$).

Table 1
Composition of PVC membrane types used for sensors construction

Membrane type	Membrane composition (% mass ratio)			
	Solvent mediator	PVC	DB30C10	KT*p*-ClPB
I	65.5	30.9	1.8	1.8
II	66.7	31.5	1.85	–
III	65.5	30.9	–	3.6