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Hydrogen peroxide biosensor utilizing a hybrid nano-interface of iron oxide nanoparticles and carbon nanotubes to assess the quality of milk

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ABSTRACT

A hybrid interface was developed using nano iron oxide and carbon nanotubes and this architecture offered an improved performance for the detection of hydrogen peroxide. Nano iron oxide was synthesized by a simple thermal co-precipitation technique and it was dispersed in nafionic solution. To this mixture added the catalase enzyme adsorbed multi-walled carbon nanotubes and this solution was used for the modification of the electrode. The morphology of the prepared nanocomposite was observed using FE-TEM and the electrochemical studies were carried out using cyclic voltammetry and amperometry. The linear range of the prepared amperometric sensor was found to be between 1.2 and 21.6 μM with a quick response time of less than 1 s. The interference, reproducibility and stability studies were carried out with satisfactory results. The limit of detection and limit of quantification were found to be 3.7 nM and 12.2 nM respectively. With the convincing results obtained in terms of the performance of the biosensor, this platform was successfully upgraded for the determination of hydrogen peroxide in the presence of milk samples.

1. Introduction

Quantification of hydrogen peroxide is of immense value in clinical diagnostics and food quality monitoring [1]. Milk and milk-based commodities are one among the highly consumed global food product, and especially by young children. But milk is easily susceptible to spoilage and in order to prevent its spoilage or extend its longevity, hydrogen peroxide (H_2O_2) is added in small quantities. This strategy is effective in controlling the microbial growth that leads to milk spoilage [2]. However, excessive quantities of H_2O_2 can lead to the arousal of ill-effects due to its propensity in enhancing the oxidative stress and the reports demonstrate the detrimental effect of the carbohydrate-digesting enzymes found in the small intestine [3]. Additionally, the expulsion of H_2O_2 was observed during the colonization of pathogenic microbes such as *Staphylococcus aureus* [4]. Recently, quantification of H_2O_2 has been utilized as a valuable tool in the detection of *S. aureus* contamination in milk [5]. Thus an accurate measure of H_2O_2 levels can be employed for monitoring the quality of milk and therefore, the health of consumers. Currently, the detection of hydrogen peroxide is mainly carried out using a variety of techniques namely electron spin resonance spectroscopy (ESR), UV–vis spectrophotometry, chemiluminescence, titrimetry, etc. [6–9]. But these methods suffer a major trade-off: costly, time consuming and tedious. But, the electrochemical method offers a quick and efficient data acquisition with minimal interference apart from its cost effectiveness and the simple preparative procedures involved [10–14].

Now, the main focus of research in electrochemical sensors relies on prolonging the sensing range, speed and limit of detection by the way of designing appropriate working electrodes. In this context, the integration of biosensors and nanointerfaces has led to superior detection capabilities. Biosensors with their unmatched specificity are the most preferred for detection of analytes, from the samples that contain many closely related components [11–15]. In the similar lines, the immobilization of the enzyme onto the electrode surface offered an enhancement in their reusability and reduces the cost of analysis. However, the immobilization techniques adopted will either cause an alteration in the function of the biomolecule and thus resulting in reduction of sensing capability [16] or retarding the diffusion of the substrates. These factors will affect the transfer of electrons and results in a poor response

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times [17]. Catalase enzyme has been extensively employed in the design of many biosensors for the efficient detection of H_2O_2 owing to its high turnover number and substrate specificity [18]. The introduction of a nanointerface on the electrode surface has been recently explored for improving the response time. The nature of the nanointerface decides the rapidity of signal generation and therefore the response times as low as a few microseconds [19].

A wide variety of biosensors has been reported that utilizes a single nanointerface namely ceria, gold nanoparticles, multi-walled carbon nanotubes and iron oxide [20–23]. A nanointerface unites the activity of the sensing element in a spatial manner and it results in a sharp signal. An improvement in the performance of the sensor in terms of higher sensitivity, relatively lesser time for analysis and a lower detection limit has been reported with the introduction of a nanointerface [17].

Recently, use of multiple interfaces on the surface has emerged as a new paradigm in biosensing. This multiple interface can offer promising results in the several other fields namely solar cell and supercapacitor application in a cost-effective manner. Carbon nanotubes in conjunction with a room temperature ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate, has been used as an interface in the construction of a catalase-based sensor and it exhibited an improved electron transfer from the reaction medium to the electrode surface [24]. Recently, a multi-walled carbon nanotube-nano nickel oxide composite has been reported as an effective interface in the design of catalase-based sensor [25]. A novel cobalt tetraphenyl porphyrin and reduced graphene oxide nanocomposite electrode has been developed for the enzyme-free detection of H_2O_2 [26]. Apart from the size and electron transfer properties of the nanoparticles, their ability to immobilize the enzyme without adversely affecting its catalytic ability and diffusion of substrates is a critical aspect that needs to be taken into account while designing the hybrid interface. Iron oxide (Fe_3O_4) nanoparticles are attractive candidates as nanointerfaces for sensing applications due to their biocompatibility, high surface area-to-volume ratio and excellent electron transfer characteristics [27]. Our earlier work on the development of a H_2O_2 sensor utilizing catalase and iron oxide confirmed the promising effect of iron oxide nanoparticles as a nanointerface [23]. Carbon nanotubes (CNTs) are another category of nanomaterials that are well explored for their excellent electron transfer properties in electrode modification and biosensor field. Moreover, their high aspect ratio could provide sufficient active sites for the immobilization of catalase enzyme that in turn auger for superior detection ranges. Additionally, owing to their versatile characteristics such as the large active surface area, biocompatibility, high thermal and chemical stability and high electrical conductivity they are grabbed for the improvisation of electrocatalytic performance [28]. Few of the research works implies the importance of the nanocomposites of CNT with conducting polymers by the way of increasing the active surface area and thus, promotes the electron transfer [29,30]. The mutual integration of the CNT and metal oxide nanoparticles proved as an effective electrochemical biosensor due to their synergistic effect [31]. The higher surface activation areas of CNTs' allow for the quick mass transport of ions either through the electrolyte of electrode interface and therefore lead to the rapid electrochemical reactions [32]. Fe_3O_4 nanoparticles are dispersed in the polymeric nafion solution of Naf–CNT in order to avoid the problem of aggregation and thus the Naf–Fe_3O_4–CNT hybrid nanocomposite is prepared. The nafionic polymer solution aids for the wrapping and stabilization of the nanomaterials with a minimal aggregation. The presence of iron oxide nanoparticles in the nanocomposite helps to increase the electroactive surface area of the matrix and thus an elevated level of stability can be attained. It also increases the electron transfer kinetics between the electrolyte solution and that of the electrode [33,34]. The combination of a nano-Fe_3O_4 and CNT hybrid interface along with the catalase enzyme has not been explored for sensing applications thus far. The present work envisages the development of a biosensor based on catalase using a Fe_3O_4–CNT interface for detection of H_2O_2 in milk samples.

2. Materials and methods

2.1. Reagents and materials

Iron (II) chloride, catalase, hydrogen peroxide and nafion solution (5 wt%) were purchased from M/s Sigma Aldrich Co., Ltd., India. CNT's were purchased from M/s Nanostructured and Amorphous Materials Inc., USA. Analytical grade reagents sodium dihydrogen phosphate, disodium hydrogen phosphate, iron (III) chloride, ascorbic acid, uric acid, glucose were purchased from Merck, India and used as such without further purification. All solutions were prepared using deionized water.

2.2. Characterization techniques

The morphology of the nanocomposite was characterized using Field Emission Transmission Electron Microscopy (FE-TEM) (Model JSM 2100F JEOL, Japan). Cyclic voltammetry and amperometric experiments were performed using an electrochemical workstation (Model CHI600C, CH Instruments, USA). Cyclic voltammograms (CV) were recorded at a scan rate of 0.1 V/s in pH 7.4 phosphate buffer solution for successive additions of 1.2 μM hydrogen peroxide solution to 5 mL of the buffer. All measurements were recorded using a three electrode system comprising of the CAT/Fe_3O_4–CNT/Au (catalase/iron oxide–carbon nanotube/gold) working electrode, Ag/AgCl in 0.1 M KCl as reference electrode and platinum wire as the counter electrode. Sonication was carried out using probe sonicator (VibracellTM, Sonics, USA) of 130 W, 20 kHz.

2.3. Synthesis of iron oxide nanoparticles

Fe_3O_4 nanoparticles were synthesized by co-precipitation of Fe^{2+} and Fe^{3+} aqueous solutions in presence of ammonium hydroxide, a base according to the method reported by Kouassi et al. [35]. Briefly, iron (II) chloride and iron (III) chloride were dissolved in deionized water at a ratio of 1:2 and chemically precipitated at room temperature (298 K) by adding ammonium hydroxide solution (30%, v/v) at a controlled pH (10–10.4). The suspensions were heated at 353 K for 35 min under continuous stirring. The impurities were removed by centrifuging several times in water and then in ethanol at 2800 rpm. The resulting particles were dried in a vacuum oven at 343 K.

2.4. Preparation of the nanocomposite

The nanocomposites of iron oxide and carbon nanotubes were prepared as follows. Briefly, 10 μL of the catalase enzyme solution (1 mg/mL) was added to 1 mg of pre-weighed CNTs and incubated at room temperature for 1 h. This was followed by the addition of 100 μL nafionic solution (0.5 wt%) of Fe_3O_4 and the mixture was ultrasonicated for few seconds using a probe sonicator (VibracellTM, Sonics, USA). This forms the coating for the working electrode.

2.5. Fabrication of CAT/Fe_3O_4–CNT/Au electrode

To obtain a clean and reproducible surface, the gold electrode was polished with α-alumina powder of 1.0 μm, 0.3 μm using nylon polishing pads and with 0.05 μm γ-alumina powder using microcloth polishing pad. It was then ultrasonically cleaned with 1 mL acetone and then with distilled water for 10 min