

# Circumventing Traditional Conditioning Protocols in Polymer Membrane-Based Ion-Selective Electrodes

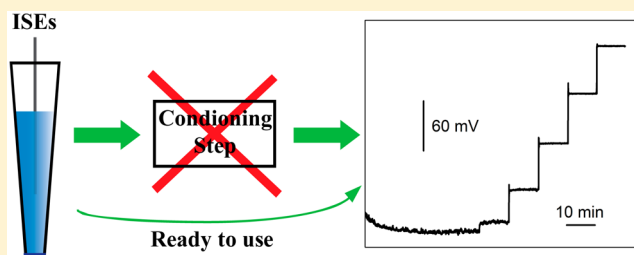
Michelle Rich,<sup>†</sup> Lukasz Mendecki,<sup>‡</sup> Samantha T. Mensah,<sup>†</sup> Enrique Blanco-Martinez,<sup>†</sup> Stephanie Armas,<sup>†</sup> Percy Calvo-Marzal,<sup>†</sup> Aleksandar Radu,<sup>‡</sup> and Karin Y. Chumbimuni-Torres<sup>\*,†</sup>

<sup>†</sup>Department of Chemistry, P.O. Box 25000, University of Central Florida, Orlando, Florida 32816-2366, United States

<sup>‡</sup>Lennard-Jones Laboratories, Birchall Centre, Keele University, Keele, Staffordshire ST5 5BG, United Kingdom

## Supporting Information

**ABSTRACT:** Preparation of ion-selective electrodes (ISEs) often requires long and complicated conditioning protocols limiting their application as tools for in-field measurements. Herein, we eliminated the need for electrode conditioning by loading the membrane cocktail directly with primary ion solution. This proof of concept experiment was performed with iodide, silver, and sodium selective electrodes. The proposed methodology significantly shortened the preparation time of ISEs, yielding functional electrodes with submicromolar detection limits. Moreover, it is anticipated that this approach may form the basis for the development of miniaturized all-solid-state ion-selective electrodes for in situ measurements.



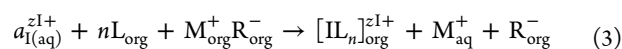
Scientific research on ion-selective electrodes (ISEs) has gained momentum within the last few years due to improvements in the limits of detection and selectivity, becoming applicable now for trace-level measurements through understanding of transmembrane ion fluxes.<sup>1</sup> The response of ISEs can be described by the phase boundary potential,  $E_{PB}$ , according to the equation

$$E_{PB} = E^0 + \frac{RT}{z_1 F} \ln \frac{a_{I(aq)}}{a_{I(org)}} \quad (1)$$

where  $a_{I(aq)}$  and  $a_{I(org)}$  are the activities of the primary ion ( $I^+$ ) of charge  $z$  in aqueous and organic phases, respectively, and  $E^0$ ,  $R$ ,  $T$ , and  $F$  are the standard potential, gas constant, temperature, and Faraday constant, respectively. When  $a_{I(org)}$  is kept constant, eq 1 reduces to the well-known Nernst equation

$$E_{PB} = E^{0'} + \frac{RT}{z_1 F} \ln a_{I(aq)} \quad (2)$$

For an ion-selective membrane to be rendered functional, the ionophore and lipophilic ionic sites are required. One of the major roles of the ionophore is to make relatively strong complexes with the primary ion, thereby establishing their constant activity in the membrane.<sup>2</sup> For more details, see eqs 1–5 in the Supporting Information. The role of the lipophilic ionic sites is to provide ion-exchange properties. For a cation selective membrane, this process could be described by the equilibrium



where  $L$  is a ligand (ionophore) that forms an ion–ionophore complex with ion  $I^+$  of stoichiometry  $n$ .  $M_{org}^+ R_{org}^-$  is a lipophilic ion exchanger composed of lipophilic anion  $R^-$  and its counterion  $M^+$ . Partitioning of  $I^+$  from aqueous sample into the membrane results in its exchange with  $M^+$ . Anion  $R_{org}^-$  remains in the membrane, thereby rendering the membrane permselective while preserving the charge balance.<sup>3</sup> In a typical experimental protocol for the preparation of ion-selective membranes, the ion-exchange process is obtained by conditioning (soaking) the membrane in an aqueous solution containing the ion  $I^+$  (traditional protocol).<sup>4</sup>

Significant effort in the ISEs field has been spent on researching ways to miniaturize<sup>5–9</sup> and optimize/simplify the preparation of ISEs.<sup>10–14</sup> Reducing or eliminating the need for the conditioning step prior to the use of the electrodes is an important step for devising a simple, practical protocol for mass production of ISEs. This is mainly for the preparation of single-use ISEs and their application for in situ analysis. As a result, sample degradation, contamination, and aging can be prevented by avoiding sample transport.<sup>15</sup> Furthermore, such a system would enable nontrained personnel to use ISEs quickly and reliably.

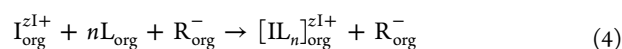
In this work, we propose a simple alteration of the sensor's conditioning protocol. Instead of placing the ISEs in a solution of primary ions  $I^+$ , the solution is added directly into the membrane cocktail prior to its casting. The concentration of that solution is calculated to allow for stoichiometric exchange of  $I^+$  and  $M^+$ . Consequently, ions  $I^+$  are present in the

Received: April 19, 2016

Accepted: August 15, 2016

Published: August 15, 2016

membrane, facilitating the formation of an ion–ionophore complex according to the equilibrium



Therefore, upon casting, the membrane is already preloaded with complexed ions  $I^+$  in the form of the ion–ionophore complex  $[IL_n]_{\text{org}}^{zI+}$ . Satisfaction of stoichiometry implies that the concentration of the complex in the membrane is constant, thus fulfilling the requirements for application of eq 2. The well-formed Nernstian responses are therefore expected for these nonconditioned ISEs.

## EXPERIMENTAL SECTION

**Reagents.** 4-*tert*-Butylcalix[4]arene-tetraacetic acid tetraethyl ester (sodium ionophore X), *o*-xylylenebis(*N,N*-diisobutylidithiocarbamate) (copper(II) ionophore I), potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (KTFPB), sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), tridodecylmethylammonium nitrate (TDMANO<sub>3</sub>), bis(2-ethylhexyl)sebacate (DOS), high molecular weight poly(vinyl chloride) (PVC), and tetrahydrofuran (THF), all of Selectophore grade, were purchased from Sigma-Aldrich. [9]-Mercuracarborand-3 (MC3) was synthesized in house as described previously.<sup>16</sup> The salts AgPF<sub>6</sub>, AgNO<sub>3</sub>, NaNO<sub>3</sub>, NaI, and KCl were also obtained from Sigma-Aldrich. All aqueous solutions were prepared in ultrapure water obtained with a Purelab Ultra water purification system (18 MΩ cm resistance).

**Membranes.** The Na<sup>+</sup>-selective membrane contained 10.0 mmol kg<sup>-1</sup> of sodium ionophore X and 5.0 mmol kg<sup>-1</sup> of NaTFPB. The Ag<sup>+</sup>-selective membrane contained 10.0 mmol kg<sup>-1</sup> of copper(II) ionophore I and 5.0 mmol kg<sup>-1</sup> of NaTFPB.<sup>17</sup> The I<sup>-</sup>-ISEs contained 1.0 mmol kg<sup>-1</sup> of MC3 and 0.75 mmol kg<sup>-1</sup> of TDMANO<sub>3</sub>. Ion-selective membranes were prepared by dissolving the components mentioned above together with PVC (33.3 wt %) and DOS (66.6 wt %) in 2 mL of THF, and the resulting cocktail was vortexed for 30 min.

To avoid traditional conditioning steps, 25 μL of 0.1 M AgNO<sub>3</sub> solution was added to the Ag<sup>+</sup>-selective membrane, and 1.8 μL of 0.1 M NaI solution was added to the I<sup>-</sup>-selective membrane before vortexing. No additional solution was added for the Na<sup>+</sup>-selective membrane. Control membranes were also prepared: For Ag<sup>+</sup>-selective electrodes, no AgNO<sub>3</sub> solution was added; for I<sup>-</sup>-selective control membranes, no NaI solution was added. For Na<sup>+</sup>-selective electrodes, KTFPB was used as ion-exchanger instead of NaTFPB. Volume ratio of primary ion solution (aqueous to the cocktail solution (THF)) is approximately 1% v/v. Each solution of primary ions was prepared by dissolving the specified primary ion salt directly in ultrapure water. The resulting membrane cocktail was cast into a glass ring (26 mm inner diameter) secured to a glass plate, and the solvent was allowed to evaporate overnight. This resulted in a membrane film with a thickness of approximately 200 ± 10 μm (obtained using a caliper).

**Electrodes.** The ISEs were prepared as follows: First, a 3.2 mm diameter disk was cut from the parent membrane film and adhered to the end of a PVC tube (2 cm length, 1.6 mm i.d.) using THF. The other end of the tube was fixed to a 1 mL pipet tip that held the inner filling solution. Inner filling solutions for the Na<sup>+</sup>-ISE, Ag<sup>+</sup>-ISE, and I<sup>-</sup>-ISE were 1.0 × 10<sup>-3</sup> M NaNO<sub>3</sub>, 1.0 × 10<sup>-3</sup> M AgNO<sub>3</sub>, and 1.0 × 10<sup>-3</sup> M NaI, respectively. A

diaphragm separated the internal filling solutions from the reference half-cell (Ag/AgCl in 0.1 M KCl).

**EMF Measurements.** Potential measurements of the nonconditioned ISEs began immediately after adding the inner filling solution to the electrode. Measurements were carried out in 100 mL polypropylene beakers. A high-input impedance (10<sup>15</sup> Ω) EMF-16 multichannel data acquisition system (Precision Electrochemistry EMF Interface, Lawson Laboratories) was used to monitor the potentials at room temperature (22 °C) and under constant stirring. A double junction Ag/AgCl/3 M KCl/1 M LiOAc (Metrohm AG) was used as the reference electrode. All EMF values were corrected for liquid-junction potentials according to the Henderson equation, and activity coefficients were calculated by the Debye–Hückel approximation.

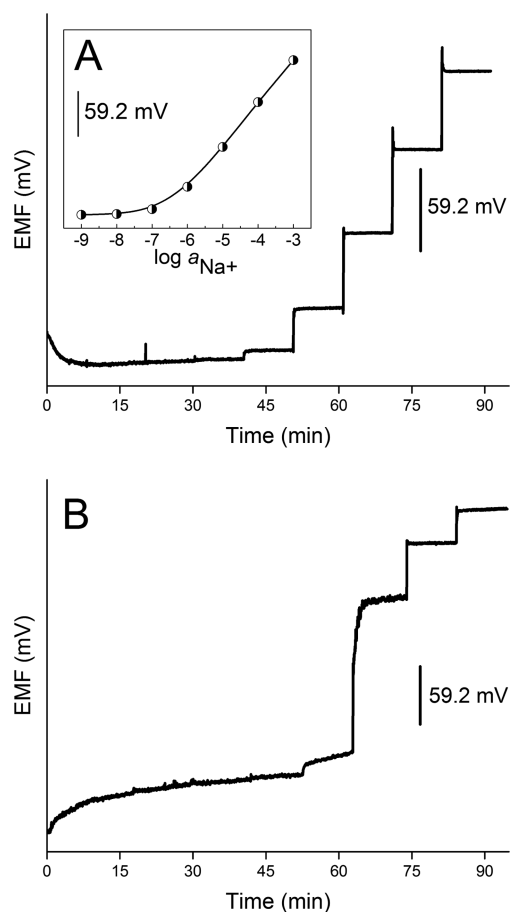
**Selectivity Measurements.** ISEs for unbiased selectivity measurements were prepared according to the protocols described in *Membranes* and *Electrodes* with some modifications. For Na<sup>+</sup>-ISEs, lipophilic ion exchanger KTFPB was used instead of NaTFPB. Ag<sup>+</sup> cocktail solution was loaded with 5 mmol kg<sup>-1</sup> of NaNO<sub>3</sub>, and 5 mmol kg<sup>-1</sup> of KNO<sub>3</sub> was added to the I<sup>-</sup> membrane solution. Inner filling solutions for the Na<sup>+</sup>-ISE, Ag<sup>+</sup>-ISE, and I<sup>-</sup>-ISE were 1.0 × 10<sup>-3</sup> M KNO<sub>3</sub>, 1.0 × 10<sup>-3</sup> M NaNO<sub>3</sub>, and 1.0 × 10<sup>-3</sup> M KNO<sub>3</sub>, respectively. Responses toward all ions were recorded according to a separate solution method as described by Bakker.<sup>18</sup>

## RESULTS AND DISCUSSION

**Response Characteristics.** Establishment of the ion–ionophore complex equilibrium is key to create the potential gradient at the interface of the electrode while maintaining constant activity of analyte ions inside the membrane.<sup>19</sup> By understanding the ion flux at the membrane/sample interface, it enables the ion-selective membrane composition to be optimized while avoiding long conditioning steps. The equilibrium between complexed and free ionophore in the membrane could be obtained during the preparation of the membrane by adding the ion of interest directly to the membrane cocktail at a stoichiometrically optimized amount. In the preparation step, consideration should be given to the lipophilicity of the counterion of the loading solution (see Figure S1 and accompanying discussion).

Thus, Figure 1A shows the calibration curve of the nonconditioned Na<sup>+</sup>-ISE that contains NaTFPB as the ion-exchanger. This calibration curve displays a Nernstian slope of 57.05 ± 2.03 mV decade<sup>-1</sup> and a submicromolar limit of detection of 3.24 × 10<sup>-7</sup> ± 0.02 M, showing a similar performance as the conditioned Na<sup>+</sup>-ISEs previously reported using a similar membrane composition.<sup>20</sup> Conversely, the calibration curve of the nonconditioned Na<sup>+</sup>-ISE control membranes containing KTFPB instead of NaTFPB as the ion-exchanger (Figure 1B) demonstrated super-Nernstian behavior upon initial exposure to sodium ions. This is caused by a flux of sodium ions from the sample solution into the bulk of the ion-selective membrane as previously reported in the literature.

Similarly, the calibration curve (Figure 2A) of the nonconditioned Ag<sup>+</sup>-ISE with preloaded AgNO<sub>3</sub> solution also displays a Nernstian slope of 58.05 ± 0.77 mV decade<sup>-1</sup> and a limit of detection of 2.69 × 10<sup>-7</sup> ± 0.03 M, which are similar to the traditionally conditioned Ag<sup>+</sup>-ISE as reported in the literature, but with a higher limit of detection.<sup>21</sup> The small discrepancy in the observed and reported detection limits could

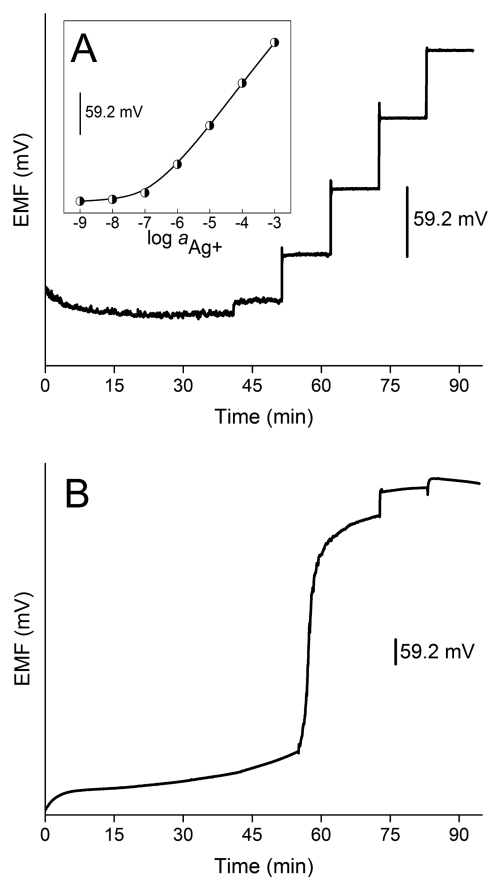


**Figure 1.** Trace lines for the nonconditioned  $\text{Na}^+$ -ISE containing (A) NaTFPB and (B) KTFPB as the ion-exchanger. (inset in A) Respective calibration curve. All other components of the membranes in A and B are the same.

be caused by the transmembrane ion flux from the inner filling solution in the direction of the sample.<sup>22</sup> This could disturb the local equilibrium at the sample/membrane interface, consequently worsening the detection limit of ISEs.

On the other hand, a super-Nernstian response was observed (Figure 2B) for the nonconditioned  $\text{Ag}^+$ -ISE in which no  $\text{AgNO}_3$  solution was added to the cocktail solution (control membrane). Similarly to the  $\text{Na}^+$ -ISE, the super-Nernstian response was also observed due to the initial exposure of ISEs to the primary ions (silver ions). In addition, control membranes were subsequently conditioned for 2, 4, 6, 8, 10, 12, and 24 h in  $1.0 \times 10^{-3}$  M of  $\text{AgNO}_3$  solution, and a minimum of 12 h was required to obtain ISEs with Nernstian response slopes (data not shown). Moreover, traditionally prepared  $\text{Ag}^+$ -ISEs exhibited similar response characteristics (lower detection limits and Nernstian behavior, Figure S2) to those conditioned according to the proposed methodology. This demonstrates the practicality of adding the solution of primary ions into the membrane cocktail to further reduce the preparation time of ISEs. In addition, electrodes conditioned using the protocol described herein retain good response behavior after at least 3 days of storage (Figure S4). Furthermore, our concept for conditioning also applies for solid contact electrodes (Figure S3).

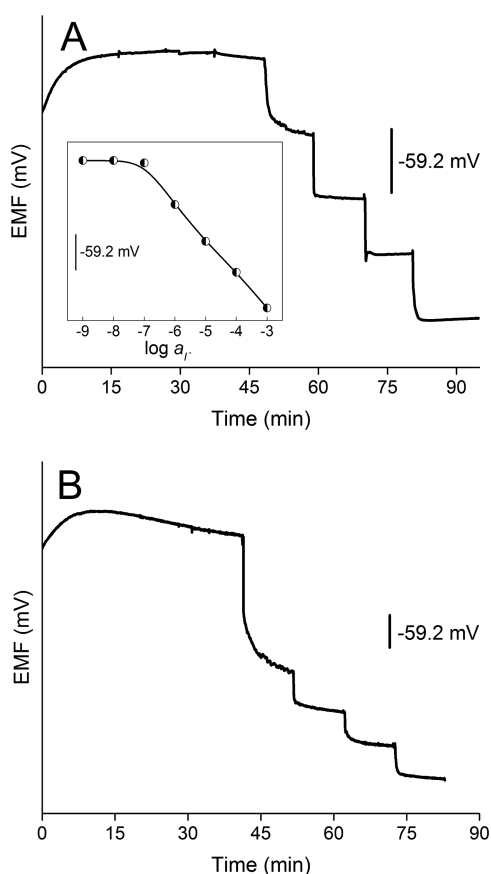
The Nernstian behavior of the nonconditioned cation-selective system that was doped with the primary ion during the membrane preparation for sodium and silver ions was also



**Figure 2.** Trace lines for the nonconditioned  $\text{Ag}^+$ -ISEs containing (A)  $\text{AgNO}_3$  solution and (B) without  $\text{AgNO}_3$  solution in the membrane. (inset in A) Respective calibration curve. All other components of the membranes in A and B are the same.

tested for an anion-selective system. As described in the Experimental Section, the  $\text{I}^-$ -ISEs were prepared with and without NaI in the membrane composition. As shown in Figure 3A, the calibration curve of the nonconditioned  $\text{I}^-$ -ISEs, where NaI solution was added to the cocktail, presents a Nernstian slope of  $57.59 \pm 2.56$  mV decade<sup>-1</sup> and a limit of detection of  $8.51 \times 10^{-8} \pm 0.07$  M. This is in good agreement with the traditionally conditioned  $\text{I}^-$ -ISEs based on the same ionophore as reported in the literature, but the observed detection limit was higher by 1 order of magnitude.<sup>23</sup> The nonoptimal behavior of MC-3-based ISEs at the lower detection end could be caused by the strong interference from the hydroxide ions.<sup>24</sup> Similarly to that in the cation system, when a solution of the analyte was not added to the cocktail solution (blank membranes for iodide), a super-Nernstian response was observed (Figure 3B).

The nonconditioned ISEs were also characterized in terms of selectivity using an unbiased method. Therefore, each electrode was conditioned in a solution of less discriminating interfering ions prior to the performed measurements.<sup>18</sup> Table 1 shows the selectivity values obtained for  $\text{Na}^+$ -ISE,  $\text{Ag}^+$ -ISE, and  $\text{I}^-$ -ISE. Additional control experiments involving the addition of pure water into the membrane cocktail indicated no difference in response due to hydration effects of the membrane (see Figure S5).



**Figure 3.** Trace lines for the nonconditioned  $I^-$ -ISEs containing (A) NaI solution and (B) without NaI solution in the membrane. (inset in A) Respective calibration curve. All other components of the membranes in A and B are the same.

**Table 1. Unbiased Selectivity Coefficients Obtained for the  $Na^+$ -ISEs (Slope:  $50.23 \pm 0.09$  mV decade $^{-1}$ ),  $Ag^+$ -ISEs (Slope:  $57.32 \pm 3.27$  mV decade $^{-1}$ ), and  $I^-$ -ISEs (Slope:  $-56.33 \pm 0.17$  mV decade $^{-1}$ ) with the Corresponding Slope Values for Interfering Ions**

ion $J^{Z+}$	$K_{Na,J}^{pot}$	slope (mV decade $^{-1}$ )
$K^+$	$-1.1 \pm 0.1$	$56.35 \pm 0.13$
$H^+$	$-4.5 \pm 0.3$	$51.61 \pm 1.80$
$Li^+$	$-5.2 \pm 0.3$	$53.03 \pm 1.39$
ion $J^{Z+}$	$K_{Ag,J}^{pot}$	slope (mV decade $^{-1}$ )
$Na^+$	$-6.9 \pm 1.2$	$53.11 \pm 1.15$
$K^+$	$-6.4 \pm 0.9$	$54.51 \pm 0.68$
$H^+$	$-7.6 \pm 0.5$	$50.58 \pm 3.23$
$Cu^{2+}$	$-10.6 \pm 0.3$	$28.43 \pm 1.43$
ion $J^{Z+}$	$K_{I,J}^{pot}$	slope (mV decade $^{-1}$ )
$Br^-$	$-1.3 \pm 0.0$	$-64.71 \pm 2.92$
$Cl^-$	$-1.3 \pm 0.0$	$-64.65 \pm 2.71$
$NO_3^-$	$-2.1 \pm 0.0$	$-55.93 \pm 4.00$

## CONCLUSIONS

In this work, a new methodology for the preparation of ISEs with the elimination of complex conditioning protocols is proposed. This was achieved by adding a small aliquot of primary ions into the cocktail solution giving rise to ISEs with submicromolar detection limits. As a result, single-use ISEs will facilitate measurements in the field, which simplify the task for

nontrained personnel. Further characterization has to be carried out to fully understand the properties of the sensor prepared according to the proposed modified protocol.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b01542.

Additional experimental details, supporting equations, and control experiments with accompanying discussion (PDF)

## AUTHOR INFORMATION

### Corresponding Author

\*E-mail: Karin.ChumbimuniTorres@ucf.edu. Fax: 4078232252.

### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

The authors thank the Office of Research and Commercialization, College of Science, and the Department of Chemistry at the University of Central Florida, the Royal Society (RG120118), and the Marie Curie IRSES Grant (IBS Network; R6810 B475) for financial support.

## REFERENCES

- (1) Ngeontae, W.; Xu, Y.; Xu, C.; Aeungmaitrepirom, W.; Tuntulani, T.; Pretsch, E.; Bakker, E. *Anal. Chem.* **2007**, *79*, 8705–8711.
- (2) Duncan, D. M.; Cockayne, J. S. *Sens. Actuators, B* **2001**, *73*, 228–235.
- (3) Woźnica, E.; Mieczkowski, J.; Michalska, A. *Analyst* **2011**, *136*, 4787.
- (4) Lazo Fraga, A. R.; Calvo Quintana, J.; Li Destri, G.; Giambianco, N.; Toro, R. G.; Punzo, F. *J. Solid State Electrochem.* **2012**, *16*, 901–909.
- (5) Anastasova-Ivanova, S.; Mattinen, U.; Radu, A.; Bobacka, J.; Lewenstam, A.; Migdalski, J.; Danielewski, M.; Diamond, D. *Sens. Actuators, B* **2010**, *146*, 199–205.
- (6) Goldberg, H. D.; Brown, R. B.; Liu, D. P.; Meyerhoff, M. E. *Sens. Actuators, B* **1994**, *21*, 171–183.
- (7) Gyurcsanyi, R. *Talanta* **2004**, *63*, 89–99.
- (8) Hart, J. P.; Crew, A.; Crouch, E.; Honeychurch, K. C.; Pemberton, R. M. *Anal. Lett.* **2004**, *37*, 789–830.
- (9) Renedo, O. D.; Alonso-Lomillo, M. A.; Martínez, M. J. A. *Talanta* **2007**, *73*, 202–219.
- (10) Bakker, E.; Bhakthavatsalam, V.; Gemene, K. L. *Talanta* **2008**, *75*, 629–635.
- (11) Bhakthavatsalam, V.; Shvarev, A.; Bakker, E. *Analyst* **2006**, *131*, 895–900.
- (12) Mendecki, L.; Chen, X.; Callan, N.; Thompson, D. F.; Schazmann, B.; Granados-Focil, S.; Radu, A. *Anal. Chem.* **2016**, *88*, 4311–4317.
- (13) Pawlowski, P.; Kisiel, A.; Michalska, A.; Maksymiuk, K. *Talanta* **2011**, *84*, 814–819.
- (14) Michalska, A.; Pyrzyńska, K.; Maksymiuk, K. *Anal. Chem.* **2008**, *80*, 3921–3924.
- (15) Eriksen, R. S.; Mackey, D. J.; Alexander, P.; De Marco, R.; Wang, X. D. *J. Environ. Monit.* **1999**, *1*, 483–487.
- (16) Zinn, A. A.; Zheng, Z.; Knobler, C. B.; Hawthorne, M. F. *J. Am. Chem. Soc.* **1996**, *118*, 70–74.
- (17) Szigeti, Z.; Malon, A.; Vigassy, T.; Csokai, V.; Grün, A.; Wygladacz, K.; Ye, N.; Xu, C.; Chebny, V. J.; Bitter, I.; Rathore, R.; Bakker, E.; Pretsch, E. *Anal. Chim. Acta* **2006**, *572*, 1–10.
- (18) Bakker, E. *Anal. Chem.* **1997**, *69*, 1061–1069.

- (19) Mathison, S.; Bakker, E. *Anal. Chem.* **1998**, *70*, 303–309.
- (20) Kimura, K.; Yoshinaga, M.; Funaki, K.; Shibutani, Y.; Yakabe, K.; Shono, T.; Kasai, M.; Mizufune, H.; Tanaka, M. *Anal. Sci.* **1996**, *12*, 67–70.
- (21) Chumbimuni-Torres, K. Y.; Rubinova, N.; Radu, A.; Kubota, L. T.; Bakker, E. *Anal. Chem.* **2006**, *78*, 1318–1322.
- (22) Ceresa, A.; Radu, A.; Peper, S.; Bakker, E.; Pretsch, E. *Anal. Chem.* **2002**, *74*, 4027–4036.
- (23) Malon, A.; Radu, A.; Qin, W.; Qin, Y.; Ceresa, A.; Maj-Zurawska, M.; Bakker, E.; Pretsch, E. *Anal. Chem.* **2003**, *75*, 3865–3871.
- (24) Radu, A.; Bakker, E. *Chem. Anal.* **2005**, *50*, 71–83.