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Potentiometric flow injection sensing system for determination of heparin based on current-controlled release of protamine

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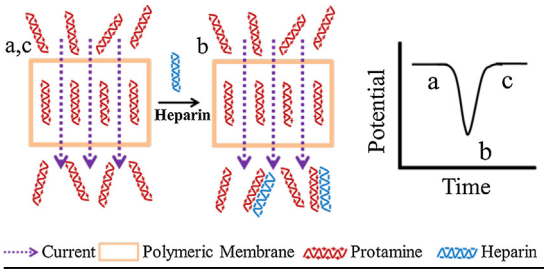
HIGHLIGHTS

- A potentiometric flow injection system for determination of heparin is described.
- An external current is applied for controlled release of protamine.
- The system has been employed for detection of heparin in whole blood.

GRAPHICAL ABSTRACT



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ABSTRACT

A flow injection system incorporated with a polycation-sensitive polymeric membrane electrode in the flow cell is proposed for potentiometric determination of heparin. An external current in nano-ampere scale is continuously applied across the polymeric membrane for controlled release of protamine from the inner filling solution to the sample solution, which makes the electrode membrane regenerate quickly after each measurement. The protamine released at membrane–sample interface is consumed by heparin injected into the flow cell via their strong electrostatic interaction, thus decreasing the measured potential, by which heparin can be detected. Under optimized conditions, a linear relationship between the potential peak height and the concentration of heparin in the sample solution can be obtained in the range of 0.1–2.0 U mL^{-1}, and the detection limit is 0.06 U mL^{-1}. The proposed potentiometric sensing system has been successfully applied to the determination of heparin in undiluted sheep whole blood.

1. Introduction

Heparin is a highly-sulfated polysaccharide with an average molecular weight of 15,000 and an average valence of 70. It is widely used as an anticoagulant drug in a variety of surgical procedures, such as kidney dialysis and open heart surgery, via the accelerating effect on inactivating coagulation factors [1,2]. Rapid and accurate measurement of heparin levels during clinical procedures are of crucial importance to avoid significant detrimental effects caused by heparin overdosing, such as hemorrhages and thrombocytopenia [3,4].

The activated clotting time (ACT) or the activated partial-thromboplastin time (aPTT) has been widely used for the quantification of heparin in clinical analysis. However, these assays are indirect and not always reliable [5]. Various methods including colorimetry [6], Raman spectroscopy [7,8], liquid chromatography [9], electrochemistry [10], and fluorimetry [11] have also been developed for measuring heparin. Unfortunately, these methods may not be suitable for whole blood analysis. In

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recent years, potentiometric sensors based on polyion-sensitive membranes have made significant contributions to heparin quantification, even in undiluted blood, via direct [12–15] or indirect [16–20] detection modes. Since the spontaneous extraction of polyions into membrane phase can form cooperative ion pairs with the lipophilic ion exchangers in the membranes, it is unfortunate that these electrodes are naturally irreversible [14,15,21]. More recently, reversible polyion-sensitive membrane electrodes based on the chronopotentiometric sensing strategy have been developed [22–26]. In this method, a lipophilic salt with a carefully matched cation and anion is added into the polymeric membrane to suppress the spontaneous extraction of polyions and a current pulse is applied to control the cation or anion fluxes into the membrane. This method can also be used for on-line rapid determination of heparin via flow injection analysis (FIA) [27]. However, the determination of heparin could be disturbed by the lipophilic anions co-existing in samples.

Recently, we developed a polycation-sensitive membrane electrode for reagentless determination of heparin by using the zero current ion fluxes of protamine released from the inner filling solution to the sample solution [28]. The electrode can be reused by conditioning at a high concentration of protamine for ca. 10 min before next measurement. Such an additional regeneration procedure will prolong the analysis time and make continuous monitoring difficult. In the present work, an external anodic current is continuously applied across the polymeric membrane to drive the ion fluxes of protamine from the inner filling solution to the sample solution, which could make the electrode membrane regenerate quickly after each measurement. By integrating the FIA and galvanostatic techniques, a rapid and continuous sensing platform for heparin has been constructed. As shown in Fig. 1a, the applied current drives the fluxes of protamine through the polycation-sensitive membrane with a stable potential baseline. When heparin is injected into the flow cell with the carrier solution, it can electrostatically bind to protamine released at the sample–membrane interface. The consumption of free protamine could facilitate the stripping of protamine out of the membrane surface via the ion-exchange process with sodium ions, thus decreasing the membrane potential (Fig. 1b) [28]. With the current-controlled reagent delivery, the membrane electrode can be regenerated on line shortly after heparin flows out of the flow cell, which allows continuous sensing of heparin in the flow injection mode (Fig. 1c).

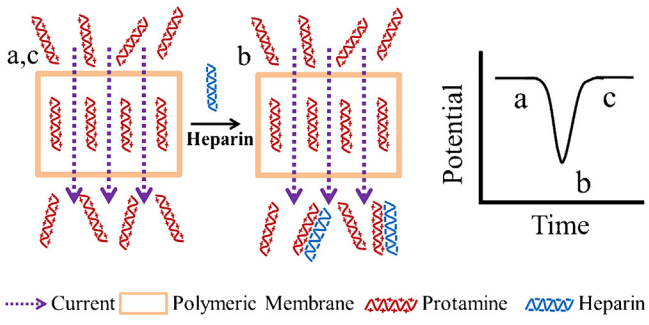

Fig. 1. Illustration of potentiometric flow injection determination of heparin with a polycation-sensitive membrane electrode based on the current-controlled release of protamine from the inner filling solution to the sample solution: (a) potential baseline (without heparin); (b) sample injection (with heparin); (c) membrane recovery (without heparin).

2. Experimental

2.1. Reagents

Dinonylnaphthalene sulfonic acid (DNNS) as a 50% solution in heptane, protamine sulfate from herring, heparin sodium salt from bovine intestinal mucosa (198 U mg^{-1}), Trizma base (Tris) were purchased from Sigma–Aldrich (St. Louis, MO, USA). High molecular weight poly(vinyl chloride) (PVC), 2-nitrophenyl octyl ether (*o*-NPOE) and tetradodecylammonium tetrakis(4-chlorophenyl) borate (ETH 500) were purchased from Fluka AG (Buchs, Switzerland). All the chemicals were of selectophore or analytical grade. Aqueous solution was prepared with freshly deionized water (18.2 MΩ cm^{-1} specific resistance) obtained with a Pall Cascada laboratory water system. Unless stated otherwise, 0.05 mol L^{-1} Tris–HCl buffer (pH 7.4) containing 0.12 mol L^{-1} NaCl was used as sample medium and carrier solution.

2.2. Apparatus

The FIA system for determination of heparin was controlled by a flow injection analyzer (FIS-D, Xi'an Remex Analyze Instrument Co., Ltd., China). The system contains two peristaltic pumps (1.8 mL min^{-1}), a six-way injection valve (500 μL loop) and a wall-jet flow cell (60 μL) [29]. The detection chamber has a three-electrode system. The working electrode (polycation-sensitive membrane electrode, i.d. 4 mm, o.d. 6 mm) and the reference electrode (Ag/AgCl electrode with an inner filling solution of 3 mol L^{-1} KCl, i.d. 2 mm, o.d. 4 mm) were embedded in the cell body, with a distance of 10 mm. A platinum wire as the counter electrode was placed between the working and the reference electrodes. Tygon and PTFE tubes were used to assemble the flow-through system. A CHI-660C electrochemical workstation (Shanghai Chenhua Apparatus Corporation, China) was used to perform potentiometric measurements.

2.3. Membranes and electrode preparation

The polycation-sensitive membranes contained 3 wt% DNNS, 6 wt% ETH 500, 30 wt% PVC and 61 wt% *o*-NPOE. The membranes (~100 μm) were obtained by casting a membrane cocktail (386 mg) dissolved in 6.0 mL THF into a glass ring of 50 mm diameter fixed on a glass plate and evaporating the solvent overnight. Disks of 6 mm diameter punched from the parent membrane were glued to plasticized PVC tubes (i.d. 4 mm, o.d. 6 mm) to fabricate the polycation-sensitive membrane electrodes. 0.05 mg mL^{-1} protamine in 1 mL of 0.05 mol L^{-1} Tris–HCl buffer (pH 7.4) containing 0.12 mol L^{-1} NaCl was used as the inner filling solution. During the polarization, a stable potential on the inner side of the membrane can be obtained in the presence of a high concentration of sodium chloride. Before measurements, all the electrodes were conditioned in the solution identical to the inner filling solution for 12 h at 25 ± 1 °C, which ensures the stable ion fluxes of protamine from the inner solution to the sample solution.

2.4. EMF measurements

Ion-selective chronopotentiometry was used in this work. An anodic current of 20 nA was applied across the polymeric membrane polycation-sensitive electrode to release protamine from the inner filling solution into the sample solution to get a stable baseline. Potentiometric measurements of the interactions of protamine with injected heparin at the membrane–sample interface were performed at room temperature in the galvanic cell: Ag/AgCl/3 mol L^{-1} KCl/sample solution/polycation-sensitive membrane/inner filling solution/AgCl/Ag. A 0.05 mol L^{-1} Tris–HCl buffer