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Zeolites-AuNPs assembled interface towards amperometric biosensing of spermidine

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ABSTRACT

Detection of the spermidine level (Spmd) present in a sample is a sensible indication of the standard of foodstuffs. A new biosensing electrode having polyamine oxidase (PAO) as the biorecognition element immobilized on the surface of chitosan (CHIT)/zeolites-gold nanoparticles (AuNPs) modified gold (Au) electrode was prepared and applied for determination of Spmd contents in fish homogenates. Zeolite nanocrystals and AuNPs were synthesized via hydrothermal and biological (using *Ocimum sanctum* leaf extracts) routes respectively and hybrids of zeolites-AuNPs were synthesized by high temperature calcination. Transmission Electron Microscopy (TEM) was used to determine the size of AuNPs and their efficient impregnation onto zeolites was confirmed by Energy Dispersive X-ray (EDX) Spectroscopy studies. Thus synthesized zeolites-AuNPs hybrids, when used with chitosan (CHIT/zeolites-AuNPs), offered a porous structure with high surface area having an activated surface for attaching. This retains the enzyme along with better electron transport properties. Electrochemical Impedance Spectroscopy (EIS) and Scanning Electron Microscopy (SEM) were used to confirm the modification of Au electrode with PAO loaded CHIT/zeolites-AuNPs composite film. Cyclic voltammetry (CV) was used to study the activity of the immobilized PAO electrochemically. Under optimum conditions, the proposed electrochemical sensor showed wide linearity ranging from 0.2–200 μM and a detection limit (LOD) of 0.1 μM (S/N = 3) with favorable reproducibility and stability.

1. Introduction

The polyamines such as agmatine, cadaverine, putrescine, spermidine (Spmd) and spermine are naturally occurred in the foodstuffs and are involved in growth, cell proliferation and cell death [1]. However, improper refrigeration and preservation increase concentration of these polyamines in the food caused by amino acids decarboxylation through microbes [2]. In the presence of nitrates, these amines are converted into the carcinogenic nitrosamines and promote tumor growth in the presence of histamine. Hence, determination of polyamines in food items is essential not only to safeguard human and animal health but also can be used as an indicator of the freshness or spoilage of food [3,4].

Conventional methods for polyamine determination such as HPLC with UV-visible detection and derivatization reaction require long sample preparation time and do not satisfy all the criteria like reliability, selectivity and operational sensitivity [5–7] unlike biosensors [8]. Amongst the biosensor devices, the amperometric sensing based devices utilizing direct or mediated electron transfer between electrode and enzyme are the most promising with response to sensitive and linearly [9]. In this study, the electrode for polyamine determination is based on the electrochemical/amperometric detection of H_2O_2 produced in the enzymatic reaction or oxidation of polyamines by polyamine oxidase (PAO).

$$\text{Spermidine(polyamine)} + O_2 + H_2O \xrightarrow{\text{PAO}} 4-\text{aminobutanal} + 1,3-\text{diaminopropane} + H_2O_2$$

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^- (current)$$

In the earlier studies, PAO was immobilized onto Prussian blue modified Screen-printed electrodes (SPEs) [10], cellulose acetate membrane [11] and carbon paste matrix [12]. However these biosensors also bear certain drawbacks including improper electron communication, complex immobilization process and fast instability of enzyme. It is often desirable to improve stability

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and loading of the enzyme on the electrode surface. Gold nanoparticles (AuNPs) and zeolites are very promising in this regard and nowadays increasingly used to develop enzyme electrodes for potentiometric/amperometric sensing [13] as they are hydrophilic [14], physically stable, biocompatible, impose minimal diffusional limitations and have large surface areas [15,16]. Furthermore, good electrical conductivity of AuNPs ensures more effective electrical wiring of the immobilized enzyme to the electrode [17–22]. Though both the zeolites and AuNPs, as a immobilizing support, have been used previously for the immobilization of pepsin [23] and hemoglobin [24], but have never been used for PAO immobilization. A hybrid support prepared by embedding AuNPs in the zeolites matrix is expected to work synergistically to improve the overall performance of the present biosensor.

Herein, we have developed a new electrochemical enzyme biosensor for Spmd determination by immobilizing PAO onto chitosan/zeolites-AuNPs modified Au electrode. Spmd is synthesized from putrescine and is itself a precursor of spermine. Hence, determination of Spmd content will give a fair idea about the presence of spermine and putrescine. Thus prepared PAO/chitosan/zeolite-AuNPs modified Au electrode showed an excellent electrochemical response towards Spmd, a low limit of detection, wider linear range and prolonged stability. Analytical applications of the biosensor for determination of Spmd contents in real fish samples are included to explore its suitability for monitoring of polyamines in foodstuffs.

2. Experimental

2.1. Chemicals and reagents

PAO was synthesized, extracted and purified (386 units/mg protein) from 10 days old oat seedlings by the method of Li et al. (1993) [2]. Spermidine from Sigma-Aldrich, U.S.A.; tetra-ethyl-orthosilicate (TEOS, 98%), tetra-methyl-ammonium hydroxide (TMAOH), aluminium isopropoxide and NaOH from Merck India and $HAuCl_4.3H_2O$ and chitosan (CHIT) from Sisco Research Laboratory, Mumbai, India were procured. Au wire ($1.5 \times 0.05\ cm^2$, 23 carat) was purchased from the local market. Only deionized water (DW) was used in each experiments.

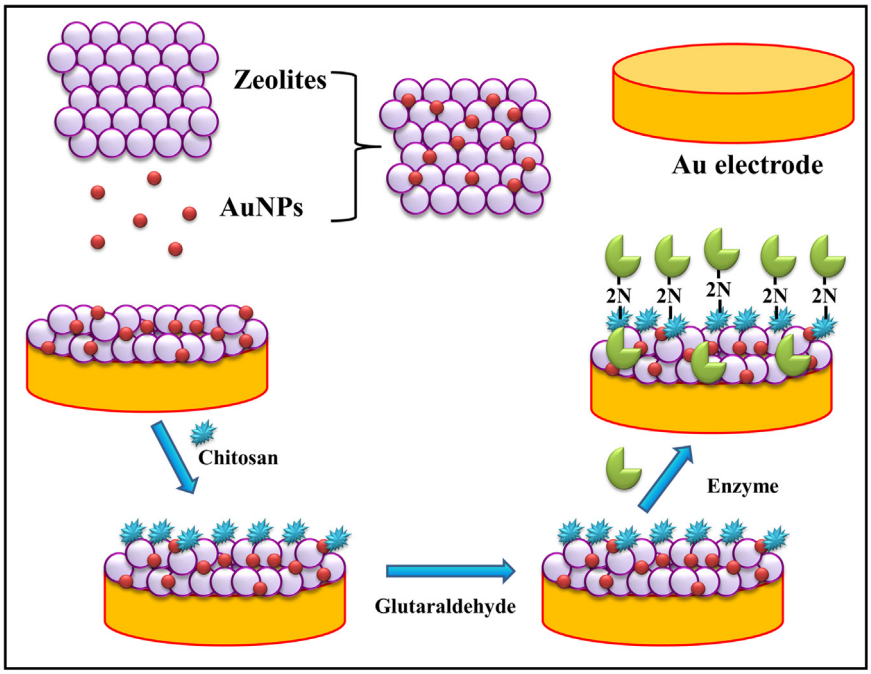
2.2. Synthesis of AuNPs

AuNPs were synthesized by reduction of $HAuCl_4.3H_2O$ with *Ocimum sanctum* leaf extract. 10 g of the leaves were washed with deionized water, finely cut, stirred with 200 mL deionized water for 1 min at 28 °C and filtered to get the extract. 10 mL extract was added to a vigorously stirred 30 mL aqueous solution of $HAuCl_4.3H_2O$ (0.5×10^{-3} M) and stirring was continued for 2 min. The beaker was left covered and appearance of light violet color within 10 min indicated the formation of AuNPs [25]. Size of the prepared AuNPs was confirmed by Transmission Electron Microscopy (TEM; 300 kV, JEOL 3010) at CPMU, JNCSAR, Bangalore, India

2.3. Preparation of AuNPs embedded TMA-A zeolite nanocrystals

TMA-A zeolite nanocrystals of size between 60 – 150 nm were synthesized by hydrothermal method, using tetra-ethylorthosilicate (TEOS), aluminum isopropoxide Al(i-pro)3, tetra-methyl-ammonium hydroxide (TMAOH), and sodium hydroxide (NaOH) in a ratio of 1:2.2:2.4:0.3 in 200 parts of water. The prepared reaction mixture was then kept at 100 °C for three days and removed from the oven at predetermined times to arrest the reactions. The crystallized TMA-A zeolites were obtained after centrifugation and repeatedly washed with distilled water to remove the remaining reaction mixture until the pH of the dispersion medium was close to 7. Further, the crystalline TMA-A zeolites were collected by drying for 4 h at 80 °C [26].

To synthesize zeolite-AuNPs hybrids, 0.3 g TMA-A zeolites were soaked in 20 ml of AuNPs solution for 3 h at room temperature so as to give sufficient time to AuNPs to permeate completely into the zeolites matrix. The resultant hybrids were distinguished by



Scheme 1. Schematic of the electrochemical sensing platform (PAO/zeolite-AuNPs/Au electrode) construction where PAO was covalently linked to the zeolite-AuNPs modified Au electrode through glutaraldehyde coupling.