Polymeric membrane ion-selective electrodes with anti-biofouling properties by surface modification of silver nanoparticles

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ABSTRACT

Polymeric membrane ion-selective electrodes (ISEs) have been widely applied in environmental analysis. In many cases of practical relevance, however, when these sensors are applied in complex samples, they could suffer from problems of biofouling (related to microorganisms and biofilms) which may shorten the sensors' lifetimes and cause the measurement errors. Herein, we describe a robust and effective method to endow the ISE membranes with anti-biofouling properties based on surface modification of silver nanoparticles (Ag NPs). The ISE membrane is modified with the hydrophilic polydopamine (PDA) followed by *in-situ* formation of Ag NPs. The PDA modification enhances anti-adhesive properties of the ISE membrane by increasing the surface hydrophilicity, while Ag NPs impart strong anti-microbial properties to the membrane. A classical polymeric membrane K⁺-ISE has been chosen as a model. Compared to the unmodified K⁺-ISE, the Ag NPs modified K⁺-ISE exhibits significantly improved anti-biofouling properties in terms of the low viability and adhesion rates of bacteria while retaining its original potentiometric ion response properties. Moreover, Ag NPs on the sensor surface show an excellent long-term stability. We believe that the proposed approach can be extended to improve the anti-fouling abilities of other polymeric membrane-based electrochemical sensors for detection in complex environmental samples.

1. Introduction

Nowadays, polymeric membrane ion-selective electrodes (ISEs) have become a powerful analytical tool in a variety of fields [1–5]. Especially in environmental monitoring, such electrodes play an important role in real-time and accurate analysis of some environmental parameters, such as pH, the concentrations of Ca²⁺, NH₄⁺, and carbonate [6–8]. Extensive attentions have been attracted to develop sensors with reliable performances [9–11]. However, note that, when these sensors are applied in the complex environmental samples (e.g., wastewater), they may suffer from problems of biofouling which refer to the undesirable attachment of microorganisms and their development into biofilms on sensors' surface [12]. In particular, for the tropical countries, this situation becomes more serious because the high climate temperature and the high

contents of nutrients are suitable for microorganism growths [13,14]. To date, using protein as a model fouling agent, considerable efforts have been devoted to investigate the biofouling of various sensors which involves the fouling influences on ISEs [15,16], fouling kinetics [17,18], and mitigation methods [19], but the biofouling caused by microorganisms and biofilms has rarely been studied.

Generally, the formation of biofilm is a complex dynamic process. When an material is immersed in sample, an electric double layer can be established at the surface through adsorption of water molecules and ions, which can mediate the adsorption of biomolecules (e.g., polysaccharides and proteins) [20]. This adsorbed layer acts as a conditioning film to facilitate the settlement of microorganisms [21]. As suspended bacterial cells approach the membrane surface, they can be attached to the surface via electrostatic and hydrophobic interactions

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[22]. Once the bacterial cells bind to the surface, they begin to change phenotype and excrete extracellular polymeric substances (EPS). Over time, the bacteria proliferate and continue to excrete EPS forming a dense biofilm. The mature biofilms can release more bacteria cells to foul another surface of sensors [23]. Such biofouling may affect the electrochemical performance of sensors by perturbing the analyte mass transfer to the sensing interface, and cause the loss of the lifetime and measurement errors [12].

During the past decades, surface modification has been proven as an effective method to improve the anti-biofouling properties of the polymeric membrane ISEs [24]. A few strategies have been successfully used to mitigate the biofouling of the ISEs by surface modification of the anti-adhesive materials (e.g., hydrophilic polymers, hydrogels and silicone rubber) [19,25,26]. However, it should be noted that these anti-adhesive methods cannot completely inhibit the non-specific adhesion of the foulants (e.g., bacteria). In this case, the biofilm would be generated in a relative long term, and thus finally cause fouling of sensors. An alternative strategy is to construct anti-microbial membrane surfaces that can actively inhibit microbial growth. Recently, our group proposed an anti-biofouling polymeric membrane ISE using 6-chlorindole as the antibacterial additive [27]. Although the antibacterial material can kill the bacteria on membrane surface, it may have the limited role to reduce the initial adhesion of bacteria. In recent years, the combination of the anti-adhesive and anti-microbial materials has exhibited a great potential to dramatically change this situation. The surface coatings with the dual functionality of both anti-adhesive and anti-microbial properties have been fabricated for anti-adhesion and bacterial inactivation, respectively. Although these bifunctional coatings have showed excellent biofouling resistance, the use of them in the polymeric membrane potentiometric sensors has received little attention. Our group developed an anti-biofouling polymeric membrane ISE, which makes use of both anti-adhesive and anti-microbial properties of graphene oxide (GO) [28]. Nevertheless, GO is a kind of contact-killing anti-bacterial material with relative low anti-bacterial activities [29]. The accumulation of foulants (e.g., bacteria and bacteria debris) on such anti-bacterial materials may affect their anti-fouling properties.

Herein, a robust and effective method is proposed to improve the biofouling-resistance properties of the polymeric membrane ISEs by surface modification of silver nanoparticles (Ag NPs). Ag NPs have attracted growing interest due to their broad-spectrum and high-effective anti-bacterial properties [30,31]. The anti-bacterial mechanisms of Ag NPs include causing cell membrane damage, generating toxic reactive oxygen species (ROS) and disrupting cell metabolisms, such as DNA replication [23]. The released silver ions from Ag NPs can not only kill the bacteria on the surface of materials but also inhibit the development of biofilms [32,33]. In order to achieve surface modification of Ag NPs, a hydrophilic polydopamine (PDA) layer is coated on the surface of the ISE through the self-polymerization of dopamine, which can reduce silver ions to Ag and achieve *in-situ* synthesis of Ag NPs on different substrates without any other reducing agent [34]. Note that, the O- and N-sites of PDA can serve as the anchors for Ag NPs to enhance the durability of Ag NPs on the surfaces via metal coordination [35,36]. This can effectively avoid the loss of anti-bacterial nanomaterial. As a proof-of-concept experiment, a solid-contact polymeric membrane K^+ -ISE has been selected as a model sensor. It will be shown that the Ag NPs-coated K^+ -ISE exhibits excellent anti-biofouling performances in terms of the low viability and adhesion rates of bacteria.

2. Experimental

2.1. Reagents

Poly(vinyl chloride) (PVC), *o*-nitrophenyl octyl ether (*o*-NPOE), sodium tetrakis [3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), poly (sodium 4-styrenesulfonate), (NaPSS, Mw:~70 000), tridodecylmethylammonium chloride (TDMACl), 3,4-ethylenedioxythiophene (EDOT,

97 %) and fluorescent stains including SYTO9, Concanavalin A (Con A) and propidium iodide (PI) were obtained from Sigma-Aldrich. The potassium ionophore III was purchased from Nanjing Jingjie Biotechnology Co., Ltd., China. Deionized water with a specific resistance of 18.2 M Ω cm was obtained using a Pall Cascada laboratory water system. All other chemical reagents were purchased from Sinopharm Chemical Reagent with analytical grade and were used as received.

2.2. Preparation of K^+ -ISE

The membrane solution was prepared according to our previous study [37]. Briefly, 360 mg of membrane components containing 1.0 wt % potassium ionophore III, 0.6 wt% NaTFPB, 32.8 wt% PVC, and 65.6 wt% *o*-NPOE were dissolved in 3.6 mL THF. After vigorous stirring for 2 h, a transparent membrane cocktail was obtained. A poly(3, 4-ethylenedioxythiophene) : poly(sodium 4-styrenesulfonate) (PEDOT : PSS) layer was used as the intermediate layer of the solid-contact K^+ -ISE [38,39]. The PEDOT : PSS layer was deposited onto the surface of a polished glassy carbon electrode (GCE, 3.0 mm in diameter) by electrochemical polymerization in a solution containing 10 mM EDOT and 100 mM NaPSS. A constant current of 0.014 mA was applied to produce the polymerization for 714 s. The obtained electrodes were rinsed with deionized water and dried overnight. Finally, 80 μ L of the membrane cocktail was drop-cast onto the pretreated GCE with a circular support to K^+ -ISM (0.6 cm in diameter) and dried overnight to generate an ion-selective membrane (ISM) with a thickness of approximately 100 μ m.

2.3. Surface modification of K^+ -ISE

Surface modification of Ag NPs onto the surface of K^+ -ISEs was performed according to the previous work [33]. The modification process is shown in Scheme 1. The K^+ -ISEs were immersed in 10 mM Tris-HCl buffer (pH 8.5) containing 2 mg/mL dopamine for 12 h to generate PDA coating. After that, the PDA coated K^+ -ISEs (K^+ -ISE/PDA) were rinsed with deionized water and then immersed in 50 mM silver nitrate aqueous solution for 8 h to modify Ag NPs on the electrode surface. Finally, the Ag NPs modified K^+ -ISEs (K^+ -ISE/PDA/Ag NPs) were thoroughly rinsed with deionized water for further measurements.

2.4. Characterization

The surface morphology of the obtained ISMs was observed by using a scanning electron microscope (SEM, S-4800, Hitachi). The elemental analysis was performed by using an energy dispersive X-ray micro-analyzer (EDX, EX-350, Horiba). To avoid burning the ISMs, all samples were sputter coated with Pt before SEM and EDX observation. The water contact angle (WCA) was measured by using an optical instrument (ADS300, Data Physics). The amount of Ag NPs on the K^+ -ISM was quantified by an inductively coupled plasma mass spectrometer (ICP-MS, ELAN DRC II, PerkinElmer) after dissolution by using 3.5 % HNO_3 solution for 48 h. The leaching of silver ions from the K^+ -ISE/PDA/Ag NPs was investigated by immersing the electrode with an effective area of 0.20 cm² in 10 mL deionized water. After 24 h, the solution was analyzed by using ICP-MS, and refilled with deionized water for prolonged release study.

2.5. EMF measurements

The electromotive force (EMF) values were measured by an electrochemical workstation (CHI 760D, Shanghai Chenhua Apparatus Corporation) in a galvanic cell: Ag / AgCl / 3 M KCl / 0.1 M LiOAc // sample solution / ISM / PEDOT-PSS / GCE. For K^+ detection, the electrodes were conditioned in 1 mM KNO_3 solution overnight, while for measurement of experimental selectivity coefficients, the electrodes were conditioned in 1 mM $NaNO_3$ solution overnight. The successive

Scheme 1. Schematic representation for *in-situ* formation of Ag NPs onto the polymeric membrane ISEs. The ISE membranes were firstly coated with PDA, followed by *in-situ* reduction of silver by immersing the PDA coated membrane into an AgNO_3 solution of 50 mM.

addition method was used to make the calibrations and the measurements were carried out in deionized water. The response times of electrodes were obtained when the K^+ concentration was increased from 1×10^{-4} to 1×10^{-3} M. Selectivity coefficients were determined by using the separate solution method [40]. The ion activity coefficients were calculated by the Debye-Hückel equation, and the EMF values were corrected with the Henderson equation.

2.6. Biofouling study

The anti-biofouling performances of the K^+ -ISE/PDA/Ag NPs were evaluated by determining the bacteria adhesion and biofilm formation on the surface of the sensing membrane. A monoculture of *P. aeruginosa* (ATCC 27853) was firstly cultivated overnight at 37 °C in Luria-Bertani broth. Then, the bacteria cells were collected via centrifugation and washed with the sterile deionized water. The obtained bacteria cells were re-suspended in sterile artificial wastewater (containing 8.0 mM NaCl, 0.15 mM MgSO_4 , 0.5 mM NaHCO_3 , 0.2 mM CaCl_2 , 0.2 mM KH_2PO_4 , 0.4 mM NH_4Cl , and 0.6 mM sodium citrate, pH 7.5 [41]) to reach a concentration of approximately 10^8 CFU mL^{-1} . The experiments of the bacteria adhesion were performed by incubating a circular K^+ -ISM (0.6 cm in diameter) with 30 mL bacterial suspension for 2 h at room temperature. The K^+ -ISM was then collected and gently rinsed by the sterile 0.9 % NaCl solution. To form biofilm on the K^+ -ISM, the experiments were carried out under the same conditions except for increasing the incubation time to 24 h. The live bacteria, dead bacteria and EPS were stained with SYTO9 (3.34 μM), PI (20 μM) and Con A (50 μM), respectively. The bacteria and biofilms were finally observed by using a confocal laser scanning microscope (CLSM, FV1000). A Z stack scanning with a slice thickness of 2 μm was performed to obtain the three-dimensional structure images of biofilm. The obtained images were analyzed by using Image J Pro software (National Institutes of Health, MD) to quantify the number of bacteria.

3. Results and discussion

3.1. Optimization of the loading amount of Ag NPs

It has been reported that the loading amount of Ag NPs on the substrates depends not only on the concentrations of dopamine and the silver salt in the polymerization solution but also on the polymerization time [42]. The surface modification of Ag NPs with excellent anti-biofouling properties can be achieved by polymerization of 2 mg/mL of dopamine and 50 mM of AgNO_3 [42,43]. Therefore, these experimental conditions were used in this work. The effect of the polymerization time on the loadings of Ag NPs was further investigated by using ICP-MS. As shown in Fig. S1, the loading amount of Ag NPs increases with prolonging the polymerization time from 2 h ($7.6 \pm 0.5 \mu\text{g}/\text{cm}^2$) to 8 h ($25.5 \pm 4.7 \mu\text{g}/\text{cm}^2$) and then reaches a plateau with further prolonging the polymerization time to 12 h. This is probably ascribed to the fact that the catechol groups of PDA could be gradually consumed by the continuous reduction of silver ions to Ag NPs [36]. Thus, 8 h was selected as the optimal polymerization time.

3.2. Characterization of the obtained Ag NPs modified membrane electrode

The surface morphology of the obtained ISM was observed by using SEM. As shown in Fig. 1A, the control K^+ -ISM shows a smooth surface. After PDA coating and Ag NPs modification, numerous bright nanoparticles with a size distribution of 22–70 nm and an average of 41 nm are found on the surface of the K^+ -ISM (Fig. 1B). In addition, the elemental analysis was performed to verify the modification of Ag NPs. Compared to the control K^+ -ISM, there is an obvious increase in the intensity of Ag peak for the K^+ -ISM/PDA/Ag NPs (Fig. 1C and 1D), which further confirms the successful modification of Ag NPs on the sensor surface.

The surface hydrophilicity was characterized by measuring the WCA of the sensing membranes. The results of the WCA measurements are shown in Fig. S2. As illustrated, the membrane surface becomes hydrophilic with a decrease in the WCA from ($82.3 \pm 1.5^\circ$) to ($68.5 \pm 2.1^\circ$) after PDA functionalization. Indeed, PDA can be effectively used to improve the surface hydrophilicity of the polymeric membrane K^+ -ISEs. In addition, no obvious change in the WCA is observed after further modification of Ag NPs on the PDA functionalized electrodes. These results suggest that the enhanced hydrophilicity of the modified membranes is mainly attributed to the presence of the PDA films [35,44].

3.3. Influences of the surface modification on the sensor's performance

The general consideration of the surface modification of the ISEs is to improve their fouling-resistance properties without sacrificing their analytical performance [24]. Therefore, the response slope, response time and selectivity coefficients of the K^+ -ISEs before and after Ag NPs modification were evaluated to investigate the influences of the surface modification on the sensor's analytical performance. As shown in Fig. 2A, the pristine K^+ -ISE exhibits a Nernstian response slope of 54.2 ± 1.3 mV/decade over the range from 10^{-5} to 10^{-2} M. Being similar to the pristine electrode, the K^+ -ISE/PDA and the K^+ -ISE/PDA/Ag NPs show the response slopes of 54.4 ± 0.9 mV/decade and 52.5 ± 1.8 mV/decade, respectively. A similar detection limit of about $10^{-5.4}$ M is observed for all these electrodes. These results illustrate that the surface modification indeed does not impair the final thermodynamic extraction equilibrium state of K^+ , which is consistent with our previous reports [28,44].

Moreover, no obvious changes is observed in the sensor's response time after PDA and Ag NPs modifications (Fig. 2B), which is different from the previous observations [28,44]. Previously, it has been found that the response times usually increase with the successive coverage of the modification layers. This is probably due to the fact that the surface modification layers could affect the ion diffusion process of the target ions (e.g., Ca^{2+}) between the polymeric sensing membrane and the aqueous layer via non-covalent interactions (e.g., the chelating interactions between the phenolic hydroxyl and secondary amine groups of the PDA layer and Ca^{2+}). Thus, the response time can be changed. However, compared to Ca^{2+} , K^+ has much weaker chelating interactions with the PDA layer. These may lead to the fact that the ion diffusion process of K^+ could not be influenced by the modification layers. Hence, the response time is not changed obviously.

Fig. 1. SEM images of the K^+ -ISM (A) and K^+ -ISM/PDA/Ag NPs (B). EDS spectra of both electrodes (C) and the enlarged EDS spectrum of Ag peaks (D).

Fig. 2. Calibration curves (A) and potential response times (B) of the K^+ -ISE, K^+ -ISE/PDA and the K^+ -ISE/PDA/Ag NPs. The response times of electrodes were measured in deionized water when the K^+ concentration was increased from 1×10^{-4} to 1×10^{-3} M. Error bars represent the standard deviation for three electrodes. Statistical significance was determined by a Student t test with a confidence interval of 95 %.

Table 1
Potentiometric selectivity coefficients of the K⁺-ISE/PDA/Ag NPs.^a

Ion j	$\log K_{K,j}^{pot}$	
	K ⁺ -ISE	K ⁺ -ISE/PDA/Ag NPs
Na ⁺	-3.60 ± 0.06	-3.43 ± 0.09
Ca ²⁺	-7.19 ± 0.24	-7.07 ± 0.12
Mg ²⁺	-7.94 ± 0.10	-7.78 ± 0.10
Li ⁺	-4.08 ± 0.15	-4.08 ± 0.10
Ag ⁺	-2.72 ± 0.19	-2.52 ± 0.02

^a Average value of three determinations ± standard deviation.

The selectivity coefficients of the proposed sensors were measured by using the separate solution method, and metal ions including Na⁺, Ca²⁺, Mg²⁺, Li⁺ and Ag⁺ were selected as the interfering ions. As shown in Table 1, no obvious change in the selectivity coefficients of all these ions is observed before and after modification, which suggests that the surface modification could not influence the selectivity coefficient based on the thermodynamic equilibrium response. These results can further confirm that the modification cannot affect the extraction equilibrium state of K⁺. Notably, although the prepared K⁺-ISE/PDA/Ag NPs has a relative poor selectivity to silver ions, the silver ions may have neglectable influence on detection of K⁺, because the K⁺ concentration in the natural water sample is at the levels of millimoles per liter and the concentration of the silver ions released from the sensor surface is ultralow (about 10⁻⁸ M as determined by the ICP-MS).

To explore the generality of the proposed method, the chloride-selective electrode (Cl⁻-ISE) was prepared and used to investigate the influences of the surface modification on the sensor's performance. As shown in Fig. S3, the unmodified Cl⁻-ISE exhibits a Nernstian response of 56.9 mV/decade over the concentration range of 10⁻⁵ to 10⁻² M with a detection limit of 10^{-5.4} M, while the Ag NPs modified electrode shows a similar Nernstian slope (52.0 mV/decade) and detection limit (10^{-5.0} M) as well. This can confirm that the leaching of silver ions could not obviously influence the potentiometric responses of both cation and anion sensitive electrodes.

3.4. Evaluation of the anti-adhesion and anti-bacterial performances

The release-based anti-bacterial properties of Ag NPs modified ISMs were investigated by using the classical diffusion inhibition zone test. As shown in Fig. S4, abundant bacteria grow on the agar plate around the K⁺-ISM and the K⁺-ISM/PDA. In contrast, a clear bacterial-free area is observed around the K⁺-ISM/PDA/Ag NPs, suggesting the excellent releasable anti-bacterial properties of Ag NPs.

To further illustrate the abilities of the anti-adhesion and anti-bacterial, the ISMs were incubated with the bacteria suspensions (*P. aeruginosa*) for 2 h. The attached bacteria were then stained and intuitively observed by using a CLSM. Live and dead bacteria were stained with SYTO9 (green), and PI (red), respectively. As shown in Fig. S5A, an intense green fluorescence and a weak red fluorescence are observed on the surface of the pristine K⁺-ISM, while the K⁺-ISM/PDA/Ag NPs membrane surface exhibits a significantly decreased green fluorescence and an enhanced red fluorescence. These results suggest that the live bacteria are predominant on the surface of the unmodified K⁺-ISM, but the majority of the adhered bacteria on the Ag NPs modified K⁺-ISM surface are dead. These images were then quantitatively analysed by using Image J Pro software and the results are shown in Fig. S5B. As expected, compared with the pristine K⁺-ISM, Ag NPs modification obviously reduces the cell viability from 84.6 % to 6.7 %. It is worth noting that this anti-bacterial property is much higher than that of our previous GO modified ISEs (anti-bacterial efficiency, 93.3 % vs 53.1 %). Moreover, the total number of bacterial cells attached onto the surface of the modified ISMs decreases by 32.1 % (3.8 × 10⁵ cells per cm² for the K⁺-ISM and 2.6 × 10⁵ cells per cm² for the K⁺-ISM/PDA/Ag NPs), which is similar to that on the GO modified electrode [28].

Previous studies have demonstrated that the bacterial attachment is favorable with the hydrophobic surface [23]. Hence, the reduced number of bacterial cells is mainly owing to the presence of the PDA layer which can effectively improve the surface hydrophilicity of the ISM. Such PDA layer can also resist the adhesion of other bio-foulants (e.g., lipids and proteins) [44]. Therefore, it can be concluded that the K⁺-ISM/PDA/Ag NPs exhibits both excellent anti-adhesive and anti-bacterial properties.

3.5. Evaluation of the anti-biofouling performances

It has been well known that biofouling is a dynamic process which involves not only the attachment of the microorganisms and but also their further development into a biofilm [20]. The biofouling process of electrode was explored by measuring the short-term and long-term time-potential curves of the K⁺-ISE and K⁺-ISE/PDA/Ag NPs during the fouling experiments. The bacterial cells were thoroughly washed by using artificial wastewater and then used for the fouling experiments. As shown in Fig. S6A, no potential response is observed for the unmodified K⁺-ISE or the K⁺-ISE/PDA/Ag NPs after the addition of bacteria in the solution. The potential responses are stable within the 180 min for both electrodes (Fig. S6B), which indicates that the bacterial adhesion has little influence on the drift of electrodes. With prolonging the time to 1200 min, an upward drift is observed for both electrodes. Such drifts of electrodes are probably due to the fact that the attached bacteria could produce cations during their metabolisms. However, it should be noted that the drift of K⁺-ISE/PDA/Ag NPs in 70000 s (5 mV) is smaller than that of K⁺-ISE (10 mV), which indicates that the Ag NPs functionalization improved the stability of the sensor via suppression of biofilm.

The influence of Ag NPs on the formation of the biofilm was further investigated. For this purpose, the ISMs were immersed in the bacterial suspension with a high concentration (approximately 10⁸ CFU mL⁻¹) for 24 h. The biofilms formed on the sensing membrane surfaces were stained by using the fluorescent dyes. Fig. 3 shows that the biofilm has been formed and aggregated on the unmodified ISE membrane surface uniformly after 1 day. There are abundant live cells and EPS, and a few dead cells on the membrane surface. These results suggest that the pristine ISM has a high risk of biofouling problem. In contrast, although a slight biofilm has also been generated on the Ag NPs modified ISE membrane, the number of living bacteria and the amount of EPS are far less than those on the unmodified membrane. These results further confirm the excellent anti-biofouling property of Ag NPs modification.

Finally, the effects of biofouling on the sensor's analytical performance were evaluated by measuring the response slopes before and after immersing the control and modified electrodes in the bacterial suspension at a rather high concentration (10⁸ CFU mL⁻¹) for 7 days. As illustrated in Fig. 4, a response slope loss of 17.1 % is observed for the unmodified K⁺-ISE after 7-day immersion in the bacteria suspension. Under the same conditions, the response slope of the K⁺-ISE/PDA/Ag NPs decreases only by 2.0 %. These results indicate that the Ag NPs modified K⁺-ISE still maintains its effective biofouling-resistance properties even after a relatively long-time contact with the bacterial suspension at a rather high concentration. Therefore, the proposed Ag NPs modified strategy provides a promising potential for applications of the polymeric membrane ISEs in complex environmental samples. To explain the loss of sensor's analytical performance, the three-dimension structure of biofilms after 7 days were observed using CLSM. As shown in Fig. S7, the biofilm on the pristine ISM has a dense and uniform morphology with a thickness of 88 μm. It should be noted that the unmodified membrane has been completely covered by the biofilm after 7 days. In contrast, the biofilm on the Ag NPs modified ISM is loose and non-uniform with a thickness of 60 μm. These results demonstrate that the biofilm on the pristine ISM is more thriving than that on the Ag NPs modified ISM. In this case, the electrode would respond to the concentration of K⁺ within this biofilm rather than its concentration in the sample. [45] It can be concluded that the dense biofilm on the sensing

Fig. 3. Confocal laser scanning microscopy images of the biofilms on the K^+ -ISE and the K^+ -ISE/PDA/Ag NPs. Biofilms were stained with SYTO9 (green), PI (red) and Con A (blue) for live cells, dead cells and extracellular polymeric substances (EPS), respectively.

Fig. 4. Response slopes of the K^+ -ISE and the K^+ -ISE/PDA/Ag NPs before and after incubation with the bacterial suspension (10^8 CFU mL $^{-1}$) for 7 days. Error bars represent the standard deviation for three electrodes.

membrane is responsible for the loss of sensor's analytical performance.

3.6. Stability of Ag NPs on the membrane surface

The stability of Ag NPs on the membrane surface was investigated by measuring the leaching of silver ions from the K^+ -ISE/PDA/Ag NPs. As shown in Fig. 5, the release of silver ions from the sensor surface is rapid within the first 3 days. It has been well known that silver ions can effectively kill bacteria cells and destroy the biofilm structure. Therefore, the initial rapid release of silver ions from Ag NPs may be the reason for the effective prevention of the formation of the biofilm on the Ag NPs modified membrane surface after 24-h immersion as demonstrated above. After 3 days, the release becomes slowly and almost constant. Such slow release is favourable for maintaining the long-term anti-bacterial abilities of the proposed sensor. It should be noted that the concentrations of released silver ions as determined by ICP-MS are

Fig. 5. Accumulative amount of released silver nanoparticles from the K^+ -ISE/PDA/Ag NPs and the concentrations of silver ions released in the sample solution on different days.

between 6.19×10^{-8} M and 0.33×10^{-8} M. Therefore, the presence of silver ions at low concentrations in the sample would hardly cause interferences to the proposed sensor. Meanwhile, the low-level of released silver ions could also ensure the environmental friendly property of the electrode [46]. In addition, the amount of Ag NPs modified on the electrode is measured to be $5.10 \mu\text{g}$. After 7 days, the released amount of Ag NPs is only $0.17 \mu\text{g}$, which is calculated to be 3.3 % of the original amount of Ag NPs. Previous study also shows that the Ag NPs based on the same modification method remain effective after leaching of silver ions in water for a month [42]. Hence, it can be concluded that Ag NPs are stable on the sensor surface and applicable for sustainable anti-biofouling.

4. Conclusions

In this work, a polymeric membrane K^+ -ISE with excellent anti-biofouling properties is proposed which is based on the successive

modification of hydrophilic PDA and anti-bacterial Ag NPs. Compared to other polymeric membrane protection strategies (e.g., surface modification of the hydrophilic anti-adhesive materials), the proposed method can not only prevent the adhesion of bacteria but also kill the bacteria, and can further inhibit the development of biofilms on the membrane surface. The analytical performances of the K^+ -ISE were not obviously influenced by the surface modification. Moreover, compared to the control sensor, the Ag NPs modified sensor shows superior sensing abilities after contact with bacterial suspension for 7 days. Since the proposed Ag NPs modified strategy has the flexibility of employing different substrates, we envision that this novel concept can be extended to improve the anti-fouling abilities of other polymeric membrane-based electrochemical sensors for the analysis of the targets in complex environmental samples.

CRediT authorship contribution statement

Longbin Qi: Conceptualization, Methodology, Investigation. **Tianjia Jiang:** Conceptualization, Methodology, Writing - review & editing. **Rongning Liang:** Formal analysis, Writing - review & editing, Supervision. **Wei Qin:** Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.snb.2020.129014>.

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