J Solid State Electrochem (2009) 13:149–155
DOI 10.1007/s10008-008-0602-7

ORIGINAL PAPER

A miniaturized and integrated galvanic cell for the potentiometric measurement of ions in biological liquids

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Received: 14 March 2008 / Accepted: 27 May 2008 / Published online: 28 June 2008

Abstract A procedure for an all-plastic electrochemical cell comprising miniaturized planar indicator and reference electrodes is described. All electrodes are based on conducting polymers, are fully integrated, and contain no internal electrolyte. The reference microsensors were deposited via electrochemical polymerization from a water solution containing the monomer 3,4-ethylenedioxythiophene (EDOT) or 1-methylpyrrole (MPy) and a biochemical buffer 3-(*N*-morpholino) propanesulfonic acid [MOPS], 2-(*N*-morpholino) ethanesulfonic acid [MES], or 2-hydroxy-5-sulfobenzoic acid [SSA]). Ion-sensitive microelectrodes were prepared by the deposition of the ion-sensitive membrane solution (Ca^{2+}, K^{+}, and Cl^{-}) directly onto the mediating poly-EDOT (PEDOT), PEDOT–SSA, PEDOT–MES, PEDOT–MOPS, or poly-MPy–MOPS layers.

Keywords Ion-selective electrode · Reference electrode · Galvanic cell · Potentiometry · Clinical analysis

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Introduction

The majority of recent research in potentiometric ion sensors has been devoted to developing robust and miniaturizable ion sensors that retain the good analytical response of conventional electrodes. This trend necessitates the elimination of the internal solution, which is achieved by the application of mediating layers, e.g., conducting polymers (CPs).

Technological progress in the use of ion sensors as indicating electrodes was recently supplemented by a revival of interest in redesigning reference electrodes to make them integrated and/or technologically compatible with ion sensors [1–6].

This is a natural consequence of the reference electrode being an equal partner with all of the indicator electrodes in potentiometry and open-circuit sensor technology. The failure of the reference electrode means the failure of the entire system, so that none of the indicator electrode measurements can be collected. Thus, the quality of the reference electrode is critical in potentiometric measurements where multiparameter analyses are performed, for instance in the analysis of biological fluids.

The demand for the miniaturization of sensors adds additional technological challenges. In this paper, due to the obvious analytical targets such as interstitial liquid or small volumes of blood, the integration of the electrode to avoid the necessity of an internal solution is a priority. As shown before, this can be achieved, e.g., by the application of CPs, both in the case of indicator electrodes and the reference electrode.

Miniaturized potentiometric ion sensors for the determination of charged and neutral analytes have been of interest since the beginning of ion-selective electrode (ISE) history.

They are also recently described in a number of papers [7–16]. CPs have often been reported to be very useful for manufacturing electrochemical microsensors [12–14]. For example, CPs have been deposited on small-diameter (6–7 μm) carbon fiber substrates in the preparation of microsensors applied to the in situ monitoring of nitrate [13]. The concentrations of Ca^{2+}, Ag^{+}, and Na^{+} ions have also been successfully determined by means of a monolithic capillary electrode prepared without CPs. An inner filling solution was, however, necessary for the construction of this electrode [16].

An important advantage offered by the application of CPs is the structural simplicity of the sensors, their mechanical integration and physicochemical similarity, possibility of modification of the deposition/deposits, and the subsequent possibility of sensor miniaturization. CPs exhibit ionic, redox, or mixed sensitivity, which can be adjusted during the electrodeposition and soaking stages [17–19]. Enhanced cationic sensitivity and improved selectivity of CP films can be obtained by doping with bulky metal-complexing ligands [20], e.g., metalochromic indicators such as calcion [21–23], calcon, tiron, arsenazo [24, 25], kalces [20], or calmagite [26]. In contrast, poly (3,4-ethylenedioxythiophene) (PEDOT) films deposited in the presence of biochemical pH buffers containing a sulfonic group (e.g., 3-(*N*-morpholino) propanesulfonic acid [MOPS], 2-(*N*-morpholino) ethanesulfonic acid [MES], or 2-hydroxy-5-sulfobenzoic acid [SSA]) are characterized by a hampered ionic and redox sensitivity and an insensitivity to pH and ions. The effect of redox inactivity is ascribed to the presence bulky anions of the pH buffers in the CP films, while pH insensitivity is the result of pH buffering in the vicinity of the CPs interface controlled by the buffers dispersed in the films. The biological buffers were recently used by this group for the fabrication of macroreference electrodes [1].

In this report, we present how the above approach is used to obtain a flat, maintenance-free, and multiparameter galvanic cell based on integrated microelectrodes.

Experimental

Chemicals

The SSA p.a. $C_7H_6O_6S{\cdot}2H_2O$ (Fluka, Germany), was used as received. The MOPS, $C_7H_{15}NO_4S$ (Sigma Chemicals), was used as received or after the trace of chloride ion removal.

The 3,4-ethylenedioxythiophene monomer (EDOT, >97%) was obtained from Bayer AG (Germany) and used as received. The 1-methylpyrrole (MPy) from Merck (Germany) was purified by double distillation under argon gas and then stored under argon at low temperature and protected from light.

All other chemicals used were analytical grade and were obtained from POCh, Merck, Fluka, or Sigma Chemicals and used as received. Doubly distilled and deionized water (resistance 18,2 MΩ, Millipore, France) was used.

All the solutions with a concentration lower than 0.01 mol dm^{-3} were prepared immediately prior to use.

Three pH/electrolyte standards mimicking those used in blood electrolyte analysis with the following pH and concentrations of main electrolytes (mmol dm^{-3}) were prepared:

- STD1: pH=7.00 (by MOPS) and Na^{+}=120.0, K^{+}=6.0, Ca^{2+}=0.58, Cl^{-}=93.4
- STD2: pH = 7.40 (by TES) and Na^{+}=140.0, K^{+}=4.5, Ca^{2+}=1.25, Cl^{-}=113.1
- STD3: pH = 7.80 (by HEPES) and Na^{+}=150.0, K^{+}= 3.0, Ca^{2+}=2.23, Cl^{-}=118.2.

For simplicity, hereafter in the text, mol dm^{-3} is denoted as M.

Lyophilized serum Nortrol (lot 981043) supplied by Thermo Fisher Scientific with the mean levels of ionized potassium 3.8 (SD=0.08), chloride 97.9 (SD=2.43) and total calcium 2.12 M (SD=0.043) was used.

Instrumentation

The electrochemical polymerization of EDOT or MPy was carried out using an Autolab PGSTAT 100 potentiostat/galvanostat (Eco Chemie B.V., The Netherlands) or an EA-9C type electrochemical analyzer (MTM Poland). The electropolymerization was performed in a single-compartment, three-electrode electrochemical cell, utilizing a Ag/AgCl/3 M KCl reference electrode connected to the cell via a bridge filled with supporting electrolyte solution. A Pt wire or sheet with an area of about 2 cm^2 was used as the auxiliary electrode.

In the studies described below, CPs were deposited on commercial glassy carbon (GC) electrodes with a surface area of 0.07 cm^2, Pt disks with a surface area of 0.03 cm^2, and on Au microelectrodes with a geometrical surface area of 0.0007, 0.002, 0.004, or 0.008 cm^2. In the last case, arrays containing four to ten single microelectrodes were used.

The microelectrodes were produced by the following methods:

- By the circuit printing method on an epoxy resin or aluminium oxide (alund) base (obtained from the Institute of Microelectronics of the Warsaw Technical University, see Fig. 1a)
- By microprinting on a polypropylene substrate (donated by Maj-Zurawska, University of Warsaw)
- By embedding gold wires of a different diameter in a chemo-setting or UV-setting resin (e.g., Translux D180

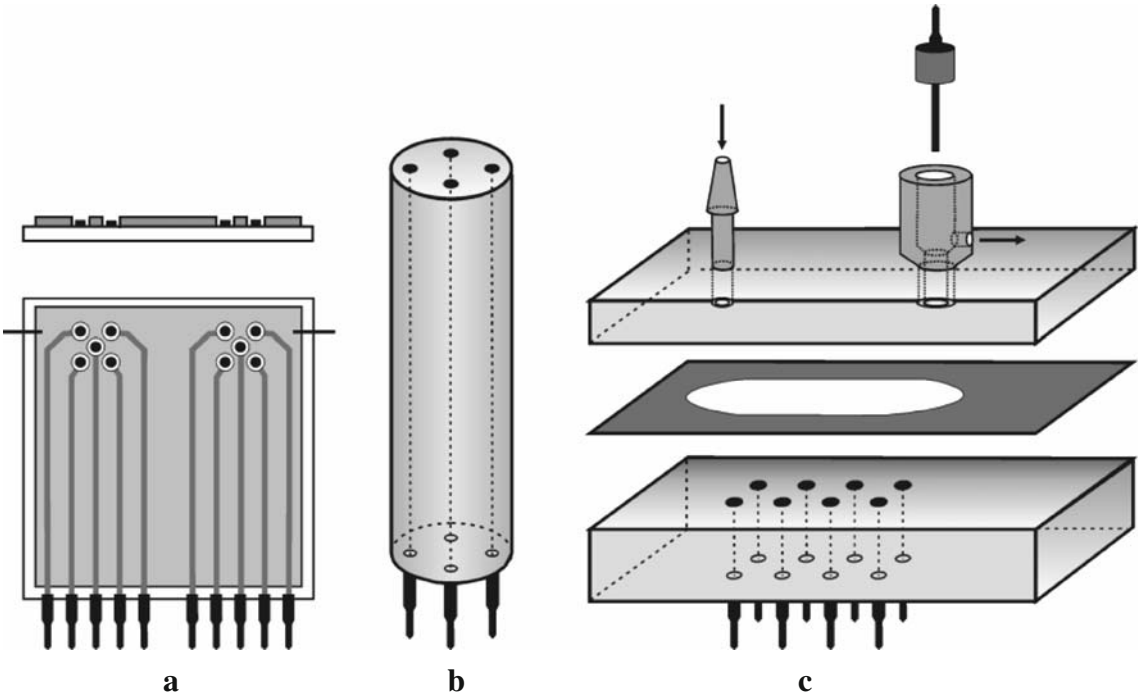

Fig. 1 Arrays of electrode substrates (pads) used for constructing microgalvanic cells, **a** obtained by the circuit printing method, surface of exposed Au surface 0,0007 cm^2, **b**, **c** obtained by embedding gold wires of a different diameter in a UV-setting resin, Au surface 0.002–0.008 cm^2

from AXSON France, 3M™ESPE™Protemp™ from 3M ESPE, USA, SpeciFix from Struers, Germany); in this way, multielectrode disks were obtained (self-made, see Fig. 1b,c)

In the case of printed circuits (Fig. 1a), the best results were obtained when the alund base and glass mask were additionally covered with a chemo-setting resin. If a bare glass mask was used, after a few hours, it was wetted by the electrolyte, resulting in shunts between the pads, which made further potentiometric use redundant. The mask obtained with nonwettable du Pont resin adhered poorly to the alund base, resulting in the formation of a water layer between the base and mask and shunts. The microelectrodes made on the epoxy base and coated with the typical light-setting mask were not suited for application because of the leakage of unidentified components from the base and mask. The exudates were electrochemically active in the range of the potentials used for the oxidation of the monomers, and the oxidation currents were even a few hundred times bigger than those obtained from pure monomer solutions. In all of these cases, a Au microelectrode was placed in a catheter formed by mask material, which hindered any accidental damage to the deposited film and facilitated the deposition of the poly(vinyl-chloride) (PVC) membrane cocktail (for solid-contact ISEs).

A detailed description and pictures of the fabrication of foil-based microelectrodes has been provided by Zielińska et al. [27].

The third type of electrodes (see Fig. 1b)—i.e., the flat-disk multielectrode array—contained four to eight gold electrodes (99.999%) of 0.5–1 mm diameter placed in a cylindrical body with a 8–12 mm external diameter. In this case, the greatest source of difficulty was the poor adhesion of the resin applied to the Au wires, which could result in water uptake due to capillary forces and to "memory effects." This problem was circumvented by applying subsequently three different resin layers with different adhesive properties (as well as different chemical resistance) or, alternatively, by the use of long bodies made from the most chemically resistant epoxy resin. In another design (see Fig. 1c), eight Au microelectrodes of 0.5 mm diameter were placed in holes drilled in a polymethacrylate body and glued with UV-setting dentistry resin to create a flow-through cell. In this design, a flow-through tubular silver chloride electrode was used on the exit from the cell. Measuring with a 50 μl sample volume, a fast response and minimal carryover was obtained.

All potentiometric measurements were performed using a homemade 16-channel setup. The input impedance was greater than 10^{13} Ω, and the input current was lower than 0.1 pA for each of the 16 inputs as well as for the reference electrode input. The multichannel potentiometer was

coupled to a personal computer equipped with a 16-bit resolution data acquisition card PCI-DAS 6014 (Computer-Boards) and custom-made software. A Ag/AgCl/3 M KCl or double-junction Ag/AgCl/3 M KCl/1 M KNO_3 electrode was used as the reference electrode. All the experiments were performed at 22±2 °C.

Electrode preparation

Before polymerization of the EDOT or MPy, the commercial GC, Pt, or Au electrodes as well as arrays of home-made microelectrodes (see Figs. 1b,c) were polished with 0.3-μm alumina and carefully rinsed with water. The electrode to be covered with a CP was then placed in an ultrasound bath and cleaned with 0.2 M KOH in methanol, 0.1 M HNO_3, as well as with deionized water and then immediately immersed in the solution to be used for electropolymerization. The arrays of the screen-printed microelectrodes were cleaned in an ultrasound bath in water, methanol, and once again in water. The arrays were then immediately immersed in the solution to be used for electropolymerization.

The electropolymerization conditions for the poly-MPy (PMPy) and PEDOT films doped with biological buffer ligands were selected according to the disk type (GC, Pt, or Au) and to their surface areas (0.07 or 0.03 cm^2) and extend to other electrode surfaces in the range 0.0007–0.01 cm^2.

The PMPy films doped with MOPS ligands were deposited from 1.4 M MOPS containing 0.05–0.02 M of the monomer. The best electrodeposition conditions were found in serial potentiostatic experiments, in which the polarizing voltage was incrementally increased by using 500-s intervals. In Fig. 2, typical changes in currents registered for +700 mV vs. Ag/AgCl are shown. In the current–time curves, the current minimum was always observed at the beginning of the electrodeposition. This was why the trials that applied the voltammetric method for setting the optimal electrodeposition potential resulted in values of up to 250 mV higher than needed. Therefore, the PMPy–MOPS films were deposited in two stages. In the first stage, the potentiostatic electrodeposition was performed for 500 s at +700 mV, and in the second stage, it was continued via the galvanostatic method over 2,000–3,000 s. In this way, by allowing for initial film formation under potentiostatic conditions, the abrupt increase in initial potential, which was observed when only the galvanostatic method was used, was avoided. Under identical electrodeposition conditions, the charge density was always bigger for microelectrodes, 0.33–0.46 C/cm^2, while for (commercial) electrodes with a larger surface, it was 0.14–0.18 C/cm^2. The PMPy–MOPS films were then prepared to work as reference electrodes by soaking them in a 0.1 M sodium salt of MOPS, pH=7.4.

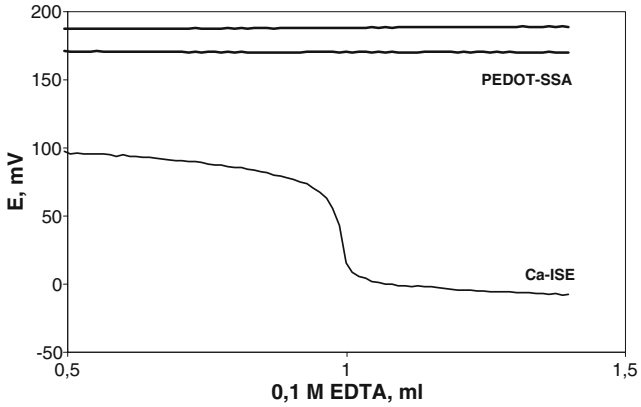

Fig. 3 The reference PEDOT–SSA films and solid-contact Ca-ISE in a potentiometric titration of $CaCl_2$ (80 ml, 0.00125 M) with EDTA (0.1 M). The potential is measured vs. an external Ag/AgCl/3 M KCl electrode

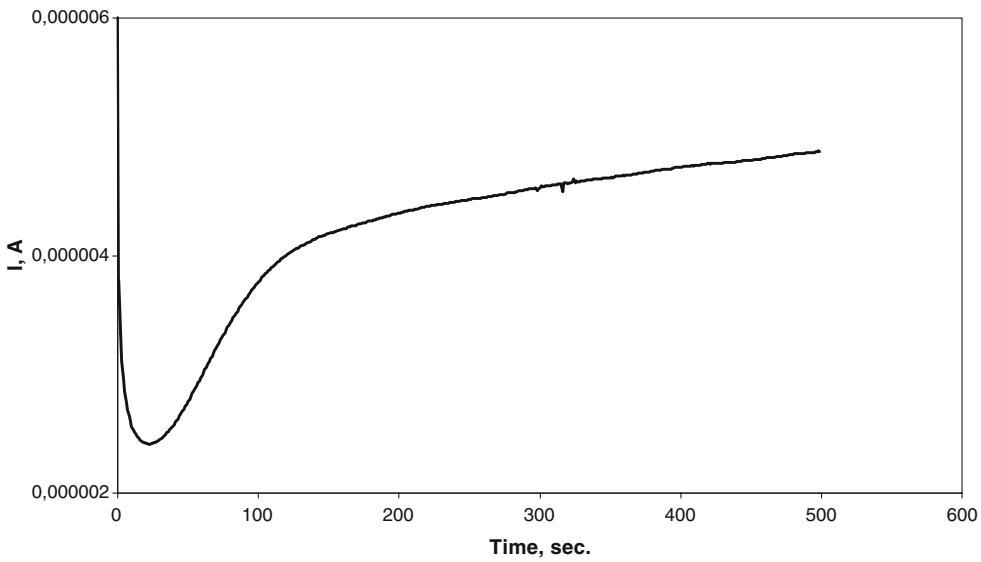

Fig. 2 Current vs. time during the first phase of PMPy–MOPS deposition onto a 0.07-cm^2 GC substrate. E=700 mV vs. Ag/AgCl, 1.4 M MOPS, 0.2 M MPy

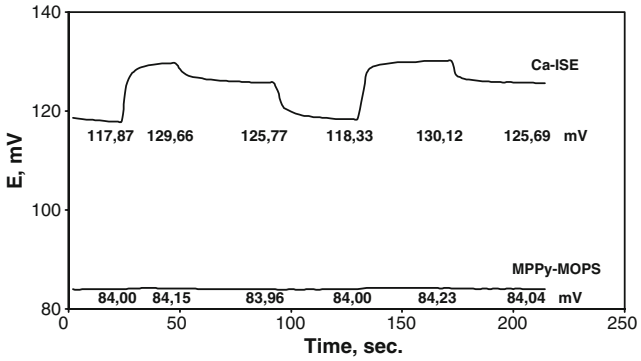

Fig. 4 Response of the PMPy–MOPS reference films (*thick line*) and solid-contact Ca-ISE (*thin line*) in the calibration with clinical standards. The response is measured vs. an external Ag/AgCl/3 M KCl electrode

The PEDOT–SSA films were deposited onto Au microelectrodes from 3M SSA solutions, containing 0.01 M EDOT. The electrochemical conditions applied were the same as described before, i.e., 20 mV/s, voltage range 500–850 mV, and 20 full cycles [1].

After deposition, the films were rinsed with deionized water and allowed to dry. The quality of the deposited films was inspected under a microscope. The PEDOT–SSA reference films were soaked in 0.1 M SSA solutions with the pH value adjusted to 11.6 via addition of NaOH.

Ion-selective electrode preparation

The Ca^{2+}-, K^{+}-, Na^{+}-, and Cl^{-}-sensitive membranes were made from the appropriate cocktails containing the following electroactive components obtained from Fluka: ETH 129 for calcium, valinomycin for potassium, ETH 2120 for sodium, and tridodecylmethyammonium chloride (TDMACl) for chloride. The active compounds (1% *w*/*w*), potassium tetrakis (4-chlorophenyl) borate (0.5% *w*/*w*), bis (2-ethylhexyl) sebacate (65.5% *w*/*w*), and high-molecular-weight PVC (33% *w*/*w*) were dissolved in freshly distilled tetrahydrofuran in the case of cation-sensitive membranes. In the case of the chloride-sensitive membrane, TDMACl was dissolved together with PVC (20% *w*/*w*).

To prepare the membranes, 0.5 μl of the cocktails were deposited on the surfaces of the selected microelectrodes covered with PMPy–MOPS or PEDOT–SSA films. Prior to use, the microcells with PMPy–MOPS films were soaked in a mixture containing 0.1 M $CaCl_2$, 0.1 M KCl, and 0.1 M sodium salt of MOPS at pH=7 for 24 h. The calcium-sensitive microcells with PEDOT–SSA films were soaked in a mixture of 0.1 M $CaCl_2$ and 0.1 M SSA, with the pH value adjusted to an appropriate value with NaOH.

Results and discussion

To check the applicability of a four-microelectrode Au disk array, calcium ion-sensitive cells were used with a PEDOT–SSA solid-contact film and a reference electrode film as previously described [1]. Following the deposition and 24-h conditioning in 0.1 M SSA, pH=11, all four PEDOT–SSA films exhibited an insensitivity to pH changes in the range 9–11. The Ca solid-contact electrodes were then prepared by drop casting. After evaporation of the solvent, the microcell was conditioned for 24 h in 0.1 M $CaCl_2$ and

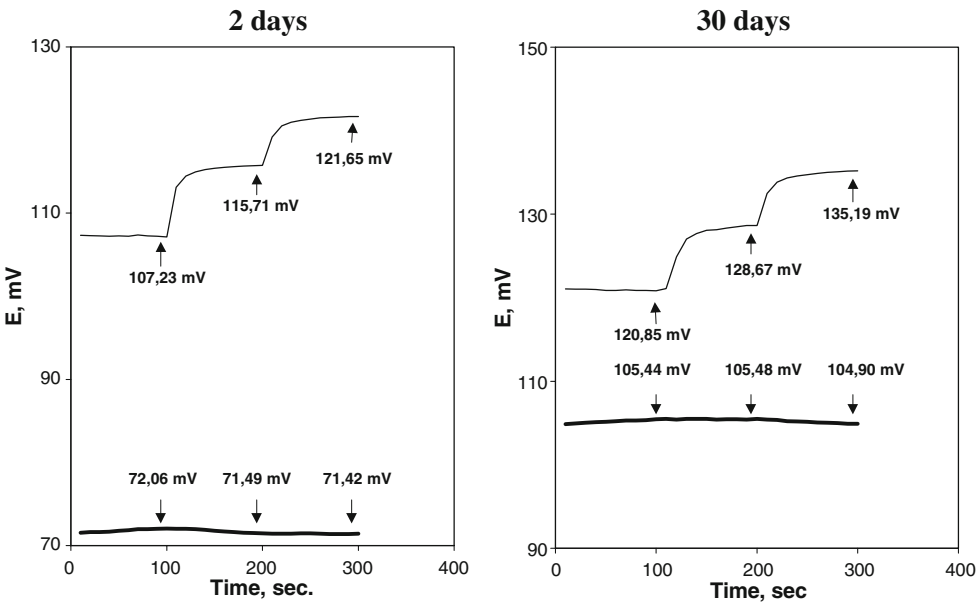

Fig. 5 Response of the cell (as in Fig. 4) after 2 and 30 days of use

0.1 M SSA at pH=11 (adjusted by NaOH). The microcell was subsequently used for end-point detection in the titration of calcium by ethylenediamine tetraacetic acid (EDTA) in a borate buffer (pH 9.2). In the first titration, the potential changes of two "solid-contact" calcium microelectrodes and the remaining two "plastic" PEDOT–SSA-based reference electrodes were measured against a Ag/AgCl external electrode, as shown in Fig. 3. The observed differences against this reference electrode were less than 1 mV for both PEDOT–SSA films. Therefore, in the following titrations, the potential of the indicator calcium microelectrodes was measured against the PEDOT–SSA-based reference electrodes.

The PMPy–MOPS films were conditioned in 0.1 M solution of MOPS (pH 7.4 adjusted with NaOH) and calibrated in clinical calibrating solutions, which also have pHs in the pH range of the MOPS buffer. The reproducibility of the potential in three subsequent calibrations was less then 1 mV in each standard. The best performing films maintained similarly stable potentials after tenfold dilutions of each standard. These films were further used either as the reference electrode or as the solid contacts for ion-selective membranes obtained via drop casting. In this way, an integrated and flat microgalvanic integrated cells (MGICs) were made. After conditioning the cells in STD1, the properties of the MGICs were first tested by measuring the responses of the reference electrodes (films) and ISEs vs. a Ag/AgCl reference electrode. In Fig. 4, the responses of the PMPy–MOPS film and Ca-ISE in clinical calibrating solutions are shown. In subsequent calibrations, the potential changes of the PMPy–MOPS films did not exceed 0.27 mV. The following measurements were therefore performed without the Ag/AgCl reference. In Fig. 5, to illustrate the stability of the MGIC, with the Ca-ISE/PMPy–MOPS electrodes, the response on the second and 30th day of use are shown. In both cases, a good reproducibility of the reference electrode potential (difference of 0.64 and 0.58 mV) and MGIC readouts (14.42 and 14.34 mV) were observed.

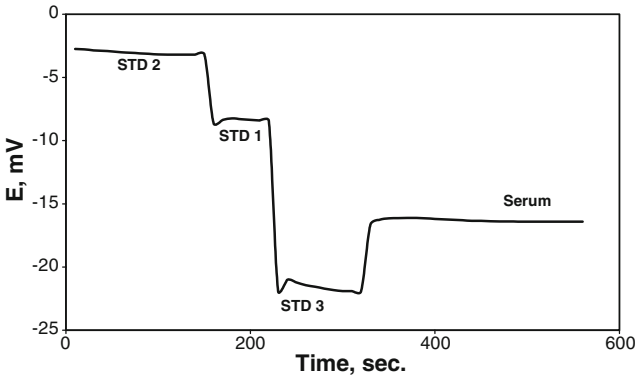

Fig. 7 Potential changes of the MGIC with K-ISE and with PMPy–MOPS as the reference electrode material during the calibration in clinical standard solutions and measurement in serum (1 ml)

In Fig. 6, the changes of four solid-contact micro-ISEs (measured against the PMPy–MOPS-based reference electrode) during calibration in clinical standards in the flow-through system are presented. Both the reference electrode films and the ISE CP-based mediating films were deposited onto Au electrodes of 500 μm diameter, enabling a cell dead volume of 50 μl.

One of the eight Au electrodes lacking a deposited polymer was used to ground the solution, while four of the remaining electrodes were converted into solid contact ISEs and three into CPs based reference electrodes. The slopes obtained were: 26.7 mV/p[Ca], 54.6 mV/p[K], 49.5 mV/p[Na], and 40.4 mV/p[Cl], respectively. The three reference

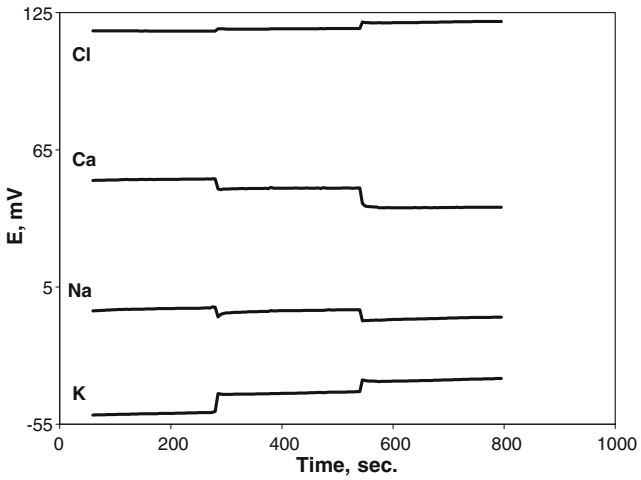

Fig. 6 Response of the MGIC cell with K-, Na-, Cl-, and Ca-ISEs measured during the calibration in the flow-through cell. The reference electrode material is PMPy–MOPS

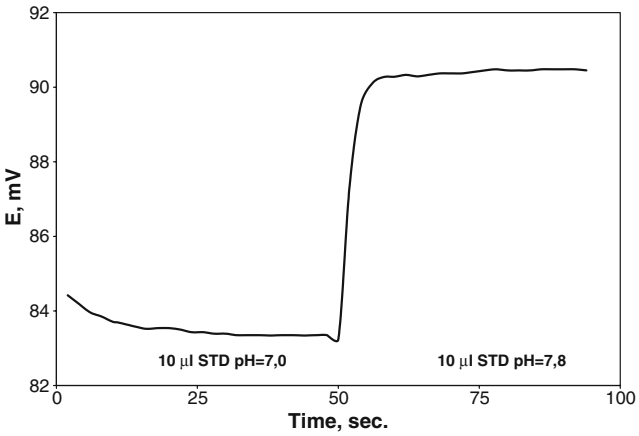

Fig. 8 Potential changes of the MGIC with a Ca-ISE and PMPy–MOPS as the reference electrode material during the calibration in clinical standards by using 10 μl solution volume

electrodes exhibited similar potentials difference, within ±0.2 mV.

In Fig 7, some examples of practical application of the MGICs are shown. In this case, the cells with the K-ISE-, Ca-ISE-, and PMPy–MOPS-based reference electrodes were used for determination of calcium and potassium ions in lyophilized serum samples. The MGIC was first calibrated in clinical standards, and then potassium in 1 ml of serum sample was assessed. The potassium and calcium measured were 1.0±0.01 and 3.7±0.03 mmol/L, respectively (n=3), and remained in good agreement with the respective values given by the manufacturer.

In Fig. 8, MGICs with a Ca-ISE were used for similar measurements but in 10μl samples. This measurement illustrates the goal of the ongoing research in which we apply described MGICs for biological measurements with living cultures of the cells.

Conclusions

It is shown that through the tailored use of conductive polymers, it is possible to integrate (i.e., to remove internal liquid contacts) and miniaturize indicator and reference electrodes while maintaining the characteristics of macro-conventional potentiometric sensors. Furthermore, the electrodes can be placed on a flat surface, which facilitates the integration of this galvanic cell in flow-through modules and for direct applications in small volumes and/or for the monitoring of ion fluxes at biological membranes.

Acknowledgments Financial support from the Polish State Committee for Scientific Research (KBN), project number 3T08E 085 30 and R15 005 03, is gratefully acknowledged. Mrs. Anna Lingenfelter is acknowledged for improving the English of this text.

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