



Blood heparin sensor made from a paste electrode of graphite particles grafted with molecularly imprinted polymer



Yasuo Yoshimi*, Yuto Yagisawa, Rina Yamaguchi, Maki Seki

Department of Applied Chemistry, Shibaura Institute of Technology, 3-7-5 Toyosu, Koto-ku, Tokyo 135-8548, Japan

ARTICLE INFO

Article history:

Received 23 September 2017

Received in revised form

12 December 2017

Accepted 13 December 2017

Available online 14 December 2017

Keywords:

Molecularly imprinted polymer (MIP)

Heparin

Graphite paste electrode

Voltammetry

ABSTRACT

A real-time heparin monitor could be used to optimize the dosage of heparin during extracorporeal circulation procedures. This report describes the development of a graphite-paste (GP) electrode with molecularly imprinted polymer (MIP) grafted onto it. Heparin-imprinted poly (methacryloxyethyltri-ammonium chloride -co- acrylamide -co- methylenebisacrylamide) was grafted directly onto graphite particles. The grafted particles were thoroughly mixed with oil to fabricate the MIP-GP electrode. Traditional cyclic voltammetry was performed with the electrode in physiological saline or bovine whole blood containing 5 mM ferrocyanide and 0–8 units/mL heparin. The current intensity increased with heparin concentration, due to expansion of the effective surface area resulting from heparin-promoted mobility of the oil in the MIP-GP electrode. No significant difference was found in the sensitivity of the current to unfractionated heparin among the electrodes fabricated because of the electrode homogenization resulting from thorough mixing of the MIP-grafted particles and oil. (A previous MIP-grafted indium tin oxide electrode exhibited lower sensitivity in blood than in saline.) Only 60 s were needed to stabilize the current. The current at the MIP-GP electrode was also sensitive to low-molecular-weight heparin in blood, but insensitive to chondroitin sulfate C (CSC), which is a heparin analog. The non-imprinted polymer (NIP)-grafted electrode was insensitive to heparin. Thus, the MIP-GP electrode, which operated through a new heparin-sensing mechanism, is an excellent candidate for application as a disposable sensor to monitor heparin levels in blood.

© 2017 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

In extracorporeal therapy (e.g., hemodialysis, cardiopulmonary bypass), blood contacts artificial materials in the blood vessels, creating a risk of clotting. The clots can plug tubes used for extracorporeal perfusion, or block blood capillaries. Clotting is generally prevented by administering anticoagulants, among which heparin is the most widely used injectable anticoagulant and is metabolized rapidly by the human body. Controlling anticoagulation during a medical procedure is very important because a heparin overdose could induce bleeding, while an underdose could cause clotting. The administered dose of heparin and its antidote, protamine sulfate, are usually determined by measuring the “activated clotting time” (ACT). However, the ACT of heparin added to blood during extracorporeal therapy is approximately 600 s [1], which is too long to be of practical use in surgery. In addition, ACT is insensitive to low-molecular-weight heparin (LMWH) [2], which produces a lower

risk of bleeding than conventional unfractionated heparin (UFH) [3]. Thus, a sensor that could detect heparin, including LMWH, in real time is needed to monitor heparin dosage.

A new sensor has been developed for the quantification of heparin based on a heparin-specific molecularly imprinted polymer (MIP), which are synthetic polymers that contain specific binding sites formed by imprinting the structure of the target molecule, referred to as the template, during the polymerization process. This heparin sensor was developed by grafting the MIP layer with the heparin template onto the surface of a flat indium tin oxide (ITO) electrode [4,5]. The oxidative current of hexacyanoferrate (II) (or ferrocyanide) at the MIP-grafted ITO (MIP-ITO) electrode was sensitive to LMWH as well as UFH in whole blood [5]. The sensor current could be stabilized in less than 30 s, indicating that the electrode is practical for application as a real-time heparin sensor. Sensors for the diagnosis of whole blood samples are usually disposable for hygienic reasons and therefore must exhibit high reproducibility because the sensor can be used for only one sample. However, large variations in sensitivity existed among the fabricated electrodes, so actual use of the MIP-ITO electrode as a disposable sensor was very difficult. This was likely due to difficulty in ensuring uniform radical

* Corresponding author.

E-mail address: yosimi@sic.shibaura-it.ac.jp (Y. Yoshimi).

graft polymerization at the surface of the electrode. Thus, the MIP layer tends to be heterogeneous, and reproducibility of the sensitivity of the grafted electrode is very poor. In addition, sensitivity toward heparin in blood was also poor due to inhibition by plasma proteins.

In the present study, a new method to improve the quality of MIP-grafted electrodes was developed by using a fluidized bed for graft polymerization, which enabled uniform chemical reactions [6], and a carbon pasting method that allowed mass production of the electrodes with high homogeneity [7]. First, the graphite carbon particles were coated with a radical polymerization photo-initiator, and this was followed by radical polymerization to graft the MIP onto the fluidized initiator-coated graphite. Finally, the grafted graphite was mixed thoroughly with oil to fabricate homogenous carbon paste electrodes. The heparin-sensitivity and the reproducibility of the output current at the MIP-grafted graphite paste (MIP-GP) electrode were evaluated in whole blood and in saline. The sensing mechanism of the MIP-GP was examined using the sensitivity of the contact angle of saline on the MIP-GP electrode to the presence of the template.

2. Materials and methods

2.1. Chemicals

Spherical graphite particles 8 mm in diameter (SG-BH8) were donated by Ito Graphite Co., Ltd. (Kuwana, Japan). Ethanol, *N,N*-dimethylformamide (DMF), sulfuric acid, hydrochloric acid, and acetic acid were purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan). Zinc chloride, sodium diethyldithiocarbamate, formaldehyde, sodium salt of UFH (180 units/mg; from porcine intestinal mucosa), acrylamide AAm, *N,N*-methylenebisacrylamide (MBAA), potassium hexacyanoferrate (II) (or ferrocyanide) trihydrate, potassium nitrate, and sodium chondroitin C (from shark cartilage) were purchased from Wako Pure Chemical Industry Co. Ltd. (Osaka, Japan). (2-(Methacryloxy)ethyl) trimethyl ammonium chloride acrylamide (METMAC) was purchased from Sigma-Aldrich Inc. (St. Louis, USA). Dalteparin sodium (LMWH) was purchased as Fragmin® from Pfizer Inc. (New York, USA). Bovine whole blood was purchased from a meat processing company (Tokyo Shibaura Zoki KK, Tokyo).

2.2. Introduction of radical photopolymerization initiator on the graphite surface

The diethyldithiocarbamate methylene group, which is a radical photopolymerization initiator, was introduced onto the surface of graphite particles *via* chloromethylation using a procedure described by Samuels et al. [8], as shown in Fig. 1. A three-neck, 200-mL, round-bottom flask was charged first with 5 g of the graphite, then 0.25 g (1.9 mmol) of zinc chloride, and finally with 100 mL of 1:1 (v:v) concentrated hydrochloric acid and acetic acid. After complete dissolution of the zinc chloride, the graphite suspension was cooled in an ice bath and bubbled with argon gas for one hour. Next, the argon stream was stopped and 10 mL of formaldehyde aqueous solution (37%) was added to the suspension. The suspension was placed in an ice bath and bubbled with hydrogen chloride gas produced by dropwise addition of concentrated hydrochloric acid into concentrated sulfuric acid in another flask for 4 h. The hydrogen chloride gas at the outlet was absorbed by a saturated aqueous solution of sodium hydrogen carbonate. When bubbling ceased, the suspension was stirred overnight at room temperature. The treated graphite was collected by suction filtration, washed with 500 mL of distilled water, and dried under vacuum overnight. The dried particles were suspended in a 0.12 M ethanolic solu-

tion of sodium diethyldithiocarbamate for 24 h to introduce the diethyldithiocarbamate methylene group used as an initiator of radical polymerization. Progress of the reaction was confirmed by the turbidity of the supernatant due to NaCl crystals produced by the reaction. The initiator-coated graphite was washed with distilled water followed by methanol, and then dried under vacuum. The dried initiator-coated graphite was packed in a brown bottle covered in aluminum foil and stored in a refrigerator.

2.3. Graft polymerization of the graphite particle surface

The METMAC was recrystallized in acetone before use. The UFH sodium (0.16 g), METMAC (0.87 g), and AAm (1.00 g) were dissolved in 6 mL of distilled water, the MBAA (1.00 g) was dissolved in 18 mL of DMF, and the two solutions were mixed together. The initiator-coated graphite (0.7 g) was suspended in the combined solution, and the suspension was placed in a quartz crystal test tube (26.5 mm inner diameter) and bubbled vigorously for 20 min with nitrogen gas to remove dissolved oxygen. While being bubbled with nitrogen gas and stirred with a magnetic stirrer, the suspension was irradiated with light from a xenon lamp (LC-5, Hamamatsu Photonics, Co. Ltd., Hamamatsu, Japan) guided by an optical fiber. The treated particles were collected by suction filtration and washed with 1:3 (v:v) water and DMF, followed by washing with 1 M aq. sodium chloride to remove the heparin from the grafted layer. The isolated particles were washed with distilled water, dried under vacuum overnight, and stored in a desiccator. The particles were observed using a scanning electrode microscope (SEM) (JSM-6010LV, Jeol, Akishima, Japan). A non-imprinted polymer (NIP) was also prepared using the same procedure described above, but without the UFH template.

2.4. Preparation of MIP-GP electrode

The grafted particles (7 mg) and 3 mg silicone oil (KF-96-300CS, Shin-Etsu Chemical Co., Ltd., Tokyo) were mixed and ground into a paste in a polytetrafluoroethylene mortar and a pestle for 20 min. The graphite paste was packed into the tip of a hematocrit capillary (Hirschmann Labogeräte GmbH & Co. KG, Eberstadt, Germany) with an inner area of 1 mm². The particles were packed to a length of approximately 1 mm using a glass rod. The tip of the packed graphite paste was polished using paraffin paper (Hakuai Co., Ltd. Tokyo), and a copper (diameter 0.8 mm) wire was inserted into the graphite paste through the other end of the capillary to maintain the electrical connection.

2.5. Electrochemical sensing

Samples containing 0–8 unit/mL heparin and 5 mM potassium hexacyanoferrate (II) (or ferrocyanide) in physiological saline or in bovine whole blood were added to an electrochemical cell VC-4 (BAS Inc., Tokyo). The prepared carbon-paste electrode, platinum wire, and Ag/AgCl electrode (RE-1B, BAS Inc.) were inserted as the working, counter, and reference electrodes, respectively. The electrodes were connected to an electrochemical analyzer HZ-5000 (Hokuto Denko, Tokyo). Traditional cyclic voltammetry was performed with a scan rate of 0.2 V/s. Scanning was continued until the current stabilized. The relation between the peak anodic current of hexacyanoferrate (II) and the heparin concentration was determined.

2.6. Measurement of contact angle

The MIP-GP or NIP-GP was packed into a 1 cm × 1 cm square hole in a 100- μ m-thick poly(ethyleneglycol terephthalate) (PET) film that was pasted onto a glass plate on which a conductive

Download English Version:

<https://daneshyari.com/en/article/7140991>

Download Persian Version:

<https://daneshyari.com/article/7140991>

[Daneshyari.com](https://daneshyari.com)