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Application Of Polyaniline Nanostructure Based Biosensor For Glucose And Cholesterol Detection.

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ABSTRACT

Qualitative and quantitative measurement of food components is important because of high costs of conventional methods and also tendency to precise and sensitive detection of these compounds. From those components that their determination is very common are glucose and cholesterol. Many procedures have been used for detection and determination of these compounds, but need for accurate measurements with high sensitivity of these compounds, especially in food safety issues have been caused biological methods developments, especially biosensors. Among these, biosensors based on conducting polymers nanostructures especially polyaniline have recently got attention because of their exclusive properties. In this paper, studies on polyaniline based biosensors for glucose and cholesterol detection are reviewed.

Keywords: Biosensor, Cholesterol, Glucose, Polyaniline Nanofiber, Polyaniline Nanoftube

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INTRODUCTION

Nowadays, detection of food composition has received great attention in food industry. Traditional methods are limited due to time consuming, low precision and sensitivity. Therefore, several studies are still being carried out to obtain faster and more accurate and more sensitive and low cost methods of analysis in food industry.

Common analytical instruments in food industry are time-consuming and require experienced operators, high purity chemicals and a long time separation. Enzymatic analysis overcomes most of these limitations. However, new food industries are needs of small analytical equipment which can be used easily in organic solution as well as control simultaneously one or more characteristics during food process. Enzymatic electrodes meet these requirements and increase the recovery and product quality.

Although, these electrodes must be inexpensive, reliable, strong and superior compared other techniques. Cardiovascular diseases in people are increasing day by day and one of the most important reasons is hypercholesterolemia i.e. the increased concentration of cholesterol in the blood. Cholesterol is present in egg yolk, dairy products, etc. HPLC and gas chromatography (GC) methods used for the determination of total cholesterol offer sensitivity and selectivity but are neither suitable for rapid nor cost effective detection. Enzymatic procedures based on cholesterol oxidase and cholesterol esterase have been development due to the advantages of simplicity, rapidness and cost effectively.

Glucose is one of the most important explosives and its detection has high priority in the quality control of food products. Up to now, several methods have been applied for detection of glucose, especially in the fermentation syrup, such as high-performance liquid chromatography (HPLC). Recently, enzymatic methods were developed for the deamination of food proteins.

The determination of these compounds in the in vivo samples with high selectivity and good repeatability as the major interest of researchers involved in food applications. This interest lead to the development of biosensors. Indeed biosensors account for about 85% of the entire biosensor market.

Sensors are devices that register a physical, chemical, or biological change and convert that into a measurable signal.The sensor contains a recognition element that enables the selective response to a particular analyte or a group of analytes, thus minimizing interferences from other sample components. Another main component of a sensor is the transducer or the detector device that produces a signal. A signal processor collects, amplifies, and displays the signal.

Electrochemical biosensors, a subclass of chemical sensors, as indicated by high specificity of biological recognition processes. These devices contain a biological recognition element (enzymes, proteins, antibodies, nucleic acids, cells, tissues or receptors) that selectively reacts with the target analyte and produces a response.

The transducer converts the response into an electrical signal (voltage, current or impedance) that is related to the concentration of the analyte being studied. According to the high specificity of biological recognition elements, the biosensors yield concentration and quality evaluation of analytes. A schematic overview of a biosensor is shown in Fig. 1.

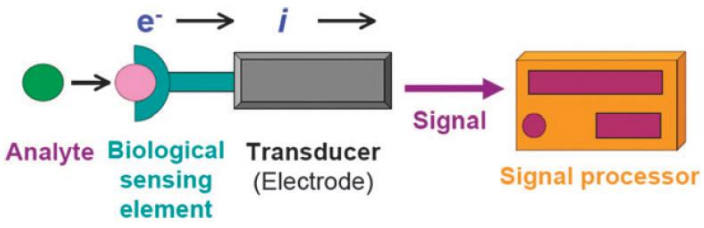

Fig 1: A schematic overview of a biosensor [8].

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The objective is to have an intimate contact between the enzyme and the transducer's sensing surface without blocking the active site of the enzyme or drastically altering the enzyme geometry.2 Immobilization methods are considered successful if the biosensors prepared are stable, reusable, and maintain the selectivity of the enzyme.

Conducting Polymers

Conducting polymers are a class of organic materials that exhibit electrical conductivity.Conducting polymers combine many advantages of organic materials, eg flexibility, with the additional advantage of electrical conductivity either in the metals. The advantage of conducting polymers is their process ability, mainly by dispersion and melting. Conductive polymers are generally not thermoform able. Additionally, the electrical conductivity can be fine-tuned.

The electrical conductivity of these polymers is based on the presence of conjugated double bonds along the polymer. Structures of some typical conducting polymers have been shown in Fig. 2.

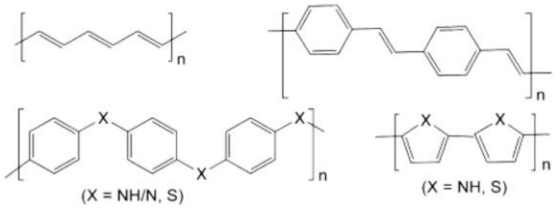

Fig 2: Chemical structures of some conductive polymers. polyacetylenes; polyphenylene vinylene; polypyrrole (X = NH) and polythiophene (X = S); and polyaniline (X = NH/N) and polyphenylene sulfide (X = S).

Among various conducting polymers, polyaniline (PANI) has been widely studied as it has controllable electrical conductivity, excellent environmental stability, interesting electrochemical properties and easy process ability. In the recent years the electrical conductivity of PANI has improved significantly. An increase in the specific surface area of polymer resulted in an improvement of its process ability. As a result, the application of PANI has been expanded in many fields, especially in food science and technology. Up to now, various types of nanostructured PANI films were prepared such as nanoparticles, nanofibers, nanowires, nanotubes and nanobelts.

The nanostructured conducting polymers have attracted much interest as an immobilization matrix on the transducer surface. PANI nanoparticles, naofibers or nanotubes were employed in fabrication of biosensors. The use of nanostructured PANI in the fabrication of biosensors will be discussed further.

Biosensors

As mentioned, biosensors to be selective towards the specific target analyte to prevent the interference by other substance. Immobilization of bio receptors on the bio transducer provides specific response to analyte. Thus, biosensors are excellent approach, in terms of selectivity.

In the biosensors, a change in signal was occurred due to the interaction between recognition element and analyte. The change converts to readable signal.

Methods for immobilizing enzymes to electrode surfaces

Enzyme electrodes have been studied extensively and various physical and chemical schemes have been used to immobilize enzymes on the electrochemical transducer. The objective is to have an intimate contact between the enzyme and the transducer's sensing surface without blocking the active site of the enzyme or drastically altering the enzyme geometry.

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Common enzyme immobilization methods include enzyme entrapment against a matrix such as electropolymerized film, cross-linking, and covalent attachment.

Covalent bonding between protein and biorecognition element provides high stability of the biorecognition element. Covalent bonding to the transducer links functional groups on the enzyme such as NH2, COOH, OH, and SH that are not necessary for the performance of the biological reaction.

Adsorption includes the forces linking the biorecognition element to the transducer. The lifetime of a sensor prepared using adsorption is rather limited. However, adsorption is very easy because it does not require any reagents or clean-up and is less disruptive to the enzymes.

Biotin–avidin binding immobilizes the enzyme through the electrostatic induction between positive or negative charge and transducer surface by applying potential.

Using conductive polymer nanostructures in immobilization of biorecognition compounds are advantageous due to increased electrode surface area, electrical conduction, living environment compatibility, environmental sustainability

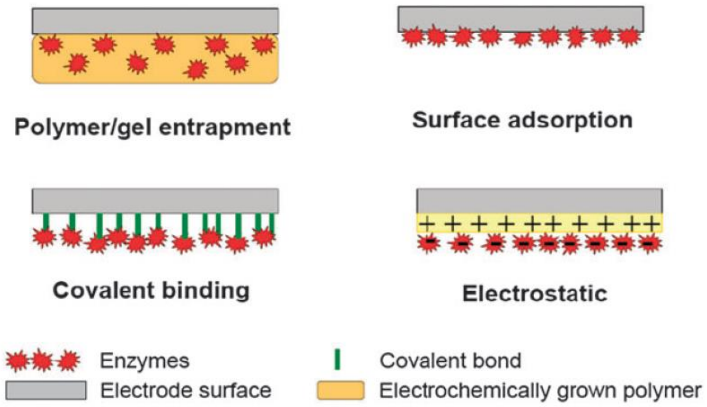

Fig 3: Common methods of immobilizing enzymes onto an electrode surface [8].

Cyclic Voltammetry

Cyclic voltammetry is an electrochemical technique used to characterize electro-active species in solution. As biosensors work based on electrochemical reactions, cyclic voltammetry technique is suitable for the characterization of analyte. The transducer of biosensor was used as the working electrode. For electrochemical investigations three electrodes (working, reference, and auxiliary) are used. The reference electrode is an electrode which has a stable and well-known electrode potential. It is often used Ag/AgCl as reference electrode. Auxiliary electrode is often fabricated from platinum. Also the working electrodes, consisting of coated polymer and transducer was used.

The reversible potential is applied between the working and reference electrodes and the response such as current is measured between the working and auxiliary electrodes. The current was plotted versus applied potential and a current-voltage curve was achieved. When potential become positive, an oxidation peak was observed, which indicates the oxidation ofelectro-active species.

When potential is reversed, electrochemical reduction is occurred for electro-active species that can be reduced. Thus a peak current is observed. Since each species has a specific redox potential, qualitative analysis can be a qualitative analysis can be carried out. Consequently cyclic voltammetry is the most important method for evaluation of transducer and bio receptor. Fig. 4. Shows a scheme for cyclic voltammetry, applying potential and its cyclic voltammogram.

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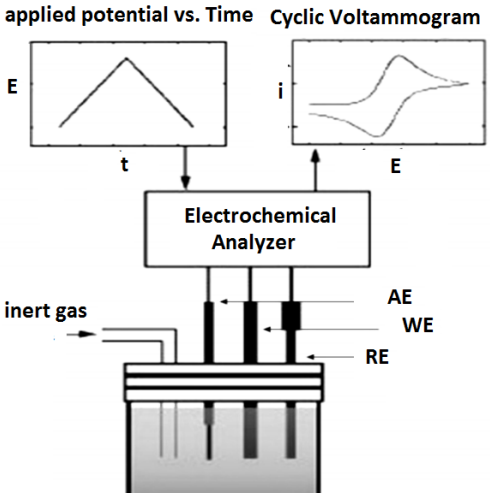

Fig 4: A scheme for cyclic voltammetry, applying potential and its cyclic voltammogram [11].

Glucose Detection

In the Glucose biosensorsthe following reactions were occurred on the bio-electrode (working electrode):

$$Glucose \xrightarrow{GOX} C_6H_{10}O_6 + H_2O_2$$

$$H_2O_2 \xrightarrow{GOX} H_2 + O_2 + 2e^-$$

The enzyme biosensor based on the glucose oxidase (GOx) incorporated polyaniline nanowires described by Horng et al.First of all, aniline monomers was polymerized with ammonium persulfate, a film was deposited on the carbon electrode surface. At the nucleation stage of PANI particles, the linear potential sweep voltammetry was performed for aniline-polymerization. Subsequently, NWs-growth was formed by applying constant current.

Then, the as-grown PANI-NWs were subjected into a phosphate buffer solution (PBS) (pH = 7.2) and by applying the constant potential reduction process was occurred. In order to remove the anions and physically adsorption of GOx, the mentioned process was carried out. Then PANI-NWs/CC was transferred to GOx (2.5 mg/ml) solution in PBS, and initially subjected to oxidation at 0.25. The immobilization of active GOx was induced via electrostatic interaction between the positive-charged NWs-surface and the negative-charged GOx molecules. Fig. 5 presents a schematic diagram for the fabrication of GOx/PANI-NWs/CC electrode.

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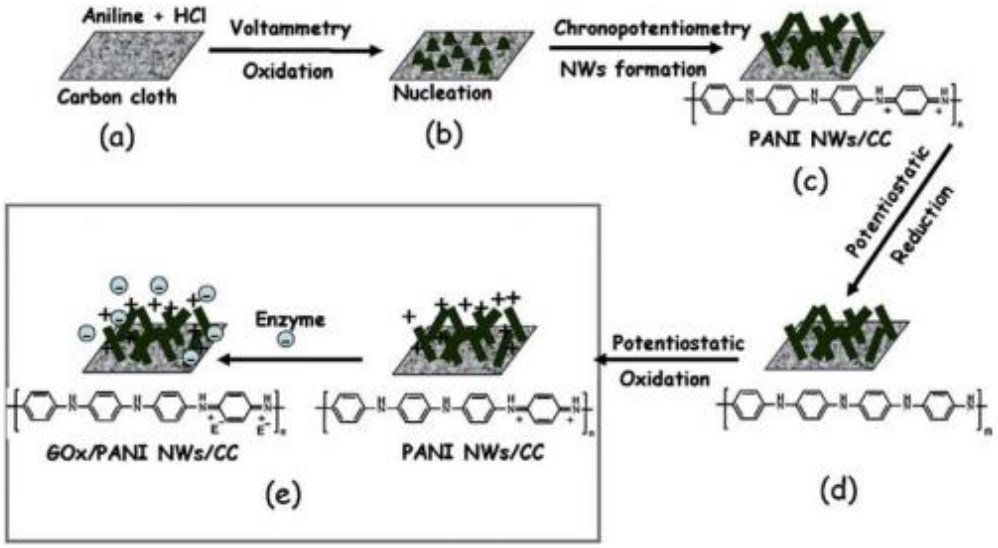

Fig 5: The schematic of Fabrication of GOx/PANI-NWs/CC electrode [12].

In order to study the viability of electrode as a potential glucose-sensor, CV measurement was performed. The as-prepared electrode was studied in the presence and absence of glucose. The cyclic voltammogram illustrated that, the as-prepared electrode shows activity in presence of glucose oxidase; while it exhibited almost no activity in the absence of enzyme (Fig. 6.). This observation was presented to investigate biosensor performance.

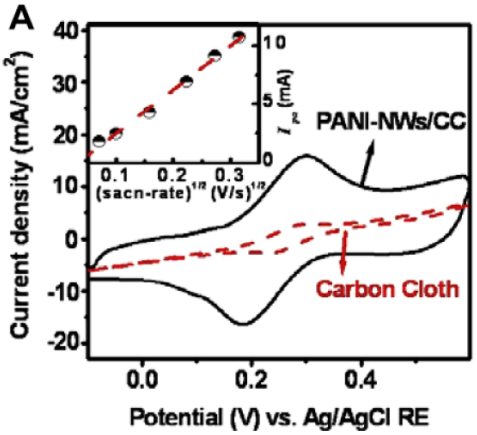

Fig 6: Corresponding CV of the mentioned work [12].

In another published work Wang et al.glucose oxidase immobilized by adsorption on polyaniline nanotubes. In this study, the anodic aluminum oxide (AAO) membrane with the pores size of 200-250 nm in diameter and the average interpore distance of 100 nm, was used for the synthesis of the polyaniline nanotubes.

First, one side of the AAO membrane was coated with a layer of platinum film with thickness of ~10 nm by evaporation. The electrical contact was made to the Pt-coated AAO membrane using copper wire. Then Pt film and the copper wire were coated to avoid contact of solution with them. After that, the working was subjected in 0.5 M H2SO4 solution containing 0.2 M aniline. During the potential scanning, the electropolymerization of aniline occurred in the pore of the AAO membrane and the nanoPANi was formed.

For immobilization of GOx, the nanoPANi was first reduced in phosphate buffer solution at -250 mV in order to remove anions in the nanoPANi as completely. In order to electrostatic entrapment of glucose oxidase, the nanoPANi was oxidized in phosphate buffer solution (containing 7 mg/mL of GOx) under a potential of +750 mV. During the oxidation process, negatively charged GOx were electrostatically entrapped

onto the inner wall of nanoPANi. The resulted electrode was rinsed with doubly distilled water to remove any loosely entrapped enzyme. For electrode characterization cyclic voltammetry was applied.

Cholesterol Detection

In the Cholesterol biosensors the following reactions were occurred on the bio-electrode:

$$Cholesterol + O_2 \longrightarrow Cholesterol - ten - 30ne + H_2O_2$$
$$H_2O_2 \longrightarrow 2H^+ + O_2 + 2e^-$$

Malhotra et al. utilized Polyaniline nanotubes (PANI-NT) for covalent immobilization of lipase (LIP) in cholesterol sensor. In this study, PANI-NT had been chemically synthesized by oxidative polymerization of aniline (Fig. 7). Then, the nanotubes film was electrophoretically deposited onto indium–tin–oxide (ITO) electrode. The electrode was placed into glutaraldehyde (Glu) solution. Glutaraldehyde has two carbonyl groups at its two ends. Glu covalently immobilizes lipase on the polyaniline film surface through binding of aldehyde group with nitrogen of polyaniline film as well as aldehyde group with nitrogen groups of lipase. This electrode is then transferred into Glu solution, followed by drying in temperature room. The aim of this work was investigation of electrode performance using cyclic voltammetry. Three electrodes including PANINT/ITO, PANI/Glu /ITO and LIP/Glu/PANI-NT/ITO electrode were compared to each other in environments with and without cholesterol. The accuracy of electrode performance was confirmed by existing of peaks related to lipase and cholesterol reaction for one electrode and absence of peaks in others. In the same study, a cholesterol biosensor based on polyaniline film coated onto indium-tin-oxide (ITO) electrode had fabricated electrochemically by Khan et al. In this work, polyaniline was coated onto indium-tin-oxide (ITO) electrode electrochemical polymerization and then all the steps which made by other researchers were repeated.

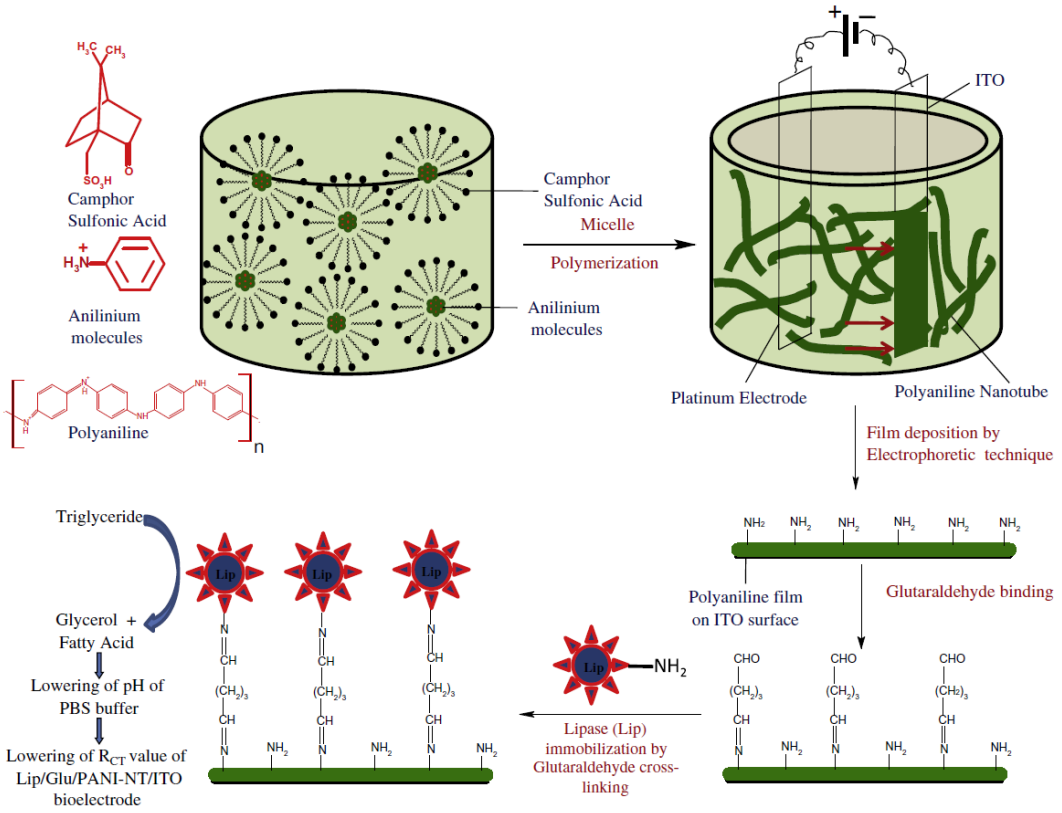

Fig 7: Schematic showing the fabrication of LIP/Glu/PANI-NT/ITO bioelectrode and electrochemical reaction involved in triglyceride detection [14].

Singh et al. fabricated a cholesterol biosensor through co-immobilization of cholesterol oxidase and cholesterol esterase on the electrochemically prepared polyaniline films. At the first, polyaniline was

chemically polymerized onto indium-tin-oxide (ITO) electrode. Then, 1% glutaraldehyde (Glu) solution was dispersed on polyaniline film and dried. In the next step, cholesterol esterase was dispersed on the dried electrode surface. After drying, cholesterol oxidase was dispersed and dried naturally. Finally, this polyaniline–enzyme film was rinsed with deionized water to remove any unbound or loosely bound enzyme molecules. The cyclic voltammetry results confirmed the existence of cholesterol and cholesterol oleate.

CONCLUSION

Cholesterol and glucose detections play an important role in quality control tests. Although, analytical methods used for detection of these compounds must be accurate, sensitive, fast and inexpensive, common methods are time consuming and require experienced operators. Biosensors, a class of chemical sensors, with specific performance overcome these limitations. Biosensors based on conductive polymers, especially aniline polymers have received most attention because of high electrical conductivity, high specific surface area and easy process ability. Biosensors have introduced as the most important measurement tools due to the proper operation.

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