Carbon Based Nanosensor for the Determination of Total Cholesterol in Food Samples

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ABSTRACT

A new electrochemical biosensor was designed for the cholesterol determination based on immobilization of cholesterol esterase (ChEt) and cholesterol oxidase (ChOx) onto gold electrode and glutaraldehyde as a cross-linker. Chitosan nanofibres (CSNFs), sol-gel derived from zinc oxide (ZnO), multi walled carbon nanotubes (MWCNTs) and bimetallic alloy nanoparticles of gold (Au) and platinum (Pt) were used as a matrix for immobilization. Electrochemical measurements utilizing a Potentiostat/Glavanostat conduct using a three electrode configuration. A modified gold electrode was used as working electrode, while Pt and Ag/AgCl as the counter and reference electrode, respectively. Optimization of the physiochemical factors such as buffer concentration, pH, scan rate, working potential, enzyme loading and operating temperatures were also implemented. Finally, cholesterol biosensor can be used for the estimation of total cholesterol in food samples.

Keywords: *Cholesterol Biosensors; Multi Walled Carbon Nanotubes; Chitosan Nanofibre; Sol-Gel Zinc Oxide.*

INTRODUCTION

Determination of cholesterol in food samples is important since increasing cholesterol levels in the blood may result in the potential of cardiovascular disease (Basu *et al.*, 2007). Cholesterol determination by spectrometry and high performance liquid chromatography (HPLC) suffer from poor specificity, instability and standardization difficulties. Utilization of enzyme biosensor has become an attractive technique due to its remarkable performance of high sensitivity, easier instrumentation and cost effectiveness (Zou *et al.*, 2008).

The biosensor consists of biological recognition element that reacts with the analyte and a transducer for measuring electrical signals. The fabrication of cholesterol biosensor involves the use of cholesterol esterase and cholesterol oxidase as a biological recognition element and a solid support for holding sensing molecules. This study aims to develop a cholesterol biosensor by leveraging the advantage of gold electrode, chitosan nanofibres, sol-gel derived zinc oxide, multi-walled carbon nanotubes and bimetallic alloy nanoparticles of gold and platinum. Optimization of physiochemical factors such as buffer concentration, influence of pH to the current response, enzyme loading, scan rate, working potential and operating temperatures must be implemented before determination of cholesterol in food samples.

Cholesterol

Cholesterol is a waxy and fatty substance and is the most abundant steroid in mammalian cells. Cholesterol is mainly located in the phospholipid bilayer within the cellular membrane and in the organelle membrane serving as building blocks. Cholesterol also acts as a physiological regulator of steroid hormones, sexual hormones production and as a precursor of bile acids and vitamin D. The interest in determination of cholesterol content in foods has been driven by the awareness of the association between dietary cholesterol and human disease (Dinh *et al.*, 2011). High cholesterol levels may cause diseases such as arteriosclerosis, hypertension, myocardial infraction, diabetes, cerebral thrombosis and strokes (Wang *et al.*, 2012). Gallstones (Cholelithiasis) in the gallbladder or bile duct is composed primarily of cholesterol (more than 50%) due to excessive excretion of cholesterol from the liver (Dowling, 2000). In contrast, reducing cholesterol levels in the blood always result in hyperthyroidism, anaemia and wasting Syndrome.

Biosensors Configuration for the Determination of Cholesterol in Food Samples

A biosensor is an analytical tool for the detection and analysis of biomaterial sample to gain an understanding of their bio-composition, structure and function. The fabrication of biosensors basically comprises of four elements namely the biological recognition element (enzyme, antibodies, DNA and cell receptors), matrix for immobilization, method of immobilization and a transducer selection (Arya *et al.*, 2008).

Matrix for Immobilization

Matrix for immobilization is a solid support that is used to hold a biological recognition element. Excellent electrical conductivity of the surface must be implemented as electrodes for biosensor applications like electrode materials based on metals such as gold, platinum and silver. A gold electrode provides more inertness thus assure less prone to the formation of stable oxide films and surface contamination (Uslu and Ozkan, 2007). The immobilizations of cholesterol oxidase onto gold electrodes for biosensor application have been reported in the literature (Umar *et al.*, 2009; Saxena *et al.*, 2011).

Chitosan (CHIT) is derived from the transformed of linear hydrophilic polysaccharide obtained by deacetylation of natural chitin (Zeng *et al.*, 2009). CHIT has excellent properties such as biodegradable, nontoxicity and hydrophilicity for enzyme immobilization (Bansal *et al.*, 2011). The immobilizations of enzyme within CHIT for cholesterol biosensor application have been reported in the literature (Solanki *et al.*, 2009a; Safavi and Farjami, 2011). Due to its desirable properties, chitosan nanofibres (CSNFs) are able to enhance the features now available to chitosan fibres. CSNFs provide high surface area for enzyme loading, high porosity and nanometer scale diameter of fibre (Gomathi *et al.*, 2011).

Immobilizing using silica sol-gel matrices have become an attractive technique due to capability for entrapment large amount of enzymes, cost effectiveness, high mechanical strength and simplicity of formation (Yang *et al.*, 2010). Numerous applications of silica sol-gel matrix for cholesterol biosensor have been reported in the literature (Tiwari *et al.*, 2008; Solanki *et al.*, 2009a). Nevertheless, disadvantages of using silica sol-gel such as the brittleness and aggressive chemical environment lead to enzyme denaturation consequence giving negative impact to the biosensor performance. Hence, non-silica sol-gel materials have been developed for the immobilization of enzyme. Sinha *et al.* (2010) reported an AChE biosensor based on zinc oxide sol-gel for the detection of pesticides with excellent operational stability, reproducibility and retained enzymatic activity. Yang *et al.* (2010) designed a glucose biosensor by the synthesis of ZnO via sol-gel route. The cholesterol biosensor based on ZnO sol-gel has been reported by Solanki *et al.* (2009b).

Previous studies on MWCNTs highlighted the advantages of electrochemical sensor materials over other fibres due to its superior mechanical strength, excellent electrical conductivity, low detection limit, high sensitivity and high surface area (Vashist *et al.*, 2011). MWCNTs have been widely used for the construction of biosensors and published recently for the detection a variety of substances such as cholesterol (Yang *et al.*, 2011; Wang *et al.*, 2012), glucose (Wang *et al.*, 2008; Chen *et al.*, 2012), hydrogen peroxide (Upadhyay *et al.*, 2009), DNA (Thuy *et al.*, 2012; Yang *et al.*, 2013) and ethanol (Das *et al.*, 2013).

The electrodeposition of noble metals such as gold (Au) and platinum (Pt) provides an effective approach to improve the performance of the biosensor (Wang *et al.*, 2012). Bimetallic alloy nanoparticles have higher catalytic efficiencies than their monometallic counterparts due to the strong interaction between the metals (Singh and Xu, 2013). Moreover, this bimetallic alloy has high sensitivity, fast response time, wide linear range, resistance to deactivation, better selectivity and reproducibility (Chen *et al.*, 2013).

Method of Immobilization

The immobilization technique is the most critical step in the biosensor performance towards the development of stable enzymes with retained activity for long time use (Grieshaber *et al.*, 2008). This technique involves the process of attachment of biological recognition element onto the surface of matrix.

Common methods used for enzymes immobilization are physical adsorptions, entrapment and covalent binding via cross-linking. The physical adsorption method for immobilization of enzymes involves direct adsorption of enzyme onto the desire surface of matrix. The drawback of this method is that it allows the leaching of enzyme that may contaminate the substrate solution (Spahn and Minteer, 2008). Meanwhile, the entrapment method involves entrapping of enzyme within a gel matrix or in polymer membranes (Vidal *et al.*, 2001). This method improves stability and reduces the leaching of enzymes but limit exposure to the active site of the enzyme (Spahn and Minteer, 2008). Covalent binding method involves formation of a covalent bond between functional groups on the surface of the enzyme and functional groups on the surface of matrix (Elnashar, 2010). Covalent bonds via cross-linker provide strong linkages between the enzyme and the surface of matrix.

Transducer for Cholesterol Biosensor

The electrical signal of transducer resulting from the interaction of the analyte with the biological element is amplified by a detector circuit using the appropriate reference and sent for processing by computer software. The data obtained can be further converted into a meaningful physical parameter describing the process being investigated (Grieshaber *et al.*, 2008). Electrochemical measurements such as electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and differential pulse voltammetry (DPV) can be performed with a Potentiostat/ Glavanostat.

CONCLUSION

In conclusion, the enzymatic procedure for the determination of cholesterol is more effective based on practical and able to replace traditional chemical methods for exceptional performance as better analytical sensitivity, selectivity, reliability, simplicity and cost-effective fabrication.

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