Registration of small changes of calcium ion concentration by the constant potential coulometric method

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1. INTRODUCTION

Clinical analysis requires control of ionized calcium in blood. Potentiometric sensors (ion-selective electrodes, ISEs) used in clinical analyzers are successfully used for measurements of Ca^{2+}. It is, however, desirable to improve the sensitivity of the measurements, given the range of Ca^{2+} in human blood is narrow: 1.16 - 1.32 mM. The constant potential coulometry (CPC) allows for improved sensitivity [1, 2], and is therefore a promising alternative to zero-current potentiometry. In this work we used the CPC method for Ca^{2+} quantification with sensitivity of 0.1%, utilizing solid-contact Ca^{2+}-ISE with PVC membrane containing ETH 1001, KClTPB, ETH 500 and oNPOE.

2. ELECTROPOLYMERIZATION
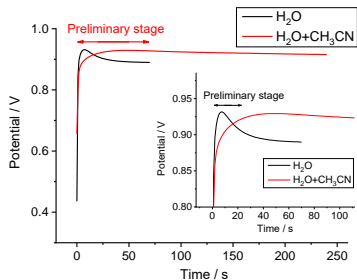
The dissolution of EDOT in water is very slow, typically 10-12 hours with continuous stirring. To speed up the procedure, we used a mixed water-acetonitrile solution. The process of galvanostatic polymerization from a mixed solution, per se, is slower than from aqueous solution, but the entire procedure for obtaining a PEDOT-PSS film, including solution preparation, takes about 30 minutes.

3. NEW MEASUREMENTS PROTOCOL

Stirring is necessary to equalize the concentration throughout the sample volume after dilution or addition. However, stirring causes noise. Therefore, we modified the measurement procedure: cell OFF before addition/dilution, stirrer ON for 20 s, then stirrer OFF and cell ON. The noise was decreased roughly 50 times.
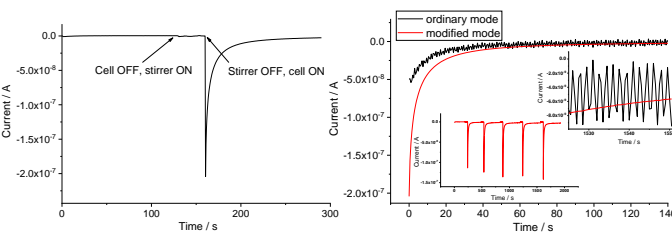
4. Ca^{2+} INTERACTION WITH BSA

Kragh-Hansen/Vorum's model [3]:

$K_1 = [CaBSA^{(x-2)-}]/(a_{ca} \cdot [BSA^{x-}])$

$K_2 = [Ca_2BSA^{(x-4)-}]/(a_{ca}^2 \cdot [BSA^{x-}])$

$K_1 = 1513, K_2 = 647$

$$[BSA] = \frac{[BSA^{tot}]}{(1 + a_{ca} \cdot K_1 + a_{ca}^2 \cdot K_2)}$$

BSA^{tot}	$[Ca^{2+}]$	$[BSA]$	$[Ca^{tot}]$
мM	мM	мM	мM
0.150	1.98	0.038	2.10
0.301	1.81	0.080	2.03
0.451	1.66	0.128	1.98
0.602	1.56	0.179	1.98
0.752	1.43	0.237	1.95

BSA^{tot} varied, $[Ca^{2+}]$ measured potentiometrically, [BSA] calculated, $[Ca^{tot}]$ calculated for control (target value 2 mM).
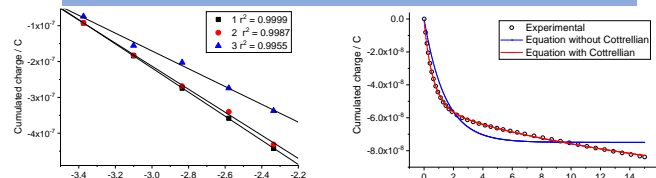
5. SERUM SAMPLE

Registration of 0.1 % changes in the content of ionized calcium in blood serum sample, whereas the $[Ca^{2+}]/[Ca]^{tot}$ ratio remains constant and equal to 0.7868.

$Q = 2.36 \cdot 10^{-10} - 1.05 \cdot 10^{-7} \cdot \Delta C_{Ca}$
$r^2 = 0.9994$
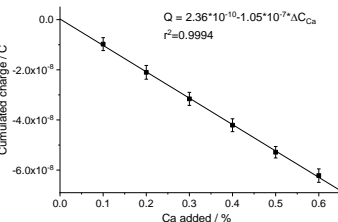
Linearity to changes in concentration, not the logarithm of concentration, is determined by the fact that additions are small compared to the initial value of the Ca^{2+} concentration.
The extrapolation of the graphs to zero additive corresponds to an almost zero charge value, which is consistent with the idea of constant potential coulometry.

6. CALIBRATION vs. FITTING

$$Q = Q^0 + S_q log(a_{ca}^{ini}/a_{ca}^{fin})$$

$$Q_{(t)} = \frac{RT}{z_1 F} ln \frac{a_i^{ini}}{a_i^{Fin}} \left[C_{CP} \left(1 - e^{-\frac{t}{R_{Mem}C_{CP}}} \right) + N\sqrt{t} \right]$$

Equation containing the Cottrellian term allows nice fitting of the charge curve. It is therefore possible to perform analysis using (i) calibration curves, or (ii) fitted parameters. Both methods provide fairly good results.
Some of the measurements was performed with an electronic capacitor with value of 10 μF in series with the ISE.

| Target, mM	Measured, mM	Recovery, %	Method
0.400	0.403	101.8	fitting
no capacitor	0.387	96.7	calibration
0.400	0.395	98.2	fitting
with capacitor	0.368	92.1	calibration
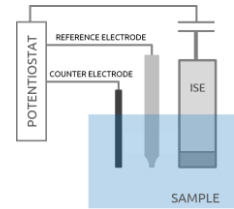
7. CLASSICAL ISE, "ARTIFICIAL" SIGNAL
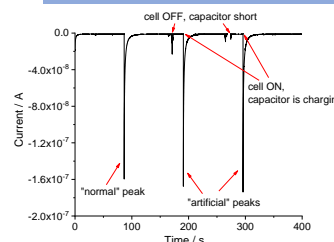
Potentials of classical ISEs with internal solution, normally, are more stable over time than that of solid-contact ISEs. For CPC measurements classical ISE must connected with a capacitor in series. Once a "normal" current peak is recorded, the following procedure: cell OFF, capacitor discharge, cell ON, allows obtaining "artificial" current peaks, the same as the "normal", and forming the same calibration plot.

8. SIMULATION OF NON-IDEALITIES

The potential setting by a potentiostat cannot be absolutely ideal, neither ideal is the stability of the E^0 of the ISE (drift). The respective errors, in the case of small additions/dilutions may cause inversed current peaks. The possible impact from these non-idealities was simulated, the experimental results are consistent with the calculated. The current depends linearly on the inaccuracy of the OCP setting, whether due to instrument imperfections or drift of the standard electrode potential.
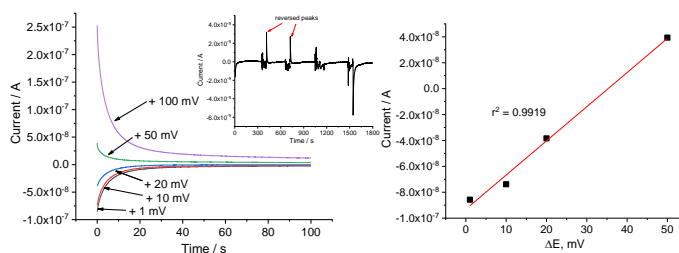
9. CONCLUSIONS

A novel measurements protocol allows decreasing electrostatic noise significantly, making it possible to register 0.1% changes of Ca^{2+} concentration.
Simple approach can be applied to the calculation of the ionized calcium content in samples with known total calcium and total BSA concentrations.
Measurement of Ca^{2+} concentration can be performed with calibration curves and also using the charge curve fitting parameters.
"Artificial" current peaks can be also used for analysis.
Possible impact of the ISE potential drift and non-ideality of the potential setting by the instrument is simulated.
A convenient procedure is developed for deposition of PEDOT-PSS on glassy carbon surface.

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[6] A.V. Bondar, K.N. Mikhelson, Constant Potential Coulometric Measurements with Ca^{2+}-Selective Electrode: Analysis Using Calibration Plot vs. Analysis Using the Charge Curve Fitting. Sensors 2022, 22(3), 1145.