

Biosensors for detection of hydrogen peroxide, L-ascorbic acid and catechol in form of student experiments

Abstract

Background: A biosensor is an analytical device, used for the detection of an analyte that combines a biological component with a physicochemical detector. The aim of this paper is to demonstrate the possibilities of simple nanotechnology application in the form of biosensors for the detection of H₂O₂, L-ascorbic acid and catechol. By performing these experiments, students gain experience, be innovative, and understand biosensors. The experiments used amperometric detection of the sample with modified carbon paste and print electrodes, which we modified ourselves. The experiments demonstrated the success of the biosensor application and the ability to interpret the results. The emergence of unnecessary peaks stemming from background current, as a disturbance, can be avoided with longer time stabilization of the method, using cleaner chemicals and preventing the appearance of bubbles.

Keywords: amperometric detection, modified carbon paste and print electrodes, biosensors, background current, student experiment

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Introduction

There are different types of biosensors based on the sensor devices and the biological materials and some of them are discussed below (based on literature number 1, 2 and 3).

Electrochemical biosensor

Electrochemical biosensors is a simple device. It measures the measurement of electronic current, ionic or by conductance changes carried by bio-electrodes (Figure 1).

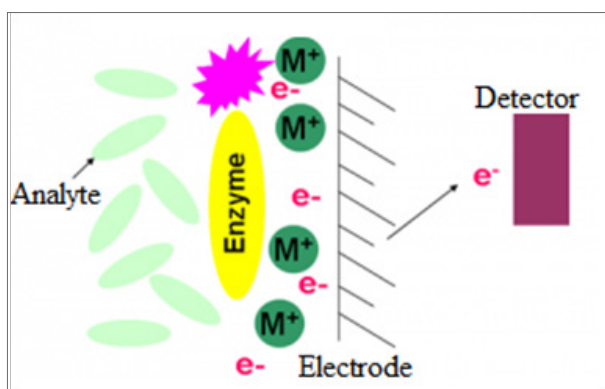


Figure 1 Electrochemical biosensor.

Amperometric biosensor

The biosensors are based on the electrons movement, i.e. electronic current determination as a reaction of enzyme-catalyzed redox reaction. Generally a normal contact voltage passes through the electrodes to analyze. In the enzymatic reaction which produces the substrate or product can transfer the electrons with the surface of electrodes to be reduced. As a result an alternate current flow can be

measured. The substrate concentration is directly proportional to the magnitude of the current. The reduction of oxygen is acquired through the oxygen electrodes and it is a simple way to form an amperometric biosensor (Figure 2). The example is the determination of glucose by glucose. The above description is about the first generation of amperometric biosensor and it has a direct transfer of electrons which are released from the electrodes are having some difficulties. The second generation amperometric biosensors are developed in a mediator takes the electrons and transfer to the electrodes.

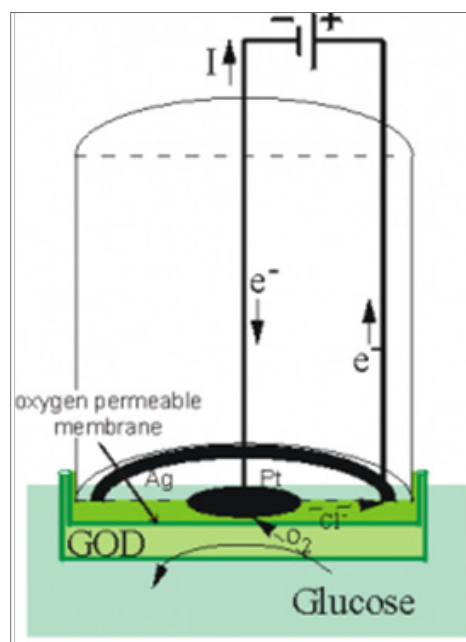


Figure 2 Amperometric biosensor.

Blood glucose biosensor

The blood glucose biosensors are used widely throughout the world for diabetic patients. It has a single use disposable electrodes with glucose oxidase and derivatives of a mediator (Ferrocene) and the shape of the blood glucose biosensor looks like a watch pen. With the help of hydrophilic mesh electrodes are converted. The blood glucose biosensor is a good example of amperometric biosensor. Swelling of the film and enzymatic reaction are followed by an electrochemical reaction (Figure 3). In these sensor films the active components are immobilized in a matrix consisting of various polymers and other additives.¹

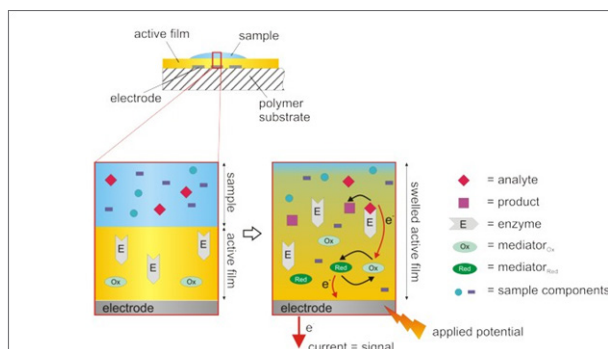


Figure 3 Schematic drawing of an amperometric blood glucose sensor.

Potentiometric biosensor

In this type of biosensors changes the concentration of ionic is determined by the ion-selective electrodes in this pH electrodes are used most commonly. Hence a large amount of enzymatic reactions is involved in the release of hydrogen ions. Ammonia-selective and carbon dioxide selective electrodes are some other important electrodes. The potentiometric electrode and the reference electrode can be measured with the help of potential difference and it is directly proportional to the substrate concentration. The potentiometric biosensors is the sensitivity of enzymes to ionic concentration like H^+ and NH_4^+ .

Conduct metric biosensor

In the biological system there are several reactions that change the ionic species. The electronic conductivity can be measured with the help of an ionic species. The example of the conduct metric biosensor is the urea biosensor which utilizing the immobilized areas.

Thermometric biosensor

There are many more biological reactions are connected with the production of heat and it forms the basis of thermometric Biosensors.

Optical biosensors

The optical biosensor is a device, it utilizes the principle of optical measurements like fluorescence, absorbance and etc. The optical biosensors are safe for non electrical remote sensing of materials. In the transducer elements primarily optical biosensors involves in the enzymes and antibodies. Usually the biosensors is not required any reference sensors and the comparative signals are generated by using the sampling sensor.

Fiber optic lactate biosensor

The working of the fiber optic lactate biosensor is based on the

measurement of change in oxygen concentration, molecular by identifying the effects of oxygen in fluorescent dye. The following reaction is reduced by the enzyme lactate mono-oxygenase. The oxygen depends on the amount of fluorescence generated by the dyed film this is because of oxygen has a reducing effect on the fluorescence. In the reaction mixture the concentration of lactate is increased, oxygen is utilized and as a result, there is a proportional decrease in the quenching effect. Hence there is an increase in the fluorescence output can be measured (Figure 4).

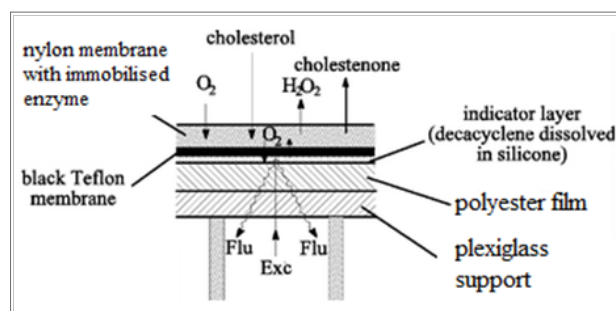


Figure 4 Fiber Optic Lactate Biosensor

Piezoelectric biosensors

The principle of piezoelectric biosensor is used in sound vibrations, hence it is called acoustic biosensors. The basics of the biosensors are formed by the piezoelectric crystals and the characteristic frequencies are trembling with the crystals of positive and negative charge. By using the electronic devices we can measure the certain molecules on the crystal surface and alters the response frequencies using these crystals we can attach the inhibitors. The biosensors for cocaine in the gas phase has been developed by attaching the antibodies cocaine to the surface of the crystal (Figure 5).

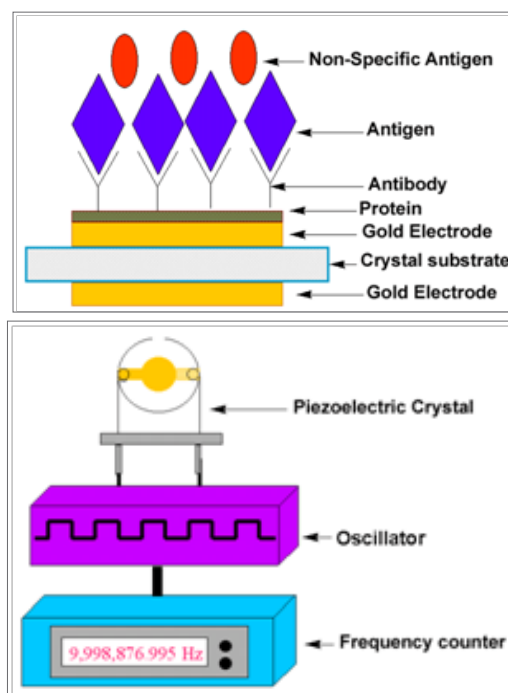


Figure 5 Piezoelectric Biosensors

Immuno-biosensors

The immune biosensors works on the principle of immunological specificity and mostly coupled with measurement on the potentiometric biosensors. There are different configuration of probabilities for immune biosensors some of them are given below and the Figure 6 shows the description.

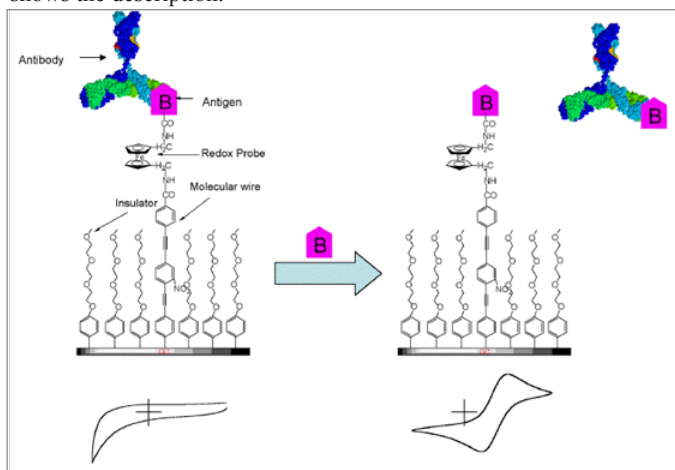


Figure 6 Immuno-Biosensors

- I. The immobilized antibody can directly combine through the antigen
- II. The immobilized antigen can combine with the antibody which can twist to a second free antigen.
- III. The immobilized antibody combined with the free antigens and enzyme labeled antigen in opposition.

I hope the given information in the article is helpful to give some good information and understanding the biosensors. There are several applications of biosensors in food analysis. In the food industry, optics coated with antibodies are commonly used to detect pathogens and food toxins. Commonly, the light system in these biosensors is fluorescence, since this type of optical measurement can greatly amplify the signal. The sensitive biological element,

Table I Display the main experimental parameters and results

Method	I electrode	II electrode	III electrode	Buffer	Potential	Stabilization time	Detection
Amperometric	Ag / AgCl	Pt	MnO ₂	Phosphate buffer of 7.5	0,44 V	3000 s	Hydrogen peroxide
Amperometric	Ag / AgCl	Pt	MnO ₂	even at pH 5	44 V	4000 s	L-ascorbic acid
(Chrono) amperometric	Ag / AgCl	Pt	Banana	Phosphate buffer of 7.5	- 0,8 V	5000 s	Catechol from beer

Hydrodynamic amperometry (HA) was performed with an electrochemical workstation PAR 273A potentiostat/galvanostat (EG&G Princeton Applied Research, Princeton, NJ, USA). A magnetic stirrer and a Teflon-coated stirring bar (approx. 300 rpm) provided the convective transport. A platinum wire served as a counter electrode and an Ag/AgCl electrode (Model 6.0733.100; Metrohm, Switzerland) as a reference electrode. Buffer solutions and fresh measuring solutions were purged with for at least 20 min with helium (99.995%) via a glass tube. Display graphs were recorded on

e.g. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc., is a biologically derived material or biomimetic component that interacts, binds, or recognizes with the analyte under study. The biologically sensitive elements can also be created by biological engineering. A biosensor typically consists of a bio-recognition site, biotransducer component, and electronic system which includes a signal amplifier, processor, and display. Transducers and electronics can be combined, e.g., in CMOS-based microsensor systems.^{2,3} The recognition component, often called a bioreceptor, uses biomolecules from organisms or receptors modeled after biological systems to interact with the analyte of interest. This interaction is measured by the biotransducer which outputs a measurable signal proportional to the presence of the target analyte in the sample. The general aim of the design of a biosensor is to enable quick, convenient testing at the point of concern or care where the sample was procured. However, students tend to develop their understanding of science concepts in accordance with formal teaching as well as their everyday experience. Therefore, students could have conceptions that do not agree with scientific laws and theories, and teaching itself can be one cause for them.⁴ They can be caused by teachers who use inadequate instructional methods,⁵ by some representations of particulate nature of matter in existing textbooks.⁶ Also, teachers without sound scientific background can introduce misconceptions to their students.⁷ Ausubel (1961)⁸ states that students' cognitive structure of knowledge impacts on interpretation of new knowledge. When students are exposed to new information, the misconceptions that are already incorporated in their cognitive structure affect the integration of new knowledge. This causes weak or wrong understanding of new concepts.^{8,9} One of the teaching strategies that showed good results in teaching science is conclusion based on own experiment.

Methods

Apparatus

The method is based on the application of the trowel system (Table 1):

- A. Ag / AgCl (I electrode)
- B. Pt electrode (II electrode)
- C. Working electrode (III electrode)

a personal computer with an appropriate interface for analogue-to-digital conversion.

Reagents and solutions

Analytical reagent grade chemicals and double-distilled water were used. Phosphate buffer was prepared by mixing 12.62 mL 0.05 M Na₂HPO₄ and 2000 mL 0.05 M NaH₂PO₄. A buffer stock standard solution containing 10 g L⁻¹ ascorbic acid was prepared freshly each day. Working standard solutions of lower concentrations were

prepared immediately before use. L-ascorbic acid analytical grade was obtained from Fluka. All other compounds were obtained from Merck.

Fabrication of the electrode

For each working electrode we prepared different mixture of component. In experiment of amperometric detection of L-ascorbic acid we used modified carbon ink (5%). It was prepared by thoroughly mixing 4.75 g carbon ink (UK) with 0.25 g manganese dioxide (Merck). The MnO_2 modified carbon ink was sonicated for 20 min and used immediately for electrode fabrication. The working electrodes were screen printed on inert laser preetched ceramic supports (Coors Ceramic GmbH, Chattanooga, TN, USA). The preparation consisted of applying thick layers (0.05 mm) of the ink onto the substrates through an etched stencil with the aid of a screen printing device (SP-200, MPM, Franklin, Ma, USA). The resulting plates were dried at 60°C for 1h. In experiment for amperometric detection of H_2O_2 we prepared biosensor based on the incorporation of glucose-oxidase as biocomponent and MnO_2 as a mediator for carbon paste electrode. In experiment of amperometric detection of catechol from beer, the working electrode is prepared by mixing paraffin oil, carbon and bananas as a modifier.

Analysis of samples

Pharmaceutical tablets containing L-ascorbic acid were bought from the pharmaceutical company. Samples were dissolved in 49.5 mL 0.05 M phosphate buffer pH 5.0 and 1 mL of that solution was diluted to 10 mL. Quantitative determinations were made with hydrodynamic amperometry by adding standard solutions and samples solution successively.

Amperometric detection of H_2O_2 with modified carbon paste electrode

Stabilization of the method is done at a potential of 0,44 V for a duration of 3000 s. In the solution (in which the electrodes) is a phosphate buffer of 7.5, then H_2O_2 is added. If we use HPLC instrument, the sample flow will be continuous. The instrument sensitivity is up to nanoampere, it is excellent for preliminary tests. The biosensor based on the incorporation of glucose-oxidase as biocomponent and MnO_2 as a mediator for carbon paste electrode.

Amperometric detection of L-ascorbic acid with modified carbon paste electrode

The method is based on the application of the trowel system of electrodes: Ag / AgCl, Pt and working electrode. The method used the MnO_2 like modifier for carbon electrode in the three-cell system. We used a HPLC that pushed a continuous sample through the system, through a buffer, and the result was a Figure 8. The working electrode is in the form of a print electrode, and the same properties as described in the previous exercise, gives a linearly dependent response to the concentration, the other electrodes are the same. The sensor electrode is very stable even at pH 5, and its composition is MnO_2^+ carbon paste. The used potential was 44V, the stabilization time was 4000 s, at the set flow rate of 0,2 mL / min. We used sodium L ascorbate as a test sample.

Amperometric detection of catechol from beer with modified carbon paste electrode

Argon was introduced through beer to remove dissolved O_2 and CO_2 . The working electrode is prepared by mixing paraffin oil, carbon and bananas as a modifier. The result is a very sensitive sensor. An electrochemical biosensor containing an enzyme polyphenol oxidase, and it catalyses the oxidation of polyphenol as an antioxidant. The biosensor can be tested with catechol from beer. The three-cell system immersed in phosphate buffer, pH 7.5. We used the potential of -0.8 V, the stabilization time of 5000 s. We used chronoamperometric method. The quantities of inserted samples were: 100, 50, 50, 10, 100 μL . A summary of the all set parameters is shown in Table 1.

Results

Graphics from experiment, which we made, are similar. We decided to show the most characteristic of them, which are most interesting in student discussion. In chronoamperometric method, in experiment with banana like biosensor, dopamin + buffer added to the beaker to induce a leap, or amperometric response to the receptor, and obtain the following Figure 7.

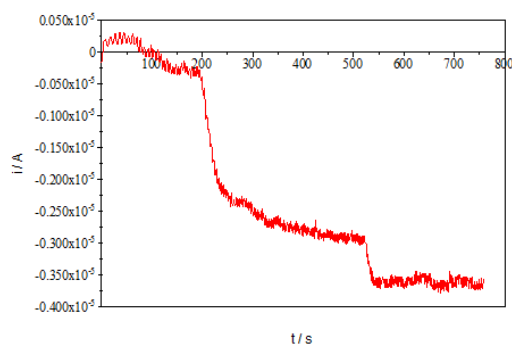


Figure 7 Specific chronoamperometric graph with banana like biosensor.

The same samples used for HPLC determination were 100, 50, 50, 10, 100 μL and we obtain the following Figure 8.

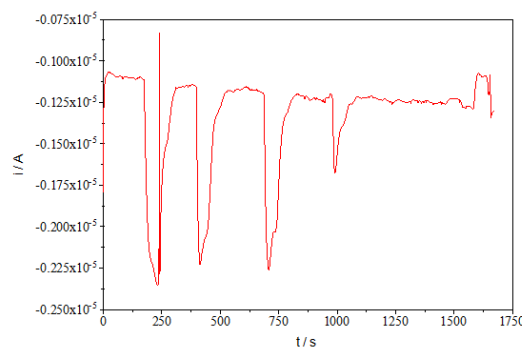


Figure 8 Specific amperometric graph with banana like biosensor.

Discussion

The biosensor is used to detect the analyte so the biosensor is an analytical device and it gathers the biological components with a physicochemical detector. The sensing biological elements are biometric components interact with the recognize and analyze the study and the components like tissue, microorganisms, antibodies, nucleic acids and etc. The sensitive elements of biological can also generate by the biological engineering. The detector elements transform the signals from the interface of analyte with the biochemical elements into other signals like transducer and it can be measured more easily and qualified. The biosensor devices are associated with the electronics and the signal processors and they are generally responsible for the display of the results and they are user-friendly. The biosensor research has a significant role in the development of modern electronics.^{1, 10, 11} The method is based on the application of the trowel system (Table 1): Ag / AgCl like reference electrode; Pt electrode like counterelectrode (it closes the electron circuit) and working electrode like tile sensor, which can be of different composition. The key element is that students build a working electrode. One of recepture was showed in Method-part. For the detection of H_2O_2 we used biosensor as for glucose, as shown by the following reaction on Figure 9, and the biosensor based on the incorporation of glucose oxidase as a biocomponent and MnO_2 as a carbonate paste modifier (on carbon print or carbon paste electrode). The second experiment has the same essence of the test, and the ultimate result is that students gain the impression of creating a new biosensor. When working on HPLC, it took a lot of time to look for optimal parameters from which the clarity of the final graphics depends. The reactions relevant to the second experiment are simplified in Figure 10. The electrochemical oxidation of L-ascorbic acid (H_2A) at various electrode surfaces has been a subject of great interest because of the significance of this compound

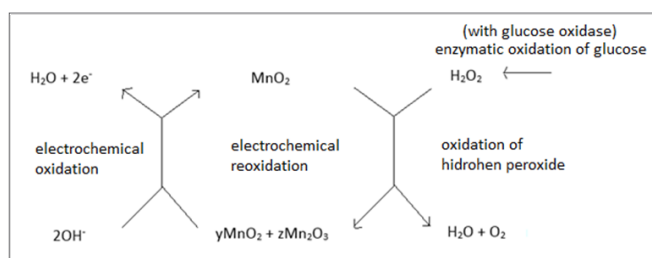


Figure 9 Key reactions for the hydrogen peroxide detector.

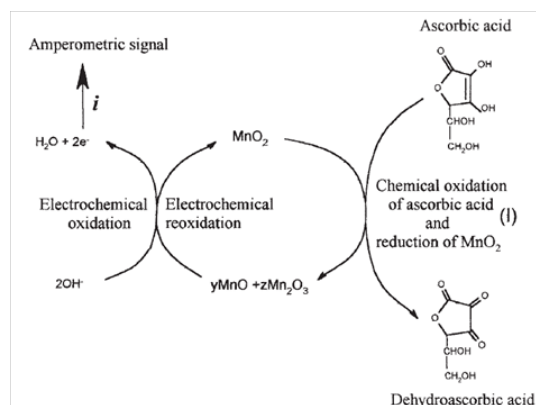


Figure 10 Reaction mechanism of ascorbic acid.

in biological-biochemical (e.g., neurochemistry) and electrochemical (e.g., electrocatalysis) settings.¹² The reaction mechanism of ascorbic acid at an MnO_2 modified sensor based on heterogeneous carbon material is shown in Figure 10 which demonstrates¹² chemical and electrochemical reactions occurring in the recognition layer of the sensor.

Ascorbic acid reacts chemically with MnO_2 producing manganese species at lower oxidation states (Figure 11) which can be electrochemically reoxidized to Mn^{4+} (Figure 10). The oxidative current can be directly related to the ascorbic acid concentration. The sensor for amperometric detection of L-ascorbic acid could be operated at pH 5.0 (0.05 M phosphate buffer) and exhibited excellent reproducibility and stability.¹² The concentration of MnO_2 in the screen-printed electrode has a significant influence on the voltammetric signal. Presumably more MnO_2 at the electrode surface reduces the amount of conductive areas (carbon particles). The sensor response showed that it is sensitive to L-ascorbic acid. Figure 7 & Figure 8 describe the basic graphics that occur when testing new sensors and are key to defining the performance of the experiment. Figure 8 show unnecessary pik (on 250 s), and we suggest to make a similar spike with the intent to discuss its cause with students. Difference in the potentials and stabilization time which we see in Table 1 talking about the originality of the (bio)sensors. Each (bio) sensor has different parameters and it is necessary to detect these parameters on the exercises, together with the students. The peak from Figure 8 is triggered by background current, disturbances, and can be avoided with the longest time of stabilizing the method, the use of cleaner chemicals and paying attention to the occurrence of bubbles that interfere with the process. The effect of temperature was not investigated in this paper.

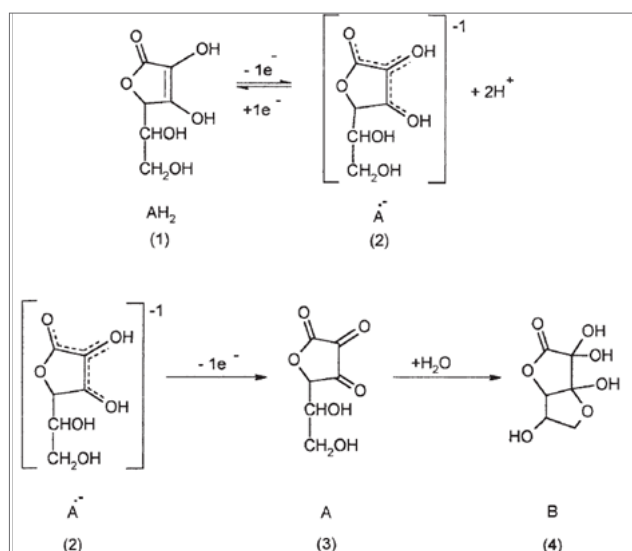


Figure 11 Oxidation of ascorbic acid.

Conclusion

Biosensors are extremely small, they use incomparably less energy for their functioning, or drawing it from the environment in which they operate. Their miniature dimensions allow the installation of

nanosensors in an extremely wide range of applications, from the surfaces of walls and large structures, metal surfaces, to the human body. The manganese dioxide modified screen paste electrode shows long term stability. We find them in nature and we have the ability to develop many detectors based on them. Simple exercises and group discussion can awaken the passion for innovation in students. Innovative biosensor-based student exercises increase understanding of the biosensors.

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Conflict of interest

Authors declare that there is no conflict of interest.

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