Biosensors and Bioelectronics 87 (2017) 587–592

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Characterization of a new ionophore-based ion-selective electrode for the potentiometric determination of creatinine in urine

Tomàs Guinovart [a], Daniel Hernández-Alonso [b], Louis Adriaenssens [b,c], Pascal Blondeau [a], F. Xavier Rius [a], Pablo Ballester [b,d], Francisco J. Andrade [a,*]

[a] Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili (URV), C/ Marcel·lí Domingo 1, 43007 Tarragona, Spain
[b] Institute of Chemical Research of Catalonia (ICIQ), The Barcelona Institute of Science and Technology, Av. Països Catalans, 16, 43007 Tarragona, Spain
[c] School of Chemistry, University of Lincoln, Brayford Pool, Lincoln, LN6 7TS, UK
[d] Catalan Institution for Research and Advanced Studies (ICREA) Pg. Lluís Companys 23, 08010 Barcelona, Spain

ARTICLE INFO

Article history:
Received 13 June 2016
Received in revised form
29 July 2016
Accepted 9 August 2016
Available online 10 August 2016

Keywords:
Creatinine
Ion-selective electrode
Calix[4]pyrrole
Ionophore
Membrane
Sensor
Potentiometry

ABSTRACT

The optimization, analytical characterization and validation of a novel ion-selective electrode for the highly sensitive and selective determination of creatinine in urine is presented. A newly synthesized calix[4]pyrrole-based molecule is used as an ionophore for the enhanced recognition of creatininium cations. The calculation of the complex formation constants in the polymeric membrane with creatininium, potassium and sodium confirms the strong selective interactions between the ionophore and the target. The optimization of the potentiometric sensor presented here yields an outstanding analytical performance, with a linear range that spans from 1 μM to 10 mM and limit of detection of $10^{-6.2}$ M. The calculation of the selectivity coefficients against most commonly found interferences also show significant improvements when compared to other sensors already reported. The performance of this novel sensor is tested by measuring creatinine in real urine samples (N=50) and comparing the values against the standard colorimetric approach (Jaffé's reaction). The results show that this sensor allows the fast and accurate determination of creatinine in real samples with minimal sample manipulation.

1. Introduction

Creatinine is a normal metabolic byproduct generated by the body during energy production (Burtis et al., 2012). Since its accumulation is toxic for the cells, a fine-tuned mechanism of excretion to avoid harmful levels of this substance is essential to sustain life. For this reason, creatinine is transported by bloodstream mostly to the kidneys, where it is filtered out and excreted via urination. Therefore, monitoring the levels of creatinine in both blood and urine is of outmost importance. As a key indicator of the kidney function (Israni and Kasiske, 2012), the levels of creatinine are an essential parameter to diagnose and monitor both chronic and acute kidney diseases such as infections, chronic failures, drug effects, etc. It is widely known that the concentration of creatinine in blood beyond certain threshold is a life threatening condition (Davila and Gardner, 1987). In chronic kidney disease (a condition that affects almost 10% of the world population (Eknoyan et al., 2013)) the evolution of the patient is monitored through the blood levels of creatinine. Urinary levels (in particular 24 h urinary excretion) are also important to evaluate the creatinine clearance. Last but not least, from an analytical standpoint the concentration of creatinine in urine is used as a normalization factor to minimize variability due to volume dilution (Viau et al., 2004; Wagner et al., 2010). For all these reasons, the determination of creatinine is among the most common routines of the clinical laboratory.

It may then come as something of a surprise that, despite of this relevance, current methods used for the determination of creatinine show so many drawbacks (Jacobs et al., 1991) that have raised serious concerns among the medical community (Delanghe et al., 2008). Today, the most widely used approach to determine creatinine is based on its reaction with picric acid (Jaffé reaction (Pizzolante, 1989)), a method that has been reported more than a century ago. Alternative enzyme-based colorimetric approaches are also used. However, most of these colorimetric methods are subject to errors deriving from sample color and common interferences like acetone and glucose that can perturb the color formation (Jacobs et al., 1991).

Potentiometry is a very attractive option for the clinical lab, mostly due to its robustness and simplicity of operation and instrumentation. Nowadays, potentiometric methods using ion-selective electrodes (ISEs) are part of the routine toolkit for the determination of pH and ions in biological fluids (Bakker and

* Corresponding author.
E-mail address: franciscojavier.andrade@urv.cat (F.J. Andrade).

http://dx.doi.org/10.1016/j.bios.2016.08.025
0956-5663/

Meyerhoff, 2007). As a further proof of their advantages, their use has been even extended to point-of-care approaches (Novell et al., 2014). Bühlmann and co-workers introduced an ISE for the direct determination of creatinine (Bühlmann et al., 2001). This ISE did not use any specific receptor for creatinine. Instead, it used a polymeric membrane with an ion-exchanger and chloroparaffin as the plasticizer. While creatinine could be determined in aqueous solutions, the biofouling produced by electrically neutral lipids becomes a serious limitation in any practical application. Evidently, the way to improve the selectivity of an ISE is the incorporation of an ionophore, *i.e.,* a synthetic receptor capable of binding the analyte via selective host-guest interactions. A creatinine ISEs based on this approach has been proposed some decades ago, but –once again- the performance obtained was not good enough to be applied to the analysis of real samples (Elmosallamy, 2006; Hassan et al., 2005; Kelly et al., 1995).

Recently, we reported, for the first time, an ISE capable of determining creatinine in real samples (Guinovart et al., 2016). In this work, we provide novel insights regarding the characterization, optimization and analytical performance of this novel sensor, together with the validation through the determination of creatinine in real urine samples. This ISE hinges on the use of a phosphonate-bridged calix[4]pyrrole ionophore that shows excellent selectivity for creatinine. Here, we report the complex formation constants of the ionophore with the targets in the polymeric membrane, which confirms its superiority compared to the previously reported ionophores. We further demonstrate the influence of the pH on the detection parameters. Lastly, particular emphasis was given to the real sample analysis and on the approach to reduce the matrix interference. Dilution appeared as a crucial way to afford reliable results, so that 50 real urine samples were analyzed using the potentiometric sensor and provided values that correlated excellently with the standard Jaffé's method.

2. Experimental

2.1. Electrode preparation

Glassy carbon (GC) (Sigradur-G, Germany) rods (length: 4 cm, diameter: 0.3 cm) were introduced into a Teflon body (length: 2 cm, diameter: 0.6 cm). One end of the GC was first polished with an abrasive paper (Carbimet 600/P1200, Buehler, USA) and afterwards with alumina of different grain-size (1 and 0.03 mm, Buehler, USA).

In the Supporting Information it is detailed the composition of the membrane selective for creatinine and the membranes composition used for the calculation of the complex formation constants.

2.2. Instrumentation

Electromotive force (EMF) was measured at room temperature (24 °C) with a high-input impedance (10^{15} Ω) EMF16 multichannel data acquisition device (Lawson Laboratories, Inc. Malvern). A double junction Ag/AgCl/KCl 3 M reference electrode (type 6.0726.100, Metrohm AG) containing 1 M LiOAc electrode bridge was used in all the potentiometric experiments.

The complex formation constants were obtained using a Philips IS-561 (Electrode Body ISE, Selectophore) from Sigma-Aldrich (Spain). The procedure described by Bakker and co-workers (Mi and Bakker, 1999) was followed to calculate the binding constants (see SI for more information). It has to be pointed out that for the calculation of these constants, both ion-pairing effects and membrane-internal diffusion are not taken into account.

2.3. Determinations in real samples

Urine samples were diluted with 50 mM HOAc/$Mg(OAc)_2$ at pH 3.8 in order to ensure the creatinine is in its positively charged state (creatininium cation). Samples were diluted at 1:2, 1:10 and 1:100 ratio in order to evaluate the influence of the matrix components. The results obtained were compared against the standard Jaffé method. Best predictions were obtained when samples were diluted 1:100, as it will be discussed below. Therefore, this dilution factor was used for the determination of creatinine in all the urine samples. The EMF was recorded until stable potential readings are obtained (typically less than a minute) and afterwards, the electrodes are thoroughly cleaned with double distilled water.

Under optimized conditions, the colorimetric determination of creatinine using the Jaffé's reaction was carried out using 2 mL of 10 mM of picric acid in strongly basic media (250 mM NaOH) in a cuvette where 2 μL of the standard (or the sample) were added and let react for 30 min. The absorbance of the product was followed at 505 nm. Considering the actual concentration in the cuvette (*i.e,* a dilution factor of 1:100 for standards and sample), the linear range spans from 1 μM to 100 μM of creatinine.

3. Results and discussion

3.1. Calix[4]pyrrole ionophore

The calix[4]pyrrole molecule used as ionophore in this study (Fig. 1b) has been recently reported by our group (Guinovart et al., 2016). There are multiple host-guest interactions that make the synthetic receptor ideal to entrap creatinine inside its cavity. These can be seen in the crystal structure calculated by X-ray of the host-guest complex featuring the ionophore and neutral creatinine (Fig. 1c). The cationic species, the creatininium cation (Fig. 1a), binds to the ionophore differently, making complexes with 2:1 guest: host stoichiometry. However, the binding mode within the deep cavity of the calix[4]pyrrole has been shown to be similar. This is relevant in potentiometry since charged species are

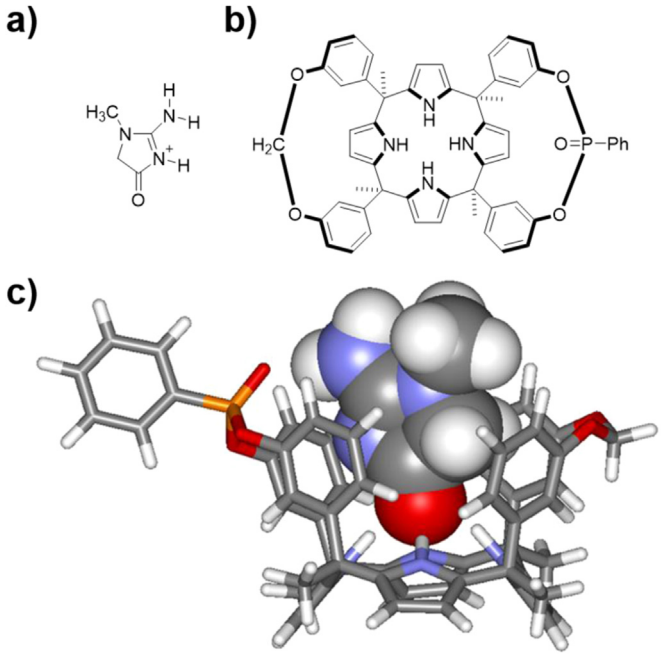

Fig. 1. Molecular structure of a) creatininium cation, b) calix[4]pyrrole molecule and c) X-Ray crystal structure of the calix[4]pyrrole ionophore with creatinine inside the cavity.