Applications of carbon nanotubes-based electrochemical biosensors

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Abstract. Electrochemical biosensors are newly developed devices and used for various purposes, which is applied in plenty of fields, including medicine, agriculture, food production, and other fields that require the detection of certain particles. Carbon nanotubes (CNTs) are useful nanomaterials in many fields and can be further divided into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). These CNTs with special structure and property make many things possible, such as for development of a diverse of different sensitive electrochemical biosensors. This research aims at investigating the applications of carbon nanotube-based electrochemical biosensors in various fields. In the introduction, some basic information about electrochemical biosensors is outlined. The composition and classification of electrochemical biosensors are provided in detail, and the advantages of using carbon nanotubes in electrochemical biosensors are explained specifically. This research concludes with the working principle of electrochemical biosensors and principles of enzyme immobilization. Applications of carbon nanotubes-based electrochemical biosensors are introduced, where specific reaction equations and more detailed applications are also mentioned in this section. The current limitations of electrochemical biosensors are provided at the end of this research.

Keywords: carbon nanotubes, electrochemical biosensors, enzymes, application.

1. Introduction

Since there is the need for a fast and sensitive diagnosis of diseases, especially those that need early treatment, and the need for detection of different substances in drugs, food and the environment, different biosensors with excellent performance are developed [1]. Biosensors are more portable and simpler devices used to detect and recognize various biomolecules, compared to another large instrument analysis. These biosensors have significant roles in medicine, biochemistry, environmental protection, and food production. Biosensors can recognize a specific kind of biomolecules in a sample that contains more than one kind of biomolecules. There are three main components in the biosensors: a recognizing component, a detecting component and a signal processor [2]. Recognizing components are responsible for binding the target molecules, usually causing a chemical reaction or change, and producing a biological, chemical, or physical signal [3]. Detecting components will recognize and receive the signals produced by the recognizing components. Signal processors will turn these signals into digital signals, so the signals could be displayed on a screen, and the researcher can read them. Biosensors can be categorized into several types due to the difference in the detecting component, and this research will mainly focus on electrochemical biosensors [4]. In addition, biosensors can also be categorized by recognizing components.

Electrochemical biosensors can be further classified as conductimetric biosensors, potentiometric biosensors, and amperometric biosensors. Amperometric biosensors are the most common choice of scientists [5]. Theoretically, electrochemical biosensors couple the reaction of recognizing components with the sample to its transducer to get the chemical signals, and then these signals will be turned into electrical ones. Generally, electrochemical biosensors include three different electrodes: a working electrode, a reference electrode, and a counter electrode [6]. There are also electrochemical biosensors with only two different electrodes [7]. Electrochemical biosensors are the more popular detecting devices nowadays since they are relatively cheaper to produce, smaller in size, and easier to use. Electrochemical biosensors allow the detection of the sample that is without preparation, which means similar molecules or the molecules that can react with the substances in recognizing components in the sample will not affect the result.

Carbon nanotubes (CNTs) are hollow carbon structures with one or more walls, so there are single-walled CNTs (SWCNTs) and multi-walled CNTs (MWCNTs) based on the number of walls [8]. CNTs have advantages in absorbing enzymes, so amperometric electrochemical biosensors tend to combine enzymes with CNTs to form the electrodes. CNTs have few advantages compared with other materials, including making quicker and more sensitive detections possible, and greater strength and conductivity. More sensitive detection means that it is possible to detect the samples with low target molecule concentrations. Moreover, CNTs can reduce fouling effects during the experiment, provide a larger stability, and be used for a longer time. To be more specific, SWCNTs have a special combination of several physical properties, enabling their wide-range applications. They can interact more sensitively with more molecules due to their large surface area. CNTs also enable the invention of highly sensitive nano-biosensors, which can be used to monitor the metabolites in different cells. As a result, CNTs have been widely used to develop electrochemical biosensors.

Generally, CNTs-based electrochemical biosensors can be divided into the following main categories: oxidases-based electrochemical biosensors, dehydrogenases-based electrochemical biosensors, enzymes-based electrochemical biosensors, DNA aptamer-based electrochemical biosensors, and antibody-coated electrochemical biosensors [6]. Oxidase-based electrochemical biosensors can be considered a special type of enzyme biosensors. Oxidases-based electrochemical biosensors can be further classified according to oxidases: glucose oxidases, cholesterol oxidases, and other CNTs-oxidases. Glucose oxidases-based electrochemical biosensors are essential in medicine; since there are plenty of diseases related to blood glucose concentration, and food production. Cholesterol is detected for various heart and brain problems. Other oxidases are important for fields like drugs and agriculture. Basically, the above electrochemical biosensors are created based on the oxidation reaction of related oxidases. Dehydrogenases-based electrochemical biosensors have a similar working principle as oxidases-based electrochemical biosensors. Alcohol-dehydrogenase electrochemical biosensors are one of the typical applications. It can be used directly to recognize the alcohol in different drinks, like wine, and food. Immobilization approaches used in oxidase-based electrochemical biosensors are also applied in enzyme-based electrochemical biosensors. Various substances can be detected using enzyme-based electrochemical biosensors depending on the type of enzymes. DNA-based electrochemical biosensors work by detecting the nucleic acid in the sample and can be used to diagnose gene-related diseases. MWCNTs can be used to capture specific DNA sequences.

This research will focus on the main applications of carbon nanotube-based electrochemical biosensors. In section 2, this research will briefly introduce the mechanism of carbon nanotube-based electrochemical biosensors. In section 3, this research will talk about the details of some common applications of carbon nanotube-based electrochemical biosensors, including enzyme-based electrochemical biosensors, oxidase-based electrochemical biosensors and DNA-based electrochemical biosensors. In section 4, this research will summarize the main idea of this work and outline the current limitations of electrochemical biosensors.

2. Mechanism of electrochemical biosensors

2.1. Working principle of electrochemical biosensors

Generally speaking, the reaction that happened in an electrochemical biosensor can generate or change one of the following things to allow the monitoring of signals: conductivity between two electrodes, or potential or current that can be measured. Conductimetric biosensors work by measuring the changes in the conductivity of the testing sample. Since reactions are happening in the sample, the conductivity changes due to the change in the chemical properties of its components. Potentiometric biosensors work by detecting the potential across two reacting phases while applying current to them.

Amperometric, or voltametric biosensors, are the most commonly used type of electrochemical biosensors. These electrochemical biosensors are used to detect the electron transfer between two electrodes [9]. For amperometric biosensors, a fixed potential is applied to a working electrode [10] [11]. The current, which is produced by the reduction or oxidation reaction that happed at the working electrode, between working and reference electrodes are is measured. The change in current according to the change in time is also measured. Voltametric biosensors have a similar working principle as amperometric ones. The only difference is that the potential applied to voltametric biosensors is fixed. Therefore, the change in current in voltametric biosensors is measured by the change in voltage.

2.2. CNTs functionalization

Functionalization means the walls of CNTs are attached by certain types of chemical functional groups or molecules without changing their properties that are wanted by people. There are two ways of functionalizing carbon nanotubes: covalent and noncovalent. The biggest difference between these two methods is that there are chemical bonds formed in the covalent method, but there are only interactions in non-covalent methods. The CNTs can functionalize with different molecules to satisfy different needs of solubility in different samples [12] [13]. Figure 1 shows several types of functionalization of CNTs with different molecules.

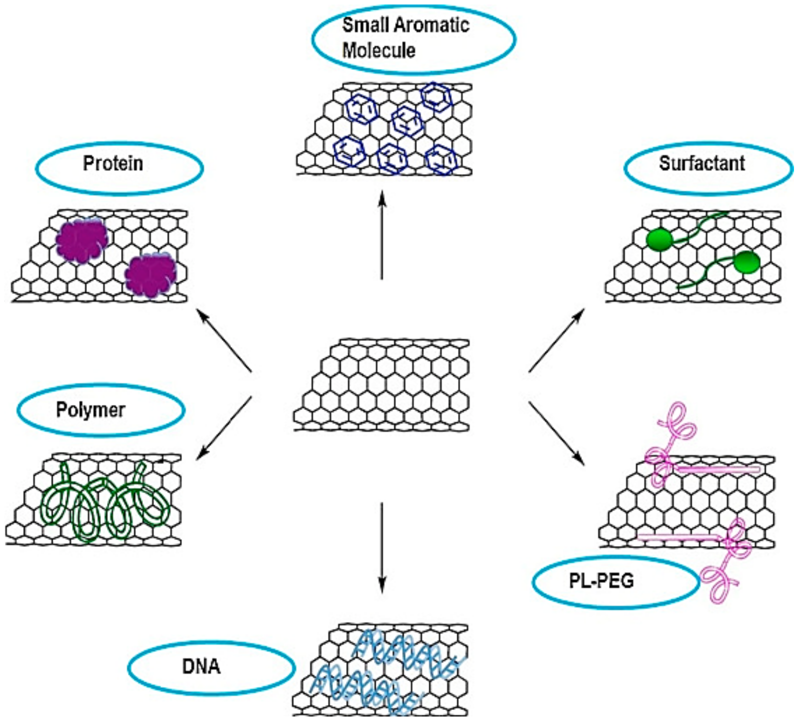

Figure 1. Different types of functionalization of CNTs with different molecules [5].

Covalent functionalization can also be further categorized as follows: electrochemical, oxidized, and photochemical methods. This means that molecules can be attached to CNTs through electrochemical reactions, oxidation reactions, and photochemical reactions. Compared with non-covalent methods, covalent methods usually need further modification since salts, which are used to deal with charge screening effects, are involved in the reactions. Another advantage of the non-covalent method is that it can prevent reducing the electrical abilities and breaking the structure of CNTs.

2.3. Immobilization of enzymes

To immobilize an enzyme means to move it to a specific position to ensure further usage [14]. The number of enzymes immobilized should be enough for an electrochemical biosensor to work. This process should be done carefully since the activity of enzymes may be lost when the chemical environment is not suitable for them. Immobilization of enzymes can be done either physically or chemically. The biggest difference is that covalent bonds will form between enzymes and the electrodes in chemical methods, but enzymes are only entrapped in the electrode surface in physical methods. Figure 2 shows four common methods of enzyme immobilization.

The relatively easy way is the entrapment approach. The adsorption method is also quite easy to be done. However, it is a relatively unstable way of immobilizing enzymes [15]. The reason is that enzymes and electrode surface is connected by weak intermolecular forces, and there are sometime hydrogen bonds that are also not stable. The covalent method usually requires certain conditions, and the biosensors made by the covalent method will have a longer lifetime than those made by the adsorption method.

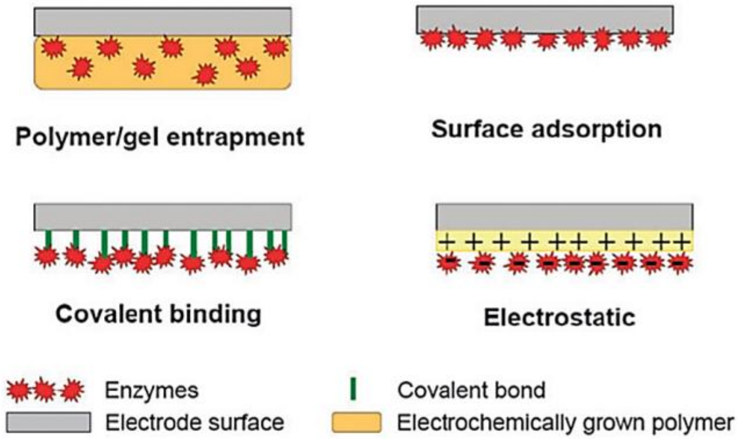

Figure 2. Four common methods of enzyme immobilization in electrochemical biosensors [7].

3. Applications of electrochmical biosensors

3.1. Enzymes-based electrochemical biosensors

Enzymes-based electrochemical biosensors are popular for commercial use. Enzyme-based electrochemical biosensors are comparably more sensitive and more selective [16]. As shown in Figure 3, enzymes-based electrochemical biosensors have been used to detect lots of biomolecules, including glucose.

3.1.1. Glucose detection

Blood glucose is a relatively important indicator of human health. Glucose biosensors are widely used in hospitals for the monitoring of blood glucose since some diseases are caused by high blood glucose. More than half of the biosensors in the market are glucose biosensors. Glucose Oxidase (GOx) is used in glucose biosensors [6].

Glucose reacts with oxygen to form gluconolactone and hydrogen peroxide. GOx acts as a catalyst in the whole process. Flavin adenine dinucleotide (FAD) is a molecule contained in the GOx. The overall reaction above can be divided into the following two steps. Glucose and GOx-FAD$^+$ first react to form gluconolactone and GOx-FADH2. Then GOx-FADH2 turns into GOx-FAD and hydrogen peroxide.

GOx-FADH2 acts as a mediator in the whole reaction. The amount of glucose can be determined by H_2O_2 produced. An electrode would oxidize the H_2O_2, and electrons would be released. Therefore, the concentration of glucose can be found by measuring the number of electrons released [17]. Hydrogen peroxide can be turned into oxygen, hydrogen ions, and electrons.

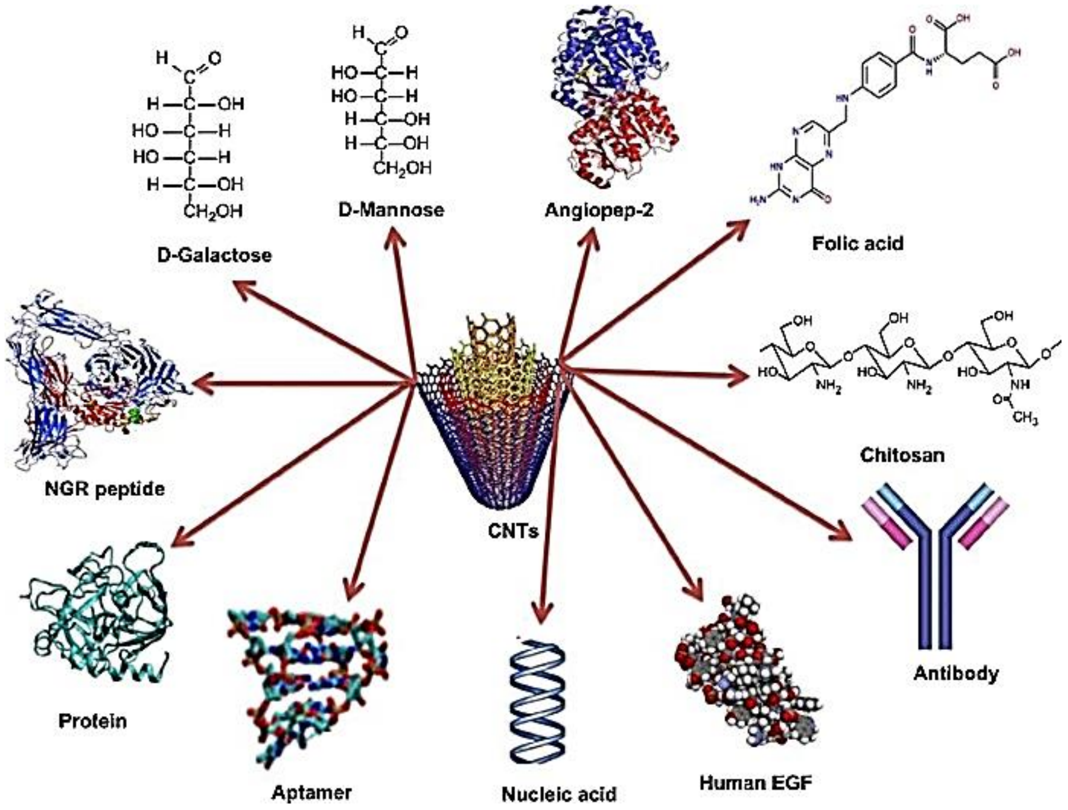

Figure 3. Applications of different biomolecules to CNTs [6].

The measurement can also be done by detecting the oxygen used in the reaction, or the electrons transfer of GOx. Chen et al. made a glucose biosensor using a multi-walled carbon nanotube-based electrochemical biosensor [18]. Silver nanoparticles (AgNPs) are combined with carbon nanotubes to form AgNPs-MWNT, which is a special application of CNTs. As described above, GOx, a kind of enzyme used to react with glucose, has been immobilized on the surface of the carbon nanotubes. GOx attached to the AgNPs-MWNT is beneficial for the reaction between glucose and GOx. Another point is that AgNPs-MWNT can improve carbon nanotubes' ability to reduce the reaction. It has also been mentioned in the article that AgNPs-MWNT has great conductivity and can carry a large amount of protein. This characteristic enhances the direct transfer of electrons of GOx, making the transfer quicker. The last two remarkable advantages of AgNPs-MWNT electrochemical biosensors are that they can preserve the activity of enzymes well, and they can be reproduced.

3.1.2. Cholesterol and dehydrogenase detection

Cholesterol is also very important for monitoring human health. Cholesterol oxidases (ChOx) are used in cholesterol biosensors. Cholesterol reacts with oxygen to give hydrogen peroxide and 4-Cholesten-3-one. ChOx also reacts as a catalyst. The determination of cholesterol can also be done by detecting the amount of H_2O_2 produced.

Dehydrogenase biosensors have a similar working principle as glucose and cholesterol biosensors. Many dehydrogenases can be used to make a dehydrogenase biosensor [6]. The dehydrogenase biosensor also works by measuring the current produced in the reaction that happened inside the biosensor. The substrate reacts with NAD+ with the presence of dehydrogenase to form a product and NADH. Dehydrogenase here is the enzyme used to detect the target molecules.

Alcohol dehydrogenase biosensors, for example, can be used directly to detect the alcohol in alcoholic drinks, including wine and beer.

3.1.3. Other enzyme-based biosensor

Other enzyme-based biosensors have been used in lots of other fields too. They have important usage in diagnosing diseases and solving environmental problems. Some of the biosensors can also be used in food production to monitor the concentration of certain molecules or be used to detect the concentration of certain molecules in pesticides. The immune biosensor is another widely-used type of biosensor in the real world. It has been mainly used in hospitals. Immune biosensors basically use the combination between antigen and antibody to detect target molecules. Different antigens would be combined with different antibodies. Therefore, different chemical signals would be produced, and different molecules could then be recognized. The application of CNTs enables the accurate detection of various biomolecules, such as proteins. Furthermore, single-walled carbon nanotubes enable the accuracy of the detection, making more precise detections possible.

3.2. DNA-based electrochemical biosensors

DNA-based electrochemical biosensors are used to diagnose gene-related diseases. These biosensors work by finding the nucleic acid in the testing samples. The reaction would take place in the electrochemical biosensor. This reaction would then turn into signals and be received by the signal component. CNTs allow the improvement in their responsiveness. DNA-based electrochemical biosensors can be used to detect lots of molecules, including protein and various metal ions. Since DNA aptamer is relatively stabler, many electrochemical biosensors would use it instead of protein and other molecules. MWCNTs can be used to recognize certain DNA strands or sequences. The structure of SWCNTs and MWCNTs is shown in Figure 4 below.

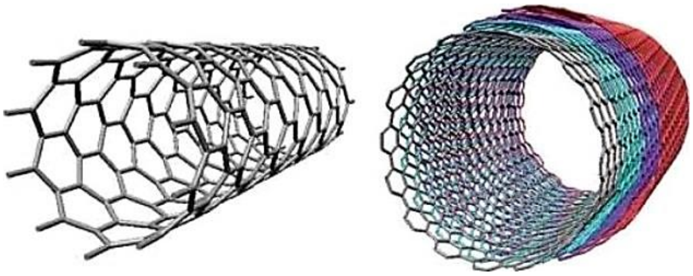

Figure 4. The structures of SWCNTs and MWCNTs [6].

3.3. Existing limitations

There are still some limitations of carbon nanotube-based electrochemical biosensors, although they are popular materials for biosensors. The first limitation is that the electrochemical biosensors require CNTs of a certain size [19]. However, it is quite difficult to turn CNTs into a specific size. Secondly, CNTs-based electrochemical biosensors have requirements for purity. Some of these biosensors need a purity of 99.99%, which is hard to achieve. Therefore, currently, CNTs-based electrochemical biosensors are hard to be mass-produced for commercial use. Finally, CNTs usually need enzymes immobilization. The problem is that immobilization may affect come desirable properties of CNTs, and even damage them. This will eventually affect the usefulness and effectiveness of CNTs-based electrochemical biosensors [20].

4. Conclusion

In this research, several kinds of CNTs-based electrochemical biosensors are introduced and explained. Enzyme-based electrochemical biosensors and DNA-based electrochemical biosensors are the two main types of electrochemical biosensors introduced in the paper. These electrochemical biosensors are classified due to the molecules attached to these biosensors, but they can also be classified due to the way that the enzymes are immobilized. Simple working principles and reactions involved in different biosensors are also mentioned in the corresponding section. Working principles of CNTs-based electrochemical biosensors are also explained in this paper. The research mainly focuses on enzymes-based electrochemical biosensors, and a specific example is provided. At the end of the research, some limitations of electrochemical biosensors are mentioned and explained roughly.

5. Authors' Contributions

Aiwei Jia completed the work design and article writing.

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