Journal of Hazardous Materials 173 (2010) 253–257

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Spectroscopic investigation of sulfonate phthalocyanine to probe enzyme reactions for heavy metals detection

Shweta Chaure, Deepen Paul, Pankaj Vadagma, Asim K. Ray*

School of Engineering & Material Science, Queen Mary, University of London, London E1 4NS, UK

ARTICLE INFO

Article history:
Received 28 April 2009
Received in revised form 15 August 2009
Accepted 18 August 2009
Available online 25 August 2009

Keywords:
Membrane formulation
Hydrogel beads
Water quality
Trace-metals
Public health
Entrapment
Sensor
Exposure
Dimerization

ABSTRACT

Optical absorption and Raman spectra of the sulfonated copper phthalocyanine (CuTsPc) layer were exploited for detection of cadmium (Cd) contaminants in water. Acetylcholine esterase was immobilized by freely suspending them in calcium alginate microbeads and this gel was then spincoated on the drop cast sulfonated copper phthalocyanine film on a glass substrate to form a bilayer. The inhibition of catalytic reaction between acetylcholine chloride and enzyme due to Cd contaminants was monitored by recording changes in spectra of drop cast CuTsPc as an indicator. The detection limit of cadmium content in water was found to be 1 ppm.

1. Introduction

The heavy metal contamination of water poses hazardous risk to humans and aquatic life. Pollution can occur in a variety of ways. For example, food products including vegetables, grains and fruits become contaminated by accumulating metals from pullulated soil and water. In a recent study on weekly spatial and temporal fluctuations of metal concentrations over two years in the Ten Mile Creek (South Florida), the surface runoff from agricultural lands and urban wastewater, geological backgrounds and tidal flow were identified as the principal sources of river sediments pollution. The heavy metal contents in this region were found to be well over the U.S. Environmental Protection Agency prescribed limits [1]. Similar studies were undertaken in Thailand to investigate the effect of cadmium concentrations in water and sediments in Chao Phraya River on fish industries [2]. The effects of long-term storage of heavy metals within the river sediments were determined by examining contaminants in the groyne fields built along the River Odra (Poland) during the industrialisation in the coal mine districts. It is reported that long period sediment contamination levels could be 60 times larger than local geochemical background [3]. Mining and smelting also increase heavy metal contaminations of soils. For example, the target hazard quotients and estimated daily intakes for cadmium (Cd) and lead (Pb) of rice and vegetables grown around the Dabaoshan mine, South China were found to exceed the FAO/WHO permissible limit of 0.001 mg/l calculated at hardness 100 for Warm Water Aquatic Habitat [4,5].

Using the atomic absorption spectrometry, it was possible to determine the concentrations of heavy metals as low as 1 ng L^{-1} of heavy metals. The technique is based upon adsorption of trace metals on the polymeric resin MCI GEL CHP 20Y after treatment with 2-(2-quinolinil-azo)-4-methyl-1,3-dihydroxidobenzene [6]. The limit as low as 1 ppb was achieved for detection of cadmium (Cd^{2+}) and lead (Pb^{2+}) ions by employing the technique of total reflection at the interface between the planar silicon nitride waveguide and sensing membrane containing composite polyelectrolyte self-assembled films of urease or acetylcholine esterase and cyclotetrachromotropylene as enzyme and indicator, respectively. The catalytic activities of enzymes were inhibited by the presence of metal ions. Individual enzyme reactions as well as their inhibition by metal ions were studied by monitoring the intensity of light output from the planar waveguide [7,8].

Calcium alginate beads were successfully used to immobilize α-Amylase enzyme [9] and *Oryza sativa* L. peroxidase [10]. In the present investigation, acetylcholine esterase (AChE) enzyme was freely suspended in porous spun films containing uniformly dis-

* Corresponding author. Tel.: +44 (0)20 7882 7767; fax: +44 (0)20 7882 3390.
E-mail address: a.k.ray@qmul.ac.uk (A.K. Ray).

0304-3894/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2009.08.077

tributed calcium alginate beads. The purpose of this article is to present an experimental investigation into the effectiveness of sulfonated copper (II) phthalocyanine (CuTsPc) as an indicator for enzyme reactions with acetylcholine chloride (AChCl) solution. The phthalocyanine molecules are known to exhibit different molecular organisations in the aggregates depending on the neighborhood pH value [11,12]. The split Q absorption bands and the Raman peaks were chosen to monitor enzyme reactions during the initiation and inhibition processes for heavy metals detection.

2. Experimental

In order to fabricate the bilayer structure in Fig. 1(a), a 20 nm thick CuTsPc layer was deposited by drop casting of a very small volume of CuTsPc (chemical structure shown in Fig. 1(b)) dissolved at 1 mg/ml concentration in deionized water of 18 MΩ cm on an ultrasonically cleaned glass substrate. The AChE enzyme was dissolved in a 1 mg/ml concentration in a 0.05 M Trizma base buffer at pH 7.4 and a 4% (w/v) solution of sodium alginate was added to the enzyme solution in deionized water of 18 MΩ cm. This gel was then spin coated on the CuTsPc film using the Chemat technology spin coater KW-4A at the speed of 1000 rpm. The sodium alginate layer was then exposed to the 2% (w/v) calcium chloride solution in order to obtain calcium alginate micro beads with enzymes freely suspended inside pores. The membrane was estimated to be approximately 80 nm thick.

Using the PerkinElmer Lambda 950 spectrophotometer within the range 450–800 nm at 1 nm resolution, UV–vis absorbance spectra were recorded for CuTsPc/(calcium alginate-AChE) bilayer at room temperature. A monochromatic beam of intensity I_0 was allowed to be incident perpendicular to the plane of the films. An uncoated substrate was used as a reference for absorption, and a surface silvered mirror for reflection, so that the outputs I_t were solely in terms of the transmission characteristics of the films. Using Beer's law, values of $\ln(I_0/I_t)$ was recorded as absorbance [13]. Raman spectra were recorded on a Nicolet Almega XR dispersive Raman spectrophotometer, equipped with a green argon 514 nm laser accumulated 128 number of scans and an exposure time of 2 s at 4 cm^{-1} resolution. The spectra were recorded in the range of 300–1600 cm^{-1}. In order to investigate the enzyme activities, the membrane was dipped in acetylcholine chloride solution (pH 7.4 by diluted sodium hydroxide aqueous solution) for 15 min at 37 °C in an incubator and initial enzyme activity was then measured by recording both UV–vis absorption and Raman spectra for the sample. The bilayer membrane was subsequently exposed to water contaminated with cadmium of four different concentrations varying from 100 ppm to 1 ppm for 15 min. Residual enzyme activity was finally determined by repeating optical absorption and Raman spectroscopic measurements, A Digital Nanoscope III, Atomic force microscope was used to study the surface morphology of the spun calcium alginate films on silicon substrate. Films were imaged in tapping mode AFM along with silicon cantilevers [14].

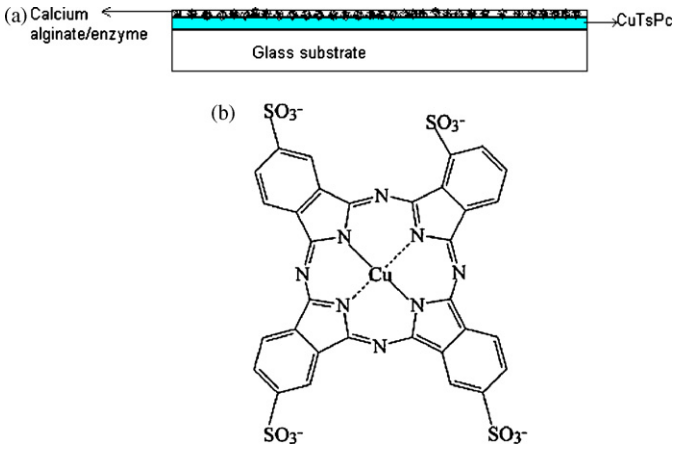

Fig. 1. (a) Schematic diagram of CuTsPc/(calcium alginate-AChE) bilayer and (b) chemical structure of copper phthalocyanine tetrasulfonate (CuTsPc).

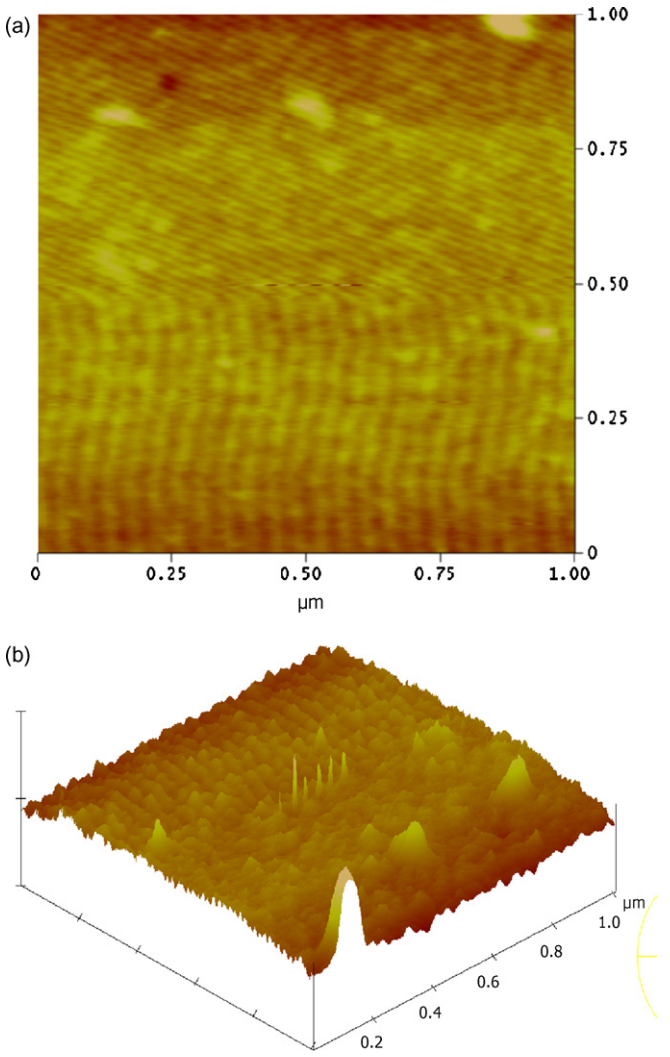

Fig. 2. (a) Two and (b) three dimensional AFM micrograph of top surface of calcium alginate on the silicon substrate.

3. Results and discussions

The AFM micrographs in Fig. 2 show that the top surface of the calcium alginate on the silicon substrate was covered with spherical beads, largely self-organised, of 40–50 nm in diameter without intervening spaces. The surface was found to be uniform and the average roughness was estimated to be 8 nm.

As shown in Fig. 3(a), essential characteristics of the UV–vis absorption spectra of the CuTsPc/(calcium alginate-AChE) bilayer on the glass substrate are similar to those reported for CuTsPc film the appearance of the Soret band at ~350 nm due to the transition between $\pi(b_{2u})$ and $\pi^*(e_g^*)$ levels and the split Q bands at ~550 nm (Q_1) and 678 nm (Q_2). Q_1 represents the dimerisation while monomers are associated with the peak Q_2 [15]. The spectra were not expected to be influenced by the presence of the top layer since the absorption peak is reported to have occurred at 250 nm for calcium alginate which is transparent in the range between 350 nm