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Short communication

Primary-ion-conditioned polymeric membrane electrodes for sensitive detection of polyions

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ABSTRACT

A novel operation mode for development of sensitive and renewable polymeric membrane polyion-sensitive electrodes has been explored in this paper. In contrast to the traditional polyion sensors which are conditioned with highly discriminated ions, the proposed polycation-sensitive membrane electrode with dinonylnaphthalene sulfonate as the ion-exchanger is conditioned with the primary ion (protamine), and activated with the discriminated ion (Na^+) before measurement. Using this protocol, the membrane with protamine in the membrane bulk but with discriminated ions rich at the membrane outer surface can be prepared. The protamine inside the membrane efficiently suppresses the fluxes of protamine from the sample–membrane interface into the membrane bulk, thus decreasing the detection limit toward protamine by one order of magnitude. With the reproducible protamine fluxes obtained by the conditioning and activation steps, the protamine-sensitive electrode could be reused without significant signal deterioration. Titrimetric analysis of heparin with this novel technique has been demonstrated. The proposed polyion sensing platform offers much promise in the detection of polyions as well as in other polyion-involved bioanalyses.

1. Introduction

During the past two decades, polyion-sensitive polymeric membrane electrodes have attracted special attention in the field of modern potentiometry owing to their unique capability of detecting polyionic macromolecules [1,2]. Using polyion-sensitive electrodes (PSEs), a large number of polycations (e.g. protamine, foldamer, and dendrimer) [3–5] and polyanions (e.g. heparin, DNA, dextran sulfate, and chondroitin sulfate) [6,7] have been detected via direct potentiometry or potentiometric titration. Additionally, antigen–antibody interaction [8], polyion cleavage enzymes [9] and their inhibitors [10,11] as well as activators [12] can also be monitored via polyion sensors. The working principle of PSEs is spontaneous polyion extraction from the aqueous sample solution into organic membrane via cooperative ion pairing interaction of polyions with corresponding ion exchangers [13,14]. A quasi-steady-state response in proportional to the analyte polyion concentration can be established when polyion fluxes into and out of the sample–membrane interface are equal. According to this mechanism, the sensitivity of PSEs is greatly affected by the relative magnitude of polyion fluxes in aqueous and organic phases, while the reproducibility of polyion fluxes governs the reversibility of PSEs. Therefore, many efforts have been made to improve the performances of PSEs with focus on the control or modulation of polyion fluxes.One advance in decreasing the detection limit toward polyion is the introduction of rotating electrode configuration, by which the ion flux from sample bulk to membrane surface is largely enhanced. Rotation of the PSEs at 5000 rpm results in detection limits toward protamine and heparin one order of magnitude lower than their conventional counterparts with magnetic stirring [15]. However, such a rotation design requires complex instrument and may suffer from larger potential noises. Another great advance in polyion flux control is the development of pulsed chronopotentiometric polyion electrodes, firstly for protamine [16,17] and lately for polyanions [18]. Using this technique, polyion fluxes are repeatedly controlled by sequential electrochemical pulses imposed on inert electrolyte-based polymeric membranes, and fully reversible detection of polyions can be fulfilled. However, the sensitivity of those polyion pulstrodes is moderate as compared to their potentiometric counterparts especially in physiological electrolyte solutions [18].

In this work, we explore a novel technique to improve the performance of PSEs via controlling the polyion fluxes within the organic membrane under open-circuit conditions.

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By conditioning the dinonylnaphthalene sulfonate (DNNS)-based protamine-sensitive electrode (PrSE) with protamine and then activating it with discriminated ions, the inward protamine fluxes from the membrane–sample interface into the membrane bulk are better defined and, consequently, both the sensitivity and regenerability of polyion electrodes can be significantly improved.

2. Experimental

2.1. Reagents

Dinonylnaphthalene sulfonic acid (DNNS) as a 50% solution in heptane, 2-nitrophenyl octylether (*o*-NPOE), high molecular weight poly(vinyl chloride) (PVC), protamine sulfate (from herring), heparin sodium salt (from bovine intestinal mucosa, 149 units/mg) were purchased from Sigma–Aldrich. Sodium chloride and tetrahydrofuran (THF) were purchased from Sinopharm Group Co. Ltd. All chemicals and reagents were of Selectophore or analytical reagent grade. Aqueous solutions were prepared with freshly deionized water (18.2 MΩ cm specific resistance) obtained with a Pall Cascada laboratory water system.

2.2. Preparation of protamine-sensitive electrodes

Protamine sensitive membranes (*ca.* 100 μm in thickness) containing 49.5 wt% PVC, 49.5 wt% NPOE and 1.0 wt% DNNS were prepared by solvent-casting technique with THF as the casting solvent. Membranes were then glued to plasticized PVC tubes (i.d. 6 mm, o.d. 9 mm) to fabricate PrSEs.

The PrSEs were conditioned in 100 μg/mL protamine in 0.12 M NaCl for *ca.* 12 h in ambient conditions and the same solution was used as the inner filling medium. Subsequently, the thoroughly rinsed electrode was moved to 0.12 M NaCl for *ca.* 20 min (without stirring) to finish activation. Potential measurements were then carried out in the same 0.12 M NaCl with constant stirring. Electrodes were stored at 4 °C in the conditioning solution when not in use. For traditional PrSEs with the same membrane formulation, 0.12 M NaCl was used as the conditioning and inner filling solutions.

2.3. EMF measurements

The electrode potentials were measured using a CHI 760D electrochemical workstation (Shanghai Chenhua Apparatus Corporation, China) in the galvanic cell: Ag, AgCl/3 M KCl/1 M LiOAC/test solution/PrSE membrane/inner filling solution/Ag, AgCl. All EMF measurements were conducted at room temperature and a Faraday cage was used to lower the noise.

3. Results and discussion

3.1. Operational principle of the proposed PrSE

For classical ion selective electrodes, primary ion conditioning is the prerequisite of the thermodynamic Nernstian response, whereas PSEs usually avoid contact with primary ions before measurement to ensure the counterdiffusion of primary ions with discriminated ions. For the present work, however, high concentration of protamine was chosen to condition the DNNS-doped membranes, yielding a protamine-occupied membrane boundary layer. Thus an equilibrium response of *ca.* 3 mV/decade toward protamine is anticipated from the Nernst equation, which is too small to be analytically useful. To make the electrode responsive to protamine in a super-Nernstian format analogous to the traditional PrSE, protamine in the membrane boundary layer must be depleted. To this end, an additional activation step was employed, in which 0.12 M NaCl was used to strip protamine from the outside membrane layer via ion-exchange process. The effectiveness of this step was validated by the large potential drop of about 120 mV during activation of 20 min, as shown in Fig. 1A. According to the phase boundary potential theory, an increase in the concentration of Na^+ in the membrane boundary layer by about two orders of magnitude is predicted, which facilitates the following extraction of sample polyion into the membrane. On the other hand, such an ion-exchange process released primary ion into the contacting stagnant aqueous layer. In classical ionophore-based ion selective electrodes, the leached primary ion was shown to considerably deteriorate the detection limit and selectivity [19] and even be significant enough to be analytically utilized [20,21]. However, this contamination effect appeared to be much less pronounced for PrSE according to the subsequently obtained large and sensitive response to

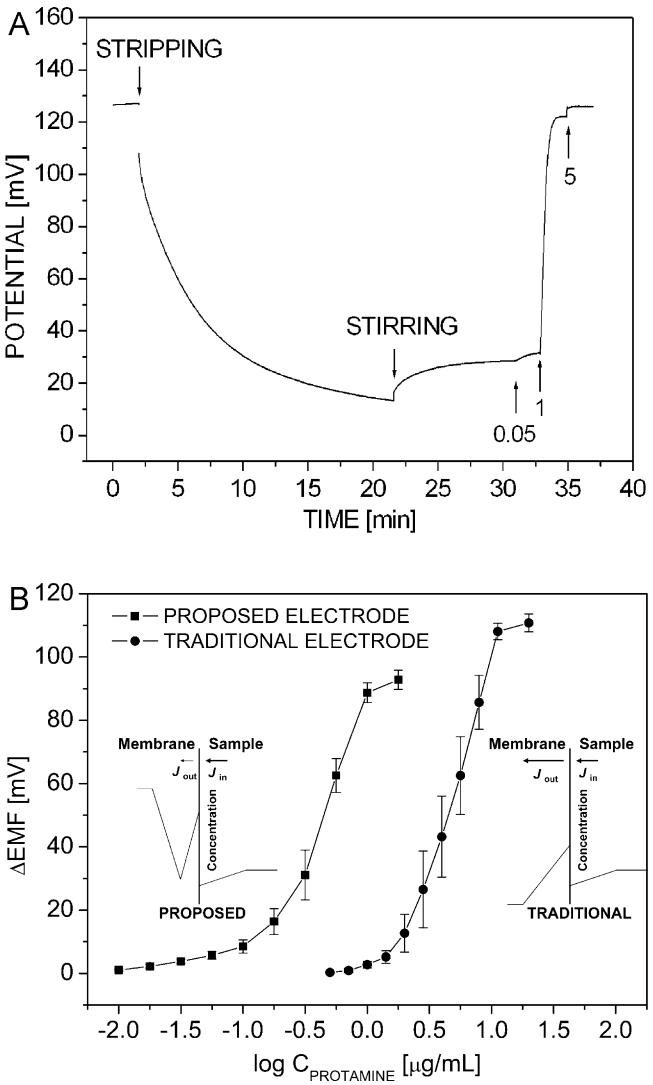

Fig. 1. (A) Potential–time trace of activation and measurement processes of the proposed electrode in 0.12 M NaCl. The numbers below the plot represent concentrations of added protamine (μg/mL). (B) Protamine calibration curves with traditional PrSE and proposed PrSE in 0.12 M NaCl under otherwise identical conditions. Potential readings were taken 2 min after each addition of protamine. Each data point represents an average ± standard deviation for three measurements. Insets: the expected protamine concentration profiles of the traditional PrSE (right) [14] and proposed PrSE (left) near the sample–membrane interface. The regions near the interface with different protamine concentrations from the bulk phase represent the diffusion layers. J_{in} and J_{out} denote the protamine flux from the aqueous bulk into the sample–membrane interface and that from the interface into the membrane bulk, respectively.

analyte protamine. This discrepancy might be related to the different response mechanisms and the smaller diffusion coefficients of polyions as compared to small ions in the polymeric membrane.

3.2. Sensitivity of the proposed PrSE

Fig. 1 shows the potential–time profile (A) and calibration curve (B) of the novel PrSE. The calibration curve of a traditional PrSE was also plotted for comparison. As can be seen, the proposed primary ion conditioning protocol shifts the dynamic range of PrSE to about one order of magnitude lower than its sodium ion conditioning counterpart.

Polyion response in the dynamic range is a quasi-steady-state response occuring when polyion flux from the sample bulk into the sample–membrane interface is equal to that further into the membrane bulk [14]. For protamine electrodes, the response can be formulated by:

$$\Delta\mathrm{EMF} = -\frac{RT}{F}\ln\left(1 - \frac{z}{R_T}\frac{D_a\delta_m}{D_m\delta_a}c_{\mathrm{bulk},a}\right)$$

where ΔEMF is the change in membrane potential upon spiking the aqueous solution with protamine to give a bulk concentration of $c_{\mathrm{bulk},a}$; R_T is the total concentration of DNNS in the membrane; D_a and D_m are the diffusion coefficients of protamine in the aqueous and membrane phases, respectively; δ_a and δ_m are the thicknesses of stagnant diffusion layers in the aqueous and membrane phases, respectively; R, T, and F have their usual meanings, and z is the average charge number of protamine. Although this equation is deduced for PrSE with a zero background of primary ion in the membrane before measurement, considering the requirement of qualitative interpretation and to facilitate the comparison with other established approaches affecting polyion responses, this equation is employed here to get an insight into the response characteristics of primary-ion-conditioned PrSEs.

Two factors including R_T and D_m are examined to account for the improved sensitivity in the novel PrSE. Firstly, given the limited ion exchange constant and the existence of polyion flux from the membrane bulk into the membrane surface, the content of protamine in the membrane boundary layer is certainly not zero after activation; i.e. available DNNS in the outer membrane surface is lower than R_T. According to the response equation, a lower R_T would yield a lowered detection limit, which was demonstrated previously [22]. However, it was found that for the Na^+-conditioned PrSE, reducing the amount of DNNS to 0.2% in the membrane formulation to give a comparable sensitivity to the proposed electrode could yield a much smaller potential change of about 60 mV (Fig. S1 in supplementary materials). Hence the improved detection limit should not be mainly attributed to the decreased ion exchanger sites. In addition, the reversibility test of Na^+-conditioned PrSEs also demonstrates that the improvement in sensitivity after contact with protamine was not concomitant with large losses in total potential changes in the first several measurement cycles (Fig. S2 in supplementary materials). All of these results imply that protamine present in the membrane bulk during conditioning or measurement plays a decisive role in the enhanced sensitivity. It is well known that a higher ratio of polymer to plasticizer is usually chosen in polyion sensitive membrane to yield a smaller ion diffusion coefficient [14]. With smaller D_m, a lower concentration of polyion is needed to elicit the same potential response according to the response equation (i.e. decreased detection limit can be achieved). For the novel PrSE with protamine in the membrane, although D_m is actually not changed with given membrane formulation, a similar suppression effect of inward polyion flux in the membrane is also expected. Relative to a membrane void of any protamine, the transport of analyte protamine from the sample–membrane interface into the membrane bulk becomes unfavorable in protamine-loaded membrane. Consequently, the accumulation of analyte protamine in the membrane boundary layer is enhanced, and higher sensitivity can be obtained for the novel PrSE (Fig. 1B). Notably, since protamine in the membrane is crucial to the response, the sensitivity toward analyte is influenced by the activation time that dictates the protamine stripped layer in the membrane (Fig. S3 in supplementary materials). With shorter activation time, the membrane protamine can occupy a relatively large number of anionic sites in the surface layer and the total potential response to analyte protamine is smaller. While, with longer activation time, more protamine is stripped from the membrane bulk and its accumulation effect will be reduced, thereby deteriorating the detection limit. Therefore, it is necessary to control the activation time to ensure reproducible sensitivity.

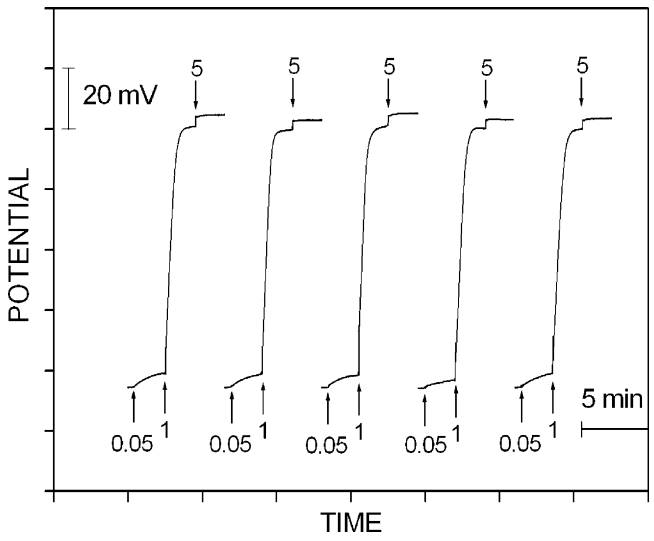

Fig. 2. Electrode regenerability of the proposed technique. Before each measurement, the electrode was thoroughly rinsed and allowed to be activated in 0.12 M Na^+ for 20 min; after each measurement a new round was performed without the reconditioning step. Protamine concentrations (μg/mL) are indicated on the traces.

3.3. Regenerability of the proposed PrSE

Owing to the intrinsic non-equilibrium nature of polyion response, the reversibility of PSEs has been an issue of concern. The repeatability of membranes with tridodecylmethylammonium chloride [23] or tetraphenylborate salt [24] as active components has been reported to be acceptable after stripping with concentrated saline solution. With the aid of pH indicator, renewable tridodecylmethylammonium chloride-based electrode suitable for automated heparin monitoring was also obtained [25]. However, to the best of our knowledge, DNNS-based zero-current polycation-sensitive electrodes, one of the most reliable and common PSEs, are always for single use. Our experiments also demonstrate that, upon contact with protamine of moderate concentration, the DNNS-doped membrane conditioned with Na^+ showed serious memory effects when stripping with either 0.12 M or 2 M NaCl for 20 min (Fig. S2 in supplementary materials). Such memory effects can be ascribed to gradually penetrated protamine in the membrane during measurements, which is hard to be fully stripped. The protamine residue makes the protamine flux deviate from that developed in the fresh membrane void of any protamine and thus yields varied potential response. In contrast, an obviously better repeatability of the proposed PrSE that was conditioned with protamine and activated with Na^+ was observed in consecutive measurements illustrated in Fig. 2, indicating that more reproducible protamine fluxes were obtained in the membrane with

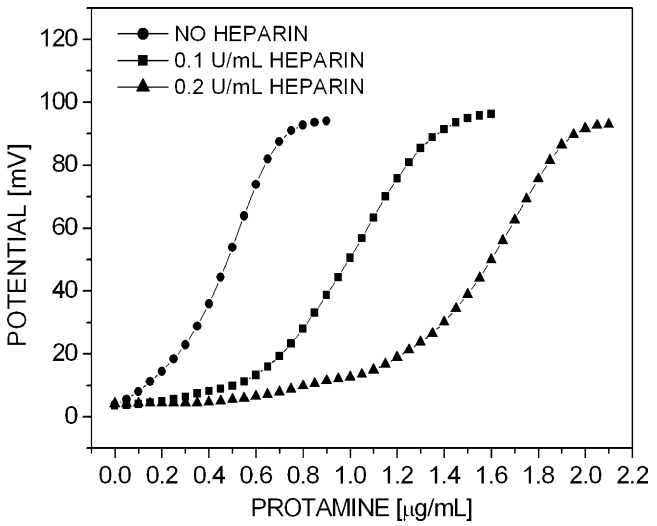

Fig. 3. Potential curves for the titration of heparin in 0.12 M NaCl with protamine using the same proposed PrSE. The titration interval was 30 s.

similar membrane protamine background and stripped layer after activation. Regenerating electrode just needs refilling the stripped layer with polyion to shift the membrane to Nernstian-response region, which is much easier than thoroughly removing stubborn polyion residue from the membrane. Typically, 5 μg/mL protamine was used for 2 min with magnetic stirring to fulfill the regeneration. For ten consecutive measurements, the standard deviation of the potential responses for 1 μg/mL was 2.3 mV. For titrimetric heparin detection (see Section 3.4), accomplishing a titration cycle could refill the membrane boundary layer with protamine and no additional reconditioning step was needed.

3.4. Potentiometric titration of heparin

Like traditional PrSEs, the ability of the proposed PrSE to discriminate free protamine from its complex form with polyanion should be examined. As a prelude to whole blood heparin measurements, the novel PrSE was applied to detect heparin in a background medium of 0.12 M NaCl. As is shown in Fig. 3, different levels of heparin were clearly discriminated by the proposed electrode, which demonstrates the indication capability of the proposed PrSE for polycation–polyanion binding events. In terms of real-world heparin measurement, especially when continuous measurements are required, the necessity of activation of 20 min prior to measurement appears to be a troubling problem. However, by using higher concentration of NaCl (e.g. 1.2 M NaCl) as activation solution, faster ion exchange of protamine in the membrane phase with Na^+ in the aqueous phase could be obtained (Fig. S4 in supplementary materials). Also, changing the activation solution several times over a shorter period would lower the protamine concentration at the sample–membrane interface and facilitate the ion exchange (data not shown). These approaches would shorten the activation time and render the proposed electrode more suitable for practical application.

4. Conclusions

In summary, we have explored the operational principle and response characteristics of DNNS-based protamine sensors conditioned by the primary ion rather than the discriminated ion. With suppressed primary ion fluxes in the membrane phase, PrSE with protamine ready in the membrane shows a detection limit of one order of magnitude lower than the traditional Na^+-conditioned PrSE and the renewal of the proposed polyion electrode can be readily fulfilled. This novel potentiometric sensing protocol is promising in the detection of polyions as well as in many other bioanalytical applications such as enzyme activity screening and immunoassays using PSEs as signal transducers. Further investigations on the mechanism and applications of polyion-conditioned electrodes are underway in our laboratory.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.snb.2011.10.043.

References

[1] M.E. Meyerhoff, B. Fu, E. Bakker, J.H. Yun, V.C. Yang, Polyion-sensitive membrane electrodes for biomedical analysis, Anal. Chem. 68 (1996) A168–A175.
[2] E. Bakker, E. Pretsch, Modern potentiometry, Angew. Chem. Int. Ed. 46 (2007) 5660–5668.
[3] E. Bakker, M.E. Meyerhoff, Ionophore-based membrane electrodes: new analytical concepts and non-classical response mechanisms, Anal. Chim. Acta 416 (2000) 121–137.
[4] S.A. Nevins Buchanan, L.P. Balogh, M.E. Meyerhoff, Potentiometric response characteristics of polycation-sensitive membrane electrodes toward poly(amidoamine) and poly(propylenimine) dendrimers, Anal. Chem. 76 (2004) 1474–1482.
[5] C. Kelly, S. Khaja, A. Vena, A. Yu, J.M. Esson, Polyion-sensitive membrane-based electrodes for heparin-binding foldamer analysis, Anal. Chim. Acta 681 (2010) 1–7.
[6] N. Durust, M.E. Meyerhoff, Determination of DNA and its binding to protamine using potentiometric polyion sensors, J. Electroanal. Chem. 602 (2007) 138–141.
[7] L. Wang, S. Buchanan, M.E. Meyerhoff, Detection of high-charge density polyanion contaminants in biomedical heparin preparations using potentiometric polyanion sensors, Anal. Chem. 80 (2008) 845–847.
[8] S. Dai, M.E. Meyerhoff, Nonseparation binding/immunoassays using polycation-sensitive membrane electrode detection, Electroanalysis 13 (2001) 276–283.
[9] H.S. Abd-Rabboh, S.A. Nevins, N. Dürüst, M.E. Meyerhoff, Electrochemical assay of protease activities based on polycation/polyanion complex as substrate and polyion sensitive membrane electrode detection, Biosens. Bioelectron. 18 (2003) 229–236.
[10] H.A. Badr, N. Ramamurthy, V.C. Yang, M.E. Meyerhoff, Electrochemical assay of proteinase inhibitors using polycation-sensitive membrane electrode detection, Anal. Biochem. 250 (1997) 74–81.
[11] Y. Xu, A. Shvarev, S. Makarychev-Mikhailov, E. Bakker, Reversible detection of proteases and their inhibitors by a pulsed chronopotentiometric polyion-sensitive electrode, Anal. Biochem. 374 (2008) 366–370.
[12] L.C. Chang, M.E. Meyerhoff, V.C. Yang, Electrochemical assay of plasminogen activators in plasma using polyion-sensitive membrane electrode detection, Anal. Biochem. 276 (1999) 8–12.
[13] B. Fu, E. Bakker, V.C. Yang, M.E. Meyerhoff, Extraction thermodynamics of polyanions into plasticized polymer membranes doped with lipophilic ion exchangers: a potentiometric study, Macromolecules 28 (1995) 5834–5840.
[14] B. Fu, E. Bakker, J.H. Yun, V.C. Yang, M.E. Meyerhoff, Response mechanism of polymer membrane-based potentiometric polyion sensors, Anal. Chem. 66 (1994) 2250–2259.
[15] Q.S. Ye, M.E. Meyerhoff, Rotating electrode potentiometry: lowering the detection limits of nonequilibrium polyion-sensitive membrane electrodes, Anal. Chem. 73 (2001) 332–336.
[16] A. Shvarev, E. Bakker, Reversible electrochemical detection of nonelectroactive polyions, J. Am. Chem. Soc. 125 (2003) 11192–11193.
[17] A. Shvarev, E. Bakker, Response characteristics of a reversible electrochemical sensor for the polyion protamine, Anal. Chem. 77 (2005) 5221–5228.
[18] K.L. Gemene, M.E. Meyerhoff, Reversible detection of heparin and other polyanions by pulsed chronopotentiometric polymer membrane electrode, Anal. Chem. 82 (2010) 1612–1615.
[19] T. Sokalski, A. Ceresa, T. Zwickl, E. Pretsch, Large improvement of the lower detection limit of ion-selective polymer membrane electrodes, J. Am. Chem. Soc. 119 (1997) 11347–11348.
[20] H.M. Guo, T.J. Yin, Q.M. Su, W. Qin, Potentiometric measurement of ascorbate by using a solvent polymeric membrane electrode, Talanta 75 (2008) 851–855.

[21] J.W. Ding, W. Qin, Potentiometric sensing of butyrylcholinesterase based on in situ generation and detection of substrates, Chem. Commun. 97 (2009) 1–973.
[22] N. Ramamurthy, N. Baliga, J.A. Wahr, U. Schaller, V.C. Yang, M.E. Meyerhoff, Improved protamine-sensitive membrane electrode for monitoring heparin concentrations in whole blood via protamine titration, Clin. Chem. 44 (1998) 606–613.
[23] S. Mathison, E. Bakker, Improving measurement stability and reproducibility of potentiometric sensors for polyions such as heparin, J. Pharm. Biomed. Anal. 19 (1999) 163–173.
[24] J.H. Yun, B. Fu, M.E. Meyerhoff, V.C. Yang, Protamine-sensitive polymer membrane electrode: characterization and bioanalytical applications, Anal. Biochem. 224 (1995) 212–220.
[25] S. Mathison, E. Bakker, Renewable pH cross-sensitive potentiometric heparin sensors with incorporated electrically charged H^+ ionophores, Anal. Chem. 71 (1999) 4614–4621.

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