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A miniaturized solid-contact potentiometric multisensor platform for determination of ionic profiles in human saliva

Marcin Urbanowicz[1] · Dorota G. Pijanowska[1] · Artur Jasiński[2] · Marcin Ekman[3] · Maria K. Bocheńska[2]

Abstract

This paper describes a miniaturized multisensor platform (MP-ISES) consisting of electrodes: a reference one (RE) and ion-selective electrodes (ISEs) for monitoring Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and SCN^- ions and pH in human saliva. Gold electrode surface was modified by deposition of two layers: electrosynthesized PEDOT:PSS forming an intermediate layer, and ion-selective membrane. The developed ISEs were characterized by a wide linear range and sensitivity consistent with the Nernst model. The entire MP-ISEs are characterized by satisfactory metrological parameters demonstrating their applicability in biomedical research, in particular in measurements concerning determination of ionic profiles of saliva. Saliva samples of 18 volunteers aged from 20 to 26 participating in a month experiment had been daily collected and investigated using the MP-ISEs assigned individually to each person. Personalized profiles of ions (ionograms) in saliva, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SCN^-, and H^+, were obtained.

Introduction

For many years, direct potentiometry and ion-selective electrodes have been useful tools for determining ion concentration in human body fluids [1–3], since then they have still been used in medical analyses for determination of Na^+, K^+, Ca^{2+}, and Cl^- ions [4–6]. The classical ion-selective electrodes (ISEs), due to their design, e.g., large dimensions, are not compatible with modern clinical applications, which aims at reducing the sample volume. For this reason, for decades, vast part of research was focused on the elimination of the classic redox system (e.g., Ag/AgCl with liquid internal electrolyte) by designing electrodes in solid-contact technology [7–24]. The use of solid-contact technology enabled design of the ISEs in planar technology, their miniaturization, and unquestionably widening range of implementation of ISEs, including clinical and biological analyses of small volume samples [25–28]. The solid-contact electrode (SCE) consists of an intermediate layer, for example, an electroconductive polymer deposited on the electrode and covered by an ion-selective membrane [29, 30]. The electrode may be platinum-, gold-, or carbon-based materials, e.g., glassy carbon.

However, eliminating the classic redox system and replacing it with the intermediate layer cause some problems. From a practical point of view, these difficulties concern the insufficient long-term stability of the potential and poor reproducibility of duplicated electrodes, which makes them difficult to exchange e.g. inside analyzers. So far, these problems have been solved for glass and crystalline electrodes, where the slope of characteristics is typically stable, regardless of whether it is a classic design or SCE type. However, changes of SCE-ISE standard potential are unpredictable causing parallel shifts of the calibration curve, which in turn reduces the accuracy of the measurements if the calibration is not frequently enough. Therefore, frequent calibration is performed to

✉ Marcin Urbanowicz
murbanowicz@ibib.waw.pl

1 Nalecz Institute of Biocybernetics and Biomedical Engineering Polish Academy of Sciences, Ks. Trojdena 4, 02-109 Warsaw, Poland

2 Department of Chemistry and Technology of Functional Materials, Chemical Faculty, Gdańsk University of Technology, Narutowicza 11/12, 80-233 Gdańsk, Poland

3 Department of Surgical Oncology, Medical University of Gdańsk, Smoluchowskiego 17, 80-214 Gdańsk, Poland

increase the accuracy of analysis. The SSE-type electrodes are very attractive when the possibility of miniaturization is considered. Nevertheless, their small size usually increases the electrical resistance of the electrode, which consequently may decrease signal-to-noise ratio and instability of the potential. The same effect may be caused by incorporating a conductive polymer, which changes properties under influence of light, and it may affect the redox systems, e.g., in the presence of oxygen or due to the change of pH [29, 31–41]. In addition, in many works confirmed, the capability of ion-selective membranes based on polyvinyl chloride (PVC) to sorb water, which affects steady-state potential, and in consequence the stability of sensors, e.g., for solid-contact PVC-membrane-based ISEs, the stabilization time is long, ranging from 3 to 48 h [42–47]. This internal aqueous solution is formed spontaneously, and its composition depends on the ambient conditions and electrolyte diffusion level through the membrane. As a result, the stability of SCE-ISE deteriorates in particular drift of the potential occurs [10, 43, 48]. Reasonably short response time of SCE-ISEs proves their usability for potentiometric measurements in various applications, such as measurements of concentration of many inorganic ions [10, 49, 50], ionic surfactants [51], and polyions [52]. Additionally, possibility of miniaturization allows placing ISEs in the needle of the syringe [53] in a way that the measurements can be performed even within the living cells [54]. Noteworthy, it is also possible to replace an electroconductive substrate, such as metal or carbon, with a special plastic PEDOT-PSS Baytron P [55]. Traditional glass-capillary microelectrodes are characterized by a very short lifetime (1 day) [53] in comparison with the solid-contact electrodes.

Maintaining the physiological concentration of ions in body fluids is considered to be one of the key factors of homeostasis [56]. Therefore, monitoring of ion concentration changes in human blood is successfully performed as a basic analysis in medical diagnostics [57]. It is known that the ionic composition of saliva may reflect the health state of the person being examined [58–62]. Due to the correlation of ion concentration with the general health status, the potentiometric measurements can be applied to the assessment of ionic profiles in saliva [62]. Therefore, personalized ion profiles of saliva may deliver new information on condition of patient/person being examined.

This paper presents the results of the research carried out using the miniature multisensor platform of own construction composed of solid-contact ion-selective electrodes. This work is a continuation of study on the use of miniature ISEs in biomedical research [63]. Our previous article focused on examining some factors influencing ISE response, including lifetime parameters, and also showed the algorithm that allows designing ionic composition of solutions for calibration of MP-ISEs. The purpose of this work demonstrates applicability of the developed MP-ISES, in particular as a useful analytical tool for simultaneous determination of ions in saliva. The MP-ISES enabled the study of changes in Na$^+$, K$^+$, Ca^{2+}, Mg^{2+}, Cl$^-$, SCN$^-$, and H$^+$ ion concentrations in human saliva. The results in the form of personalized ionograms as well as their analysis are presented.

Experimental

Chemicals

The components for electrode membranes, i.e., poly(vinyl chloride) (PVC of high molecular weight), bis(2-ethylhexyl)sebacate (DOS) $\geq 97\%$, o-nitrophenyl octyl ether (o-NPOE) $\geq 99\%$, chloroparaffin 60% chlorine basis, and potassium tetrakis(p-chlorophenyl)borate (KTpClPB) $\geq 97\%$; tridodecylmethylammonium chloride (TDMACl); ionophores selected for the studies, i.e., p-$tert$-butylcalix[4]arene-tetraacetic acid tetraethyl ester (sodium ionophore X), valinomycin (potassium ionophore I), N,N-dicyclohexyl-N',N'-dioctyldecyl-3-oxapentane-diamide (calcium ionophore IV), N,N''-octamethylene-bis(N'-heptyl-N'-methylmalonamide) (magnesium ionophore III), and tridodecylamine (hydrogen ionophore I), were purchased from Sigma-Aldrich. Tetrakis-(4-triphenyl-phosphonium-butoxy)-p-$tert$-butylcalix[4]arene tetrathiocyanate (thiocyanate ionophore) was synthesized at the Faculty of Chemistry, Department of Chemistry and Technology of Functional Materials, Gdańsk University of Technology [64, 65]. Solvent, i.e., tetrahydrofuran anhydrous $\geq 99\%$; and conducting polymer components, i.e., 3,4-ethylenedioxythiophene (EDOT) and poly(4-styrenesulfonic acid) sodium salt (NaPSS), were purchased from Sigma-Aldrich. Other chemicals such as inorganic salts, i.e., NaCl $\geq$ 99.9%, KCl $\geq 99.5\%$, CaCl$_2$·6H$_2$O $\geq 99\%$, MgCl$_2$·6H$_2$O $\geq$ 99.5%, KSCN $\geq 99.5\%$, CH$_3$COONa $\geq 99.5\%$, CH$_3$COOLi·2H$_2$O $\geq 99\%$, KOH, methanol, pH buffer (5.00 ± 0.05, $7.00 \pm$ 0.05, 9.00 ± 0.05), and nitric acid, were obtained from Avantor Performance Materials, Poland S.A. Poly(vinyl acetate) (PVA) and 2,2-dimethoxy-2-phenylacetophenone (DMPA) were from Sigma-Aldrich. Freshly deionized water for aqueous salt solutions was obtained by reverse osmosis (RO) Hydro-Lab-PL station (≤ 0.1 μs/cm).

Preparation of multisensor platforms

Two potentiometric multisensor platforms (MP-ISEs) selective to two groups of ions were developed. The two MP-ISEs were selective to Na$^+$, K$^+$, Ca^{2+}, and Mg^{2+} and to Cl$^-$, SCN$^-$, and H$^+$ ions, respectively. Each of the MP-ISEs had a 3-mm diameter and consisted of ISEs (Figs. 1 and 2). As a basic electrode material, a 500-μm-diameter gold wire (Au $\geq 99.99\%$) was used. The gold surfaces were polished with sandpapers of various grain sizes used in the following order: 600, 2000, 2400,

Fig. 1 Scheme of the multisensor platform and solid-contact RE
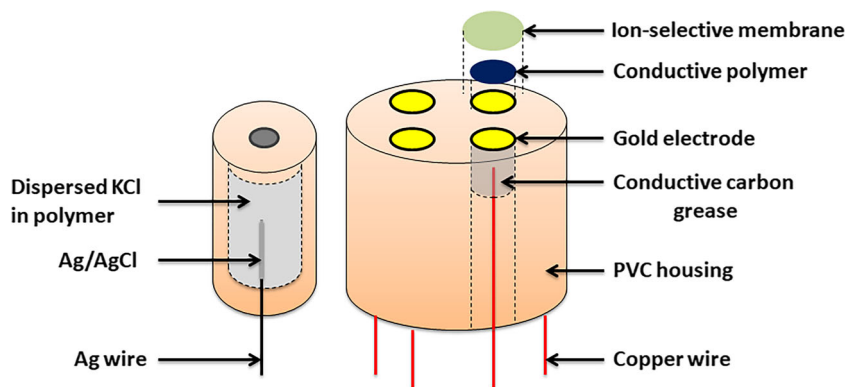
and 4000 and then with Al_2O_3 abrasive powder of a grain size of 0.3 μm distributed on a wet mesh paper. Then, the surface of electrodes was chemically cleaned by immersing in the following solutions: 1 mol dm^{-3} KOH in methanol, deionized water, 10% HNO_3 and again in deionized water [66]. Before deposition of ion-selective membranes (ISMs), except for electrodes intended to be used for SCN^- and Cl^- ion detection, the electrosynthesis of PEDOT:PSS layer on the top of Au wire surfaces was performed by galvanostatic polymerization. The electrochemical cell for the PEDOT:PSS deposition system consisted of the Au wires with polished and cleaned surfaces connected as a working electrode, an auxiliary electrode (glassy carbon rod), and a single junction reference electrode. All the electrodes were immersed in an aqueous solution of the monomer (0.015 mol dm^{-3} EDOT) and supporting electrolyte (0.1 mol dm^{-3} NaPSS). The PEDOT:PSS was deposited by applying a current density of 0.2 mA cm^{-2} to the working electrode for 714 s [67, 68]. After rinsing with deionized water and drying, the liquid membranes were deposited on the top of the PEDOT:PSS intermediate layer. For this purpose, approximately 0.3 μL of the membrane cocktail was applied onto each electrode and allowed to evaporation of the solvent for 24 h. Compositions of ion-selective membrane (ISM) cocktails, expressed in wt.%, were as follows: Na-ISM: 1.28 sodium

Fig. 2 Construction of the miniature multisensor platform used in the studies: **a** miniature solid-contact reference electrode; **b** module of four gold electrodes without ISMs
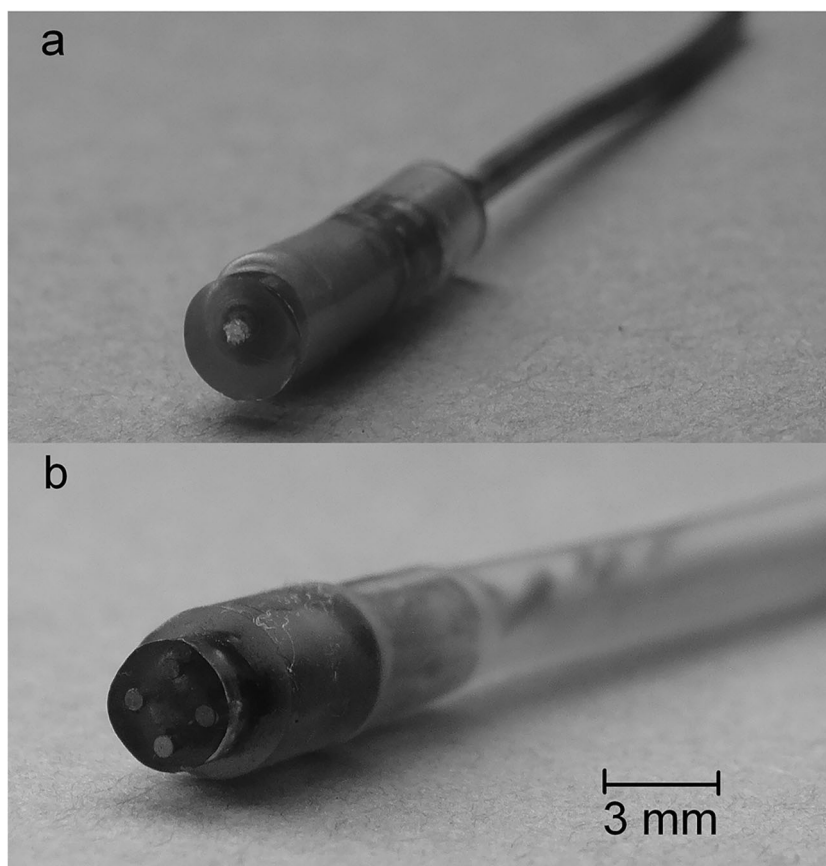
ionophore X, 0.22 KTpClPB, 65.62 o-NPOE, 32.88 PVC; K-ISM: 1.16 valinomycin, 0.56 KTpClPB, 65.24 DOS, 33.04 PVC; Ca-ISM: 1.33 ETH 5234, 0.46 KTpClPB, 64.50 o-NPOE, 33.71 PVC; Mg-ISM: 1.31 ETH 4030, 0.64 KTpClPB, 65.35 chloroparaffin, 32.70 PVC; Cl-ISM: 7.63 TDMACl, 66.49 o-NPOE, 25.87 PVC; SCN-ISM: 2.22 thiocyanate ionophore, 64.83 chloroparaffin, 32.95 PVC; H-ISM: 1.15 tridodecylamine, 0.58 KTpClPB, 65.32 DOS, 32.94 PVC.

As for all potentiometric measurements, for MP-ISEs, an integrated and miniature solid-contact reference electrode (RE) is required [69]. For many years, attempts have been made to refine or propose new concepts of RE [67, 70, 71].

In our experiments, the RE was made by dispersion of KCl crystals within the UV curable polymer [72–75]. At first, the Ag/AgCl wire was prepared by electrochemical formation of a silver chloride layer. For this purpose, the pure Ag wire was immersed in a 0.1 mol dm^{-3} HCl and then connected against platinum electrode to a power supply with a fixed voltage and current of 1.5 V and 40 mA, respectively. Next, vinyl acetate was mixed with milled and dried KCl in ratio of 1:1 wt.:wt. and then 1 wt.% of photoinitiator 2,2-dimethoxy-2-phenylacetophenone (DMPA) was added. The resulting mixture was stirred, and then, the Ag/AgCl wire was inserted and irradiated with UV light at a wavelength of 365 nm.

As a housing for the electrodes, a polyvinyl chloride (PVC) tube was used. The electrical ISE-leads were made of copper wires. In order to ensure a stable electrical connection, the MG Chemicals 8481-2 conductive carbon grease was used. As insulation of electric wires, plastic infusion tubes were utilized.

Measurements in biological samples—saliva's sampling protocol

The body fluid in the oral cavity is a mixture of saliva produced by salivary glands, gingival fluid, and serum. The saliva maintains oral homeostasis and oral health. Its primary role is to help in the formation of swallowed food and pre-digesting of some substances by enzymes, for example, α-amylase. The main components of saliva are as follows: water, enzymes, proteins, and inorganic ions. The composition of saliva depends on the type of salivary glands in which it is secreted. In the human body, three main pairs can be distinguished: parotid, sublingual, and submandibular, which produce 95% of the volume of saliva which corresponds to 1–1.5 L per day. The remaining 5% is produced by the tongue glands and other oral glands. An undoubted advantage of saliva as an analytical sample is relatively easy availability. Nevertheless, due to the complex composition of the sample and the widespread presence of microorganisms in the oral cavity, the collection, processing, and storage of saliva samples are associated with many problems. The saliva collection procedure presented below was based on Lahti et al.'s work [76].

The volume of 2-mL unstimulated saliva samples were collected from 18 healthy volunteers by sterile 10-mL-volume syringes (Polfa Lublin S.A., Poland) directly from oral cavity every day at 11 am for a course of 1 month. Single collection of a small volume of saliva minimized the risk of stimulating the salivary glands. For 30-min time interval before sampling, volunteers did not eat nor drink. Additionally, in order to limit the mixing of saliva with other fluids, e.g., from the nostrils, the volunteers made the collection of saliva samples by placing the syringe in the mouth so that the tip of the syringe was close to the submandibular gland. The sampling process lasted about 1 min during which the volunteers did not swallow saliva, did not move the tongue, and did not make any unnecessary movements that could stimulate the production of saliva. After sampling at least 2 mL of the saliva, the tip of the syringe was blinded with a sterile rubber stopper and immediately transferred for centrifugation at 6000 rpm for 6 min in order to eliminate sediments. Subsequently, the saliva samples were measured by the miniaturized MP-ISE system to determine Na$^+$, K$^+$, Ca^{2+}, Mg^{2+}, Cl$^-$, and SCN$^-$ ions and pH. The measurement was carried out in a glass cell with a rubber cover in which the MP-ISEs were placed. The use of blinding allowed limiting sample contact with air and minimizing the risk of pH changes. The volume of the test sample was 500 μL.

Characteristics of the study group

The research was carried out in a group of volunteers who have signed informed consent to participate in the study. Collection, processing, and analysis using the saliva samples were carried out under the medical supervision of the Department of Oncologic Surgery of Medical University of Gdańsk. Due to the signed agreement of all volunteers, medical supervision, and the lack of body shells violation, the bioethical committee permission was not required, in accordance with Polish law. A total of 18 healthy volunteers, including 11 women and 7 men, aged 20–25 participated in this study. Before sampling the saliva, the volunteers responded to the questionnaire. The volunteers received a questionnaire and randomly chosen part of the code containing the ID, which was marked in the questionnaire and the tube before collecting a saliva sample. In addition, 8 out of 11 women reported no problems with the regularity of the menstrual cycle.

Results

Parameters of multisensor platforms

Analytical evaluation of the multisensor platforms (MP-ISEs) included the determination of parameters such as linear range, sensitivity (slope of characteristic), and cross-selectivity

Table 1 The ISE sensitivity and linear range obtained for measurements with MP-ISE versus liquid junction and solid-contact RE ($n = 9$ repeating for each electrode)

ISE	S_{Orion} (mV/dec)	S_{Solid} (mV/dec)	Linear range for ISE versus RE_{Solid} in $-\log[a/\text{mol dm}^{-3}]$
Na^+	56 ± 1	56 ± 1	$1–7$
K^+	58 ± 1	58 ± 1	$1–5$
Ca^{2+}	28 ± 1	29 ± 1	$1–8$
Mg^{2+}	30 ± 1	30 ± 1	$1–6$
Cl^-	-53 ± 2	-54 ± 2	$1–6$
SCN^-	-52 ± 2	-53 ± 2	$1–6$
pH	62 ± 1	61 ± 1	$5–11$

coefficients for interfering ions present in human saliva. The parameters were determined for each ISE with the membrane prepared from cocktails of compositions described in "Preparation of multisensor platforms." Since the RE in the MP-ISEs is of own construction, then, the performance of ISE was compared with results obtained for measurements versus standard commercial RE (Table 1). The selectivity coefficients were calculated according to both separate solution and fixed interference methods (SSM and FIM) and compared with the values shown in the IUPAC technical report [77] and other literature data. Na-ISE obtained more favorable selectivity coefficient ($\log K_{Na/K} = -2.9$) than that declared by Cadogan [29] ($\log K_{Na/K} = -2.7$). K-ISE obtained $\log K_{K/Na} = -4.0$ while for analogous membrane composition in [77], it obtained $\log K_{K/Na} = -3.7$. Ca-ISE obtained $\log K_{Ca/Mg} = -4.0$ and was slightly worse than that declared in [78]. For Mg-ISE and Cl-ISE, selectivity coefficient did not differ significantly from those reported in the literature. However, for the SCN-ISE, the following values of selectivity coefficients were obtained: $\log K_{SCN/Cl} = -3.8$, $\log K_{SCN/NO3} = -1.9$, $logK_{SCN/}$

$_{HCO3} = -3.0$, $\log K_{SCN/H2PO4} = -4.2$, $\log K_{SCN/SO4} = -2.6$. Other values of selectivity coefficients are presented in Fig. 3. To estimate the activity coefficients, the Phreeqc Interactive 3.4.0 software was used. An algorithm of this software includes the Debye–Hückel equation and Pitzer model.

Drift coefficient of the sensor potential during calibration did not exceed 170 µV h^{-1} for cation-selective sensors and 200 µV h^{-1} for anion-selective sensors, while during the measurement of saliva samples, 315 µV h^{-1} and 430 µV h^{-1} for cation- and anion-selective sensors, respectively. Detailed data can be found in Table 2. Long-term stability corresponded with our previous research described in Urbanowicz et al.'s work [63].

The ready-to-use MP-ISEs were stabilized in the conditioning solution of the following compositions: Na^+, K^+, Ca^{2+}, Mg^{2+}, SCN^-—1 mmol dm^{-3} for each ion and 5 mmol dm^{-3} for Cl^-. *Electromotive force* (EMF) was measured versus both an Orion ROSS Ultra 800500U D/J RE and solid-contact RE of own construction (Fig. 4 and Table 1). The stability of the solid-contact RE has been examined and previously described in Jasinski et al.'s work [68]. The solid-contact RE drift of the potential measured against the classic RE for 14 h was 5 µV. The MSP (multisolution protocol) used for investigation of the stability of the electrode response in various types of aqueous solutions, indicated that the electrode is stable in a solution of 0.01 mol dm^{-3} HCl (11 mV deviation from the initial value) and 3 mol dm^{-3} KCl solution (10 mV deviation from the initial value). Additionally, positive offset with the liquid junction reference of ca. + 100 mV was obtained. These suggest that the electrode fabrication and design work very well.

The mean saliva ionic strength was modeled by using the Visual Minteq software. Therefore, to increase the accuracy of the measurements, the composition of conditioning and calibration solutions (R1 and R2) were matched to the modeled ionic strength (I) of saliva samples, i.e., $I_{R1} \approx I_{R2} \approx I_{cond}$ as

Fig. 3 The MP-ISE selectivity coefficients determined by SSM
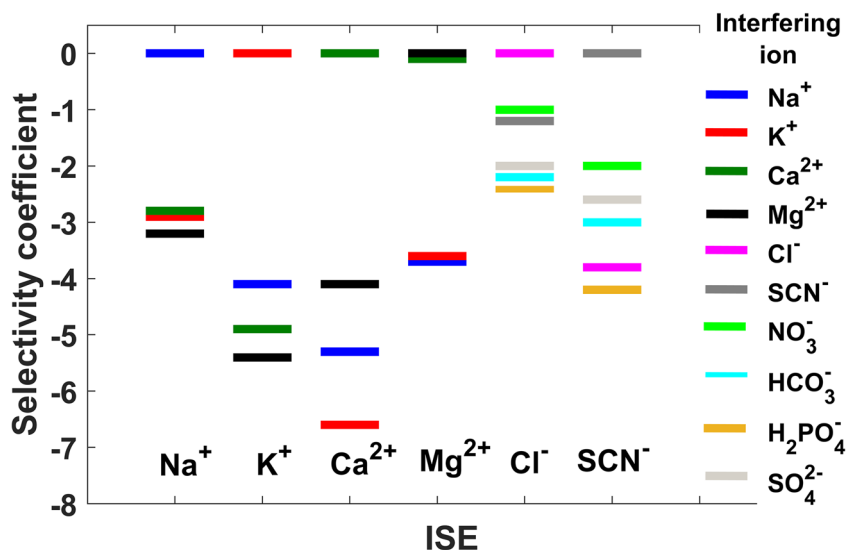
Table 2 Stability of the sensor signals during (A) calibration and (B) measurements in saliva ($n = 378$ measurements in saliva and calibration)

| ISE	S_{Solid} (mV dec^{-1})	Drift coeff. A (μV h^{-1})	Drift coeff. B (μV h^{-1})
Na$^+$	56 ± 2	123 ± 32	187 ± 41
K$^+$	55 ± 3	94 ± 27	139 ± 38
Ca^{2+}	25 ± 1	111 ± 52	221 ± 57
Mg^{2+}	25 ± 2	170 ± 60	315 ± 24
Cl$^-$	-54 ± 3	261 ± 46	430 ± 62
SCN$^-$	-54 ± 3	202 ± 37	384 ± 43
pH	58 ± 1	88 ± 25	262 ± 55

shown in Table 3. The compositions of calibration solutions R1 and R2 were designed based on literature data concerning the ionic composition of saliva. For the sake of proper measurements in saliva with the use of the MP-ISEs, the ionic strength of conditioning solution used for stabilization of electrodes was constant and close to the ionic strength of the calibration solutions.

As mentioned above, prior to measurements in saliva, the electrodes were stabilized in the conditioning solution and then calibrated using solutions R1 and R2 of the composition shown in Table 3. The compositions of the solutions were as follows: R1 10 mmol/dm^3 CH$_3$COONa, 10 mmol dm^{-3} KCl, 5 mmol dm^{-3} KSCN, 0.5 mmol dm^{-3} CaCl$_2 \cdot$6H$_2$O, 0.1 mmol dm^{-3} MgCl$_2 \cdot$6H$_2$O, 4 mmol dm^{-3} CH$_3$COOLi; and R2 1 mmol dm^{-3} CH$_3$COONa, 20.5 mmol dm^{-3} KCl, 0.5 mmol dm^{-3} KSCN, 2 mmol dm^{-3} CaCl$_2 \cdot$6H$_2$O, 1 mmol dm^{-3} MgCl$_2 \cdot$6H$_2$O. The methodology for the design of calibration and conditioning solutions was presented in the paper of Urbanowicz et al. [79].

Saliva tests

The study group consisting of 18 volunteers (23–25 years old, 11 women and 7 men) was qualified to the experiment. The volunteers received sampling kits and collected samples themselves according to the previously agreed methodology. Each participant was obliged to fill in the questionnaire.

Based on the collected data, mean ion concentrations (Table 4) were determined. The values of ion concentration correspond to those reported in literature [80]. The highest concentration was observed for Cl$^-$ and K$^+$, and the lowest for Mg^{2+}, Ca^{2+}, and SCN$^-$. The largest spread around the mean values of concentration was observed for Na$^+$. High fluctuation of sodium concentration may be caused by the influence of various types of stimuli, which has been previously described at Urbanowicz et al.'s work [75]. In the case of K$^+$, the spread of mean concentration values is significantly
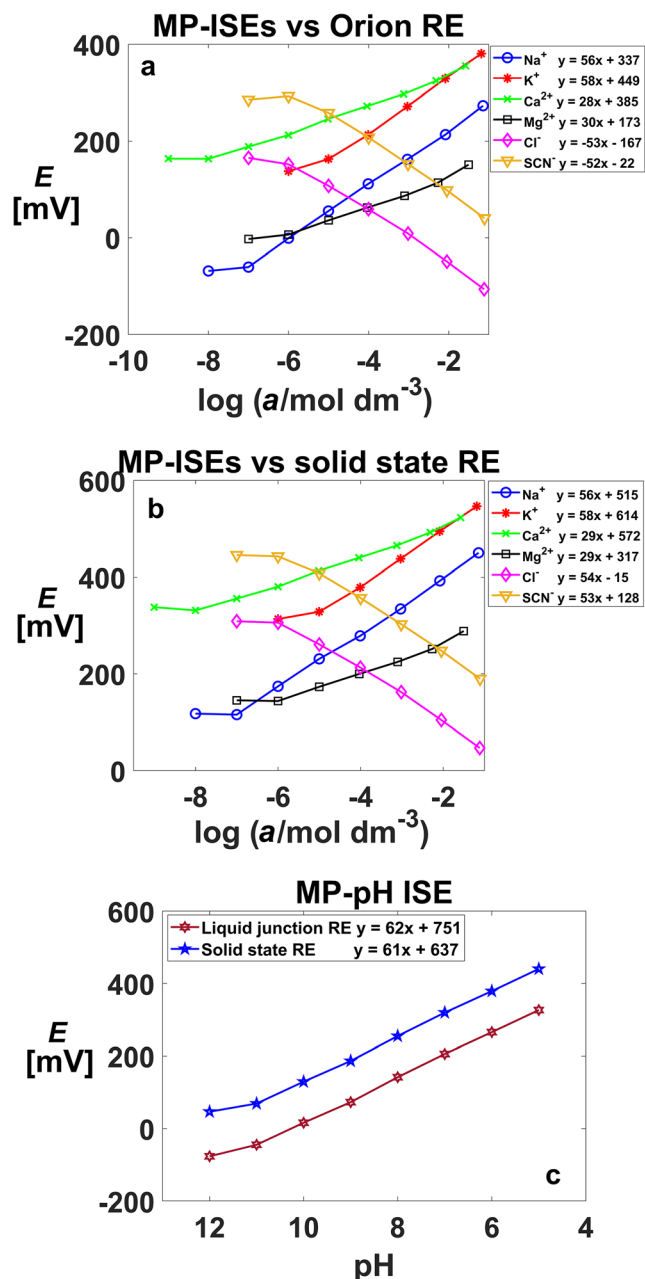
Fig. 4 The determined calibration curves for MP-ISEs. **a** Calibration curves for Na$^+$, K$^+$, Ca^{2+}, and Mg^{2+} and for Cl$^-$ and SCN$^-$ ISE versus a liquid junction RE (Orion). **b** Calibration curves for Na$^+$, K$^+$, Ca^{2+}, and Mg^{2+} and for Cl$^-$ and SCN$^-$ ISE versus solid-contact RE. **c** Calibration curves for H$^+$ ISE versus liquid junction (Orion) and solid-contact RE. Equations of calibration curves have been shown in legends

smaller than for Na$^+$. Minor standard deviations are also observed for pH and Mg^{2+}.

For all volunteers, the monthly concentration profiles of Na$^+$, K$^+$, Ca^{2+}, Mg^{2+}, Cl$^-$, SCN$^-$, and H$^+$ ions (called ionograms) were determined. Examples of ionograms are shown in Fig. 5.

Monthly ionograms for Na$^+$, K$^+$, Cl$^-$, and pH show a difference between the genders. For men, fluctuations of ion

Table 3 Ionic composition of calibration R1 and R2, and conditioning/storing solutions for MP-ISEs in saliva measurement

| Ions	R1 (mmol dm^{-3})	R2 (mmol dm^{-3})	Conditioning/storing sol. (mmol dm^{-3})
Na^+	10	1	5
K^+	15	20.5	17
Ca^{2+}	0.5	2	1
Mg^{2+}	0.1	1	0.3
SCN^-	5	0.5	1
Cl^-	11.2	26.5	17.6
Li^+	4	0	5
$CH3COO^-$	14	1	10
Ionic strength, I (mmol/dm^3)	0.303	0.303	0.298

concentration are more dynamic than for women. As it was mentioned earlier, higher dynamics of changes in Na^+ concentration for men might be caused primarily by their higher physical activity. For K^+, in the majority of cases, the concentration changes were minor and could be caused by meals rich in potassium. An interesting observation has been made for Ca^{2+} concentration in women saliva. In the saliva samples of 4 women with a regular menstrual cycle, for whom a single increase of Ca^{2+} concentration was observed. In the case of other women and men, none similar changes were observed. The obtained data suggest that there might be a relationship between the Ca^{2+} concentration in the women saliva and the menstrual cycle. However, the above hypothesis would require detailed research in this area, especially clinical trials. In the case of Mg^{2+}, no characteristic concentration changes were observed.

The parameter with minor fluctuations during the month was pH. Slight changes in pH for individual volunteers indicate that the saliva sampling procedure was carried out correctly. Long-term contact with air, contamination with external microorganisms, and overgrowth of physiological bacterial flora in the sample could have a significant impact on the sample pH, and thus could affect the ionic form of determined ions: Ca^{2+} and Mg^{2+}. For Cl^-, there was a large variability of concentration during the month for individual volunteers, which makes it difficult for interpretation. Monthly monitoring of SCN^-

Table 4 Mean, standard deviation (SD), and variance (σ) for determined ions in saliva (number of collected samples, $n = 378$)

Parameter	Na^+	K^+	Ca^{2+}	Mg^{2+}	pH	Cl^-	SCN^-
Mean (mmol dm^{-3})	5.64	16.85	0.95	0.33	6.96	23.26	0.82
SD (mmol dm^{-3})	5.41*	1.97	0.15	0.11	0.18	2.60	0.27
σ	29.24	3.89	0.02	0.01	0.03	6.78	0.07

*SD is the result of very large differences between subjects, which may result from physical activity, diet, and stressful situations [74]
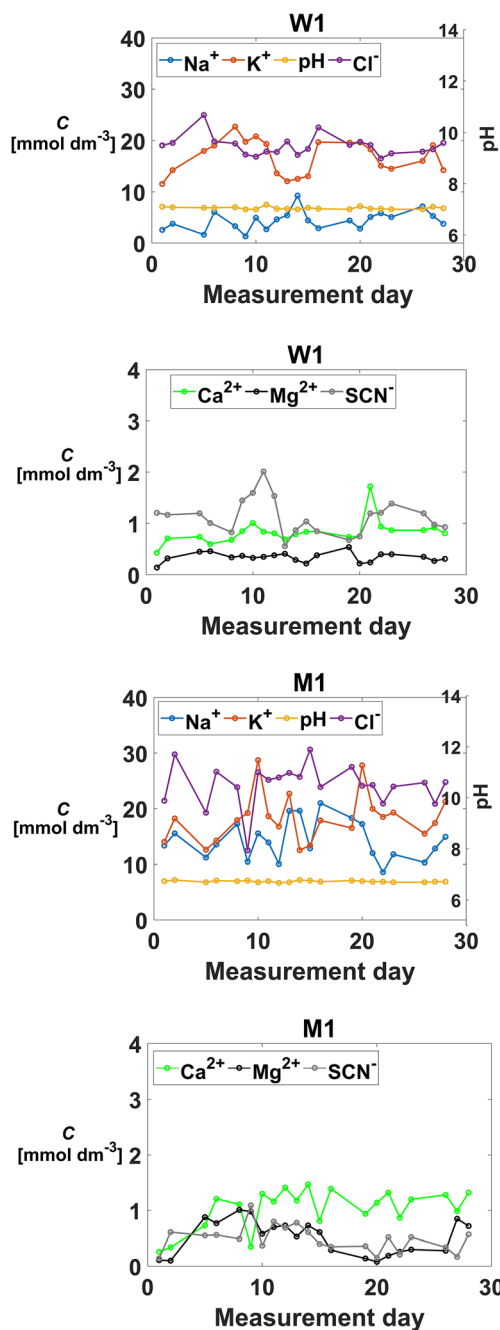
Fig. 5 Exemplary 28-day saliva ion profiles for woman and man

concentration has shown that its level can change dramatically. Between 9 and 11 measurement day for 10 volunteers, a sharp increase in SCN^- concentration was observed in saliva. None of these volunteers declared smoking. Possible reasons for a sudden increase in the SCN^- concentration in saliva could be the effect of passive smoking or prolonged staying in a place with increased smoke, for example, a walk on a crowded street.

In summary, in our research, new solid-contact potentiometric multisensor platforms with Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SCN^-, and H^+ ISEs were described. MP-ISEs were successfully applied for the determination of the abovementioned ions

in human saliva. MP-ISEs' utilization enables quick and simultaneous measurements of ion concentration.

Conclusions

In this paper, newly developed multisensor platforms (MP-ISEs) based on solid-contact ion-selective electrodes and miniaturized solid-contact reference electrode were presented. The MP-ISEs exhibited satisfactory parameters such as the sensitivity, selectivity, and linear range. The selectivity coefficients for the tested electrodes were identical or even better than those given in the IUPAC technical report [77]. The multianalyte calibration method for sensors in the MP-ISEs was successfully applied, which enabled simultaneous determination of selected ions in the small samples of complex matrix. The reference electrode without the internal liquid electrolyte was used, which allowed maintaining its size compatible in relation to the MP-ISEs. The developed MP-ISEs based on ion-selective electrodes turn out to be a reliable analytical tool for simultaneous determination of selected ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and SCN^- in human saliva presented in form of personalized ionograms for the group of 18 volunteers. The presented MP-ISEs might be potentially useful analytical tool for biomedical applications that can provide valuable and long-term data on health status.

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