An All-solid-state Polymeric Membrane Ca\textsuperscript{2+}-selective Electrode Based on Hydrophobic Alkyl-chain-functionalized Graphene Oxide

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Abstract: An all-solid-state polymeric membrane Ca\textsuperscript{2+}-selective electrode based on hydrophobic octadecylamine-functionalized graphene oxide has been developed. The hydrophobic composite in the ion-selective membrane not only acts as a transduction element to improve the potential stability for the all-solid-state Ca\textsuperscript{2+}-selective electrode, but also is used to immobilize Ca\textsuperscript{2+} ionophore with lipophilic side chains through hydrophobic interactions. The developed all-solid-state Ca\textsuperscript{2+}-selective electrode shows a stable potential response in the linear range of 3.0 × 10\textsuperscript{-7}–1.0 × 10\textsuperscript{-3} M with a slope of 24.7±0.3 mV/dec, and the detection limit is (1.6 ± 0.2) × 10\textsuperscript{-7} M (n = 3). Additionally, due to the hydrophobicity and electrical conductivity of the composite, the proposed all-solid-state ion-selective electrode exhibits an improved stability with the absence of water layer between the ion-selective membrane and the underlying glassy carbon electrode. This work provides a simple, efficient and low-cost methodology for developing stable and robust all-solid-state ion-selective electrode with ionophore immobilization.

Keywords: All-solid-state ion-selective electrode • Ionophore immobilization • Graphene

1 Introduction

All-solid-state polymeric membrane ion-selective electrodes (ISEs), as the next generation of ISEs, have recently attracted much attention, due to their durability, ease of miniaturization and low maintenance as compared to the traditional liquid-contact ISEs with inner-filling solutions [1]. However, owing to the ill-defined interface between the electronic conductor and the ion-selective membrane and the presence of the undesired water layer under the ion-selective membrane [2, 3], the long-term potential stability of the all-solid-state ISEs is unsatisfactory. In order to improve the potential stability, a variety of all-solid-state ISEs have been developed with electroactive materials as solid contacts, such as conducting polymers [4], carbon-based nanomaterials [5–10] and noble metal-based nanomaterials [11–13]. Among them, graphene is regarded as a promising solid contact for constructing all-solid-state ISEs [5, 7, 14], due to the unique characteristics, such as large specific surface area, fast electron-transfer capability, excellent conductivity and electrocatalytical activity.

As we all know, the essential membrane components of ISEs commonly contain an ionophore with exquisite selectivity, a lipophilic salt as an ion exchanger and a polymeric membrane (commonly plasticized poly(vinyl chloride) (PVC)) as a matrix [15]. However, research shows that the mobile membrane components, especially for ionophore, would slowly leach out from the ion-selective membranes (ISMs). These phenomena may not only influence the selectivity, sensitivity and lifetime of ISEs [16], but also cause toxic problems during in-vivo measurements [17]. Therefore, it is highly desired to inhibit the slow leaching of the active membrane from the sensing membrane.

In recent years, many methods have been developed to slow down the leaching processes of ionophores, as summarized in Table 1. For example, the covalent immobilization of ionophore to polymer backbones was proposed [18–22]. Unfortunately, such immobilization method suffers from problems of independently adjusting the ionophore and polymer contents of the membrane, and the polymers with highly functional groups dramatically affect the selectivity of the corresponding ISEs [22]. Gyurcsáni et al. proposed a new concept to confine ionophores in the ion-selective membrane based on the immobilization of ionophores on gold nanoparticles [23]. However, this approach requires the preparation of thiol-modified ionophore in order to anchor the molecules on the surface of gold nanoparticles, which may be disadvantages in view of the requirements of the purity and the cost of operation. Blondeau et al. synthesized a hybrid material with Pb\textsuperscript{2+} ionophore covalently binding to
carbon nanotubes and applied it to develop Pb$^{2+}$-ISEs [24]. However, due to the limited solubility and low dispersability of carbon nanotubes in most kinds of organic solvents, the dispersion reagent, poly(ethylene-co-acrylic acid), is used to obtain a homogeneous dispersion solution before preparing the all-solid-state ISEs. Such dispersion method may adversely influence the sensing properties, which is due to the introduction of the undesirable functional group, such as –COOH. Additionally, the azide-alkyne Huisgen cycloaddition (i.e., click chemistry) was proposed to immobilize K$^+$ ionophore in azide-modified poly(vinyl chloride) matrix [25]. However, the preparation process is quite complicated. Therefore, it is necessary to develop a simple, efficient and low-cost method for ionophore immobilization in order to construct robust all-solid-state ISEs without the leaching of ionophore from the membrane.

In this work, an all-solid-state Ca$^{2+}$-selective polymeric membrane electrode is constructed based on hydrophobic octadecylamine-functionalized graphene oxide (GO-ODA). The alkyl-chain-functionalized graphene oxide composite not only acts as a transduction element to improve the potential stability for the all-solid-state Ca$^{2+}$-ISE, but also is used to immobilize Ca$^{2+}$ ionophore with lipophilic side chains through hydrophobic interactions. The potentiometric performance of the proposed all-solid-state Ca$^{2+}$-ISEs is investigated.

2 Experimental

2.1 Reagents and Materials

Calcium ionophore (IV), (N,N-dicyclohexyl-N′,N′-dioctadecyl-3-oxapentanecarboxamide, ETH 5234), sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), 2-nitrophenyl octyl ether (o-NPOE), high molecular weight poly(vinyl chloride) (PVC) and octadecylamine (ODA) were purchased from Sigma-Aldrich. Graphene oxide (GO) was obtained from Nanjing XFNANO Materials Tech Co. Ltd (Nanjing, China). All other chemicals were of analytical reagent grade. Deionized water (18.2 MΩ cm specific resistance) obtained with a Pall Cascada laboratory water system was used throughout.

2.2 Preparation of the Alkyl-chain-functionalized Graphene Oxide

The GO-ODA composite was prepared according to the reported literature [26]. Briefly, GO was dispersed in deionized water by sonication, mixed with ethanol containing ODA and refluxed at 90 °C. The obtained black solution was filtrated by a Millipore membrane (nylon, 0.22 µm) and washed with ethanol to remove the excess ODA. After being dried in an oven, the resulting GO-ODA composite was stored in a dryer for further use. The content of N element for GO-ODA composite is about 2.65 ± 0.08 wt.%, which was measured by elemental analysis using a Elementar Vario Micro Cube (Germany).

2.3 Electrode Preparation

The ion-selective membrane (ISM) components for the all-solid-state Ca$^{2+}$-ISEs contain 1.3 wt% (13.6 mmol/kg) ETH 5234, 0.6 wt% (5.9 mmol/kg) NaTFPB, PVC and o-NPOE (1:2 wt/wt) together with 1.7 or 3.4 wt% GO-ODA composite. The membrane cocktails were prepared by a two-step method, i.e., dissolving 100 mg of membrane components in 1.5 ml tetrahydrofuran (THF), and then dispersing GO-ODA composite in the above THF solution by sonication for 1 h. In order to prepare the all-solid-state Ca$^{2+}$-ISEs, 90 µL of the membrane cocktail was drop cast on the bare glassy carbon (GC) electrodes and dried for at least 2 h at room temperature. Based on the different amounts of the GO-ODA composite, the above prepared all-solid-state Ca$^{2+}$-ISEs are denoted as GC/Ca$^{2+}$-ISM(I) and GC/Ca$^{2+}$-ISM(II) electrodes, respectively. For comparison, Ca$^{2+}$-ISM without GO-ODA composite was also prepared, which is denoted as GC/Ca$^{2+}$-ISM electrode. For potentiometric measurements, the electrodes were conditioned in 10⁻³ M Ca(NO₃)₂ overnight. For the selectivity measurements using separate solution method [27], the electrodes were conditioned in 10⁻³ M MgCl₂ overnight.

2.4 Apparatus and Measurements

Potentiometric measurements were performed using CHI 660C electrochemical station (Shanghai Chenhua Apparatus) at room temperature with Ag/AgCl (3 M KCl) as reference electrode in the galvanic cell: Ag/AgCl (3 M KCl) | sample solution | Ca$^{2+}$-ISM | GC. The ion activities were calculated by the Debye-Hückel equation and the potentiometric values were corrected for liquid junction potentials according to the Henderson equation. Fourier transform infrared (FTIR) spectroscopy was performed by using a Thermo Nicolet iS10 IR spectrometer with the pressed KBr pellet technique.
3 Results and Discussion

3.1 FT-IR Characterization

GO is a derivate of graphene, which consists of hydroxyl and epoxy groups in the basal planes and carboxylic and carbonyl groups at the edges of nanosheets [28]. These hydrophilic functional groups make GO well dispersed in polar solvents. It’s reported that the transformation from hydrophilic to hydrophobic GO can be performed through the grafting of ODA with a long alkyl chain onto the GO sheets based on the nucleophile substitution between the amine group of ODA and the epoxy groups of GO [29]. Moreover, the electrical conductivity of GO-ODA is found to be improved due to the \textit{in-situ} reduction during the functionalization process [26]. The hydrophobicity and the electrical conductivity of GO-ODA composite are favorable for developing all-solid-state ISEs [30].

The FT-IR spectra are used to characterize ODA-functionalized GO (Fig. 1). The typical peaks of GO appear at 3431, 1718, 1631, and 1052 cm$^{-1}$ corresponding to the presence of hydroxyl, carboxylic, C–C bonds and epoxy groups, respectively. In the spectrum of ODA, three strong peaks above 3000 cm$^{-1}$ disappear after reacting with GO, which indicates that there is a strong interaction between –NH$_2$ group of ODA and GO. In the case of GO-ODA composite, the asymmetric peaks at 2918 and 2848 cm$^{-1}$ are attributed to the C–H stretching vibrations of the alkyl group, and the new peaks at 1568 cm$^{-1}$ (C–N stretching vibration) indicate the formation of –C–NH–C– band [30]. These data demonstrate that ODA is grafted on the GO sheets. Moreover, after mixing GO-ODA composite with Ca$^{2+}$ ionophore in ethanol solution and centrifugation, the obtained composite was also characterized. As shown in Fig. 1, there is a new peak at 1647 cm$^{-1}$, which may be attributed to the C=O stretch of amide in the molecular structure of Ca$^{2+}$ ionophore. This phenomenon indicates that Ca$^{2+}$ ionophore is immobilized on GO-ODA composite through hydrophobic interactions.

3.2 Potentiometric Measurements

The schematic representation of the all-solid-state Ca$^{2+}$-selective electrode based on the GO-ODA composite is shown in Fig. 2. The proposed Ca$^{2+}$-ISM contains Ca$^{2+}$ ionophore for selectively recognizing Ca$^{2+}$, NaTFPB as an ion exchanger, PVC and o-NPOE as a matrix and the GO-ODA composite as both a transducer and a substrate for immobilizing Ca$^{2+}$ ionophore with lipophilic side chains through hydrophobic interactions.

![Fig. 2. Schematic representation of the all-solid-state Ca$^{2+}$-selective electrode based on the GO-ODA composite.](image)

After being conditioned in 1.0×10$^{-3}$ M Ca(NO$_3$)$_2$, overnight, the proposed all-solid-state GO-ODA-based Ca$^{2+}$-ISEs were measured in Ca(NO$_3$)$_2$, with the concentrations from 1.0×10$^{-3}$ to 1.0×10$^{-8}$ M. As shown in Fig. 3, all of the GC/Ca$^{2+}$-ISM, GC/Ca$^{2+}$-ISM(I) and GC/Ca$^{2+}$-ISM(II) electrodes show stable potential responses in the linear range of 3.0×10$^{-7}$–1.0×10$^{-3}$ M. The slopes of the calibration curves are 26.9±0.9, 24.7±0.3 and 22.8±1.2 (n=3), respectively. The corresponding detection limits calculated as the intersection of the two slope lines are (1.3±0.3)×10$^{-7}$, (1.6±0.2)×10$^{-7}$ and (2.5±0.1)×10$^{-7}$ M (n=3), respectively. It can be seen that both the slope and the detection limit are slightly influenced by the amounts of the GO-ODA composite that is uniformly dispersed in the Ca$^{2+}$-selective membrane.

The selectivity of the GC/Ca$^{2+}$-ISM, GC/Ca$^{2+}$-ISM(I) and GC/Ca$^{2+}$-ISM(II) electrodes were estimated by using separate solution method, respectively [27]. As shown in Fig. 4, the selectivity coefficients become slightly worse as the increase of the amounts of the GO-ODA composite, which may be due to that the presence of GO influences the formation of the complexes between Ca$^{2+}$ and three ionophore molecules in the ion-selective membrane [31].
The complex formation constants (log $\beta_{ILnI}$) of the GC/Ca$^{2+}$-ISM, GC/Ca$^{2+}$-ISM(I) and GC/Ca$^{2+}$-ISM(II) electrodes were estimated according to the equation [32]:

$$\beta_{ILnI} = \frac{K_{t,IE}(I)}{K_{t,IL}(L)/L_T - n_i R_T/Z_i}$$

where $K_{t,IE}(I)$ and $K_{t,IL}(L)$ are the respective selectivity coefficients of the ionophore-free ion-exchanger-based membrane and the ionophore-based membrane toward the tetramethylammonium ion (TMA$^+$, I), $L_T$ and $R_T$ are the total concentration of ionophore and lipophilic ionic sites, $Z_i$ is the charge number of the primary ion (I), and $n_i$ is the stoichiometry of the primary ion-ionophore complex. As shown in Table 2, the complex formation constant between the mobile ionophore and Ca$^{2+}$ for the GC/Ca$^{2+}$-ISM electrode is close to the previously published value [33], which is estimated by the sandwich membrane method. Additionally, the complex formation constants for the GC/Ca$^{2+}$-ISM(I) and GC/Ca$^{2+}$-ISM(II) electrodes are found to be smaller than that for the GC/Ca$^{2+}$-ISM electrode, which may be due to that the immobilization of Ca$^{2+}$ ionophore by the GO-ODA composite influences the complex formation between Ca$^{2+}$ and the ionophore molecules in the ion-selective membrane. Similar results are also obtained for other ionophore-immobilized ISEs [34].

The long-term potentiometric response behaviors of the GC/Ca$^{2+}$-ISM and GC/Ca$^{2+}$-ISM(I) electrodes were compared. As shown in Fig. 5, the potential performance of the GC/Ca$^{2+}$-ISM(I) electrode almost remains the same during the period of 14 days, while the slope and the detection limit of the GC/Ca$^{2+}$-ISM electrode become slightly deteriorated after this period. The result indicates that the GC/Ca$^{2+}$-ISM(I) electrode shows a better long-term stability than that of the GC/Ca$^{2+}$-ISM electrode, probably due to the presence of the GO-ODA composite in the ISM.

### 3.3 pH Effect and Redox Sensitivity

Due to the presence of the hydroxyl and carboxylic groups on GO sheets, the pH effect on the potential responses of the GO-ODA-based all-solid-state Ca$^{2+}$-ISEs was investigated in 10$^{-2}$ M Ca(NO$_3$)$_2$. The pH value of the solutions was adjusted by using HNO$_3$ or NaOH. As shown in Fig. 6, the potential responses of both the GC/Ca$^{2+}$-ISM and GC/Ca$^{2+}$-ISM(I) electrodes showed a slight pH dependence.
Ca$^{2+}$-ISM and GC/Ca$^{2+}$-ISM(I) electrodes are found to be almost constant over a wide pH range of 6.0–9.0, indicating that pH effect on the potential response is not influenced by the addition of the GO-ODA composite in the Ca$^{2+}$-ISM. The potential changes at pH < 6.0 and > 9.0 may be due to the protonation of the ionophore and the formation of calcium hydroxide, respectively. Moreover, the slopes of the proposed all-solid-state Ca$^{2+}$-ISEs are also found to be not influenced during the potentiometric measurements in the pH range of 6.0–9.0.

The redox sensitivity was investigated by measuring the potential responses of the GC/Ca$^{2+}$-ISM and GC/Ca$^{2+}$-ISM(I) electrodes in 0.1 M KCl and 1 mM Fe(CN)$_6^{3-/4-}$ with the ratio of Fe$^{2+}$/Fe$^{3+}$ from 1/10 to 10/1. As shown in Fig. 7, both the GC/Ca$^{2+}$-ISM and GC/Ca$^{2+}$-ISM(I) electrodes show stable potential responses, and no significant redox sensitivity are found, which indicates that the GO-ODA composite in the Ca$^{2+}$-ISM does not provide a direct electronic contact between the redox couple and the GC electrode, which may be due to that the PVC-based ISM is an electronic insulator [35].

### 3.4 Potential Stability and Water Layer Test

The potential stability of the GC/Ca$^{2+}$-ISM and GC/Ca$^{2+}$-ISM(I) electrodes were investigated under zero current conditions by continuously monitoring the potential responses in 10$^{-3}$ M Ca(NO$_3$)$_2$ for 10 h. As seen in Fig. 8, the GC/Ca$^{2+}$-ISM electrode shows a clearly negative potential drift of 1170 μV/h, while the GC/Ca$^{2+}$-ISM(I) electrode exhibits a potential drift of 253 μV/h. The results demonstrate that the GO-ODA composite in the ISM can improve the potential stability of the all-solid-state Ca$^{2+}$-ISE, which may be due to the fact that the GO-ODA composite with electrical conductivity influences the transducing interface between the ISM and the GC electrode.
The presence of the water layer between the ISM and the GC electrode may also influence the potential stability of the all-solid-state Ca\(^{2+}\)-ISE. Therefore, the water layer test for the GC/Ca\(^{2+}\)-ISM(I) electrode was performed by alternately measuring the potential responses in 10 \(^{-3}\) M Ca(NO\(_3\)_2) for 1 h, 10 \(^{-3}\) M Mg(NO\(_3\)_2) for 1 h and 10 \(^{-3}\) M Ca(NO\(_3\)_2) for 8 h. As shown in Fig. 9, the potential drifts are not clearly observed when changing the GC/Ca\(^{2+}\)-ISM(I) electrode from the primary ions (Ca\(^{2+}\)) to the interfering ions (Mg\(^{2+}\)) or from the interfering ions to the primary ions. These results indicate that no undesirable water layer is formed between the GO-ODA-based ISM and the GC electrode, which may be due to the hydrophobicity of the GO-ODA composite.

### 3.5 Analytical Applications

The analytical applications for the proposed all-solid-state Ca\(^{2+}\)-ISEs based on the GO-ODA composite were investigated by measuring Ca\(^{2+}\) concentrations in the diluted artificial seawater. The compositions of the artificial seawater contain 427.64 mM NaCl, 29.27 mM Na\(_2\)SO\(_4\), 10.58 mM KCl, 54.74 mM MgCl\(_2\) and 10.75 mM CaCl\(_2\). The artificial seawater was diluted to different ratios with deionized water, and the concentrations of Ca\(^{2+}\) in the diluted artificial seawater were obtained by the potentiometric standard addition method. As shown in Table 3, the proposed all-solid-state Ca\(^{2+}\)-ISEs show satisfied recoveries varying from 90 to 105\% for detection of Ca\(^{2+}\).

<table>
<thead>
<tr>
<th>Volume ratio of the artificial seawater to deionized water</th>
<th>Ca(^{2+}) concentration (M)</th>
<th>Found [a] (M)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:20</td>
<td>5.12 \times 10^{-4}</td>
<td>(5.37 \pm 0.21) \times 10^{-4}</td>
<td>104.9</td>
</tr>
<tr>
<td>1:50</td>
<td>2.11 \times 10^{-4}</td>
<td>(1.90 \pm 0.07) \times 10^{-4}</td>
<td>90.1</td>
</tr>
<tr>
<td>1:100</td>
<td>1.06 \times 10^{-4}</td>
<td>(9.95 \pm 0.41) \times 10^{-4}</td>
<td>93.9</td>
</tr>
<tr>
<td>1:200</td>
<td>5.35 \times 10^{-5}</td>
<td>(5.15 \pm 0.29) \times 10^{-5}</td>
<td>96.3</td>
</tr>
</tbody>
</table>

[a] Mean of three determinations ± S.D.

### 4 Conclusions

This work demonstrates that octadecylamine-functionalized graphene oxide can be used to immobilize Ca\(^{2+}\) ionophore with lipophilic side chain through hydrophobic interactions, but also acts as a transduction element to improve the potential stability for the all-solid-state Ca\(^{2+}\)-ISEs. The GO-ODA-based Ca\(^{2+}\)-ISEs show a stable Nernstian response within the concentration range from 3.0 \times 10^{-7}\text{–}1.0 \times 10^{-3}\) M. Additionally, the proposed GO-ODA-based electrodes exhibit an improved stability with the absence of water layer between the ion-selective membrane and the GC electrode, although the selectivity and the complex formation constants for the GO-ODA-based Ca\(^{2+}\)-ISEs may become slightly less than those for the electrodes without the GO-ODA composite.

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