Novel Solid-Contact Ion-Selective Microelectrodes for Localized Potentiometric Measurements

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Abstract
The design and properties of novel type of solid-contact ionophore-based ion-selective microelectrodes are reported. The microelectrode is based on an insulated needle-shaped metallic wire with an exposed apex. The ion-to-electron transducer is made of poly(3-octylthiophene-2,5-diyl) and placed between an ion-selective membrane and the metallic tip. The ion-selective polyvinyl chloride-based membrane is deposited atop the layer of conductive polymer. The length of the ion-sensitive part of the electrode is less than 10 μm. pH and Mg²⁺-selective microelectrodes were constructed and tested showing stable potential and fast response that are essential properties for the practical application of microelectrodes for localized scanning measurements.

Keywords: Solid contact, pH-selective microelectrode, Mg-selective microelectrode, SIET, Response time, Ionophores, Sensors

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Dedicated to Professor G. L. Starobinets on Occasion of the Centenary of His Birth and to the 75th Anniversary of the Belarusian State University/C29s Department of Analytical Chemistry

1. Introduction
Information on the spatial distribution of the ionic species consumed or released on the active sites of various solid-liquid interfaces is of primary importance for understanding many chemical and biological processes. Examples of such processes can be found in biology where living cells exchange ionic species with surrounding liquid media and in corrosion science where localized electrochemical processes occur on the metallic surfaces. The concentration of different ionic species can be assessed by using ion-selective electrodes (ISEs).

Miniaturization of conventional ISEs for application in localized techniques originated glass capillary microelectrodes with liquid ion-selective membranes (usually without polymer matrix) and inner filling solutions. The preparation and application of this type of electrodes is described in [1, 2] as well as many other publications. Different scanning apparatus and technical approaches are used in the potentiometric mode for measuring activity (concentration) of specific ions in solution. Scanning electrochemical microscopy (SECM) [3, 4], scanning ion selective electrode technique (SIET) [5, 6] self-referencing ion-selective probes (SERIS) [7, 8] and MIFE (microelectrode ion flux estimation) [9, 10] are used for corrosion electrochemistry and biomedical applications.

However, these glass capillary microelectrodes although widely used for localized potentiometric measurements suffer from several disadvantages: spontaneous leakage of the liquid membrane out of the capillary, life time limited to a maximum of one day and fragility of the glass capillary. The latter is especially critical for biological applications where the sample under study (e.g. fish) could move. Moreover the detection limit and selectivity of such electrodes are limited by the flux of primary ions from the ion-selective membrane and inner filling solution that contaminates the near boundary layer of the solution that is in contact with the ion-selective membrane.

Solid-contact electrodes contain neither liquid membrane nor inner filling solution and represent an alternative to liquid-membrane electrodes. One of the world leading scientists in the field of ISEs, Ernç Pretsch wrote in one of his recent reviews that the solid-contact electrodes “...represent the typical way of constructing the next ISE generation” [11]. Solid-contact microelectrodes have all advantages of the glass capillary microelectrodes and are free of their drawbacks.

The first work on electrodes of coated-wire type was published in 1971 by Cattrall et al. [12]. However, such electrodes suffer from a potential drift due to indefinite transition from ionic (membrane) to electronic (metallic substrate) conductivity. Later in 1992 an electrically conductive polymer was proposed to be used as an intermediate...
layer between an internal reference element and an ion-sensitive membrane by Cadogan et al. [13]. Since then considerable work has been done in the direction of stabilizing the interfacial potential between the ion-sensitive membrane and the internal conductive substrate. The main improvements were achieved by excluding the plasticizer from the polymeric matrix of the membrane and using a highly hydrophobic conductive polymer such as POT. All these aspects are critically reviewed and summarized in excellent reviews [14, 15].

Gyurcsányi et al. was the first to report on a solid-contact microelectrode for localized measurements in 1998 [16]. The K\textsuperscript{+}-selective microelectrode was used as a measuring tip in SECM. To prepare this microelectrode carbon fiber, Pt or Au wires were sealed in glass tubes with an outer diameter of about 2 mm, polished and conically bevelled around the measuring disk with subsequent deposition of polypyrrole by electrochemical polymerization and drop casting of PVC-based ion-selective membrane. Several other approaches to miniaturize solid-contact electrodes were explored since then. Gyetvai et al. [17] described a solid-contact microelectrode for the potentiometric mode of SECM. Carbon fiber coated with poly(3,4-ethylenedioxythiophene) was dipped inside the membrane cocktail placed in the tip of a glass pipette. However, this approach keeps the main disadvantages of glass capillary microelectrodes: leakage of the liquid membrane and fragility of the glass body of the electrode.

Bakker’s group [18] reported on a Ag\textsuperscript{+}-selective solid-contact microelectrode with low detection limit. The microelectrode comprised a gold wire of 100 – 200 \mu m diameter with a measuring tip in the range of 50 – 150 \mu m. The horizontal dipping was performed by using a micromanipulator (model HS6, WPI) and controlled by an optical microscope with long focus distance lenses and a built-in micro ruler.

The schematic representation of the solid-contact ion-selective microelectrode is shown in Figure 1a. The needle-shaped metallic wire finishes in a connector at the butt and in an exposed sharp apex of the conical tip at the other end. The conductive substrate is made of platinum/iridium alloy to facilitate the deposition of a gold layer and assure the stiffness of the probe for the scanning measurements or for penetration through the tissues or cells in situ biological applications. Figure 1b depicts an optical photo of the solid-contact ion-selective microelectrode. Figure 1c shows the SEM image of the tip of the microelectrode with deposited layers of conductive polymer and ion-selective membrane. The length of the entire measuring point of the solid-contact ion-selective microelectrode is equal or less than 10 \mu m. There is not any liquid in this microelectrode construction. This automatically excludes major problems inherent to traditional glass capillary microelectrodes such as leakage of the liquid membrane and fragility.

2.2. Preparation of the pH-Selective Microelectrode

The ion-sensitive membrane for H\textsuperscript{+}-selective solid-contact microelectrode was composed of 25% wt. PVC (high molecular weight), 10% wt. hydrogen ionophore 1 (tridecylamine), 55.5% wt. 2-nitrophenyloctyl ether and 9.5% wt. (90% mol relative to ionophore) potassium tetrakis(4-chlorophenyl)borate dissolved in tetrahydrofuran (THF). This membrane was deposited atop the POT layer. The microelectrodes were conditioned in buffer solution at pH 9.0 for 2 hours prior to the first calibration.

2.1. Electrode Design

Custom made needle-shaped rigid conductive substrates were obtained from Microprobe Inc. They were made of platinum-iridium alloy and insulated with a 3 \mu m thick layer of Parylene C apart from the 5 \mu m long apex of the tip that is left opened. A layer of gold (0.7 – 1.2 \mu m thick) was electrodeposited on the exposed apex of the tip to assure stable adherence of the sulfur-containing conductive polymer. The ion-to-electron transducer that enables transition from ionic to electronic conductivity is made of highly hydrophobic poly(3-octylthiophene-2,5-diyl) (POT). It was deposited from 0.7% w/v xylene solution by multiple horizontal dipping until the thickness of the POT layer reached 1.5 – 2.0 \mu m. The external ion-selective membrane was based on plasticized PVC and was also deposited on the TOP layer by horizontal dipping. The thickness of the membrane was 3 – 4 \mu m. Glass micro capillaries filled with solutions of either conductive polymer or ion-selective membrane were used as the containers where the tip of the microelectrode was dipped. The diameter of the tip of micro capillaries was in the range of 50 – 150 \mu m. The horizontal dipping was performed by using a micromanipulator (model HS6, WPI) and controlled by an optical microscope with long focus distance lenses and a built-in micro ruler.

The method of depositing the ion-sensitive membrane is depicted in Figure 1b. A thin layer of the ion-selective membrane contacted to the ion electrode was deposited on the substrate by using a micromanipulator (model HS6, WPI) and controlled by an optical microscope with long focus distance lenses and a built-in micro ruler.

A pH-selective microelectrode was constructed, characterized and used for local measurements in model systems.
2.3. Preparation of the Mg$^{2+}$-Selective Microelectrode

The ion-sensitive membrane for Mg$^{2+}$-selective solid-contact microelectrode was composed of 30.3% wt. PVC (high molecular weight), 43.2% wt. 2-nitrophenyloctyl ether, 17.4% wt. chloroparaffin, 7.0% wt. magnesium ionophore II – ETH 5214 ($N,N''-\text{octamethylenbis}(N'-\text{heptyl}-N''-\text{methylmalonamide})$) and 2.1% wt. (35% mol relative to ionophore) potassium tetrakis(4-chlorophenyl)borate. This membrane was deposited on the layer of conductive polymer. The microelectrodes were conditioned for 2 hours in dilute solution of primary ions prior to the first calibration, $\frac{1}{C_2} \times 10^6 \text{MgCl}_2$.

2.4. Response Time Measurements

The response time of the microelectrodes was measured in a specially designed “dual drop cell”, Figure 2. It comprised a Y-shape junction made of flexible polypropylene tubing of inner diameter 1.5 mm filled with agar stabilized 0.05 M NaCl solution. One drop of solution was placed on top of each of the upward branches of the Y junction. The drops contain different concentrations of the main ion. The downward branch connected the reference electrode and the two drops in the upward branches. The volume each drop was approximately 1 microliter. The solutions were not stirred to simulate scanning conditions in the course of the measurements in real systems. The potential was recorded while the microelectrode moved from one drop to another at known speed.

Two different approaches [22–24] for quantification of the response time were used. The time elapsed between the instant when the microelectrode was brought into contact with the solution in the second drop of the “dual drop cell” and the instant at which the potential/time ($\Delta E/\Delta t$) slope reached an adopted limiting value of 1 mV/min was considered as the response time of the microelectrode, $\tau_{\text{lim}}$. This limiting value of $\Delta E/\Delta t$ slope was selected because the range of response of the microelectrodes (especially the pH selective ones) and span of electrode potential are quite wide comparing to these parameters for electrodes used in biomedical applications cited in current IUPAC recommen-
2.5. Equipment and Procedures

SIET apparatus produced by Applicable Electronics Inc. and controlled by the ASET software (Scientecware) was used for all measurements. The microelectrodes were mounted on the 3D step motors with lateral resolution of 0.8 μm. A preamplifier of 10^15 Ω input impedance was used to measure the potential. The measurements were performed in a Faraday cage in order to avoid electromagnetic interference.

The microelectrodes were kept dry between the measurements. Ag/AgCl mini electrodes made in our laboratory with agar stabilized salt bridge were used as reference. Activity coefficients were calculated by the Debye-Hückel approximation. All experiments were performed at room temperature. All reagents for H^+ and Mg^2+ membrane composition were Selectophore grade products of Fluka. The aqueous solutions were prepared with salts of the composition were Selectophore grade products of Fluka. The insulation quality of the individual microelectrodes and the consistency of the deposited functional layers were controlled by scanning electron microscopy coupled with energy dispersive X-ray spectroscopy. A semi-in-lens Hitachi SU-70 UHR Schottky (Analytical) FE-SEM microscope coupled with a Bruker EDS detector was used, operating at 10 keV.

3. Results and Discussion

Calibration curves for the pH-selective microelectrode are presented in Figure 3. The pH range of linear response is 4.8–12. The slope of the linear regression is close to the Nernstian behavior, 56.8–55.8 mV/decade, during the first three days of microelectrode exploitation. The inset in Figure 3 shows the typical time dependant calibration curve. The dynamic curves were measured in pure pH buffer solutions as well as in pH buffer solutions containing 0.05 M NaCl background solution. The latter is the conventional solution for corrosion tests and a component of the isotonic solution in biomedical applications. As it could be predicted based on the selectivity coefficients published earlier [e.g. 25] and gathered in [26], the 0.05 M NaCl solution does not influence the potential of the pH selective solid-contact microelectrode based on tridodecylamine.

The random deviation of the microelectrode potential was ±0.15 mV. The long-term potential was very stable and reproducible if the microelectrodes were kept dry between the measurements or used continuously for three days (scanning the actively corroding model system). The decrease of the linear slope down to 48–46 mV/decade usually occurred for the intensively used microelectrodes in the following days. Note that if the microelectrodes were used only several hours a day once every week and kept dry between the measurements they showed the Nernstian response and reproducible values of potential beyond 1 month of use. Such robustness clearly demonstrates the advantages of the developed microelectrodes compared to the unstable and fragile glass capillary counterparts. The decrease of the linear slope of the microelectrodes and the increase of time response can be explained by washing out and/or decomposition of the ionophore and lipophilic ionic sites from the very thin ion-selective membrane. Another reason of such behavior can be the penetration of solution through the plasticized PVC membrane to the metallic substrate as it is pointed out in several recent articles [14, 15, 27, 28]. The elimination of this detrimental water layer can be achieved by changing the membrane matrix. The low propensity of the P(MMA-DMA) copolymer for water absorption and transport along with the low diffusion coefficients of ions in the P(MMA-DMA)-based membranes can provide microelectrodes with nanomolar detection limits, unbiased selectivity, high stability of the potential and longer lifetime.

One of the most important characteristics of the microelectrodes used for scanning measurements is the response time. It predetermines the acquisition time of the entire scan that often may require more than 1000 consecutive points. Semi-isochronous acquisition of all points of the scan ensures that the system under study is not changed significantly during the measurement. Figure 4 shows the potential response measured while the microelectrode moved from a solution drop with pH 9 to another drop with pH 8. E_0 is the potential recorded in the first drop and E_stat is the stationary value of the potential recorded in the second drop with pH 8. The values E_0 and E_stat were assessed.
from the dynamic response curve (Fig. 4 Inset) and used to calculate $t_{95}$. $E_{lim}$ is the value of potential at the moment when $\Delta E/\Delta t$ slope reached limiting value 1 mV/min. This instant was determined by differentiating the $E/t$ curve. The measured value of $t_{lim}$ is 0.96 ± 0.02 seconds and of $t_{95}$ is 0.33 ± 0.01 seconds. This meets the requirement for a fast responding microelectrode. However, the presented value of response time is not constant. The response time of the microelectrode measured after 4 days of continuous exploitation increased up to $t_{lim} = 2.8$ s and $t_{95} = 1.8$ s. Although the values of time response quantified as $t_{95}$ (or $t_{90}$) are often found in literature (their use was critically analyzed in the excellent review by C. Macca [24]) the differential response time $t_{lim}$ specified by the current IUPAC recommendations [22] appears to be more practical for real scanning measurements. SIET mapping implies point by point measurements of ion activities. The use of a time of acquisition for each data point of 0.33 seconds ($t_{95}$) resulted in blurring of pH maps, while the time of acquisition corresponding to 1 second ($t_{lim}$) led to sharpening of resulted images.

The applicability of the developed pH-sensitive electrodes for localized scanning potentiometric measurements was also proven employing SIET apparatus. The results of one of the model experiments are presented in Figure 5. The pH-sensitive solid-contact microelectrode scanned the surface of an aluminum/copper galvanic couple embedded into nonconductive epoxy resin. The pH mapping was acquired after about 2 hours of sample immersion in 0.01 M NaCl solution. The local activity of $H^+$ was measured about 50 $\mu$m above the surface on a 30 × 30 grid which generates 900 data points. A move-wait-measure scheme was employed for mapping. The time of acquisition of each data point was 1 s, resulting in a total scan time of about 18 min also including the time for the electrode to move from point to point.

Well defined regions of cathodic reaction with alkalization of solution over copper and anodic dissolution of aluminum with local acidification of solution, are visible. The cathodic region corresponds to the reduction reactions: 
$$2H_2O + 2e^- \rightarrow 2OH^- + H_2$$ and 
$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$$

with local alkalization of solution up to pH 11.2. The slight decrease of pH in the anodic region, down to 4.0, can be ascribed to the reaction of Al dissolution and hydrolysis:
$$Al + nH_2O \rightarrow Al(OH)_{n}^{(3-n)^+} + nH^+ + 3e^-, \quad n = 1–3.$$ Note that the pH in such model system changes sharply in a wide range, from 4 to 11.2. Nevertheless, the local activity is well resolved by the solid-contact microelectrodes making them of high potential applicability for studying localized corrosion processes.

Figure 6 shows the calibration curve for a developed Mg-selective microelectrode. The linear pMg range of response is from 1.7 to 6.3 with the slope of linear regression 29.6 ± 0.4 mV/decade that corresponds to Nernstian response. The inset presents the dynamic response curve. The stability of potential of the Mg-selective microelectrode is similar to that of the pH-selective microelectrode. The intensively used microelectrodes were stable for around 3 days with following decrease of the linear slope and increase of response time. Dry kept microelectrodes are stable for a month. The response times of the Mg$^{2+}$-selective microelectrode was evaluated as $t_{lim} = 2.58 ± 0.05$ seconds and $t_{95} = 1.10 ± 0.05$ seconds.
A model experiment involving scanning of a local source of magnesium cations was also performed. Such experiment simulates release of magnesium from a biological micro-object or from a corroding microdefect on a magnesium-containing metallic substrate. Figure 7 shows the optical micropicture and the results ($-\log a_{Mg^{2+}}$) of horizontal profiling taken over a glass capillary filled with agar stabilized 0.01 M MgCl$_2$ solution and mounted in a polished holder made of epoxy resin. The presented pMg profiling was obtained about 15 µm above the surface of the source of Mg$^{2+}$ in 0.01 M NaCl aqueous solution. The time of acquisition of each data point was 3 seconds.

It should be mentioned that only approximately two thirds of all produced microelectrodes were functional. Twenty four pH-selective microelectrodes and fifteen Mg-selective microelectrodes were produced. Twenty seven out of thirty nine microelectrodes showed reproducible potential. Twelve microelectrodes showed highly unstable and drifting potential in the course of the first calibration and were discarded. Such microelectrodes had initial defects in POT and/or PVC layers. The defects could not be noticed in an optical microscope used to control deposition of functional layers but were revealed later by SEM/EDS observations. To recycle the insulated probes with the gold-coated tips the layers of POT and PVC-based membrane can be easily washed out from the conductive substrates by an appropriate solvent, e.g. by THF. Ultrasonic agitation facilitates the dissolution of the polymers.

4. Conclusions

Novel solid-contact ion-selective electrodes with a micro-size sensitive tip have been developed. The design of the microelectrodes is based on a needle-shaped metallic rigid substrate and contains neither liquids nor highly fragile parts what make them more robust compared to traditional glass capillary analogues. Hydrophobic conducting polymer, POT, was used as intermediate electron- and ion-conductive layer. Mg$^{2+}$- and pH-selective solid-contact potentiometric microelectrodes show values of linear slope close to the Nernstian in a wide range of concentrations. These electrodes have reasonably short response time proving their applicability for localized potentiometric scanning measurements in biomedical and corrosion science applications. The life time of the solid-contact microelectrodes depends on the intensity of their use but is longer than the operating time of traditional glass-capillary microelectrodes.

The use of a polymer-based ionophore containing membrane significantly expands the applicability of microelectrodes of the present type. Different membrane formulations based on already reported [e.g. 29] and commercially available [26] ionophores and ionic additives enable fabrication of microelectrodes selective to a large number of organic and inorganic ions.

Further miniaturization of solid-contact microelectrodes is possible and is considered for future work. Moreover we believe that the life time of the microelectrodes can be
extended by using methacrylate-based membrane matrix that lowers the ionic mobility and prevents water penetration through the ion-selective membrane. Ion-selective microelectrodes of the same type with P(MMA-DMA)-based membranes are currently being developed by us.

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6. References