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Preparation and characterization of superporous agarose–reticulated vitreous carbon electrodes as platforms for electrochemical bioassays

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ARTICLE INFO

Article history:
Received 19 March 2008
Received in revised form
16 May 2008
Accepted 19 May 2008
Published on line 24 May 2008

Keywords:
Reticulated vitreous carbon
Superporous agarose
Flow-through electrodes
Sandwich assays

ABSTRACT

Three-dimensional flow-through electrodes were fabricated using superporous agarose (SPA) and reticulated vitreous carbon (RVC) composite materials that were suitable as a platform for sandwich assays. These SPA–RVC composite electrodes were fabricated by fitting a SPA–RVC composite cylinder inside a graphite tube and subsequently fixing the graphite tube onto a polypropylene micropipette tip. The electrode design allows for ease in reagent/washing steps involved in sandwich assay protocols and could easily be made portable. The electrode materials were characterized with respect to pore-size distribution, total free volume, ligament and bulk densities of the RVC, and physical structural characteristics. Coulometric detection of redox molecules such as $K_3Fe(CN)_6$ and 4-aminophenol was possible using SPA–RVC electrodes by the trapping of these redox molecules inside the SPA–RVC electrodes. Avidin affinity molecules were covalently immobilized onto the SPA matrix inside the RVC electrodes by periodate-activation followed by reductive amination. The amount of avidin immobilized inside the SPA–RVC electrodes was $(5 \pm 0.06) \times 10^{-11}$ mol, which was determined by saturating the avidin sites with biotinylated fluorescein (b-fluo) and subsequently determining the amount of immobilized b-fluo via a standard addition method using fluorescence spectroscopy. Non-specific binding of labeled enzymes such as biotinylated alkaline phosphatase (b-ALP) onto the SPA–RVC electrodes without avidin capture sites was determined to be less than 1% compared to the specific binding of b-ALP on avidinylated SPA–RVC electrodes.

1. Introduction

Applications such as point-of-care medical diagnosis [1] and in-the-field biological warfare agent detection [2] require portable sandwich bioassays which are easy to use and are cost effective. Conventional microtiter plate based sandwich bioassays (e.g. enzyme-linked immunosorbent assays, ELISA or radioimmunoassay, RIA) see limited use for such applications because they require specific skills, involve laborious and time consuming steps of washings/reagent treatment and are restricted to use inside laboratories [3]. The washings/reagent treatments involved in sandwich assays could be made simpler with the use of flow-through platforms. Various platforms such as microcapillary channels [4], filter membranes [5], planar electrodes coupled with flow-injection analyzer (FIA) have been reported for achieving sandwich assays. The above platforms have the advantage of automation but require use of pumps and in some cases bulky optical detection systems

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doi:10.1016/j.aca.2008.05.039

thereby making them less portable. Strip-based platforms like immunochromatographic lateral-flow assays have advantages of easy and rapid detection down to 50 pg mL^{-1} for several antigens, but these assays have the disadvantage of limited sensitivity and quantification ability caused by use of human eye to observe the change in color [6]. For developing portable bioassays electrochemical methods such as amperometry [7], chronoamperometry [8] and voltammetry are popular due to their prospects for sensitive detection and possible miniaturization.

Portable electrochemical bioassays would greatly benefit from electrodes that would allow for convenient reagent washing/fluid handling. A composite of reticulated vitreous carbon (RVC) and superporous agarose (SPA) material could be used to prepare flow-through electrodes. Both RVC and SPA have been previously used as flow-through electrodes [9–13] and as stationary phases in chromatographic columns [14,15], respectively. The composite of SPA–RVC material developed by Khayyami et al. was used as bioassay platform by immobilization of acetylcholine esterase enzyme onto the SPA matrix followed by detection of acetylthiocholine substrate with inhibitor responses of paraoxon pesticide [16]. The idea of using SPA–RVC composite electrodes in bioassays can be extended towards the development of enzyme-labeled sandwich bioassays on the SPA gel inside the RVC material. Immobilization of avidin affinity moieties on the SPA matrix could assist in creating the sandwich assay "constructs" by the immobilization of biotinylated capture probes on the avidins immobilized on SPA. The SPA–RVC electrode configuration has several advantages for sandwich bioassays. Firstly, the diffusion-limited geometry of the macroporous RVC electrodes provides necessary confined spaces for trapping the products of enzyme-label-catalyzed reactions within the enclosed space without the products diffusing away and becoming lost in the bulk solution. Secondly, immobilization of the capture probes for a sandwich assay on the flow-through SPA matrix would provide greater accessibility for the capture probes in capturing the analytes present in low concentration thereby reducing the incubation periods required for capturing the analytes as compared to the use of planar surfaces [17]. These advantages could show enhancement in the sensitivity and detection limits of a bioassay.

In the current work, we developed SPA–RVC flow-through electrodes as platforms suitable for use in electrochemical sandwich bioassays. The electrodes were developed on a micropipette tip that allows for easy washing and reagent treatment with the use of micropipettors. Multiple samples could be analyzed simultaneously by using multichannel micropipettors and potentiostats. Avidins were covalently bound onto the SPA matrix by creating aldehyde groups on the SPA using periodate which upon exposure to avidin resulted in covalent capture via aldehyde–primary amine coupling. The resulting imine linkage was then subjected to reductive amination to create stable secondary amine linkages [18]. The electrode materials were characterized for their physical characteristics with respect to pore-size distribution, total free volume, ligament and bulk densities of RVC, and physical structural characteristics. The SPA–RVC electrodes were tested for their ability to perform coulometric measurements using simple redox couples such as $Fe(CN)_6^{-3}/Fe(CN)_6^{-4}$ and 4-aminophenol (PAP)/quinine-imine. The amount of avidins immobilized inside the SPA–RVC electrode was determined using a fluorescence assay with biotinylated fluorescein (b-fluo). A study of the non-specific binding of enzyme labels such as biotinylated alkaline phosphatase (b-ALP) onto the SPA–RVC electrodes was also performed.

2. Experimental

2.1. Materials, solutions and equipment

The following materials were obtained from the respective suppliers and used as received, except where indicated: graphite rod (McMaster-Carr), reticulated vitreous carbon (ERG Materials and Aerospace Corp., Oakland, CA), high-impact polystyrene (HIPS) sheet (McMaster-Carr), polypropylene micropipette tips (Fisher), various sizes of polyolefinic shrink tubes (McMaster-Carr), gold wire; 0.127 mm diameter (Aldrich), agarose (MP Biomedicals), mesitylene (Acros Organics), avidin (Pierce), sodium periodate ($NaIO_4$, Fisher), sodium cyanotrihydridoborate ($NaCNBH_3$, Alfa Aesar), sodium azide (NaN_3, Mallinckrodt), conc. HCl (Fisher), Tween-20 (Acros Organics), Tween-80 (Fisher).

Electrochemical experiments were performed using a model CHI 660A electrochemical workstation from CH Instruments Inc., Austin, TX. A three-electrode cell set up was used with Pt wire as the auxiliary electrode, Ag/AgCl reference electrode and working electrodes that are indicated wherever used. The fluorescence spectrophotometer used was a custom-built instrument by Photon Technologies International (PTI). The surface area, pore size and pore volumes of the RVC materials were measured by mercury porosimetry using a Micromeretics Autopore IV porosimeter and by using a Micromeretics ASAP 2010 porosimeter via Kr gas adsorption. Densities of the carbon skeleton in RVC were measured using a Micromeretics Accupyc 1330 Helium Pycnometer. Scanning electron microscopic images for samples of RVC alone were acquired using a Hitachi S3500—scanning electron microscope; SEM images for SPA and SPA–RVC were acquired using a Hitachi S3400 variable pressure scanning electron microscope. Confocal microscopy was carried out using a Carl Zeiss 510 confocal laser scanning microscope with Plan-Neofluar 40×/1.3 oil objective. Images were analyzed using Slidebook® software developed by Intelligent Imaging Innovations and Olympus.

Tris-buffered saline (TBS) was prepared with 250 mM Tris–HCl and 150 mM NaCl followed by adjusting the pH to 8 using a 1:1 NaOH solution in DI water and measured with an Accumet® benchtop pH meter. Blocking buffer (TBS–T20–bovine serum albumin (BSA)) was prepared with 100 mM Tris–HCl, 150 mM NaCl, 1% (v/v) Tween-20, 1% (w/v) bovine serum albumin and 0.2 mg mL^{-1} NaN_3 followed by adjusting the pH to 8 using 1:1 NaOH solution in DI water.

2.2. Pretreatment of RVC material

A large RVC cylinder of about 1.0 cm diameter and 3.0 cm height was fixed on a 20 mL luer-lock type syringe with clear polyolefinic heat-shrink tubing wrapped around the cylinder