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Obtaining Nernstian Response of a Ca^{2+} -Selective Electrode in a Broad Concentration Range by Tuned Galvanostatic Polarization

Maria A. Peshkova,[†] Tomasz Sokalski,[‡] Konstantin N. Mikhelson,^{*,†} and Andrzej Lewenstam^{*,‡}

Chemical Faculty, c/o St. Petersburg State University, Universitetskij Pr. 26, 198504 St. Petersburg, Russia, and Process Chemistry Centre, c/o Centre for Process Analytical Chemistry and Sensor Technology "ProSens" and Laboratory of Analytical Chemistry, Åbo Akademi University, Biskopsgatan 8, FIN-20500 Åbo/Turku, Finland

Linear Nernstian response is obtained for a neutral ionophore-based Ca^{2+} -selective electrode down to 10^{-10} M CaCl_2 by means of galvanostatic polarization. The densities of the applied cathodic current were tuned for particular concentrations of Ca^{2+} . The procedure included recording the potential at zero current, followed by measurements when current is passed through the electrode, and then again at zero current. The respective chronopotentiometric curves included negative ohmic drop immediately after turning the current on, the polarization domain, and positive ohmic drop when the current was turned off, followed with the relaxation domain. The potentials immediately after the positive ohmic drops were used as analytical signals. These potentials make a straight line with Nernstian slope when currents are tuned (optimized) for each particular concentration. An iteration procedure is proposed which allows for simultaneous optimization of the current density and accessing analyte concentration in the sample.

After pioneering work¹ describing large improvement of the low detection limit of ionophore-based ion-selective electrodes (ISEs) works aimed at potentiometric measurements in subnanomolar, and even lower, concentration ranges became a mainstream of the ISE research. Deviations from Nernstian response of ISEs in micromolar and submicromolar concentration range are caused by local increase of the concentration of analyte ions in sample close to the sensor membrane surface. It is now generally recognized that this local increase of the analyte concentration is caused by transmembrane fluxes of ions from the internal filling solution to the sample, as well as the fluxes caused by the replacement of the primary ions in the membrane with the interfering ions due to ion exchange.^{2–4} These fluxes

have been registered experimentally in ref 5. The internal reference system of a solid contact ISE, e.g., based on a conducting polymer, also may be a source of analyte ions which eventually contaminate sample solutions, although to somewhat lesser extent than in the case of ISEs with internal aqueous solution.^{6–8}

The driving force for these fluxes is the huge difference in the activities of the target analyte in diluted samples and in internal solutions containing analyte ions in millimolar concentration or at even higher level. The first approach aimed at reduction of the transmembrane fluxes was invented and used in ref 1. It relied on maintaining the activity of the target analyte in the internal filling solution of an ISE at a very low level by means of a suitable buffer, while maintaining also a sufficiently high activity of an interfering ion. Under these conditions the analyte ions in the ISE membrane close to the internal surface are largely replaced by the interference, producing a gradient of the concentration of the analyte ions across the membrane. This gradient, ideally, eliminates ion fluxes directed to the sample and thus ensures Nernstian response of the ISE down to very low concentrations. If no suitable buffer exists, low activity of analyte in the internal solution may be kept by using, i.e., ion-exchange resins.^{9,10}

The disadvantage of this (chemical) approach is that the transmembrane fluxes of ions are eliminated, strictly speaking, at only one concentration of the analyte in the sample. From the practical viewpoint this means that the slope in even more diluted solutions is super-Nernstian because then the flux is directed toward the internal solution and the sample in the vicinity of the membrane is depleted of the analyte ions.

More advanced approaches are based on the theory of diffusion and suggest modifications of the membrane geometry or composition.¹¹ The magnitude of the transmembrane flux can be decreased by lowering the respective driving force, the gradient of the analyte ion concentration, using therefore thicker mem-

* To whom correspondence should be addressed. E-mail: konst@km3241.spb.edu (K.N.M.), andrzej.lewenstam@abo.fi (A.L.).

[†] St. Petersburg State University.

[‡] Åbo Akademi University.

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branes.^{12,13} Also, the flux can be decreased by reducing ion diffusion coefficients, i.e., by higher content of polymer in the membrane.¹⁴ Transportation of ions across membranes can be also minimized by means of dispersing of silica gel microparticles in the membrane.¹⁵ This is how the deviations from Nernstian response in diluted samples can be minimized at the cost of increasing sensor resistance. Also, when microparticles are incorporated membranes show some loss of selectivity.¹⁵ Acceleration of the ion transport in the sample phase can be achieved using a rotating disk electrode or just by stirring.¹⁶ A variety of these approaches was critically analyzed and evaluated elsewhere.⁴

An alternative way aimed at elimination of transmembrane fluxes is based on galvanostatic polarization of the ISE. This (electrochemical) approach has been proposed by the first time in ref 17 and since then was utilized by several groups of researchers.^{7,8,18–21} By applying a suitable current, one can eliminate the transmembrane ion flux. Galvanostatic polarization seems to be a more flexible approach aimed at the improvement of the low detection limit, as compared with the modification of the composition of the internal solution or of the composition and/or the geometry of the membrane. However, the net flux across the membrane is zero if the particular concentration gradient is counterbalanced by the electric field induced by the particular current. This is why this approach, as so far reported, works ideally only in a very narrow concentration range, similarly to the chemical approach which relies on buffering internal solution.

One can hope to obtain Nernstian response in a broad concentration range by applying currents of different magnitudes tuned for particular concentrations of the analyte in the sample. Another possibility is choosing a potential recorded at a particular time of the chronopotentiometric experiment as the analytical signal, so the time must be tuned for the particular concentration of the analyte. These options are challenging, and to the best of our knowledge, have been only scarcely explored.²² ISE membrane potentials at non-zero-current conditions were thoroughly analyzed recently.^{23–25} However, it is not completely clear what exactly is the voltage measured when an ISE is galvanostatically polarized. In particular, it is not clear whether only transportation

of ions is altered by passing current or also the electrochemical equilibrium at the membrane/solution interface is disturbed because of the polarization. From the practical point of view, using different currents or polarization times for different concentrations of the analyte means having two unknown variables instead of one: if the concentration is not known, the suitable magnitude of current (or suitable time) is not known either. Thus, the analytical relevance of such measurements may seem questionable.

In this work these challenges are addressed experimentally using a calcium-selective ISE based on neutral ionophore ETH 1001. This particular ISE was chosen primarily because of its excellent performance in zero-current potentiometric measurements, so this electrode is a suitable object for studying a novel measurement technique. The possibility of getting linear Nernstian response within a broad concentration range (from 0.1 to 10^{-10} M) by means of tuned galvanostatic polarization of ISEs is demonstrated here for the first time. Moreover the procedure is developed which allows for analytical application of the novel technique.

EXPERIMENTAL SECTION

Calcium-selective neutral ionophore diethyl *N,N'*-[(4*R*,5*R*)-4,5-dimethyl-1,8-dioxo-3,6-dioxaoctamethylene]bis(12-methylamino-dodecanoate)] (ETH 1001), potassium tetrakis-*p*-chlorophenylborate (KCIPB), tetradodecylammonium tetrakis-*p*-chlorophenylborate (ETH 500), solvent plasticizer *o*-nitrophenyloctyl ether (*o*-NPOE), high molecular weight poly(vinyl chloride) (PVC), and tetrahydrofuran (THF) were Selectophore grade reagents from Fluka (Switzerland). Extra pure cyclohexanone (CH) was also from Fluka. Analytical grade inorganic electrolytes KCl, CaCl₂, and NaOH were from J. T. Baker, Baker Analyzed (Holland). Ethylenediaminetetraacetic acid disodium dihydrate (Na₂EDTA) was from Merck (Germany), and HEPES was from BDH (U.K.).

All aqueous solutions were prepared with deionized ELGA Purelab Ultrawater (DI water) with resistivity 18.2 MΩ·cm.

Membrane cocktail was prepared by dissolving appropriate amounts of PVC and *o*-NPOE in THF. After that calcium ionophore ETH 1001 (23 mM), KCIPB (16 mM), and ETH 500 (17 mM) were added as appropriate aliquots of stock solutions in CH. In this way weighing small amounts was avoided, ensuring high accuracy of the membrane composition.

To obtain the membranes the cocktail was stirred for 10 min and then cast on a Teflon Petri dish with diameter of 30 mm and placed into a larger Petri dish closed with filter paper to slow down the evaporation of THF. Complete evaporation of THF and CH took 2 days, and after that master membrane with thickness of about 0.4 mm was obtained.

The electrodes were prepared by cutting disks with diameter of 8 mm from master membrane and gluing them to PVC bodies with outer diameter of 8 mm and inner diameter of 4 mm. A solution of PVC in CH was used as the glue.

Pure CaCl₂ solutions as well as CaCl₂ with 0.001 M KCl background were prepared by sequential dilution of stock 0.1 M CaCl₂ solution with DI water or with 0.001 M KCl. The dilution was performed directly in the course of calibration procedure using a Metrohm 700 Dosino automatic diluter controlled by a Metrohm 711 Liquino controller.

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Calcium ethylenediaminetetraacetic acid buffer solutions were prepared using appropriate aliquots of stock 1 M KCl, 0.1 M Na₂EDTA, and 0.1 M HEPES. The pH of the buffers was adjusted by additions of small amounts of 0.1 M NaOH under control by a Metrohm 691 pH electrode.

Before measurements the electrodes were filled with 0.01 M solution of calcium chloride and conditioned in the same solution for 2 days. Electrodes were kept in the same solution between measurements. Zero-current potentiometric measurements were performed with a custom-made multichannel potentiometric station. Chronopotentiometric curves and electrochemical impedance spectra were recorded with an Autolab 30 potentiostat-galvanostat with an FRA 2 frequency response analyzer module (Eco Chemie). The internal electrode in the Ca ISE was Ag/AgCl. The reference electrode was a single junction Ag/AgCl electrode with 3.5 M KCl, and the counter electrode for chronopotentiometric and impedance measurements was a glassy carbon rod.

Chronopotentiometric calibrations were always started from 10⁻⁵ M CaCl₂ solution. Electrodes were thoroughly rinsed with deionized water after storage in 10⁻² M CaCl₂ before measurements in 10⁻⁵ M solution. Background electrolyte (if used) was 10⁻³ M KCl and was added as an appropriate aliquot of 1 M KCl. The applied current was zero for the first 600 s, followed by the chosen current for the next 600 s, and then again zero current for the last 600 s. Impedance spectra were recorded in the frequency range of 100 kHz to 10 mHz using sinusoidal polarizing signal with the amplitude of ±5 mV. The measurements were carried out at room temperature: 23 ± 1 °C.

RESULTS

Zero-Current Measurements in Solutions Buffered with EDTA. It is now believed that the low detection limit of ionophore-based ISEs is determined by contamination of the sample with analyte ions coming from the membrane. It was therefore worth to check the value of the detection limit when solutions are buffered so that activity of calcium ions is adjusted at a certain level. This idea was exploited for the first time decades ago with Ca²⁺ ISEs based on alkylphosphoric acids^{26–29} and then also with a neutral ionophore-based electrode.³⁰ This idea assumes that the kinetics of complexation/decomplexation of Ca²⁺ by the buffering agents, e.g., EDTA, is significantly faster than the kinetics of Ca²⁺ transfer across the membrane/solution interface. Although none of these processes were studied comprehensively, we do assume here that the homogeneous binding/release reaction is at equilibrium at any actual value of the activity of ionized calcium, and everywhere in the sample solution, including the closest vicinity of the membrane. This means that transmembrane fluxes do not alter Ca²⁺ activity in buffered solution. Strictly speaking, even under this assumption measurements in buffered solutions do not necessarily deliver the ultimate detection limit because the selectivity of the ISE to the analyte ion is not unlimited, so other electrolytes present in the buffered sample also contribute to the membrane potential.

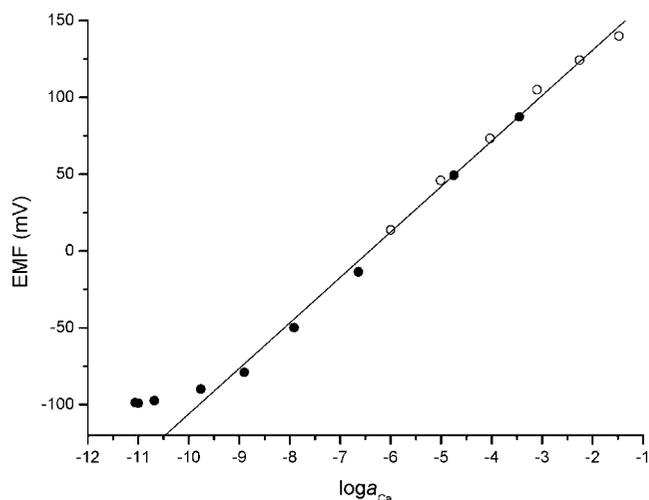


Figure 1. Calibration of a Ca²⁺ ISE in pure CaCl₂ solutions (open symbols) and in solutions buffered with EDTA (filled symbols).

The goal of this part of the study was therefore to determine the low detection limit of the Ca²⁺ ISE under particular conditions when solutions are buffered with EDTA. Calcium metal buffers contained 0.001 M CaCl₂, 0.002 M Na₂EDTA, 0.01 M HEPES, with 0.1 M KCl background. The pH of the buffers, and therefore also activity of ionized calcium, was adjusted by adding small volumes of 1 M NaOH. Calcium ion activity was calculated by solving system of equations comprising mass balance of calcium and mass balance of EDTA for known value of pH, together with Davis equation for Ca²⁺ activity coefficient at actual ionic strength. The values of the respective equilibrium constants were taken from survey.³¹ These values were as follows: log K₁ = 10.23, log K₂ = 6.18, log K₃ = 2.66, log K₄ = 2.01 for H₄EDTA association constants, and log K_{CaHL} = 3.51, log K_{CaL} = 10.59 for CaH₃EDTA and CaH₂EDTA stability constants.

The results of the EMF measurements in pure CaCl₂ solutions and in solutions buffered with EDTA are presented in Figure 1.

As can be seen, the electrode shows a linear Nernstian response over a broad Ca²⁺ activity range, from 10⁻¹ to 10⁻⁹ M, and the slope is 29.6 mV. This broad linear range is consistent with very high selectivity over K⁺ for Ca²⁺ ISEs based on ETH 1001 reported in buffered solutions: log K = -6.6.³² Deviations from Nernstian response apparently due to the high (0.1 M) KCl background were observed in solutions with Ca²⁺ activity 10⁻¹⁰ M and lower. It is important that the two sets of measured EMF values, in pure CaCl₂ and in the buffered solutions, produce the same straight line. This indicates consistency of the calculations of Ca²⁺ ion activity in pure and buffered solutions and, although indirectly, indicates the reliability of the Davis equation (third approximation of Debye–Hückel theory) for calcium at relatively high ionic strength.

Chronopotentiometric Measurements in Nonbuffered Solutions. Being assured that the ISE, in principle, is responding linearly to Ca²⁺ ions down to very low ion activities, we recorded chronopotentiometric curves applying cathodic current with density of 50 nA/cm². The electrode potential was recorded for 600 s without polarization, then current was applied for another

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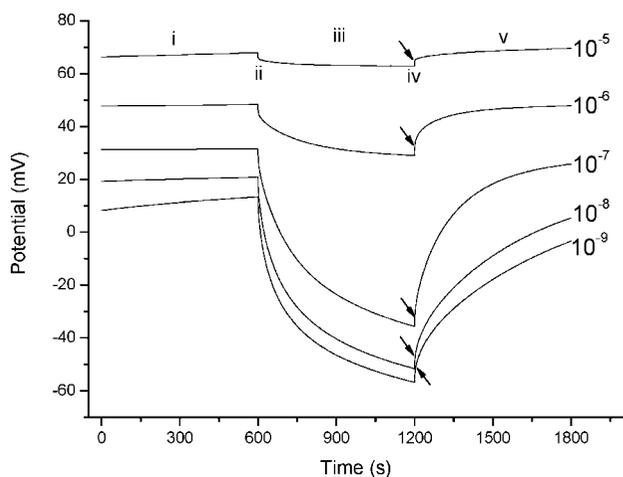


Figure 2. Chronopotentiometric curves obtained with a Ca^{2+} ISE in 10^{-5} – 10^{-9} M CaCl_2 solutions containing 0.001 M KCl background polarized by cathodic current of 50 nA/cm^2 . Arrows show beginnings of relaxation curves, immediately after positive ohmic drops.

600 s, and after that current was turned off, and the relaxation of the ISE potential was recorded for yet another 600 s. This particular protocol, 600/600/600 s, as well as the particular current density of 50 nA/cm^2 were chosen arbitrarily and are objects for optimization. The experiments were performed with pure CaCl_2 solutions and also with the same solutions containing 0.001 M KCl background.

Chronopotentiometric curves recorded for 10^{-5} – 10^{-9} M CaCl_2 solutions with KCl background are presented in Figure 2. Each curve may be divided into five domains. These are (i) the initial plateau when no current is passed, (ii) ohmic drop in negative direction when current is turned on at 600 s, followed by (iii) polarization curve. When current is turned off at 1200 s (iv) ohmic drop in positive direction appears, followed with (v) relaxation curve. The absolute values of the negative and the positive ohmic drops are the same, and these are the same also for all concentrations of CaCl_2 , within experimental error. The curves recorded in solutions without KCl background (not presented) are very similar, but the ohmic drop values increase with decrease of the concentration of CaCl_2 pointing at significant impact from the solution resistance at low concentrations.

Three sets of the data obtained from the chronopotentiometric curves shown in Figure 2 are presented in Figure 3. Potentials recorded at concentrations higher than 10^{-5} M make a straight Nernstian line and are not shown here. The first set of the data comprises potentials recorded under zero-current conditions (line 1). These values give a calibration curve which is typical for measurements with conventional ISEs, with the detection limit of about 3×10^{-6} M CaCl_2 . Obviously, transmembrane fluxes under these conditions are steady and govern the ISE response.

The second set consists of the potentials recorded immediately after the positive ohmic drop (Figure 3, line 2). The time resolution was 0.2 s, so here and below “immediate” means 0.2 s after current is turned off. These data give a curve with a super-Nernstian jump between 10^{-6} and 10^{-7} M CaCl_2 followed by sub-Nernstian response in between 10^{-7} and 10^{-8} M, and in more diluted solutions there is no response. These potentials refer to zero current but taken before the electrode relaxed after polarization. Thus, transmembrane fluxes at this moment are modified and

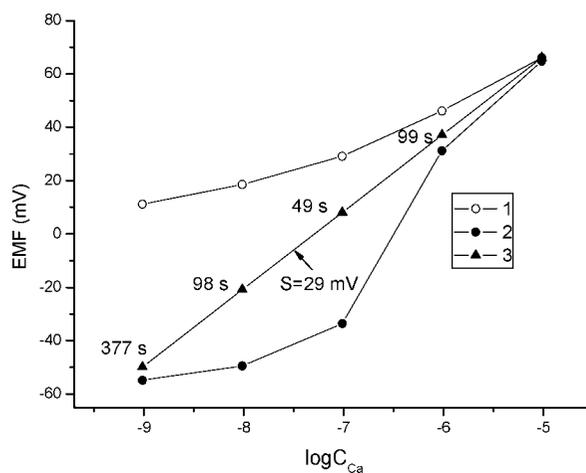


Figure 3. Calibration curves obtained from the data presented in Figure 2. Open circles (1) refer to zero-current potential values taken immediately before current was switched on. Filled circles (2) refer to potential values in the beginning of relaxation curve, i.e., immediately after the positive ohmic drop, as marked with arrows in Figure 2. Triangles (3) making a straight line with slope of 29 mV are taken from polarization curves at particular times, labeled in the figure.

differ from those at steady state. The super-Nernstian slope between 10^{-6} and 10^{-7} M CaCl_2 indicates that current of 50 nA/cm^2 is overcompensating, i.e., the solution is depleted in the vicinity of the membrane. At 10^{-8} M CaCl_2 the same current remains overcompensating (although to lesser extent), and therefore the registered potential is still more negative than the respective Nernstian value. For concentration of 10^{-9} M CaCl_2 the current of 50 nA/cm^2 is close to optimal value: the effect of transmembrane fluxes is only slightly overcompensated and the measured potential value is only in 5 mV below to the Nernstian line. The value recorded in 10^{-6} M CaCl_2 shows small negative deviation from a Nernstian straight line, whereas steady zero-current potential deviates about 18 mV in positive direction from Nernstian response. Bearing in mind the data for 10^{-7} M, one can expect that current of 50 nA/cm^2 must be overcompensating for this solution. Most probably, the depletion of 10^{-6} M CaCl_2 solution caused by polarization is smaller than the contamination by transmembrane fluxes at zero current. These considerations suggest that by passing currents smaller than 50 nA/cm^2 , one can obtain Nernstian response at 10^{-7} M and lower concentrations of CaCl_2 . The values of the suitable current densities must be different for each particular concentration. As already mentioned above, this means having two unknowns for a sample: the concentration of the analyte and the current density which makes the ISE response Nernstian at this particular concentration.

The third set of the data exploits the option based on the optimization of the length of the polarization. As shown in Figure 3, line 3, it is indeed possible to obtain a straight Nernstian line with the slope of 29 mV per decade down to 10^{-9} M CaCl_2 by plotting potential values recorded at certain times during polarization. Since response in 10^{-5} M CaCl_2 is Nernstian, the respective time is 0: no polarization is needed. For lower concentrations, except of 10^{-6} M, the polarization times required for obtaining the Nernstian value of the potential are the higher the lower is the concentration. It is not clear at this stage why the point for 10^{-6} M CaCl_2 falls out of this clear regularity.

The options based on timing possess the same kind of uncertainty as that assuming the optimization of the polarizing current: for an unknown sample the respective optimal time is unknown. In addition, this option relies on measurements when current is on, so the relevance of the recorded potential to the ordinary Nernstian treatment is even less clear than in the case when potential is taken after the positive ohmic drop. Also, since the magnitude of the drop is dependent on the sample matrix, the interpretation of the results is even less certain.

On the basis of these considerations we focused on the optimization of the polarizing current densities, using the same time protocol as before: 600/600/600 s. As analytically relevant signals, we considered the potentials recorded immediately after the positive ohmic drop, i.e., at zero current but with nonsteady fluxes across the membrane/solution system. From now on and throughout the paper we will call these potential values as current-off potentials (COP). Use of potentials measured at zero-current conditions although at nonsteady fluxes makes our approach significantly different from that used earlier^{7,8,17–21} when potentials were measured under applied current. To this effect our approach is similar to the pulsed galvanostatic technique.³³

The current density was varied within the range from 10 to 100 nA/cm². The difference between COP recorded in 10⁻⁷ and 10⁻⁶ M CaCl₂ was plotted against current density and was approximately linear (not shown here). For 10⁻⁷ M CaCl₂ solution the optimal current density delivering a Nernstian slope was found from this dependence. Other optimal currents were found after 2–3 trials in each particular solution.

An example of chronopotentiometric curves for these optimal currents is presented in Figure 4A. It can be seen that the potentials do not fully relax after the 600/600/600 s protocol in solutions more diluted than 10⁻⁸ M. However, the following chronopotentiometric measurement did not start immediately after the previous: there was a delay for 100 s for dilution and stirring. For repeated measurement at the same concentration this additional time was enough for practically full relaxation of the potential.

The respective calibration plot with slope 28.4 mV within the concentration range of 10⁻⁵–10⁻¹⁰ M CaCl₂ with 0.001 M KCl is shown in Figure 4B. Sensitivity to the current density in solutions with the concentration of CaCl₂ higher than 10⁻⁷ M is low, so the same current of 16 nA/cm² allows for obtaining a Nernstian response in these solutions. In more diluted solutions the sensitivity to the current density is bigger, and the particular current densities are somewhat different for individual electrodes, see Table 1. However, as shown in Figure 4B, current can be tuned to make the response slope Nernstian down to 10⁻¹⁰ M CaCl₂ even with relatively high background of KCl.

Electrochemical Impedance Measurements. As demonstrated above, optimization of the polarizing current for each particular concentration of analyte does allow obtaining a linear Nernstian response down to subnanomolar concentrations. This effect may be exclusively due to elimination of transmembrane fluxes but may be partly due to disturbance of electrochemical equilibrium at the sample/membrane interface. This issue was addressed by measurements of the electrochemical impedance of the electrode at open circuit potential (OCP) and at COP for

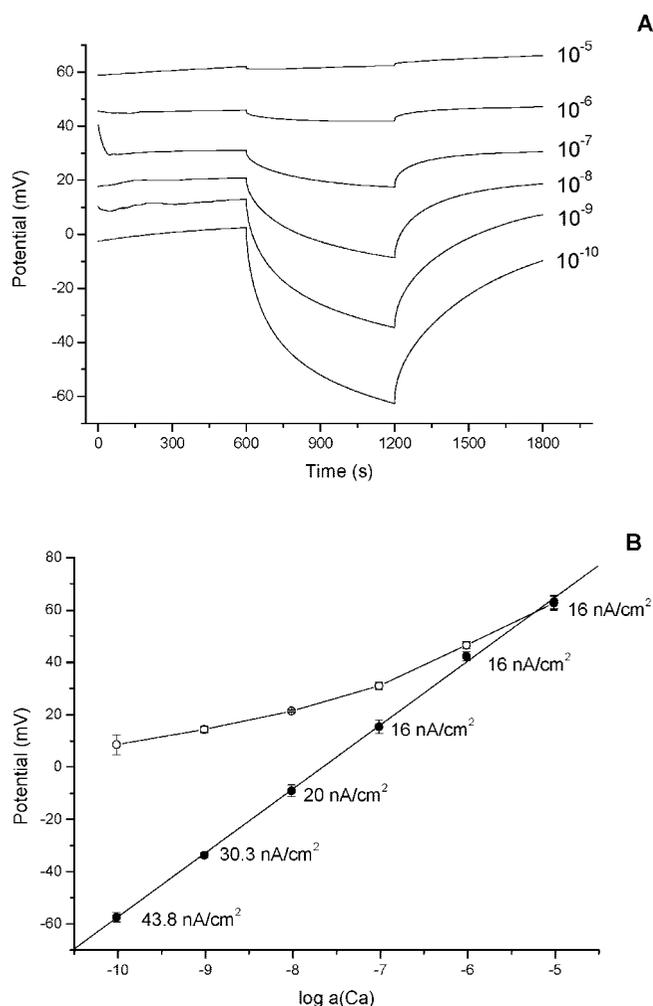


Figure 4. Chronopotentiometric curves (A) and calibration curves (B) obtained for a Ca ISE polarized by different cathodic currents, in solutions with different concentrations of CaCl₂ containing 0.001 M KCl background. In panel A: curve 1, 10⁻⁵ M, 16.0 nA/cm²; curve 2, 10⁻⁶ M, 16.0 nA/cm²; curve 3, 10⁻⁷ M, 16.0 nA/cm²; curve 4, 10⁻⁸ M, 20.0 nA/cm²; curve 5, 10⁻⁹ M, 30.3 nA/cm²; curve 6, 10⁻¹⁰ M, 43.8 nA/cm². In panel B: open circles refer to zero-current potential values (taken immediately before current is on). Filled circles refer to potential values taken immediately after the positive ohmic drop.

Table 1. Optimal Values of Polarizing Cathodic Current Densities and Resulting Slopes of Linear Calibration Curves

concn of CaCl ₂ (M)	optimal current density (nA/cm ²)		
	ISE 1	ISE 2	ISE 3
10 ⁻⁷	16	16	16
10 ⁻⁸	16	22.3	20
10 ⁻⁹	24.7	30.3	30.3
10 ⁻¹⁰	37.4	46.2	43.8
slope (mV)	31.6	28.4	24.5

the respective concentration. Results obtained in 10⁻⁸ M CaCl₂ with 0.001 M KCl background registered at OCP (21.4 mV) and at Nernstian value for 10⁻⁸ M CaCl₂ (-16 mV) are presented in Figure 5.

The spectra were successfully fitted to the circuit also shown in Figure 5. The high-frequency semicircle was the same when registered at OCP and at -16 mV: R_1 was 0.59 M Ω ; constant phase element (CPE₁) was 3.4 \times 10⁻¹⁰ F. In the low-frequency semicircle

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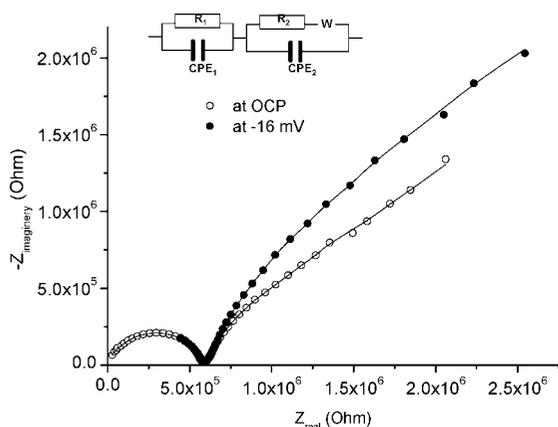


Figure 5. Impedance spectra of a Ca ISE filled with 0.01 M CaCl₂ and immersed into 1×10^{-8} M CaCl₂ with 0.001 M KCl background: open circles, measurements at OCP; filled circles, measurements at -16 mV; solid lines, results of fitting to the circuit shown in the top.

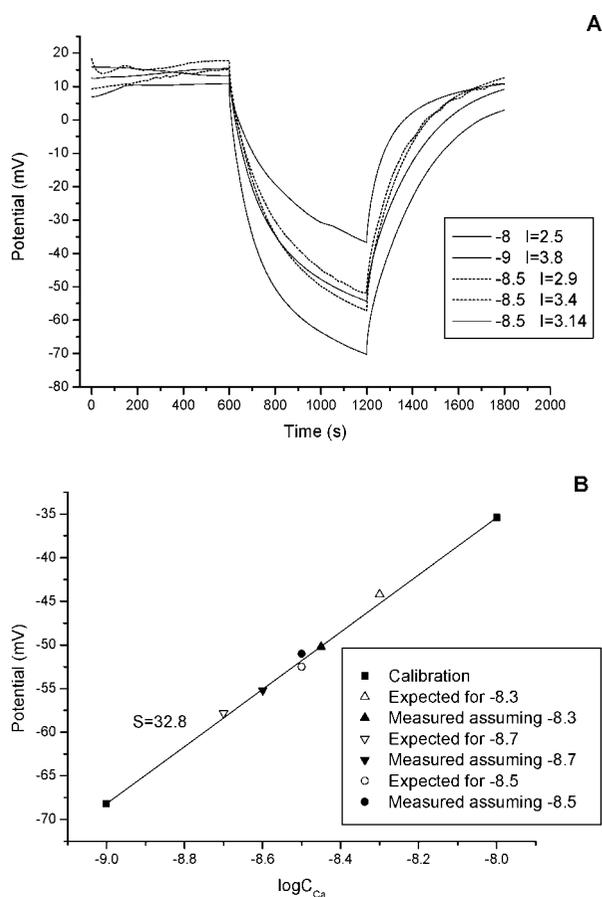


Figure 6. Analytical procedure: chronopotentiometric curves (A) and current-off potential (COP) values (B) recorded in calibration solutions of 10^{-9} and 10^{-8} M CaCl₂ and in sample solution of 3×10^{-9} M CaCl₂, all with 0.001 M KCl background.

the CPE₂ was 1.8×10^{-6} F both at OCP and at -16 mV, but the resistance R_2 was 1.9 M Ω at OCP and 4.1 M Ω at -16 mV. The respective Warburg impedance parameter W was 2.32×10^{-6} at OCP and 1.73×10^{-6} at -16 mV. The large resistance in the low-frequency semicircle which appears to be charge-transfer resistance may be caused by the low concentration of Ca²⁺ in solution. The lower value at OCP than at Nernstian potential is consistent

with the idea of the contamination of the solution in the vicinity of the membrane due to transmembrane fluxes of Ca²⁺: at OCP the surface concentration of Ca²⁺ is higher than in the bulk of the solution. The difference between the Warburg parameter values at OCP and at -16 mV can be understood in the same way: higher content of Ca²⁺ in solution in the vicinity of the interface (when transmembrane fluxes are not compensated) causes lower diffusion resistance. The CPE value of 1.8×10^{-6} F is surprisingly large being 2 orders of magnitude higher than one could expect.³⁴ The reason for this is not yet clear. The values of R_2 charge-transfer resistance and of the CPE suggest the time constant of approximately 4 s at OCP, and of 8 s at -16 mV, so the interfacial equilibrium, although initially disturbed by the current pulse, is re-established within a few seconds.

Analysis of a Sample Using Tuned Galvanostatic Polarization. We will turn now to iteration procedure which makes tuned galvanostatic polarization of ISEs analytically relevant.

The procedure is based on the following assumptions: (1) polarization with current of optimal density compensates the effect of transmembrane flux, so the respective potentials obey Nernst law; (2) current density lower than optimal undercompensates the flux, and measured potentials are therefore more positive than Nernstian values, overestimating the analyte ion activity; (3) current density higher than optimal overcompensates the flux, and measured potentials are less positive than Nernstian and provide an underestimated value of the analyte ion activity; (4) the dependence of the optimal current on the logarithm of the analyte ion activity is approximately linear. Assumptions 1–3 are strongly supported by the results above. Assumption 4 is an idealization which may be true within any relatively narrow concentration range. More research is needed for further insight in this issue.

On the basis of these assumptions, the following procedure can be implemented.

First, we assume that the true concentration of the analyte in the sample (C_x) is equal to some arbitrarily assumed concentration C_a for which we expect Nernstian potential value E_a . We polarize the electrode with current I_a which is optimal for C_a . If $C_x < C_a$ the applied current I_a is undercompensating. In this case we will register potential E_m which refers to the apparently measured concentration $C_m > C_x$ because of undercompensated transmembrane flux. However, C_m will be still lower than C_a because C_x itself is lower than C_a , and therefore measured E_m will be lower than the expected E_a , indicating that $C_x < C_a$. If the applied current I_a is overcompensating, we will obtain $C_m < C_x$ because of overcompensated transmembrane flux. However, C_m will be higher than C_a because C_x is higher than C_a , so measured E_m will be higher than the expected E_a , indicating $C_x > C_a$.

Repeating these measurements with the same sample several times we can make the difference between the assumed concentration C_a and the apparently measured C_m sufficiently small. This convergence will be indicated by the measured potential approaching the expected value: $E_m \approx E_a$. If this is achieved, then $C_m \approx C_x$ and the analysis is successfully performed.

Illustration of the analysis of an artificial sample by means of tuned galvanostatic polarization is presented below, see Figure

(34) Mikhelson, K. N.; Bobacka, J.; Ivaska, A.; Lewenstam, A.; Bochenka, M. *Anal. Chem.* **2002**, *74*, 518.

6. The sample was 3×10^{-9} M CaCl_2 with 0.001 M KCl background. It was assumed to be known that the concentration is between 10^{-9} and 10^{-8} M, so chronopotentiometric curves with respective optimal currents were recorded in 10^{-9} and 10^{-8} M CaCl_2 , containing also 0.001 M KCl. The COP were -68.2 mV in 10^{-9} M and -35.4 mV in 10^{-8} M CaCl_2 giving a slightly super-Nernstian slope of 32.8 mV. After that the ISE was immersed into the sample, and it was assumed that $C_a = 5 \times 10^{-9}$ M CaCl_2 ($\log C_{\text{Ca}} = -8.3$), so the ISE was polarized with the respective current of -23.2 nA/cm². The potential value expected assuming exact compensation of the transmembrane flux was $E_a = -44.2$ mV; however, the measured potential was $E_m = -50.2$ mV. This indicated that the target concentration was below 5×10^{-9} M CaCl_2 . It was now assumed that $C_a = 2 \times 10^{-9}$ M CaCl_2 ($\log C_{\text{Ca}} = -8.7$), and the ISE was polarized with the respective current of -27.2 nA/cm². The expected potential was $E_a = -57.8$ mV, and the measured was $E_m = -55.4$ mV indicating higher concentration of the sample. Finally, the ISE was polarized with current of -25.2 nA/cm², which is optimal for $\log C_{\text{Ca}} = -8.5$, and the expected potential was $E_a = -51.0$ mV. The actually measured potential was $E_m = -52.5$ mV, suggesting successful assay $C_{\text{Ca}} \approx 3 \times 10^{-9}$ M.

CONCLUSIONS

The results presented above clearly demonstrate the advantages of tuned galvanostatic polarization as a highly promising method aimed at improvement of the lower detection limit of ionophore-based ISEs. Moreover, it is shown that tuned galvano-

static polarization allows for successful analysis in the nanomolar concentration range.

The approach is, however, at an early stage of development, and more experimental and also theoretical studies, e.g., based on Nernst–Planck–Poisson real time and space modeling,^{23,35–37} are required. Use of classical internal filling (0.01 M CaCl_2) in combination with storage of the ISEs in the same solution showed the power of the method: Nernstian response was obtained in a broad concentration range in spite of these obviously nonoptimal conditions. It is, however, clear that use of more diluted filling and storage solution, e.g., 10^{-4} M CaCl_2 , must be more practical. The arbitrarily chosen time protocol requires significant modification: polarization time must be decreased for better throughput of the analysis. Obviously, higher polarizing currents will be needed for measurements with shorter polarization times.

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