Journal of Industrial and Engineering Chemistry 25 (2015) 180–185

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

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

Influence of load on the recycling stability of nanospheres attached platinium ion for determination of glucose

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ARTICLE INFO

Article history:
Received 18 May 2014
Received in revised form 28 September 2014
Accepted 18 October 2014
Available online 29 October 2014

Keywords:
Biocatalysis
Glucose oxidase
Nanospheres
Platinium ion

ABSTRACT

New nanospheres have been prepared with Pt^{2+} and Pt^{4+} for compare enzymatic properties of glucose oxidase enzyme (GOx). In this study nanoparticles, (aminomethyl)polystyrene (APS), 2-hydroxy-5-methylbenzaldehyde and Pt^{2+}/Pt^{4+} have been synthesized by means of template and investigated the enzymatic properties of glucose oxidase enzyme (GOx) immobilized on there. The characteristics of the immobilized glucose oxidase (APS–Pt^{2+}–GOx and APS–Pt^{4+}–GOx) enzyme showed one optimum pH value. The influence of temperature, reusability and storage capacity on the free and immobilized glucose oxidase enzyme have been investigated. It is found that nanosphere including platinium atom exhibits excellent performance as the immobilized supporter of GOx, the immobilized enzyme demonstrates perfected storage and recycling stability. APS–Pt^{2+}–GOx retains more than 30% of the initial activity after 32 successive cycles, which is a remarkable result.

Introduction

Recently there has been a considerable interest in the material chemistry of nanoparticules involving metal ion because of their potential medicine and industrial applications [1,2]. The use of nanoparticules in medicine and more specifically drug delivery is set to spread rapidly [3]. Nanoparticules involving metal ion play a very important role not only in chemical reactions (enzymatic reactions) in the human body but also in industrial chemical processes [4]. Nanoparticules have been recognized as effective enzyme loading, because they offer the special and fascinating characteristics for balancing the key factors that determine the biocatalyst efficiency, including high specific surface area [5].

Determination of Glucose is important in many areas, from clinical studies to industrial productions. Researchers are developing new methods for the last decade due to rapid determination of glucose and control is significant. In view of these, nanoparticule structures have attracted great attention in preparing advanced materials [6]. Metal nanoparticles have been extensively utilized due to their extraordinary catalytic activities for both oxidation and reduction reactions. Among them, Pt nanoparticles were one of the mostly used for immobilization of enzymes for the investigation biocatalyst due to their unique properties such as electrocatalytic ability [7].

Although glucose oxidase enzyme has attracted interests in the varying process, this enzyme is unstable due to its complex molecular structure. Therefore, a number of immobilization techniques have recently been investigated to improve its stabilities. Immobilization of enzymes onto polymeric nanosphere is the most useful strategy to improve the operational stability of biocatalysts [8,9]. Other benefits are obtained as well, such as better operational control, ease of product recovery without catalyst contamination [8].

Recently, Singh et al. reported that Hybrid xerogel as an effective carrier support for glucose oxidase. They noted that was loss in bioactivity up to six cycles of recycling of Hybrid xerogel-GOX [10]. Arslan et al., immobilized glucose oxidase (GOx) onto polyaniline-polyvinylsulfonate film without any cross linking agents or modifiers [11]. Guo and coworkers immobilized GOx on iron oxide nanoparticles [12] prepared via a coprecipitation method.

Polymers such as polystyrene, poly(vinyl alcohol), poly(ethylene oxide) can be covalently linked with enzymes, resulting in surfactant-like giant molecules that have the ability to form catalytic molecular layers at the interface of aqueous/organic biphasic systems [13–16].

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http://dx.doi.org/10.1016/j.jiec.2014.10.031
1226-086X/

Of course, the active center of the enzyme must be protected during the formation of this bond. If a polymer-based macromolecule supports have metal ions like Pt(II) or Pt(IV) may be useful for increasing the enzyme stability via covalent bonding attachment [17].

As a continuation of our study on nanoplatforms including Pt(II) and Pt(IV) ion, to investigate the effect of different charge of same atoms on biocatalysis, new two support have been prepared. To prepare such a support, the (aminomethyl)polystyrene (APS) reacted with 2-hydroxy-5-methylbenzaldehyde and Pt(II)/Pt(IV) (Fig. 1) by means of template method.

Experimental

Materials and methods

Glucose oxidase (β-D-glucose: oxygen-l-oxidoreductase, EC 1.1.3.4) from *Aspergillus niger* was purchased from Sigma Chemical Company (SIGMA, 49180). Its molecular weight and pI was 160,000 Da and 4.2, respectively. 4-Aminoantipyrene (4-AAP), phenol, (aminomethyl)polystyrene, 5-methyl-2-hydroxybenzaldehyde, $PtCl_2$ and $PtCl_4$ were purchased from Sigma (St. Louis, MO). All the other chemicals used in this work were provided by Merck and Sigma-Aldrich and used without further purification. IR spectra were recorded on a Mattson-5000 FT-IR instrument in KBr pellets. The GPC measurements were recorded on a Waters 1500 Series Gel permeation chromatography (GPC). Scanning electron microscopy of the Au-Pd-coated compounds was done by using a JEOL JEM 100 CX II scanning electron microscope (JEOL, Peabody, MA) equipped with a Link analytical system. The electron energy used was 20 keV.

1.1. Synthesis of support nanoplatforms attached Schiff bases–Pt(II)/Pt(IV)

The coordination polymer (PS–Sch–Pt(II) and PS–Sch–Pt(IV)) were prepared by reacting of (aminomethyl)polystyrene (PSA) (1 g, 1.0–1.5 mmol/g $-NH_2$ loaded, 1% cross-linked) in hot DMF (15 mL) with aldehyde (5-methyl-2-hydroxybenzaldehyde, 1.0 mmol) in DMF (10 mL) (Fig. 1). Aldehyde solutions were slowly added by the drop wise on amine solutions while stirring through 30 min. The reaction mixture was boiled and stirred under a reflux condenser *ca.* 2 h, at 70 °C. Then, $PtCl_2$/$PtCl_4$ in DMF solutions were added on this reaction mixture while stirring through 3 h. After the mixture cooling to room temperature, modified polymers were poured into the acetone. The resulting modified polymers were allowed to stand in acetone (about 30 min), was filtered and dried in the oven and kept with desiccator over anhydrous $CaCl_2$.

Immobilization of GOx on PS–Sch–Pt(II) and PS–Sch–Pt(IV)

The (PS–Sch–Pt) polymers (0.0125 g) were placed in a 15 mL DMF: water solution (9:6) of 0.010 g/L of glucose oxidase at 30 °C in a shaking water bath for 2 h. The immobilized polymer was separated and the free enzyme was removed by washing with phosphate buffer (pH: 7.0, 15 mL). The immobilized enzymes were freshly used and then stored at 4 °C. Saturation ratio was determined as % 97.13 from absorbance value in 504 nm.

Assay for enzyme activity measurement

A colorimetric method based on Trinder's reaction was used for the determination of glucose concentration [18]. Glucose is enzymatically oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The hydrogen peroxide reacts with 4-aminoantipyrene (4-AAP) and phenol to form pink colored quinoneimine dye, which has absorption maximum at 504 nm (A_{504}). The following reaction was started by adding 16 mg glucose, after pre incubating at 30 °C for 15 min. This mixture was removed after incubating the reaction mixture at 30 °C for 75 min under continues stirring. Then, was transferred quartz cuvette for measurement.

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{GOx}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{phenol} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + H_2O$$

The following recipe was used for free enzyme/immobilize enzyme assay: 4 mL studied buffer (pH 3.0–8.0) + 10 mg 4-aminoantipyrene + 20 mg phenol + 0.5 mg of horse radish peroxidase (HRP) + 0.010 g/L, 6 mL free glucose oxidase/immobilize glucose oxidase in studied buffer + 16 mg glucose.

Effect of pH and temperature on activity of free and immobilized GOx

Optimum pH for free and immobilized glucose oxidase was determined by measuring the activity of free and immobilized enzymes in buffers of different pH values ranging from 3.0 to 10.0. The buffers used were: pH: 3.0–4.0 (CH_3COONa/CH_3COOH);

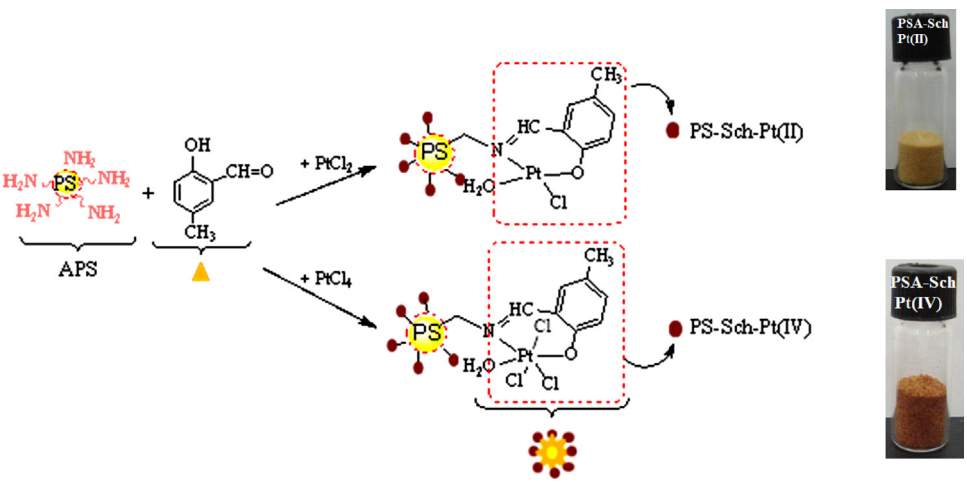

Fig. 1. Synthesis route of supports for investigation biocatalyst of GOx enzyme.