

Robust and Ultrasensitive Polymer Membrane-Based Carbonate-Selective Electrodes

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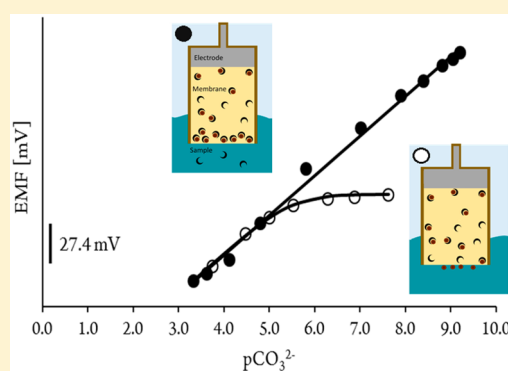
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S Supporting Information

ABSTRACT: Quantitative analysis of the carbonate species within clinical and environmental samples is highly critical to the advancement of accurate environmental monitoring, disease screening, and personalized medicine. Herein we report the first example of carbonate detection using ultrasensitive ion selective electrodes (ISEs). The low detection limit (LDL) of these electrodes was at least 4 orders of magnitude lower than the best currently existing carbonate sensors. This was achieved by a simple alteration of the sensor's conditioning protocol. This resulted in the reduction of ion fluxes across the membrane interface consequently lowering the LDL to picomolar levels. The proposed ISEs exhibited near-Nernstian potentiometric responses to carbonate ions with a detection limit of 80 pmol L⁻¹ (5 ppt) and was utilized for direct determination of carbonate in seawater. Moreover, the new methodology has produced electrodes with excellent reproducibility, robustness, and durability. It is anticipated that this approach may form the basis for the development of highly sensitive and robust ion selective electrodes capable of *in situ* measurements.



Analytical chemists are faced with a significant challenge to develop techniques and methodologies for monitoring air, water, and soil much more frequently and extensively than it is possible today while significantly lowering per-sample and per-measurement costs. Such capabilities are expected to make a significant impact in many different fields, from environmental analysis to the health, security, and manufacturing industries. Obviously, no single technique could address all these requirements. ISEs are a class of chemical sensors that in recent years went through a renaissance and showed excellent potential as tools for routine monitoring and as early warning systems. They are simple and low cost, show excellent selectivity and sensitivity, and are easily miniaturized and connected to simple communication devices. However, they suffer from the existence of zero-current membrane ion fluxes which presents as their fundamental limitation for becoming a robust tool for long-term trace level analysis.

Because of the ion fluxes, the sample/membrane interface is poisoned by the excess of primary ions which results in deterioration of the low detection limit (LDL) and selectivity of ISEs.^{1,2} In recent years, a number of approaches have been developed to reduce the fluxes and improve the selectivity and LDL by many orders of magnitude. These approaches are based on the strategies such as matching the composition of inner filling solution as close as possible to the sample³ and optimization of membrane composition^{4,5} and membrane

backside contacts.^{6,7} Interestingly, while these results brought LDL improvements of 3–6 orders of magnitude and a significant expansion of academic importance of ISEs, achieved improvements are yet to make significant practical breakthroughs.

These strategies rely on tedious and sometimes lengthy conditioning protocols. While slopes and LDL are normally stable for months, some small losses of LDL of ~0.5–1 orders of magnitude are typically observed within the first week of the electrode's shelf life. These losses can be attributed to the re-establishment of small outward ion fluxes. Despite their simplicity, low cost, and ability to determine the bioavailable fraction of ions,^{8–10} the LDL deterioration upon storage and the need for complex conditioning or preparation protocols have rendered them unreliable for *in field* applications. Notwithstanding the ISEs excellent potential for miniaturization,¹¹ their application in long-term analysis of small volumes (e.g., cells) is unsuitable due to outward ion fluxes compromising the integrity of the sample.

We report here a new methodology that allows large improvements in sensitivity and robustness of polymer

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72 membrane-based ion-selective electrodes (ISEs). This method-
73 ology allowed development of a highly robust sensor for the
74 direct potentiometric detection of carbonate ions at concen-
75 trations of 5 ppt (8×10^{-11} M) without any sample
76 preconcentration and/or instrumental signal enhancement.

77 ■ EXPERIMENTAL SECTION

78 **Materials.** Tridodecylmethylammonium (TDMACl) and
79 sodium bicarbonate were purchased from Fluka. The synthesis
80 of carbonate ionophore (diamide *N,N*-bis(2,4-dinitrophenyl)-
81 isophthalohydrazide) was modified from Jain et al.¹² Self-
82 plasticized poly(lauryl methacrylate-*r*-methyl methacrylate)
83 copolymer, copolymer D (63 mol % lauryl methacrylate
84 (LMA), 37 mol % methyl-methacrylate (MMA), was
85 synthesized via azobis(isobutyronitrile) (AIBN) initiated radical
86 polymerization according to standard procedures. All chemicals
87 were of analytical reagent grade. Solutions of metal ions were
88 prepared in ultrapure water obtained with Pico Pure 3 water
89 system. Working solutions of different activities were prepared
90 by serial dilutions of a 0.1 M stock solution. DropSens Dual
91 Carbon Screen-printed Electrodes (C1110) were purchased
92 from Metrohm USA.

93 **Electrode Preparation.** The intermediate conducting layer
94 composed of poly(3,4-ethylenedioxythiophene-2,5 diyl)
95 (PEDOT) was electrochemically polymerized onto the solid
96 contact electrodes (SCEs) by immersing the platforms into a
97 solution of 3% 3,4-ethylenedioxythiophene (EDOT), and 6%
98 tetrabutylammonium chloride in acetonitrile and using the SCE
99 as a cathode and a graphite anode. Electropolymerizations were
100 performed for 10 min at 2.0 V and 50 mA using Hewlett-
101 Packard potentiationstat (E3630A, address). The SCEs were left
102 to dry for 24 h at room temperature and then placed in a room-
103 temperature vacuum oven for 1 h before applying ion selective
104 membranes.

105 **Preparation of Carbonate-Selective Membranes.** Car-
106 bonate selective electrodes were prepared by dissolving
107 TDMACl (11 mmol/kg or 1% wt), copolymer (95% wt),
108 and if needed carbonate ionophore (76 mmol/kg or 4% wt) in
109 0.5 mL of THF. After the complete dissolution of all
110 components, the aliquot was drop cast onto the top of the
111 PEDOT layer and left at room temperature to dry overnight.

112 With the traditional protocol, the electrodes were placed in
113 0.1 M solution of NaHCO_3 for 24 h. Note that pH of this
114 solutions was ~ 8.4 resulting in $\sim 10^{-3}$ M of CO_3^{2-} .

115 The new conditioning protocol involved placing the
116 electrodes solution of 1 mg of carbonate ionophore in 0.5
117 mL of THF and 20 mL of ultrapure water to form a solution of
118 7.6×10^{-5} M of ionophore in THF + water solvent mixture.
119 Conditioning time for this step was varied between 1 h and 24
120 h.

121 **Protocol for Determination of Carbonate.** Potentio-
122 metric responses of all electrodes were recorded using a
123 Lawson Laboratories Inc. 16-channel EMF-16 interface (3217
124 Phoenixville Pike Malvern, PA) in a stirred solution against a
125 Pinnacle series H4403-2B as the reference electrode. Electrodes
126 conditioned in both traditional and new protocol were
127 immersed in 2–4 mmol/kg NaHCO_3 sample solutions
128 (ultrapure water or artificial seawater as required) followed by
129 additions 0.10 M HCl (or 0.10 M HCl in 0.70 M NaCl for
130 artificial seawater). The pH of the solution was monitored using
131 a glass VWR symphony 14002-780 Ag/AgCl pH electrode
132 which allowed calculation of activity of CO_3^{2-} . The
133 concentration of CO_3^{2-} for each solution was calculated from

the known dissolved inorganic carbon (DIC, equal to the added
134 NaHCO_3), temperature, salinity, and pH. 135

Artificial Seawater Preparation. Artificial seawater
136 (ASW) was prepared as proposed by Roy et al.,¹³ with some
137 modifications. Approximately 1 kg of distilled water was purged
138 using $\text{N}_2(\text{g})$ prior to salt addition. Supplementary Table 1 in
139 the Supporting Information demonstrates the molalities of each
140 salt used for the preparation of ASW. The appropriate amounts
141 of MgCl_2 and CaCl_2 were added from 1 M stock solutions of
142 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and anhydrous CaCl_2 , respectively. All other salts
143 were recrystallized, oven-dried overnight at 110 °C, and added
144 as solids. The seawater was adjusted to a known DIC of 2
145 mmol/kg using NaHCO_3 . ASW was kept tightly sealed and
146 covered with Parafilm to ensure no further dissolution of CO_2 . 147

148 ■ RESULTS AND DISCUSSION

149 In this paper we describe a very simple method for effective
150 removal of primary ions from the sample/membrane interface,
151 thus developing robust and ultrasensitive ISEs. Traditionally,
152 the ionophore is loaded into the membrane cocktail. Upon
153 casting, drying, and conditioning, established ion fluxes result in
154 a poisoned sample/membrane interface as illustrated in Figure
155 1A, thus determining the LDL. Our approach is analogous to

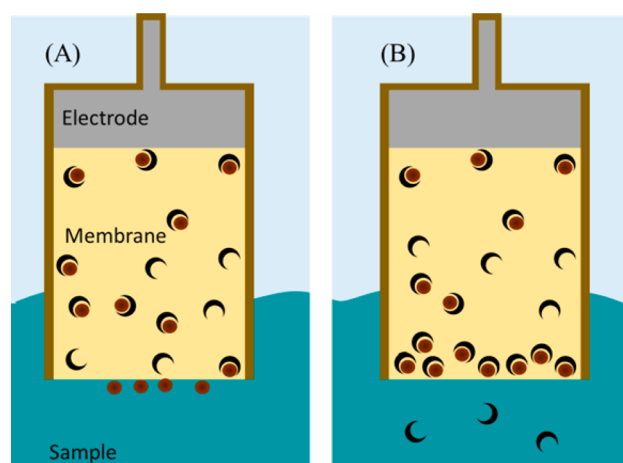


Figure 1. Schematic representation of state of ion-selective membranes after traditional conditioning (A) and after conditioning protocol suggested here (B). In the situation in part A, the membrane is loaded with ionophore (black half-moons) followed by conditioning in the solution of primary ions (brown circles). Establishment of ion fluxes results in leaching of primary ions and their accumulation at the sample/membrane interface. With the new protocol in part B, the membrane is exposed to the solution of ionophore. It complexes ions at the sample/membrane interface and partitions in the membrane thus resulting in removal of primary ions from the phase boundary.

the successful (but impractical) approach of using ion buffers in
156 the sample to maintain low and constant ion activity at the
157 sample/membrane interface.¹⁴ This new method buffers the
158 carbonate in the membrane side of the sample/membrane
159 interface by using an excess of ionophore introduced from the
160 sample side. Our approach involves a short (~ 60 min)
161 conditioning of, in principle, dry electrode in the solution of
162 ionophore. The lipophilic ionophore partitions into the
163 membrane as evidenced by monitoring of the ionophore
164 concentration in the conditioning solution (see the Supporting
165 Information, Figure S1). As it is absorbed into the membrane,
166 the ionophore complexes primary ions at the aqueous side of 167

168 the sample/membrane interface. This draws the carbonate ion
169 into the membrane, minimizing ion fluxes (see the Supporting
170 Information Figure S2 and accompanying discussion). This
171 process recovers the sample/membrane interface as illustrated
172 in Figure 1B and consequently allows significant improvement
173 of LDL.

174 This concept is demonstrated using CO_3^{2-} due to its
175 tremendous clinical¹⁵ and environmental importance.^{16–18}

176 Figure 2 shows the response of carbonate ISEs conditioned

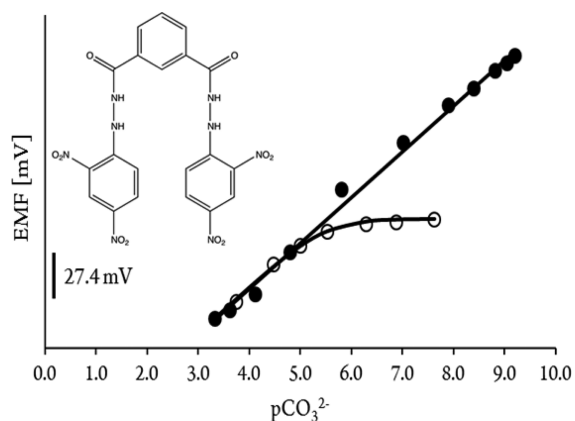


Figure 2. Response curves of CO_3^{2-} -selective electrodes prepared and conditioned in traditional fashion (open circles; slope = 7.1 mV/decade and LDL at $\text{pCO}_3^{2-}(\text{LDL}) = 5.5$) and using our methodology (closed circles). In the case of the latter, the slope was 27.4 mV/decade with $R^2 = 0.99$. LDL is dictated by the speciation of CO_3^{2-} and is observed at $\text{pCO}_3^{2-} = 9.6$. The inset presents the structure of the 3^{2-} ionophore.¹⁸

177 in the traditional fashion (open circles) and our approach
178 (closed circles). The traditional membranes were conditioned
179 in a 10^{-3} M solution of carbonate ions overnight. This
180 approach, which mimics the situation presented in Figure 1A,
181 results in suboptimal LDL ($\text{pCO}_3^{2-}(\text{LDL}) = 5.7$) due to the
182 occurrence of outward ion fluxes. On the other hand, the ISEs
183 prepared with the new approach are conditioned in a solution
184 of the carbonate ionophore. Following the complexation of
185 primary ions in the sample at the sample/membrane interface
186 and partitioning into the membrane, the phase boundary is
187 regenerated resulting in significant improvement of the LDL.
188 This situation is illustrated in Figure 1B. These ISEs showed
189 near Nernstian behavior (27.4 mV per decade) over a wide
190 concentration ($\text{pCO}_3^{2-} = 3.3\text{--}9.6$) range with optimal LDL
191 achieved under the given experimental conditions of $\text{pCO}_3 =$
192 10.10 (8×10^{-11} M or 5 ppt). Note that the LDL is here
193 dictated by the speciation of CO_3^{2-} ion and its equilibrium with
194 atmospheric CO_2 . Traditionally, the LDL of ISEs is dictated by
195 the presence of interfering ions (selectivity) and determined
196 from the typical curvilinear response curve.¹⁹ However, in this
197 case, the curvilinear region is never observed. This exciting
198 observation implies that the fundamental LDL for this system
199 has not yet been measured and that with further system
200 optimization (e.g., preventing trace amounts of CO_3^{2-} through
201 equilibrium with atmospheric CO_2), subppt levels of CO_3^{2-} can
202 possibly be determined. From our preliminary results with
203 other ions (see the Supporting Information Figures S3 and S4),
204 it seems that this methodology is valid for a wide range of
205 ionophore-based ISEs. Hence, these findings imply a high

likelihood for further improvement of already impressive LDLs
of other ions in ISE analysis.

Practical applications have been a key driving force of this work, and we have spent considerable effort studying the robustness and reproducibility of response characteristics (slope and LDL) of electrodes prepared using our approach. A large number of electrodes (150+), including both ionophore-free and optimized ISEs, were prepared according to preparation protocols explained above. They were prepared on different days using either freshly prepared or already used membrane cocktails. In addition, prepared electrodes were either used only once or stored in the air and then reused. Two types of pretreatment for the new methodology were also used: (1) electrodes were conditioned only in the solution of ionophore and (2) electrodes are dual conditioned, first in 0.1 M solution of NaHCO_3 followed by conditioning in the solution of ionophore for 1–24 h. Note that the full possible response range was not recorded for all electrodes. Rather, we focused on the range near the LDL in order to monitor the reproducibility and robustness at levels that have never been previously reached. Table 1 contains response characteristics

Table 1. Response Ranges and Slopes of Selected ISEs

electrode category	ionophore-only conditioning		dual conditioning
	new	used	new
measured range	9.6–4.1	8.9–6.9	10.0–3.50
slope	20.9 ± 0.8	20.5 ± 0.7	29.6 ± 0.4

(slope and tested concentration range) of at least four randomly selected electrodes from this large set of electrodes. Note that LDL is simply the lowest concentration measured. As explained above, the LDL depends only on the speciation of CO_3^{2-} under the experimental conditions but not on the selectivity. The electrodes were tested immediately after preparation followed by repetitions during several days, and/or after storage in air for up to several weeks. Near-Nernstian slopes at the response range of $\text{pCO}_3^{2-} > 8$ were observed in all cases (see Table 1). Retention of the slope and impressive LDLs especially in the cases of used ISEs that were later stored in air is an extremely important finding in terms of practical, *in field* application.

The ISEs were divided in three main categories: (a) dry ISEs, representing the ionophore-free electrodes conditioned only in the solution of ionophore for 1 h, b) used ISEs, representing dry electrodes that were used to record at least one calibration curve followed by the recording of next set of response after storage in air for ~ 2 weeks, and (c) optimized electrodes, representing fresh electrodes conditioned in the 0.1 M solution of NaHCO_3 for 24 h followed by their immediate conditioning in the solution of ionophore for at least 12 h.

Encouraged by these results, and upon confirmation of suitable selectivity to several major anions (e.g., Cl^- , NO_3^- and SO_4^{2-} ; see Supporting Information, Figure S2), we tested the response of these ISEs in a complex artificial seawater media. Figure 3 presents the response of carbonate ISEs in the solution of ionophore and measured in artificial seawater. The response was near-Nernstian (26.9 mV) over a wide concentration range ($\text{pCO}_3 = 3.4\text{--}8.2$), with LDL again dictated by the equilibrium with atmospheric CO_2 rather than the selectivity.

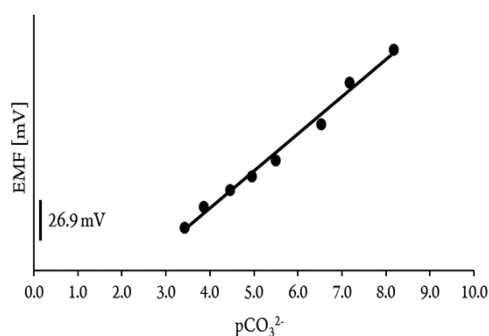


Figure 3. Response of the ion selective membrane loaded with the ionophore and ionic sites and conditioned in the ionophore solution for 24 h prior to the experiment (in artificial seawater).

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CONCLUSIONS

This work describes a new, simple methodology for reducing/eliminating membrane ion fluxes and achieving extremely low detection limits. The method was applied to carbonate selective electrodes, resulting in an improvement of the observed LOD of at least 4 orders of magnitude. To the best of our knowledge, this is the first report of ISEs that are successfully utilized for the direct determination of ppt levels of CO_3^{2-} in a highly complex matrix such as seawater. Furthermore, the sensors showed excellent robustness and durability at such low concentration ranges. Preliminary data indicate that the suggested methodology is general and can be applied for any number of cations and anions. Finally, this method eliminates a series of factors that, up until now, were considered fundamentally limiting. The use of these ISEs in situations where either the sample has a strong influence on the electrode (trace analysis in complex samples) or the electrode has strong influence on the sample (leaching of ions into the sample of ultrasmall volume (e.g., cells)) has now become a real possibility.

ASSOCIATED CONTENT

Supporting Information

Experimental details, potentiometric data, and UV–vis spectrum. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b01756.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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