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Potentiometric sensor for sensitive and selective detection of heparin

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Abstract

A polymeric membrane ion-selective electrode for determination of heparin is described in this paper. Protamine is incorporated into the organic membrane phase and functions as sensing element for selective recognition of heparin. The proposed membrane electrode exhibits high selectivity for heparin over lipophilic anions such as thiocyanide and salicylate. The potentiometric response to the concentration of heparin is linear in the range of 0.01–0.4 U/mL and a lower detection limit of 0.005 U/mL can be achieved.

Keywords: Potentiometry; Sensing element; Heparin; Protamine; Polyion sensor

Heparin, a highly sulfated polysaccharide, is widely used as an injectable anticoagulant during and after surgery. Accurate measurement of heparin levels in clinical analysis is important [1]. Polymer membrane-based potentiometric sensors have been developed to provide a rapid and direct method of analysis for heparin. These sensors using tridodecylmethylammonium chloride (TDMAC) as an anion-complexing agent can exhibit a large and reproducible potentiometric response toward heparin at clinically relevant concentrations [2,3]. In many cases of practical relevance, however, blood electrolytes such as lipophilic anions thiocyanide (SCN^-) and salicylate (Sal^-) strongly interfere with the heparin response [4]. A significant advance in heparin detection involves the use of protamine sensor as endpoint detector titration of heparin with protamine to avoid such interferences, but such titration methodology complicates the analysis procedure [5,6].

Recently, Capevila et al. demonstrated a biosensor membrane by embedding mammalian Zn_7–MT1 complexes as ionophores in the analysis of MT1, which opens up a broad range of possibilities in the use of small proteins or metalloproteins as sensing elements for potentiometric sensors [7]. In this paper, we report a novel potentiometric detection strategy that makes use of protamine as sensing element in organic membrane to offer a very promising method for simple and selective sensing of heparin.

1. Experimental

The membranes with polar plasticizer contained 0.5 wt% protamine, 42 wt% 2-nitrophenyl octyl ether (*o*-NPOE), 54.5 wt% high molecular weight poly(vinyl chloride) (PVC) and 3 wt% tetradodecylammonium tetrakis (4-

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chlorophenyl)borate (ETH 500) (membrane 1), while the membranes with nonpolar plasticizer contained 0.5 wt% protamine, 64.5 wt% PVC, 32 wt% dioctyl sebacate (DOS) and 3 wt% tetradodecylammonium tetrakis (4-chlorophenyl)borate (ETH 500) (membrane 2). The conventional heparin selective membranes contained 1.5 wt% TDMAC, 66 wt% PVC and 32.5 wt% DOS (membrane 3). Membranes were prepared as described before [8]. For all the measurements, 50 mmol/L Tris–HCl buffer containing 0.12 mol/L NaCl was used as sample background and inner filling solution. The conventional heparin selective membranes were conditioned in a solution identical to the inner filling solution overnight. All measurements of electromotive force (EMF) for the ion selective electrodes were performed at 20.0 ± 0.5 °C using a PXSJ-216 pH meter (Shanghai, China) with a Ag/AgCl (3 mol/L KCl) as the external reference electrode and a chloridized silver wire as the internal reference electrode.

2. Results and discussion

It has been well-established that the polarity of the membrane has an important effect on the response of potentiometric sensors. As shown in Fig. 1A, membrane 2 with nonpolar plasticizer (the dielectric constant of DOS is 3.9) does not yield stable potential response after soaking in the buffer solution. This is probably due to the fact that protamine is hydrophilic and leaches rapidly from the nonpolar membrane into buffer solution. On the other hand, membrane 1 with polar plasticizer (the dielectric constant of *o*-NPOE is 23.9) stabilizes protamine in the membrane phase and thus shows stable response in buffer solution. As expected, the polar membrane without conditioning exhibits a rather stable potential response when exposed in the electrolyte background, although a short equilibrium time (*ca*. 5 min) should be required after the membrane is soaked in the buffer solution (Fig. 1B). Such behaviors are attributed to the remarkably enhanced polarity of the *o*-NPOE plasticized membrane, which effectively improves the partition coefficient of protamine in the membrane phase [9]. Compared with the response of membrane 2 to high levels of heparin (0.4 U/mL), membrane 1 shows larger potential response upon addition of low levels of heparin (0.05 U/mL) into the sample background solution (Fig. 1B). The larger response is basically based on the electrostatic binding interaction between heparin and protamine. The electrostatic binding interaction decreases the concentration of protamine at the sample–membrane interface and further facilitates the stripping of chloride out of the membrane surface due to the charge balance. Therefore, a further increase in response toward heparin is observed (see Fig. 2) [10,11].

The calibration curve of the proposed electrode based on membrane 1 was depicted in Fig. 3A. Detailed analysis of the experimental results reveals that there is a linear dependence of the initial slope of the EMF change on the concentration of heparin in the range of 0.01–0.4 U/mL with a detection limit of 0.005 U/mL (Fig. 3B). The detection limit is two orders of magnitude lower than that of the conventional heparin sensor [3]. For 0.2 U/mL heparin, the relative standard deviation (RSD %) was found to be 6.0% with five measurements.

The proposed potentiometric sensor based on protamine as sensing element shows an excellent selectivity over several lipophilic anions such as SCN^- and Sal^- (Fig. 4). The minimal response to these ions makes the new electrode potentially usefully for estimating heparin levels in biological samples especially for blood samples. Moreover, the proposed membrane 1 exhibits better selectivity for heparin over lipophilic anions than that of conventional membrane

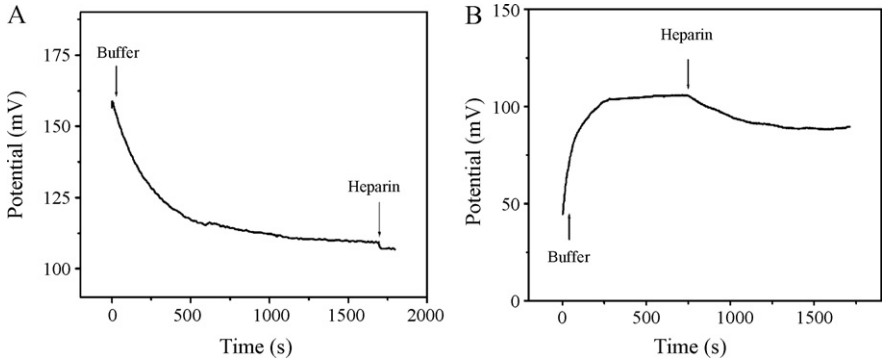

Fig. 1. Effect of the plasticizer in the electrode membrane on the potential response to heparin. (A) Nonpolar plasticizer (DOS): heparin, 0.4 U/mL; (B) polar plasticizer (*o*-NPOE): heparin, 0.05 U/mL.

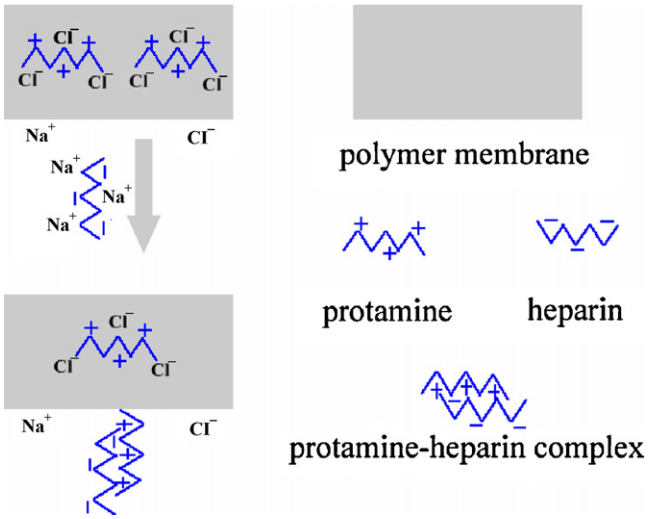

Fig. 2. Schematic mechanism of the potentiometric detection of heparin using the proposed protocol.

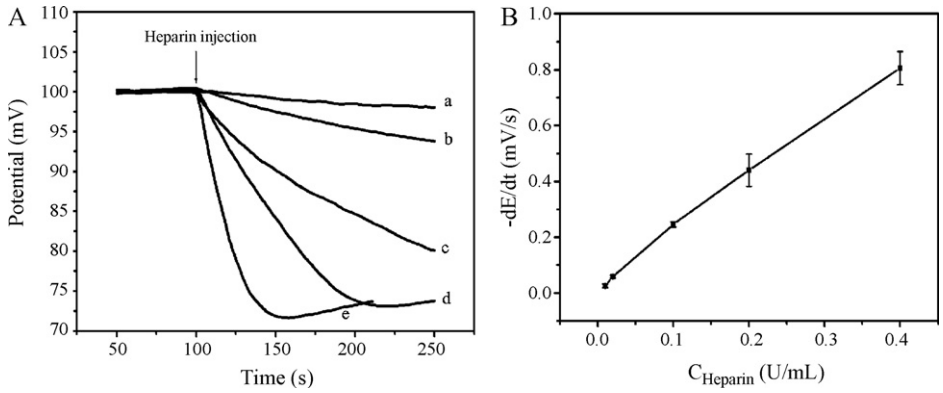

Fig. 3. (A) Typical dynamic potential responses of the sensor in sample solutions upon addition of increasing concentrations of heparin: (a) 0.01 U/mL, (b) 0.02 U/mL, (c) 0.1 U/mL, (d) 0.2 U/mL and (e) 0.4 U/mL. (B) Initial rates of the potential changes as a function of the concentration of heparin using the proposed method. Date represents an average ± standard deviation for three measurements.

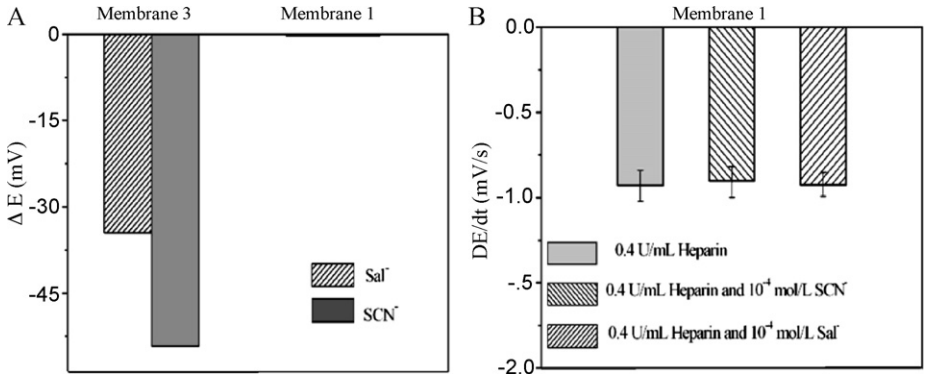

Fig. 4. (A) Potential responses of membrane 1 and 3 to SCN^- (10^{-4} mol/L) and Sal^- (10^{-4} mol/L) in 0.12 mol/L NaCl solution. (B) Initial slopes of the EMF responses to 0.4 U/mL heparin in the absence or presence of 10^{-4} mol/L SCN^- or 10^{-4} mol/L Sal^- in 0.12 mol/L NaCl solution using the proposed electrode based on membrane 1.

3 [12]. This excellent performance of membrane 1 can be attributed to the fact that protamine as sensing element can be stripped out of the organic membrane layer owing to the strong interaction between protamine and heparin, while SCN^- and Sal^- can not be easily extracted into the membrane due to lack of a lipophilic anion-exchanger in the membrane.

Acknowledgments

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