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Measurement of total calcium by flash chronopotentiometry at polymer membrane ion-selective electrodes

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ABSTRACT

Ionophore-based ion-selective electrodes are widely used for potentiometric electrolyte measurements, in which case they are known to detect the free ion activity. Total ion concentrations cannot be directly assessed by this methodology if the ion is predominantly present in a complexed form. We present here the direct measurement of total calcium using a calcium ion-selective electrode interrogated in a flash chronopotentiometric transduction mode. A high magnitude of cathodic current pulse is applied across a calcium ion-selective membrane containing the ionophore ETH 5234 but void of ion-exchanger to prevent spontaneous extraction. This induces a defined flux of calcium ions from the sample side to the membrane and results in the release of labile bound calcium and a concomitant depletion at the membrane surface at a critical current or time. This is observed as an inflection point on the potential–time curve and the square root of the transition time is linearly related to the total concentration in the sample. It is shown that the responses to solutions of labile calcium complexes of nitrilotriacetic acid (NTA) are in a good agreement with that of the same concentration of calcium chloride in saline solution with this protocol. Initial applications are aimed towards assaying extracellular calcium. Calcium binding to albumin is shown to be inconsequential with sample dilutions typical for clinical assays. Calcium calibration curves in real and artificial dilute serum are finally shown to correspond to that of calcium chloride, suggesting that the methodology is indeed capable of detecting total calcium under these conditions. The present membrane materials allow detection of up to over 0.5 mM total calcium in serum, currently requiring such samples to be diluted about 5-fold. The slopes of the square root of time–concentration dependence for the calibrations of free calcium in a background of NaCl and total serum calcium were found to be 3.857 and 3.717 $s^{1/2}$ mM^{-1}, respectively, deviating by just 3.6%. The lower detection limit (3× SD) was calculated as 12 μM.

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1. Introduction

Direct potentiometry with calcium ion-selective electrodes has been established as a reliable and precise method, especially for the detection of calcium in biological fluids [1–3]. This method measures only the ionized calcium form. Anker et al. [4] measured total serum calcium potentiometrically after releasing the bound calcium by acidifying a 20-times diluted serum sample to pH 3.5, thus giving analytical information comparable to atomic spectrometric methods. Although this work was the first to make total calcium measurements in physiological samples possible with well-established potentiometric measurement with ion-selective electrodes, the acidification step may lead to undesirable sample composition change. Another potentiometric method of measuring total calcium in blood serum using the so-called constant complexation buffer (CCB) procedure was reported [5], but still involves altering the bulk sample composition.

Preliminary work by Ceresa et al. showed the possibility of obtaining information on free and total calcium ion concentrations in the presence of labile complexes, without alteration of the sample bulk composition, using direct potentiometry with ion-selective electrodes [6]. Here, ion-selective electrodes with low ion activity in the inner filling solution were used to induce strong zero current fluxes of the measuring ions towards the inner solution. The strong inward flux resulted in ion depletion at the membrane surface and a concomitant apparent super-Nernstian response [7]. Under this non-equilibrium situation, in the presence of labile complexes, the total concentration governs the phase boundary activities and hence the electrode responses. Later, ion-selective electrodes with highly enhanced sensitivity, so-called switchtrodes

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[8], were established on the basis of ion depletion at the membrane surface at a critical ion activity. However, the ion flux in this high sensitivity super-Nernstian response region is not easily controlled in classical potentiometry and leads to non-reproducible and drifting responses [9,10].

Pulsed chronompotentiometry with polymeric membrane ion-selective electrodes [11] offers a complete instrumental control of ion fluxes owing to the lack of ion-exchanger properties of the membrane. This may result in a stable and reproducible potential response that includes the high sensitivity super-Nernstian region [12]. This advantage was utilized recently to detect calcium in diluted artificial blood serum with very high sensitivity, using differential signals obtained from pulstrode responses under consecutive pulse times [13]. In a preliminary work, information on free and total calcium ion concentration was obtained using pulsed chronopotentiometry by varying the magnitude of the applied current [14].

A new and analytically useful application of pulsed chronopotentiometry was shown very recently where a local flash titration at a polymeric ion-selective membrane electrode was performed to measure total acidity [15]. The method was utilized to determine the total acid content in different samples in a matter of seconds and proved to offer reliable quantitative information in a rapid and simple manner.

This paper presents the measurement of available calcium in physiological samples via local titrations at the surface of calcium ion-selective membrane. Given that the normal range of blood calcium is very narrow and total calcium is measured routinely in clinical laboratories, a precise, rapid, simple, and inexpensive detection method is of significant interest and the method proposed here is anticipated to be promising in this regard. More importantly, this technique may offer a unique opportunity of measuring free and total calcium levels in the same sample. Here, a cathodic current pulse applied across a Ca^{2+} selective electrode causes a strong flux of the calcium ions from the sample side to the membrane and concomitantly forces the release of bound calcium ions. A depletion of calcium ions at the membrane surface results when the electrochemically imposed ion flux can no longer be maintained by the delivery of the bound calcium from the sample. The membrane surface ion depletion, which is characterized by an inflection point in the observed chronopotentiogram, occurs at a given transition time or a critical current that is a direct function of total ion concentration as depicted by the Sand equation:

$$i = \frac{nFAc}{2}\sqrt{\frac{\pi D_{aq}}{t}} \qquad (1)$$

where i is the critical current, n is the charge on the analyte ion, A is the membrane area, c is the ion concentration, D_{aq} is the diffusion coefficient of the analyte in the aqueous phase and t is the time during which the current is applied.

2. Experimental

2.1. Reagents

High molecular weight poly(vinyl chloride) (PVC), o-nitrophenyl octyl ether (o-NPOE), tetradodecylammonium tetrakis(4-chlorophenyl) borate (ETH 500), calcium ionophore, N,N′-dicyclohexyl-3-oxapentanediamide (ETH 5234), tetrahydrofuran (THF), potassium tetrakis(4-chlorophenyl)borate (KTpClPB), nitrilotriacetic acid disodium salt (Na_2NTA) and all salts were purchased from Fluka (Milwaukee, WI). Standard solutions of sodium hydroxide and hydrochloric acid were purchased from Sigma–Aldrich (St. Louis, MO). Solution of artificial blood serum (ABS) was prepared from the respective compounds in the following concentrations: [Na^+] 140 mM, [Cl^-] 101 mM, [K^+] 5 mM, [Mg^{2+}] 1.5 mM, [CO_2]$_{tot}$ 40 mM, [Phosphates] 2 mM and bovine serum albumin [BSA] 40 g L^{-1}. Aqueous solutions were prepared by dissolving the appropriate compounds in Nanopure-deionized water (18.2 MΩ cm).

2.2. Membrane preparation

Calcium ion-selective membrane (~200 μm thick) for the chronopotentiometric sensor was prepared by solvent casting with THF as a solvent, a membrane cocktail containing 10 wt% of the inert lipophilic salt ETH 500, 40 mmol kg^{-1} ETH 5234 and PVC and o-NPOE 1:2 by weight. The membrane for the direct potentiometric measurement was formulated with polymer matrix of the same (polymer:plasticizer) weight ratio as above containing 10 mmol kg^{-1} calcium ionophore (ETH 5234) and 5 mmol kg^{-1} potassium tetrakis(4-chlorophenyl)borate (KTpClPB) as ion exchanger.

2.3. Electrodes

The membranes were cut with a cork borer (6 mm diameter) from the parent membrane and incorporated onto a Philips electrode body (IS-561, Glasblaserei Moller, Zurich, Switzerland). The outer membrane area was calculated from its geometry as 20 mm^2. The inner solution was in contact with an internal Ag/AgCl electrode. The external reference electrode was a double-junction Ag/AgCl electrode with saturated KCl as inner solution and a 1 M LiOAc bridge electrolyte. A high surface area coiled Pt-wire was used as a counter electrode in contact with the sample. The working electrodes were conditioned for at least 12 h prior to experiments and kept in the conditioning solution when experiments were not underway. The inner filling and conditioning solution was 10 mM NaCl.

2.4. Experimental setup

A conventional three-electrode setup was used for the chronopotentiometric measurements where an internal Ag/AgCl electrode acted as the working electrode and the external reference electrode and counter electrode were immersed into the sample solution. The galvanostatic measurements were conducted with an AFCBI bipotentiostat (Pine Instruments, Grove City, PA) controlled by a PCI-MIO-16E4 interface board and LabVIEW 5.0 data acquisition software (National Instruments, Austin, TX) on a Macintosh computer. The potentials were sampled at 2 ms intervals. For fixed time experiments, the potential was calculated during the last 10% of the cathodic current pulse time, with an uptake time of 1 s and a stripping time of 15 s was used throughout the experiment unless specified otherwise. A baseline potential pulse of 0 V versus Ag/AgCl was applied as a stripping potential. For the details of potentiostatic/galvanostatic control switching system, see reference [11]. Zero current potentiometric measurements were conducted with a PCI MI016XE data acquisition board (National Instruments, Austin, TX) using a 4-channel high impedance interface (WPI, Sarasota, FL). The potential of the working electrode was measured against the same reference electrode as above in the conventional two-electrode configuration. All experiments were conducted at room temperature (21–22 °C).

3. Results and discussion

Since direct potentiometry with ion-selective electrodes measures free ion activity, the method is of limited utility for the detection of total calcium levels. Fig. 1 shows a direct potentiometric calibration curve of released calcium upon acidification of 5 times

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