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The Application of Single Wall Carbon Nanotube to Synthesis Glucose Biosensor

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ABSTRACT

In this paper, the carbon nanotube was applied to prepare an appropriate bed for immobilization of Glucose Oxidase enzyme. Numerous operational advantages of glucose oxidize enzyme matrix may include: efficiency and more accurate control over enzyme reaction, protection against destruction and waste of enzymes, the use of matrix enzyme have a high efficiency and more control of enzymic reactions. For immobilization and stabilization of enzyme on the bed, the prepared matrix was washed by sulfuric acid, nitric acid and enough ionized water. The matrix surface, for restrain distort enzyme, was covered by Sistamine. The glucose oxidize enzyme was connected on the matrix by intermediate material; one side of intermediate material is amid group and another side is pyren group. For elimination of impurity, the prepared matrix was placed into Dimethylformamide (DMF) for several hours. The activity parameter of enzyme was measured by Spectrophotometer in the 460 nm wave length; the immobilized enzyme could be stabilized for long time. This matrix could be used as an electrode in sensors structure.

Keywords: single wall carbon nanotube, glucose oxidize enzyme, chemical vapor deposition, biosensor.

INTRODUCTION

Regarding the revolution which has been occurred in electronic devices specially biosensors through recent decades; it seems that many possibilities have being made to synthesize electrodes in very fine scale (nanometric sensors) by aiding of nanoscience and technology [1]

Some kind of these sensors due to their nanometric size and application in biological environment are called nanobiosensor. The nanobiosensors are minute electrodes in nanometric size and cellular dimension which are to detect specific chemical or biological species through immobilizing special enzymes on the electrode surface. Nowadays one of the most available nanostructures which are being produced in different parts of the world and Iran as well is carbon nanotube that not only its physical and chemical characteristics are easily available but also might be a good beginning for any research about biosensors, so if there is any possibility to take advantage of the abilities in this case, should be considered [2, 3].

Regarding to highly increasing level of operation in carbon nanotube specifically in SWCNT, their application in some parts of biosensors and also immobilizing the biological receptors (like enzyme) upon them could be led to: increase enzyme reacting operation, a controllable reaction, more enzymes participation in reaction, preventing destruction and waste of enzymes, having easy application and portability as well [4].

In order to prepare an appropriate bed to immobilize enzyme (in biosensors discussion), the grown up carbon nanotube should be applied upon metal bed such as gold which has more stability and availability. It should be also noted that having small settlement surface of receptor (enzyme) will cause a constant stability which is considered as an applying advantage in biosensors synthesis [5]. Through this technology, nanotube oxidation will be served to immobilize enzyme (glucose oxidase) upon nanotube wall which is already carboxided in every part. In this system, bed surface is in duty of the changes being occurred by reaction to be conveyed to Transducer device to show the signals.

Carbon nanotube is also capable of playing dual roles:
- First role as an enzyme immobilization place
- Second role as an intermediate between reactant and transducer [2].

It has been observed that in nanobiosensor technology the large proteins will be entrapped on carbon nanotube surface through a simple absorption. These junctions might be significantly appeared on the outer surface by electrostatic and hydrophilic reactions or covalence bond, therefore on the basis of these bonds the kind of intermediate component would be specified and applied [4,6-9].

EXPERIMENTAL SECTION

The operation process of this paper has three stages:
1- Preparing bed coated by SWCNT and making it ready for enzyme immobilization
2- Preparing enzyme to be immobilized upon the bed
3- Performing immobilization process by applying intermediate and stabilizer components and making the enzyme activity return.

Preparing bed coated by SWCNT and making it ready for enzyme immobilization
To prepare this bed many essential steps should be taken to make sure if its function as a base in synthesizing sensors and biosensors is appropriate.

Preparing Gold Layer

In this stage the gold layer is prepared with 10mmX10mm dimension and 1mm thickness. Since the applied gold layer is natural and has a rough surface (Fig.1) so needs to be smoothed completely before any process. This is because of making an appropriate distribution of catalyst particles over the surface, also will cause to grow up the nanotubes properly. However this preparation is accomplished by making homogenous vapors (gold vapors) to be deposited upon gold layer. After this process, all of the holes and opening being over the layer will be almost covered and catalyst is distributed over the surface in a good order.

In this case the thickness of the homogenous layer is about 4-5µm as well. The surface should be cleaned two times by solution of %98 Sulfuric Acid (H2SO4) and Nitric Acid (HNO3) and also should be completely cleaned by plenty of ionized water in order to eliminate the formed impurities and unnecessary over surface.

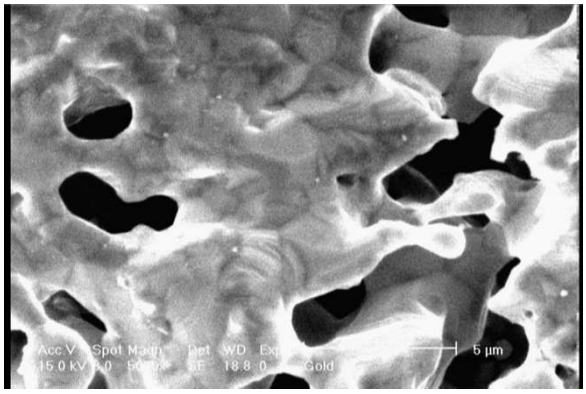

Fig.1. Gold layer scanned by SEM before preparation.

Catalyst particles positioning

After preparing gold layer, it is right time for nanotubes growth nuclei to be constructed. It should be underlined that if no nucleus construction happens, no nanotube growth could be expected even if some growth appears, that would be a quite disorderly growing.

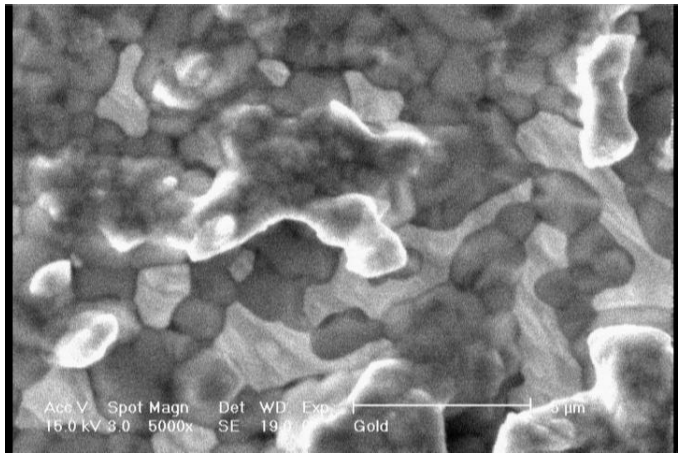

Fig.2. Gold layer scanned by SEM after positioning nanocatalyst & particles.

In this case a Nickel catalyst is used. The catalyst nanoparticles will be regularly settled on the surface through lithography method with the size about 4nm and normal compression as well. These nanoparticles will act as the beginning growth point so that their ordered positioning could absolutely effect on synthesizing a well-shaped product. This performance accomplished by incorporation of some Tehran University professors (Fig.2)

Carbon nanotubes growth

After positioning the growth nuclei upon gold layer, the nanotubes growing process would be performed. Therefore to accomplish this, first the prepared layer should be put in the vacuumed oven and then the light Hydrocarbon (in gas form) to be sprayed into the oven. After spraying the gas and occuring some chemical reactions inside the oven area, the carbon deposits will be appeared on the gold bed and cause to synthesize tube structure [6].

Because of the CVD process (Chemical Vapor Deposit) and regularl arrangement of the first nuclei upon the bed, the SWCNT would be grown over the bed vertically and in comb form under appropriate temperature and pressure conditions with 2-50nm thickness and 15-20µm height.

(This performance has accomplished in Oil Co. Research Center incorporation of some professors and experts engaged in the center) (Fig.3)

However the scanned pictures by SEM (Scanning Electron Microscope) related to the prepared bed during the recent performance would definitely complete the explanations above. (Fig.4)

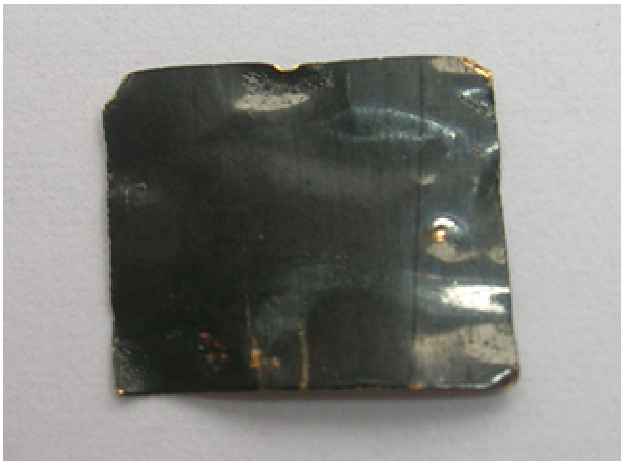

Fig.3. Gold layer coated by SWCNT.

The surface area which is being coated by these nanotubes has been measured about 10-15. Whereas each of the nanotubes plays a sensor base role, therefore it could be concluded that the sensor base growth would be about 108-109 units in total area of gold bed (100) so that the compression would be very appropriate to immobilize bioreceptors.

After preparing the SWCNT bed, the bed surface should be completely cleaned with solution of %98 H2SO4 and HNO3 also should be washed with plenty of ionized water two times. This will

cause the necessaries and surplus materials to be eliminated all around; therefore a new surface would be ready to be applied as sensor base [4].

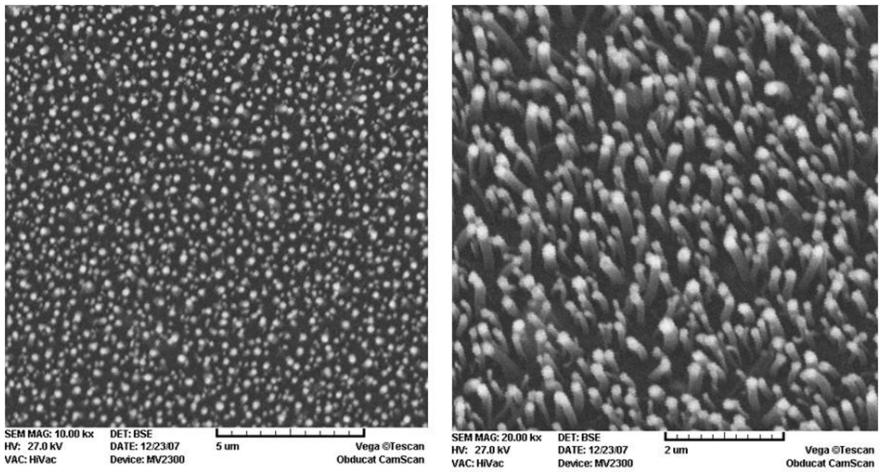

Fig.4. SEM pictures of grown up SWCNT on gold layer surface.

Preparing enzyme to be immobilized upon the bed

Many accurate and essential operations are performed (to be mentioned soon) regarding the enzyme high sensitivity to environmental and reactional conditions. Also the enzyme active points are to be protected through whole operation stages and the proper contact between prepared enzyme and bed surface (grown up nanotube) to be kept.

Preparing Glucose Oxidase Apoenzyme

With reference to above mentioned matters, first the enzyme active points should be separated and a covering should be put on the enzyme. What makes this process possible is separating FAD from enzyme structure and preparing Apoenzyme as well.

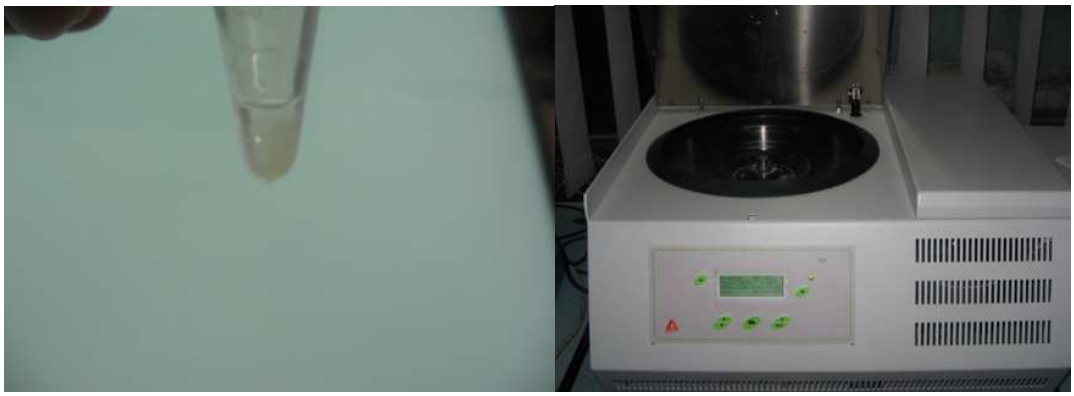

Fig.5. A, High spin centrifuge device. B, source of Apo enzyme deposit.

To carry out this job, a saturated solution of (NH4)2SO4, should be provided then the solution PH should be controlled to about 1.4 by %98 sulfuric acid in 20oC temperature. At the moment, 20 mg/ml glucose oxidase enzyme in phosphate bapher was added to 20ml solution which is

saturated in 5oC temperature (drop by drop) and then will be stirred. This solution should be kept in mentioned degree of temperature for half an hour.

After a while the upper yellow layer would be removed through 15 minutes centrifuge with 20000 rpm (Fig.5.A). Now the final deposit (Fig.5.B) has centrifuged and mustered again under the same conditions of salt and acidity PH, then should be solved at Sodium Phosphate bapher as a source of glucose oxidase Apo enzyme at the end.

Performing immobilization process by applying intermediate and stabilizer components and making the enzyme activity return while both enzyme and bed got ready to be immobilized, the immobilization process should be started in common and appropriate conditions. Since the gold electrode has been applied in this project and on the other hand enzyme direct contact to metal will make it unnatural, the electrode surface should be coated with a Cistamine layer.

However when the single-layered Cistamine possessed proton particles, in fact it has got a positive charge, therefore would be capable of creating Electrostatic traction with glucose oxidase enzyme which has negatively charged and the contact between bed and enzyme could be facilitated as well [10,11].

In order to make this contact, an intermediate component is needed which should have a Pyrene group in one side to make Vander walls bond to carbon nanotubes also should have an Amid group on the other side to make contact to enzyme.

The intermediate component applying in this project is 1-pyrenebutanoic acid succinimidyl ester. As a whole the operation process could be briefly described as follows:

First the prepared bed should be put into the solution of intermediate component (in DMF) with 2.3 mg/ml viscosity and then should be stirred for 2 hours. After spending the time the electrode should be brought out of the solution and will be cleaned with pure DMF.

Parallel to this operation, the prepared Apoenzyme will be solved in filtered and deionized water with 10 mg/ml viscosity. The cleaned bed will be in contact with the solution for 18 hours and finally would be washed with very clean water for almost 6 hours.

Since in the previous steps, the enzyme had been turned to Apo form for more safety, so it is needed to activate it again after immobilization process. If we pay attention to the enzyme structure, will find out that the presence of FAD molecule is inevitable to make the enzyme activity return again.

Therefore to achieve this purpose 200µm FAD molecule should be mixed with Apo enzyme (150µ g/ml viscosity) in potassium phosphate buffer (0.1M & PH=6.0) in room temperature and then should be incubated for 1 hour. This operation will construct the stable complex of FAD protein which is actually the same reconstructed enzyme and its dissociation constant is very small (K<10M).

After immobilization process the enzyme activity range of anchobated mixture should be measured after putting it in room temperature for 30 minutes in order to determine the activity range and also to make sure if the activity will return again .Of course this measurement is based on O-Dianizidin Method.

Through this method the prepared bed will be put in the solution of testing potassium phosphate bapher (Testing bapher is prepared by solving 0.1 ml of O-Dianizidin indicator 1% in 12 ml of phosphate bapher with 0.1 M viscosity and PH=6) with: 10µl of glucose 18% (in water), 10µl of peroxidase enzyme with 200µ g/ml viscosity, 10 µl of glucose oxidase (with dilution of 200) with 1 mg/ml viscosity and the absorption range is measured in wave-length of 460nm as well. (Measuring absorption range in fact will show us the enzyme glucose consumption range and consequently the enzyme activity range [1]). The changes range has also measured in the same wave-length of 460nm and in different intervals to study the constancy of activity.

The range of immobilized enzyme activity has been computed 280 (u/mg) at first minutes. After a while the activity range will be decreased. It should be mentioned that since the bed is put in phosphate bapher solution and is cleaned carefully, after each step of activity measurement, the affection of free (disimmobilized) enzymes presence upon the activity range would be less probable.

Therefore we can contend the measured activity will be totally related to the immobilized enzymes upon the bed.

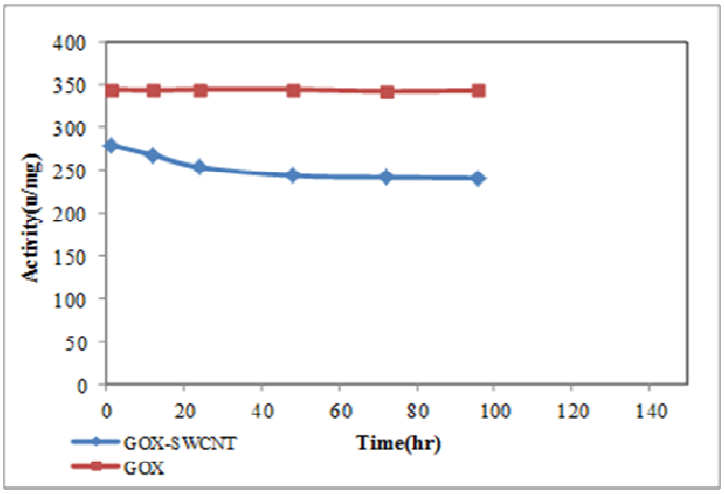

Fig.6. enzyme activity on the basis of time indicates constancy range of immobilized enzyme.

Respect to this point, we realize that studying the changes of activity range would probably show the immobilization constancy which could be led to construct a bed in nanoscale to be able to synthesize nanobiosensors at the end. On the other hand, diagram No.1 is to show us the changes of enzyme activity on the basis of time which will confirm the above mentioned points [12,5].

After performing this operation, we have accessed to a bed which consists of a highly qualified enzyme immobilized upon so that will be ready to be applied as a sensor or biosensor transducer part.

CONCLUSION

Applying nanostructures in sensors synthesis could increase the output and sensitivity and also can decrease resistivity during sensors operation. In addition to this advantage nanobiosensors could be used in very sensitive and delicate cases for they have too many options as well.

Regarding the operation level and high flexibility and conductivity of carbon nanotubes in comparison with other metals, we would get the result that nanotubes are very appropriate to immobilize the different chemical and biological receptors.

What is definitely important to synthesize the high qualified sensor is following the mentioned steps and rules due to the nanotubes growth and immobilizing the enzyme upon the bed. However making normal compression (mentioned in the text) in grown up nanotubes will have effect on the enzyme influence range and its immobilization range as well.

Although inactivating enzyme temporarily and making its activity return after immobilization process would deform some enzymes third generation but will not allow the immobilized enzymes to be inactivated permanently ,still protect them through fixation process.

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