L-Ascorbic Acid Biosensor Based on Immobilized Enzyme on ZnO Nanorods

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Abstract

In this present research, we have grown the well aligned, perpendicular to the substrate and highly dense ZnO nanorods on the gold coated substrate by using the hydrothermal growth method. The ZnO nanorods were functionalized by immobilizing the ascorbate oxidase enzyme with cross linking molecule 3-glycidoxypropyltrimethoxysilane (GPTS). The electrochemical measurements were carried out through using potentiometric technique, we observed that the output response exhibited by the biosensor to the wide linear dynamic range concentration of L-ascorbic acid from 1×10⁻⁶ to 5×10⁻⁴ M with good sensitivity of 32 mV/decade. Moreover, the biosensor showed excellent fast response time less than 10 seconds, better selectivity, repeatability, reproducibility and no significant interference to the common interfering ions such as potassium K⁺, sodium ions Na⁺, calcium ions Ca²⁺, glucose, fructose, and zinc ions Zn²⁺ etc. except slight interference with copper Cu²⁺ ions.

Keywords: Zinc oxide nanorods; Enzyme ascorbate oxidase; Potentiometric nanosensor; Nanodevices, 3-glycidoxypropyltrimethoxysilane (GPTS).

Introduction

The vitamin C which is also called L-ascorbic acid with chemical name is vital for the human health due to its determined excellent properties [1-3]. By just keeping aside its participation in making of collagen, ascorbic acid rises the soaking up of iron, plays a key role in the metabolism of folic acid, some amino acids, hormones and to behave as an antioxidant [4]. Moreover the measuring of the ascorbic acid in different synthetic and natural foods, drugs, and physiologi-cal samples is quite crucial for monitoring the level of ascorbic acid. Several techniques were used for the determination of ascorbic acid, which includes spectrometry [5,6], titration [7], the electrochemical [8], and enzyme based potentiometric. Among all above methods the potentiometric based enzyme technique has many advantages including low cost, highly selective, sensitive, and more accurate and giving very quick response to the analyte [9-11]. Currently researchers are paying more attention on electrochemical sensors based on different nanostructures of various materials. ZnO has the many favourable advantages over other nanomaterial for biological sensing because of its good properties including wide direct band gap (3.37 eV) and high exciton binding energy (60 meV). The structure of ZnO is periodic arrangement of Zn²⁺ and O²⁻ ions in tetrahedral fashion and parallel along the c-axis [12] and two planes of structure with different polarity and surface relaxation energies. ZnO has the polar surfaces, permanent dipole moment and polarization along the c-axis due to the positive charge of zinc-(0001) and negative charge of O-(0001). The size of these nanostructures is of same magnitude to that of the biological and chemical components which are to be detected, and are quite suitable for transducer to produce the electrical signals. Moreover, ZnO possess large ionic bonding (60%) and its solubility is too low at biological pH. ZnO nanostructures offer well selective adhesion with enzymes as well as membranes in many of the electrochemical bio-sensing processes. Based on such peculiar properties of ZnO nanosensor which demonstrated strong output signals, good catalytic and also allowing the quick flow of analyte through the sensors. The high surface to volume ratios of ZnO nanorods make them promising candidate for the highly sensitive nano-chemical sensors [13,14]. ZnO nanorods are n-type semiconductor nanomaterial and their electrical transport is based on the adsorption/desorption phenomenon of surface binding chemical components [15-20]. The number of one-dimensional (1D) ZnO nanostructures including nanorods, nanowires and nanotubes etc., have been prepared by various techniques and nano-devices based on these nanostructures have been used for electric field-effect switching [21], single electron transistors [22], biological and chemical sensing [23] and luminescence [24] and etc.

The enzyme ascorbate oxidase is chemically proteinases in nature with 8 g atoms per mole of enzyme [25], for binding it has three various coordination sites. In first step, copper is bonded to two imidazole groups through the nitrogen and sulphur of cysteine, due to this complex formation ascorbate oxidase possess light blue colour. In second step, copper produces bond with two imidazole groups and in step III three histidines are bonded to every copper [26-28]. The reaction mechanism of ascorbate oxidase with L-ascorbic acid in presence of oxygen is given as under [4]:

\[
\text{Enz}_\text{oxygen} \leftrightarrow \text{Enz}_\text{ascorbic acid} \\
\text{Copper} \leftrightarrow \text{Copper} + \text{Copper} + \\
\text{Ascorbate} + \text{Ascorbate} + \\
\text{H}_2\text{O} + \text{H}_2\text{O} +
\]

In above two equations, Enz is oxidized and reduced modes of enzyme; Asc represents the ascorbic acid (the substrate) and P is ascorbate radical intermediate product. Due to above chemical reaction evidence, a potentiometric biosensor may be proposed in which the change in potential can attributed to the reduction of Cu²⁺ to Cu⁺ on the enzyme. Because of the accumulated ascorbate ions on the surface of electrode, the electron density around the electrode changes, which is detected by transducer. The ascorbate oxidase in pure state is largely used in the detection of L-ascorbic acid [29-32]. Nevertheless-

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ness ascorbate oxidase is present in the fruits of the cucurbitaceous class and the amount of ascorbate oxidase enzyme is more in the epicarp of the fruit [33]. The naturally occurring source of enzyme ascorbate oxidase is cucumber which yields high amount of enzyme. There are number of methods used to immobilize the ascorbate oxidase on the surface of working biosensors including the use of PVC membrane localized onto graphite/epoxy electrode surface to immobilize the enzyme [34-36], EVA membrane fixed onto the surface of biosensor followed by the immobilization of enzyme [37-40], and covalent linkage methods. In surveying the literature about the potentiometric based biosensor for L-ascorbic acid, it is found that there are difficulties in making the sensitive membranes and also in the selectivity [41,44], but in this research work we are using the cross linking molecules 3-glycidoxypropyltrimethoxysilane (GPTS) in combination with ascorbate oxidase.

In present study, we have successfully developed a biosensor based on the functionalised ZnO nanorods for potentiometric determination of L-ascorbic with good selectivity, stability, sensitivity and fast response time.

**Experimental Section**

**Material**

The d-glucose, l-glucose-fructose, potassium chloride (KCl), sodium chloride (NaCl), potassium hydrogen phosphate (K,HPO4), sodium dihydrogenphosphate (NaH2PO4), calcium chloride (CaCl2), zinc nitrate [Zn(NO3)2], hexamethylenetetramine/ascorbic acid (A0157), and ascorbate oxidase with activity in between 1000 to 3000 units per mg protein (5960) and then, the 3-glycidoxypropyltrimethoxysilane (GPTS) purchased from sigma Aldrich Sweden. All other chemicals used were of analytical grad.

**The fabrication and preparation of sensor electrodes**

We have fabricated the sensor electrodes utilizing the glass substrates coated with gold then followed by the growth of ZnO nanorods. All processing steps for the preparation of sensor electrodes are described as: firstly the glass substrates were sonicated in ultrasonic bath for about 10 min in isopropanol then cleaned with deionizes water and in last dried by the nitrogen gas. Then these glass substrates were affixed into vacuum chamber of an evaporator instrument (Satis CR 725). After this an adhesive layer of 20 nm of titanium was evaporated after the completion of growth process, the samples were taken out from oven and washed with deionized water in order to remove the residual particles and dried by nitrogen gas. For the study of morphology of grown ZnO nanomaterial, we used the field emission scanning electron microscopy (FESEM). We observed that the grown ZnO nanorods are vertically oriented, highly dense and well aligned as shown in Figure 1a.

**Immobilization of the enzyme**

We prepared the five sensor electrodes by immobilizing the enzyme ascorbate oxidase in conjunction GPTS (a crosslinking molecule). The following immobilization procedure was carried out as: Firstly about 20% GPTS solution was prepared in toluene and drop wise this solution was dropped onto the surface of grown ZnO nanorods then the electrodes were left into oven for about one hour at 70 °C. Then unbound GPTS molecules were removed by using the 10mM phosphate buffer solution (PBS). After that the electrodes were immersed into ascorbate oxidase solution for 3 minutes. The ascorbate oxidase solution was prepared in the phosphate buffer, having concentration 2mg/ml. After immobilization process, the sensors were kept at 4 °C in refrigerator for about 20 hours in order to make sure about firm binding of ascorbate oxidase with ZnO nanorods and completion of immobilization process [45].

![Figure 1(a-c): Typical field emission scanning electron microscopy (FESEM) images of ZnO nanorods grown on gold coated glass substrate using hydrothermal growth method. Figure (a) showing the ZnO-nanorods as grown (b) with immobilized ascorbate oxidase enzyme (c) and same used biosensor.](image-url)
The FESEM images of ascorbate oxidase immobilized ZnO nanorods are shown in Figure 1b. The electrochemical measurements were performed through potentiometric technique, in which the ascorbate oxidase immobilized ZnO nanorods biosensor electrode was used as working electrode and Ag/AgCl as a reference electrode by Metrohm pH meter (model 827). All biosensor electrodes were preserved at 4 °C when not in use.

Ellipsometric analysis: The purpose of using this technique was to know about the thickness of different layers produced on the gold coated substrates. During this measurement we found that the thickness of grown ZnO nanorods onto the gold coated substrate was about 99.10±2.88 nm and it was observed that the grown ZnO nanorods are almost uniform throughout the substrates. The thickness of ZnO nanorods after the immobilization of crosslinking molecules was observed 103.12±0.18 nm. After the immobilization of crosslinking molecules onto the ZnO nanorods then the same substrate was dipped into the enzyme solution and dried, then the thickness was obtained 121.00 ±7.26 nm.

Results and Discussions

Figure 2 illustrates the electrochemical response of biosensor to different L-ascorbic acid concentrations when the proposed sensors were tested into the test electrolyte solutions and the biosensor followed very well to Nernst equation:

\[
E = E_a - 0.05916 \frac{V}{N} \log_{10} \frac{[\text{reduced}]}{[\text{Oxidized}]} 
\]

When the sensor electrodes were exposed to the test solution then the immobilized ascorbate oxidase on ZnO nanorods makes more catalytic enhancement to oxidize the L-ascorbic acid into dehydro-ascorbic acid (DAA), after that hydrogen ions and two electrons are produced into the analyte solution resulting to produce a stable response at the output. Whenever the concentration of hydrogen ions around the surface of ZnO nanorods is varied then the potential difference (EMF) between the working biosensor and reference electrode (Ag/AgCl) changed. The electrochemical response of L-ascorbic acid biosensor is mainly dependent on the efficiency of ascorbate oxidase to catalyse the L-ascorbic acid. The oxidation reaction of ascorbic acid in presence of ascorbate oxidase clearly demonstrates that the generation of hydrogen ions into the testing solution can be helpful for the detection of ascorbic acid from different samples of food and biological samples. When the concentration of produced charges varies onto the surface of immobilized biosensor then the change in potential was observed [47]. During the investigations, the electrochemical response in different concentrations of ascorbic acid test solution prepared in PBS ranging 0.5µM to 50 mM calibrated following the standard procedure as shown in Figure 2. It can be seen from Figure 2 that the proposed biosensor exhibited wide linear output response (EMF) versus the logarithm concentration of ascorbic acid solution with a sensitivity slope of 32 mV/decades form the concentration range of 1×10^{-6} to 5×10^{-2} M.

Figure 2 is showing the calibration curve of the ascorbate oxidase immobilized ZnO-nanorods biosensor electrode i.e. the electrochemical response (EMF) at different L-ascorbic acid concentrations (1×10^{-6} to 5×10^{-2} M) with Ag/AgCl reference electrode.

Performance evaluation of the sensor

In order to judge the performance of biosensor it is very necessary to examine the various properties of biosensor including concentration range of analyte, sensitivity, selectivity, stability, reusability, reproducibility, response time, and detection limit etc. The reproducibility is one of the parameters for studying the consistency in response of biosensor to analyte concentration. For this purpose we prepared five biosensor electrodes under the same set of conditions. During the measurements, it has been observed that all biosensor electrodes revealed a good reproducibility when they were exposed into the 0.05 mM ascorbic acid solution with relative standard deviation less than 5% as shown in Figure 3.

Figure 3 is representing the biosensor to biosensor reproducibility in 5×10^{-5} M L-ascorbic acid solution.

Moreover, we studied the reusability of this proposed biosensor by conducting three experiments by using the same biosensor electrode for three successive days. During the experiments, after each measurement we immersed the biosensor into PBS for 5 minutes in order to remove the attached particles from last measurement, so that it can response to next measurement an accordingly. After this biosensor electrode was dried and kept at 4°C when not in use. The proposed biosensor revealed a good repeatability working activity as shown in Figure 4.
In Figure 4 the Calibration curves representing the biosensor reusability at room temperature after 2-3 hours span in the ascorbic acid test solution from $1 \times 10^{-6}$ to $5 \times 10^{-2}$ M concentration range.

In order to study the effect of pH on the ascorbate oxidase immobilized ZnO nanorods, we observed the morphological change by using the FESEM, the ZnO nanorods after the usage were little damaged from the upper part due to the change in the pH of ascorbic acid solution during the measurement as shown in Figure 1c. For pursuing the effect of pH on the morphology of immobilized ZnO nanorods and output response of the proposed biosensor, we performed the experiments in pH range from 3 to 12. We examined that the EMF response of biosensor gradually increases up to pH 5 [10] and after that it tends to decrease because of the less stability of l-ascorbic acid in neutral or alkaline solution [47,48] as shown in Figure 5. Furthermore it is already investigated that biosensor interferes with anomeric carbon of few sugars, hence the Cu$^{2+}$ of ascorbate oxidase have the chance to react with carbonyl group of aldehyde groups an accordingly to the Fehling reaction [49]. Although the biosensor exhibited maximum response at pH 5 but we performed all experiments at pH 7 because of dissolving phenomenon of ZnO nanorods in acidic medium [50]. In addition we also observed the effect of ionic strength of buffer solution, for this task we had prepared buffer solutions of different molarity and found no significant effect on the biosensor response.

The effect of temperature on the response (EMF) of biosensor was evaluated at different temperature from 23 °C to 75 °C. It was examined that with increase in temperature, the response of biosensor also gradually increases and about at 55°C, it has shown the maximum response. However, the enzyme ascorbate oxidase showed very resistive nature at high magnitude of temperature [51], but also showed some unstable response at the output. Therefore, we carried out experimental measurements at 23 °C temperatures in order to obtain stable response and protect the enzyme from denaturation as well as for the ease in experimental set up which also enhanced the storage life of the presented biosensor.

The back bone parameter for assessing the performance of enzyme based biosensor is the selectivity towards to be sensed analyte and the response of biosensor should not be affected in the presence of interfering species. Moreover, in fruits, foods and biological samples contains potassium ions, sodium ions, zinc ions, calcium ions, glucose, fructose, copper ions etc. in considerable amount, which can influence on the response of biosensor. We demonstrated this part of our research using mixed method for the inference of calcium, potassium, zinc, glucose, sodium, copper etc., ions in mixing with ascorbic acid solution in ranging from $1 \times 10^{-6}$ to $5 \times 10^{-2}$ M and we observed no significant effect on the response of proposed biosensor except copper ion which slightly interfered with the ascorbate oxidase when the concentration of testing solution was changed from $1 \times 10^{-6}$ to $5 \times 10^{-2}$ M as shown in Figure 6. This response of ascorbic acid based on ZnO nanorods towards copper

**Table 1:** Selectivity coefficient for proposed biosensor with interfering ions.

| Interference ion | $-\log K$ |
|------------------|-----------|
| Glucose          | 4.53      |
| Ca$^{2+}$        | 3.98      |
| Na$^{+}$         | 4.87      |
| Zn$^{2+}$        | 5.12      |
| Mg$^{2+}$        | 4.75      |
| K$^{+}$          | 4.87      |
| Cu$^{2+}$        | 4.50      |
We have assessed the stability of proposed biosensor in doing the number of experiments alternatively for three weeks and biosensor were kept at 4 °C when not in use. It has been investigated that biosensor maintained enzymatic activity about 90% with respect to the actual activity and it showed a good storage stability for its long lifetime. We compared the response our proposed biosensor with already existing biosensors as given below in Table 2. By comparing the performance of present proposed ascorbic acid biosensor based on ascorbate oxidase immobilized ZnO nanorods with already reported in literature as given in Table 2. It can be visualized from Table 2 that ZnO nanorods based ascorbic acid has wide linear range of detection, fast response time and better sensitivity. The growth of ZnO nanorods was carried out onto the gold coated glass substrates with enhanced stability and there, those nanorods were exposed for favorable adsorption of ascorbate oxidase because of their high surface to volume ratios, then the adsorbed ascorbate oxidase was fully in contact with the ascorbic acid solution, due to this the efficiency of biocatalyst was highly improved. Furthermore, the ascorbic acid biosensor based on ZnO nanorods demonstrated good selectivity towards ascorbic acid, reproducibility, and stability.

**Conclusion**

In the present work, we have functionalized the ZnO nanorods with ascorbate oxidase enzyme in combination with GPTS as crosslinking molecules for ascorbate oxidase using direct physical adsorption method. It has been successfully demonstrated that the proposed biosensor has wide linear dynamic concentration range 1 × 10⁻⁶ to 5 × 10⁻² M for the detection of vitamin C and with a high selectivity towards the ascorbic acid with no significant interference in the presence of common interfering ions such as, K⁺, Na⁺, glucose, Ca²⁺ and Cu²⁺. The proposed biosensor showed a good sensitivity of 32 mV/decade and fast response time less than 10 seconds. In addition, biosensor retained the good enzymatic activity for more than three weeks due to strong electrostatic interaction of ascorbate oxidase with ZnO nanorods. All above qualities of ascorbate oxidase based ZnO nanorods biosensor for ascorbic acid make its favorable applications for the determination and monitoring of vitamin C in food, drugs and real samples.

**References**

1. Voet D, Voet JG (1990) Biochemistry: Wiley, p. 160F, 251F, 536F.
2. Tolbert BM, Ward JB (1982) Ascorbic acid, chemistry, metabolism and uses, in: P.A. Seib: Advances in Chemistry 200, American Chemical Society, Washington, DC.
3. Cleemput CAB (1989) Vitamin C, Vols. I, II, III: CRC Press, Boca Raton, Florida.
4. Rumsey SC, Levine M (1998) Absorption, transport, and disposition of ascorbic acid in humans. Nutritional Biochem 9: 116–130.
5. Wu X, Diao Y, Sun C, Yang J, Wang Y, et al. (2003) Fluorimetric determination of ascorbic acid with o-phenylenediamine. Talanta 59: 95–99.
6. Koncki R, Lenarczuk T, Radomska A, Glab S (2001) Optical biosensors based on Prussian blue films. Analyst 126: 1080–1085.
7. Sunthomsuk L, Gritsanapun W, Nikarakan S, Paachom A (2002) Quantitation of vitamin C content in herbal juice using direct titration. J Pharm Biomed Anal 28: 849–855.
8. Zen JM, Tsai DM, Kumar AS, Dhuraman V (2000) Amperometric determination of ascorbic acid at a ferrocyanide-doped tosflex-modified electrode. Electrochem Commun 2: 782–785.
9. Akylinas E, Dinkaya E (1999) A new enzyme electrode based on ascorbate oxidase immobilized in gelatine for specific determination of L-ascorbic acid. Talanta 50: 87–93.

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**Table 2**: Comparison of present proposed biosensor with the published biosensors using different techniques.

| Transducer          | concentration range (M) | Slope (mV/decade) | Detection limit (M) | Response times (min) | Reference |
|---------------------|-------------------------|-------------------|---------------------|----------------------|-----------|
| Spector Photometer  | 5.0 × 10⁻³ – 4.0 × 10⁻² | -                 | -                   | -                    | [5]       |
| Dissolved oxygen probe | 5.0 × 10⁻³ – 1.2 × 10⁻³ | -                 | 5.0 × 10⁻⁶          | -                    | [9]       |
| Ethylene vinylacetate membrane | 8.0 × 10⁻⁶ – 4.5 × 10⁻⁴ | 50.3 ± 0.6     | 8.0 × 10⁻⁶          | 5 min                | [10]      |
| ISFET               | 2.0 × 10⁻⁵, 1.3 × 10⁻⁵ | 18                | 1.0 × 10⁻⁶          | -                    | [11]      |
| Screen-printed Electrode | 2.0 × 10⁻⁵ – 1.0 × 10⁻⁵ | 13.65             | 2.0 × 10⁻⁶          | -                    | [45]      |
| ISFET               | 5.0 × 10⁻⁴ – 2.0 × 10⁻³ | 10                | 0.5 × 10⁻³          | -                    | [52]      |
| Screen-printed Electrode | 5.0 × 10⁻⁴ – 3.0 × 10⁻⁴ | -                 | 2.6 × 10⁻⁶          | -                    | [53]      |
| Oxygen Electrode    | 6.3 × 10⁻⁵ – 5.0 × 10⁻⁴ | -                 | 6.3 × 10⁻⁶          | -                    | [54]      |
| Luminol electrochemiluminescence | 2.5 × 10⁻⁵ – 1.0 × 10⁻⁵ | -                 | 2.5 × 10⁻⁶          | -                    | [55]      |
| Potentiometric (ZnO nanorods) | 1.0 × 10⁻⁴ – 5.0 × 10⁻⁵ | 32                | 1.0 × 10⁻⁶ < 10 s   | present study        |           |

**Figure 7**: Calibration curve representing the time response of the proposed sensor in 1 × 10⁻⁴ M ascorbic acid test solution.
10. Fernandes JCB, Kubota LT, Neto GDO (1999) Potentiometric biosensor for L-ascorbic acid based on ascorbate oxidase of natural source immobilized on ethylene vinyl acetate membrane. Anal Chem Acta 385: 3–12.

11. Luo XL, Xu JJ, Zhao W, Chen HY (2004) Ascorbic acid sensor based on ion-sensitive field-effect transistor modified with MnO2 nanoparticles. Anal Chem Acta 512: 57-61.

12. Wang ZL, Kong XY, Ding Y, Gao P, Hughes WL, et al. (2004) Semiconducting and piezoelectric nanostructures induced by polar surfaces. Adv Funct Mater 14: 943-956.

13. Tien LC, Sadik PW, Norton DP, Voss LF, Peartson SJ, et al. (2005) Hydrogen sensing at room temperature with Pt-coated ZnO thin films and nanorods. Appl Phys Lett 87: 222106-222108.

14. Hseuh TJ, Chang SJ, Hsu CL, Lin YR, Chen IC (2007) Highly sensitive ZnO nanowire ethanol sensor with Pd adsorption. Anal Phys Lett 91: 053111-053113.

15. Li QH, Gao T, Wang YG, Wang TH (2005) Adsorption and desorption of oxygen probed from ZnO nanowire films by photocurrent measurements. Appl Phys Lett 86: 123117-123119.

16. Huang XJ, Choi YK (2007) Chemical sensors based on nanomaterials. Sensor Actuator B-Chem 122: 659-671.

17. Li CC, Du ZF, Li LM, Yu HC, Wan Q, et al. (2007) Surface-depletion controlled gas sensing of ZnO nanorods grown at room temperature. Anal Phys Lett 91: 032101-032103.

18. Ghosh R, Dutta M, Basak D (2007) Self-seeded growth and ultraviolet photoresponse properties of ZnO nanowire arrays. Appl Phys Lett 91: 073108-073110.

19. Qiu YF, Yang SH (2007) ZnO nanotetrapods: Controlled vapour-phase synthesis and application for humidity sensing. Adv Funct Mater 17: 1345-1352.

20. Park JY, Song DE, Kim SS (2008) An approach to fabricating chemical sensors based on ZnO nanorods arrays. Nanotechnology 19: 105503-105508.

21. Kim GT, Mustier J, Krstic V, Park JG, Park YW, et al. (2000) Field-effect transistor made of individual V2O5 nanofibers. Appl Phys Lett 76: 1875-1877.

22. Stone NJ, Ahmed H (1998) Silicon single electron memory cell. Appl Phys Lett 73: 2134-2136.

23. Cui Y, Wei Q, Park H, Lieber CM (2001) Nanowire nanosensor for highly sensitive and selective detection of biological and chemical species. Science 293: 1289-1292.

24. Huang MH, Mao S, Feick H, Yan H, Wu Y, et al. (2001) Room-temperature ultraviolet nanowire nanolaser. Science 292: 1897-1899.

25. Nakamura T, Makino N, Ogura Y (1968) Purification and properties of ascorbate oxidase from cucumber. J Biochem 64: 189-195.

26. Meyer TE, Marchesini A, Cusanovich MA, Toffin G (1991) Direct measurement of intramolecular electron transfer between type I and type III copper centres in the multi-copper enzyme ascorbate oxidase and its type II copper-depleted and cyanide-inhibited forms. Biochemistry 30: 4619-4623.

27. Cole JL, Avilgiano L, Morpurgo L, Solomon El (1991) Spectroscopic and chemical studies of the ascorbic acid oxidase trinuclear copper active site: comparison to lactase. J. Am. Chem. Soc 113: 9080-9089.

28. Mariano L, Kohzuma T, Suzuki S, Marchesini A (1996) Stability of ascorbate oxidase extracted from Cucurbit. J Biochem 41: 349-351.

29. Esaka M, Suzuki K, Kubota K (1985) Determination Method for L-Ascorbic Acid in Foods with Immobilized Ascorbate Oxidase. Agric Biol Chem 49: 2955-2960.

30. Stevanato R, Avilgiano L, Finazzi-Agro A, Rigo A (1985) Determination of ascorbic acid with immobilized green zucchini ascorbate oxidase. Anal Biochem 149: 537-542.

31. Greenway GM, Ongomo P (1990) Determination of L-ascorbic acid in fruit and vegetable juices by flow injection with immobilised ascorbate oxidase. Analyst 115: 1297-1299.

32. Matsumoto K, Yamada K, Osajima Y (1981) Ascorbate electrode for determination of L-ascorbic acid in food. Anal Chem 53: 1974-1979.

33. Bin Saani N, Fujita S, Miyazoe R, Okugawa M (1995) Distribution of ascorbate oxidase activities in the fruits of family cucurbitaceae and some of their properties. J Food Biochem 19: 321-327.

34. Glab S, Koniak R, Kopczewsza E, Walcerz I, Hulanicki A (1994) Urea sensors based on PVC membrane pH electrode. Talanta 41: 1201-1205.

35. Innelli RM, Yacymych A M (1983) Urea sensor based on iridium dioxide electrodes with immobilized urease. Anal Chem Acta 146: 249-253.

36. Alexander PW, Joseph JP (1981) A coated-metal enzyme electrode for urea determinations. Anal Chem Acta 131: 103-109.

37. Jammal AE, Boukloose AA, Patriarche GJ (1991) Use of Ethylene-Vinyl-Acetate as a New Membrane Matrix for Calcium Ion-Selective Electrode Preparation. Talanta 36: 929-935.

38. Rover L, Garcia CAB, Neto G, Kubota LT, Galemebeck F (1998) Acetylthioacetic acid determination in pharmaceutical samples by FIA-potentiometry using a salicylate-sensitive tubular electrode with an ethylene-vinyl acetate membrane. Anal Chem Acta 366: 103-109.

39. Thomas JDR (1986) Solvent polymeric membrane ion-selective electrodes. Anal Chem Acta 180: 289-297.

40. Fiedler U, Ruzicka J (1973) Selectrode-the universal ion-selective electrode. Part VII. A valinomycin-based potassium electrode with nonporous polymer membrane and solid-state inner reference system. Anal Chem Acta 67: 179-193.

41. Li J, Shen G, Yu R (1995) Reversible potentiometric ascorbic acid solid-state membrane sensor based on 4,4',4",4"′-tetra-tert-butylphthalocyanine cobalt(II). Analyst 120: 2259-2263.

42. Li J, Hu M, Yu R (1996) Pressed-pellet solid potentiometric sensor for ascorbic acid based on derivatives of cobalt(II) phthalocyanine doped with iodine. Sensors and Actuators B 30: 65-69.

43. Janda P, Weber J, Dunsch L, Lever ABP (1996) Detection of ascorbic acid using a carbon fiber microelectrode coated with cobalt tetrachloroporphyrin azine. Anal Chem 68: 960-965.

44. Tse YH, Janda P, Lever ABP (1994) Electrode with electrochemically deposited N. N', N", N"′-tetramethyltetra-3, 4-pyridinoporphyrazino- cobalt (I) for detection of sulfide ion. Anal Chem 66: 384-390.

45. Jung CC, Yen HT, Chien CC (2000) Development of a Disposable All-Solid-State Ascorbic Acid Biosensor and Miniaturized Reference Electrobe Fabricated on Single Substrate. IEEE Sensors Journal 8: 1571-1577.

46. Al-Hilli SM, Al-Mofarji RT, Klasen P, Willander M (2008) Zinc oxide nanorods grown on two-dimensional macroporous periodic structures and plane Si as a pH Sensor. J Appl Phys 103: 014302.

47. Garret RH, Grisham CM (1995) Biochemistry: Saunders College Publishing, Orlando.

48. Sunyaver SP (1956) Bioquímica: editorial Paz Montalvo, Madrid.

49. Fernandes JCB, de Oliveira Neto G, Kubota LT (1998) Use of column with modified silica for interfering retention in a FIA spectrophotometric method for direct determination of vitamin C in medicine Anal Chem Acta 366: 11-22.

50. Fulati A, Usman Ali Syed M, Riaz M, Amin G, Nur O, Willander M (2009) Miniaturized pH Sensors Based on Zinc Oxide Nanotubes/Nanorods. Sensors 9: 8911-8923.

51. Maccarrone M, D’Andrea G, Salucci ML, Avilgiano L, Finazzi-Agro A (1993) Temperature, pH and UV irradiation effects on ascorbate oxidation. Photochemistry 32: 795-798.

52. Volotovsky V, Kim N (1998) Ascorbic acid determination with an ion ensitive field effect transistor-based peroxidase biosensor. Anal Chim Acta 359: 143–148.

53. Veltisistas PG, Prodromidis MI, Efthathiou CE (2004) All-solid-state potentiometric sensors for ascorbic acid by using a screen-printed compatible solid contact. Anal Chim Acta 502: 15–22.

54. Marques ET Jr, Lima Filho JL (1992) Ascorbic acid biosensor using ascorbate oxidase immobilized on alkylamine glass beads. Appl Biochem Biotechnol 32: 73-78.
trochemiluminescent biosensor for vitamin C based on inhibition of luminol electrochemiluminescence on graphite / poly (methylmethacrylate) composite electrode. Electrochimica Acta 53: 5113-5117.

56. Marques ET Jr, Lima Filho JL (1992) Ascorbic acid biosensor using ascorbate oxidase immobilized on alkylamine glass beads. Appl Biochem Biotechnol. 32: 73-78.