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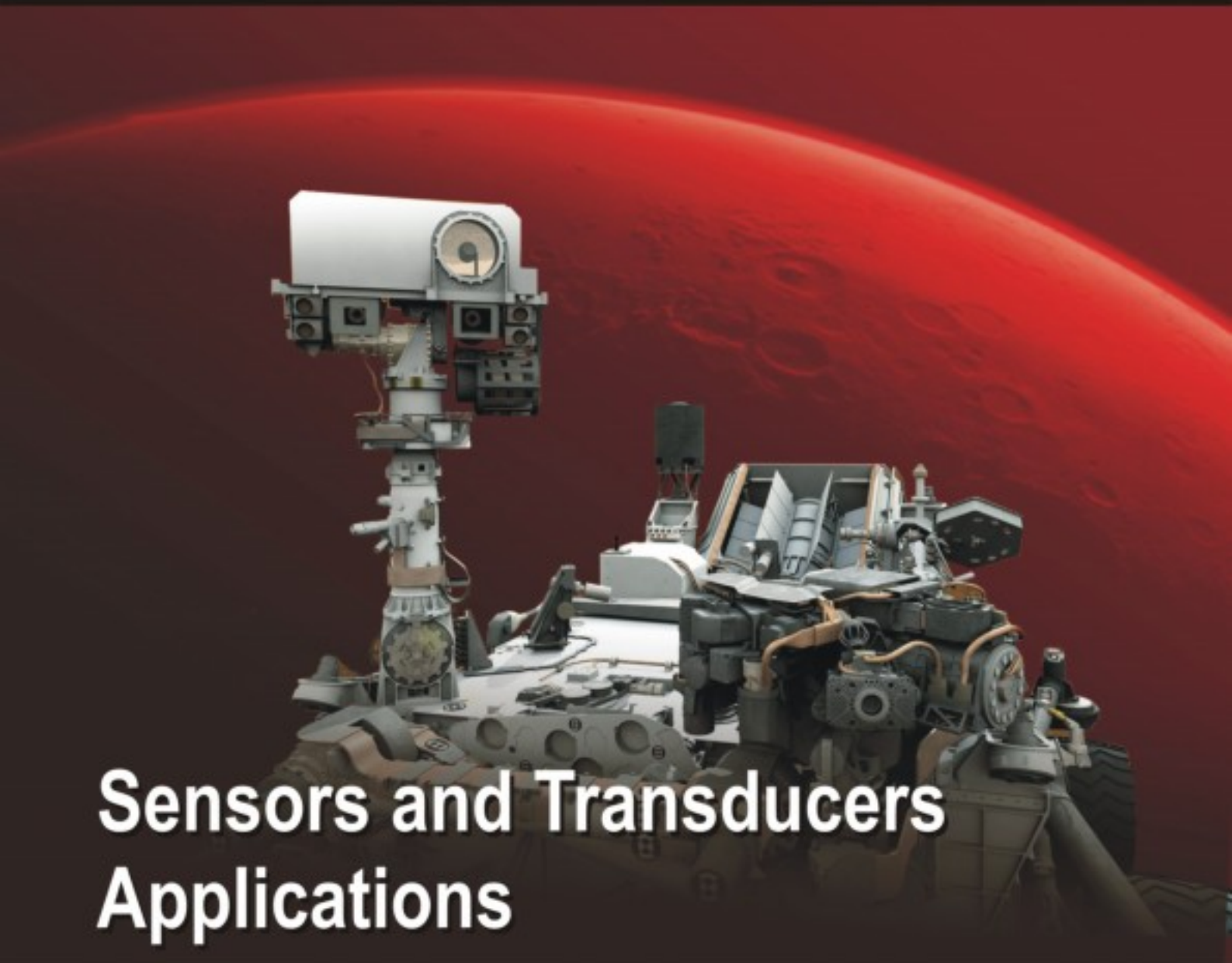

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Electrochemical Based Diagnosis of Ascorbic Acid

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Abstract: With rising healthcare costs and to improve patient health, diagnostic laboratories have been challenged to develop new tests that are easy, reliable, cost–effective and accurate and to optimize existing protocols by making them faster and more beneficial. Determination of ascorbic acid in sample is one of the most vital biochemical parameters in healthcare. With the availability of new materials used for developing new sensing techniques has led to remarkable innovations in the design and construction of ascorbic acid biosensors. The present review describes the specifications of most of the electrochemical ascorbic acid biosensors reported till date. *Copyright © 2012 IFSA.*

Keywords: Electrochemical, Ascorbic acid, Dehydroascorbate, Antioxidant, Electrode, Ascorbate oxidase.

1. Introduction

In 1928, Nobel Prize winner, Dr. Szent-Gyorgyi isolated Vitamin C, also known as ascorbic acid, is an essential nutrient for humans and certain other animal species. Ascorbate (an ion of ascorbic acid) is involved in the wide range of essential metabolic reactions in all animals and plants for proper functioning of the body. It is synthesize by almost all organisms except bats, guinea pigs, capybaras, tarsiers, monkeys and apes, including human beings and hence essential vitamin needed from outside sources. Some examples of good sources of vitamin C are green leafy vegetables, berries, citrus fruits, guavas, tomatoes, melons, papayas, Cabbage, broccoli, cauliflower, leaf lettuce, tomatoes, potatoes, and beans [1]. The vitamin C properties and its importance for human health have been studied in detail. It participates in a variety of biochemical reactions and also important for every body process from bone formation to scar tissue repair [2]. Vitamin C is required in the synthesis of collagen in

connective tissue (3), neurotransmitters [2], steroid hormones [2], carnitine [4], conversion of cholesterol to bile acids [5] and enhances iron bioavailability [6]. Ascorbic acid is a water-soluble antioxidant [7] and helps in the protection of body against pollutants like free radicals commonly known as Reactive Oxygen Species. Ascorbic acid act as a biological reducing agent, so it is also linked to prevention of degenerative diseases - such as cataracts [8], certain cancers [9] and cardiovascular diseases [10]. Ascorbic acid also promotes proper calcium absorption [11] and healing of wounds [3]. It plays an important role in the prevention of blood clotting and bruising, and strengthening the walls of the capillaries [12]. Vitamin C is needed for healthy gums that helps to protect against infection, and also involved in clearing up the infections and is also believed to enhance the immune system. Deficiency disease caused due to vitamin C is scurvy in humans [13]. However, a shortage of vitamin C in the body may result in "pinpoint" hemorrhages under the skin and a tendency to bruise easily, poor wound healing, soft and spongy bleeding gums and loose teeth. Edema also occurred due to prolonged shortage of vitamin C in the body [12].

The dosage recommended by the Recommended Dietary Allowance (RDA) is 60 mg/day. This amount helps in providing the prevention against scurvy. Recent studies in this field suggest that an intake of 200-500 mg per day may be the most beneficial for healthy people. The recommend dosage of vitamin C for pregnant or lactating women is 75-95 mg per day. Daily intake of up to 100 mg of vitamin C is 80 to 90% absorbed. If there is any excess of vitamin C found in the body, it will lose through urine because vitamin C is water-soluble. When extremely large amounts of vitamin C are taken, it arises several gastrointestinal problems but it will come back to normal level when the intake of ascorbic acid is cut or reduced. It is difficult to determine a level where a person may experience discomfort. There are some people who can easily stomach up to 25,000 mg per day, while others start showing problems at 600 or 1,000 mg per day.

Due to its immense importance, vitamin C is also widely used as a food additive for providing complete nutrition to peoples. Apart its great importance in diet, there are several factors which destroy this vitamin are air, heat, water as well as prolonged storage, overcooking and processing. It creates problem to the individuals who do not take sufficient amount of vitamin C as recommended by RDA. This problem can be overcome by estimate/determine the amount of vitamin C in food stuffs. There are several methods which are used to determine ascorbic acid level in food includes- Colorimetric method [14], UV spectrophotometric method [15], biosensor based on enzyme electrode [16], High-Performance Liquid Chromatography [17], enzymatic colorimetric method [18], sequential injection spectrophotometry [19]. Among these methods, enzymatic methods are comparatively better and mostly preferred over other methods, because they are simple, sensitive, accurate and selective. The enzyme used for these methods is ascorbate oxidase which oxidizes L-ascorbic acid into dehydroascorbic acid by adding molecular oxygen to ascorbic acid [20]. The oxidation of ascorbic acid occurs as follows:

At anode (working electrode):

$$\text{L-Ascorbate} \xrightarrow[\text{Voltage: 0.6 V}]{\text{Ascorbate oxidase}} \text{Dehydroascorbate} + 2H^{+} + 2e^{-} \quad (1)$$

At cathode (Ag/AgCl electrode):

$$2AgCl + 2e^{-} \longrightarrow 2Ag^{0} + 2Cl^{-} \quad (2)$$

The reaction involved dehydrogenation of ascorbate to dehydroascorbate by ascorbate oxidase and capture of released electrons by platinum electrode. These released electrons are relayed to

electrometer in which it is read as current in mA (milliampere). The electrons then reach Ag/AgCl electrode, where Ag^+ ions are reduced to Ag metal. This forms the basis of Ascorbic acid determination by electrochemical biosensor techniques. The ascorbate oxidase in pure state is mainly used in the detection of L-ascorbic acid [21]. The 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 [22]. A theme containing ascorbate oxidase has been isolated from *Pleurotus ostreatus* [23]. However there are almost no reports on the oxidation of ascorbic acid in microorganisms even though the occurrence of ascorbate oxidase was reported in *Myrothecium verrucaria* [24]. There is a report on the purification and characterization of enzyme from the culture filtrate of *Acremonium* sps [25]. However, the methods based on enzyme are not economical due to high cost of enzyme. This problem can be overcome by enhancing the reusability of enzyme through immobilization of enzyme onto some insoluble support.

2. Biosensors Based on Immobilized Enzyme onto Different Insoluble Supports

The reusable biosensors based on the immobilized ascorbate oxidase are developed for the determination of L-ascorbic acid level in pharmaceutical, clinical and food stuffs. The versatility in the methods of enzyme immobilization and different support used for the immobilization of ascorbate oxidase are described below with some of the ascorbic acid biosensors reported to date.

Matsumoto *et al*. [26] developed an enzyme electrode with immobilized ascorbate oxidase on collagen membrane for the determination of ascorbic acid (vitamin C).

Stevanato *et al*. [16] developed biosensor in which ascorbate oxidase was immobilized on CH-Sepharose via carbodiimide. The immobilized ascorbate oxidase was utilized in a flow-through system equipped with a polarographic detector which monitors the oxygen depletion due to the reaction: ascorbic acid + $^1/_2$ O_2 $\xrightarrow{\text{ascorbate oxidase}}$ dehydroascorbic acid + H_2O. The response of which is linear between 3×10^{-7} and 5×10^{-4} M ascorbic acid. The biosensor was used to measure the ascorbic acid in biological samples such as human plasma and fruit juices at a rate of about 60 determinations every hour with a standard deviation lower than 5%.

Macholan and Chmelikova [27] developed a Plant tissue-based membrane biosensor for L-ascorbic acid by coupling of a slice of the mesocarp of squash (*Cucurbita pepo*) or cucumber (*Cucumis sativus*) to a Clark-type oxygen electrode which allows 0.02–0.57 mmol l^{-1}L-ascorbic acid to be determined amperometrically. One tissue slice serves for 50–80 measurements at 30 °C and pH 6. The biosensor is highly selective towards ascorbic acid. The biosensor has response time of 70–90 s with the relative standard deviation of about 3%.

Marques and Lima Filho [28] developed a oxygen electrode based on immobilized ascorbate oxidase for dtermination of ascorbic acid. The detection limit of biosensor was 6.3×10^{-5} with concentration range from 6.3×10^{-5} to 5.0×10^{-4} M.

Fernandes et al. [29] developed a biosensor for the determination of L-ascorbic acid. This biosensor was based on ascorbate oxidase immobilized in a graphite/epoxy electrode by occlusion in a poly(ethylene-co-vinyl acetate) matrix. The sub-Nerntian response (slope of 50.3±0.6 mV) showed by the electrode was between 8.0×10^{-6} and 4.5×10^{-4} mol l^{-1} for ascorbate, when employing 0.1 mol l^{-1} KH_2PO_4 buffer at pH 5.0 and 25.0°C. The stability of biosensor was at least 1 month when stored in a refrigerator. The response time was about 5 min at room temperature.

Akyilaz and Dinckaya [30] fabricated the enzyme electrode for the specific determination of l-ascorbic acid in fruit juices and vitamin C tablets. Glutaraldehyde was used for the immobilization of ascorbate

oxidase with gelatin and prepared enzyme electrode by fixing it on pretreated Teflon membrane. The biosensor response depends linearly on l-ascorbic acid concentration between 5.0×10^{-5} and 1.2×10^{-3} M with a response time 45 s. The biosensor is stable for more than 2 months and the reusability was more than 200 times.

Fatibello-Filho and Vieira [31] prepared an ascorbic acid biosensor based on carbon paste electrode modified with crude extract of zucchini (*Cucurbita pepo*) as a source of *peroxidase*. This biosensor can be used for the determination of L-ascorbic acid in pharmaceutical formulations. The analytical recovery of L-ascorbic acid from five samples ranged from 98.1 to 102.1 %. The detection limit was 2.2×10^{-5} mol L^{-1}.

Greenway and Ongomo [32] developed an enzyme electrode based on ascorbate oxidase immobilized on cyanogen bromide activated-Sepharose 4B and incorporated in a flow-injection system. The calibration curve was linear in the range of 0–400 ng ml^{-1} with a correlation coefficient of 0.9994. The detection limit (2σ) in phosphate buffer (0.08 M, pH 5.5) was 4.0 ng ml^{-1}.

Jawaheer et al. [33] developed an individual enzyme-based biosensors involving three-electrode systems for the detection of ascorbic acid. Ascorbic acid is electrochemically active at the working potential chosen (+350 mV vs. Ag/AgCl), it can be measured directly.

Khorasai-Motlagh and Noroozifar [34] designed a sensitive modified glassy carbon (GC) electrode for the determination ascorbic acid. GC electrode was modified with Ni(II)-macrocyclic complex, tetrabutylammonium perchlorate and acetonitrile solutions. The calibration curve was linear up to 5 mM. The detection limit was 2.5×10^{-4} mM. This new modified GC electrode was applied to commercial pharmaceutical tablets, injections and foods.

Tomita et al. [35] constructed an ascorbic acid biosensor by using the crude ascorbate oxidase which was immobilized onto nylon net and fixed over the polypropylene membrane of the oxygen electrode by an o-ring. The ascorbic acid calibration curve was linear from 1.2×10^{-4} to 1.0×10^{-3} mol L^{-1}. The evaluation of biosensor lifetime leads to 500 injections. The biosensor monitors the oxygen depletion, which is proportional to the ascorbic acid concentration.

Paixao et al. [36] fabricated an ascorbate oxidase based positionable platinum microelectrode by electrochemical etching for measuring the ascorbic acid content. The standard deviation was found to be 3 %. The sensor allowed the evaluation of the spatial distribution of the ascorbic acid concentration in oranges by in-situ measurements.

Zhang et al. [37] developed carbon fiber microelectrodes modified with multi-walled carbon nanotubes for in vivo measurements of ascorbic acid (AA) in rat brain.

Vermeir et al. 2007 [38] fabricated a high-throughput microplate calorimetric biosensor for rapid ascorbic acid quantification in food and pharmaceutical products. The sensor is based on microplate differential calorimetry (MiDiCal) technology in which exothermic reaction occur between ascorbic acid and ascorbate oxidase causes heat generation which was differentially monitored between two neighboring wells of an IC-built wafer. The biosensor exhibited a wide functional range from 2.4 to 350 mM with a detection limit of 0.8 mM.

Zheng et al., 2009 [39] developed an ordered mesoporous carbon (OMC)/Nafion composite film for the determination of Ascorbic acid. OMC/Nafion suspension was prepared by dispersing the insoluble OMC into the ethanol in presence of 0.5 % nafion. After evaporation of ethanol, a uniform OMC/Nafion composite film-coated glassy carbon electrode (GCE) was obtained by simple casting deposition.

Pundir et al. 2010 [40] constructed an ascorbate biosensor with immobilized ascorbate oxidase onto epoxy resin "Araldite" membrane to measure ascorbic acid. The retention of initial activity of free enzyme was 79.4 %. The enzyme electrode can be reused 200 times without considerable loss of activity during the storage of 90 days when stored at 4°C.

Chauhan et al. 2011 [41] fabricated an ascorbate biosensor based on the immobilized ascorbate oxidase for ascorbic acid determination in sera, fruit juices and vitamin C tablets. An ascorbate oxidase was immobilized covalently onto a carboxylated multiwalled carbon nanotubes and the surface of Au electrode was treated electrochemically for the deposition of polyaniline (c-MWCNT/PANI) layer. Linear range, response time and detection limit were 2–206 μM, 2 s and 0.9 μM respectively. The biosensor showed optimum response at pH 5.8 and in a broader temperature range (30–45 °C), when polarized at +0.6 V. The analytical recovery was 91% and 6.5 % and 11.4% within and between batch coefficients of variation respectively for five serum samples. There was a good correlation (r = 0.98) between fruit juice ascorbic acid values by the standard 2,6-dichlorophenolindophenol (DCPIP) method and the present method. The reusability of enzyme electrode was found 200 times with storage period of 2 months, when stored at 4 °C.

Chauhan et al. 2011 [42] constructed a non-enzymatic ascorbate sensor by single-step electro-deposition method. Gold electrode was modified with copper nanoparticles (CuNPs), carboxylated multi-walled carbon nanotubes (MWCNTs) and polyaniline (PANI) composite by electro-deposition. The sensor showed optimum response within 2s at +0.4 V. The response was linear up to 600 μM ascorbic acid concentration. The detection limit was 1.0 μM and half life of electrode was 4 months.

Ibupoto et al. 2011 [43] developed a biosensor based on the functionalized ZnO nanorods for potentiometric determination of L-ascorbic acid. Ascorbate oxidase was immobilized onto the ZnO nanorods by cross linking molecule 3-glycidoxypropy-1-trimethoxysilane (GPTS). The response time was less than 10 seconds with detection limit of 1×10^{-6} M. The biosensor has linear concentration range from 1×10^{-6} to 5×10^{-2} M for the detection of vitamin C.

Yang et al., 2012 [44] developed ascorbate oxidase (AO) electrochemical biosensor based on the biocompatible poly(3, 4-ethylenedioxythiophene) (PEDOT) matrices, which was easily prepared by single-step electrodeposition technique in ionic liquid microemulsions.

3. Applications of Ascorbic acid Biosensor

(i) In pharmaceutical industry. Ongoing research is looking at the clinical use of vitamin C in the prevention and treatment of human diseases. It is necessary to determine the appropriate level of ascorbic acid for making drugs used for human diseases.

(ii) In food industry. Ascorbic acid is used as preservative in food industry. Therefore, biosensors used for the determination of ascorbic acid in food stuffs have great value in food industry.

(iii) In clinical diagnosis. These biosensors can be used for clinical diagnosis of human diseases by detecting the level of ascorbic acid present in the blood, urine etc.

(iv) In determining food nutritional value. Biosensors can also be used for determining the ascorbic acid levels in food stuffs which provide nutritional information related to these food stuffs.

Table 1. A brief account of various ascorbate biosensors.

No.	Biosensor fabrication	Characteristics	Reference
1.	Ascorbate oxidase immobilized on collagen membrane.	– – –	[26]
2.	Immobilized ascorbate oxidase on CH-Sepharose via carbodiimide.	3×10^{-7} and 5×10^{-4} M	[16]
3.	Clark-type oxygen electrode coupled with slice of mesocarp contains ascorbate oxidase	Detection limit = 0.02–0.57 mmol l^{-1}, response time = 70–90 s.	[27]
4.	Ascorbate oxidase immobilized on oxygen electrode	Detection limit = 6.3×10^{-5}, concentration range = 6.3×10^{-5} to 5.0×10^{-4} M	[28]
5.	Ascorbate oxidase immobilized in a graphite/epoxy electrode by occlusion in a poly(ethylene-co-vinyl acetate) matrix	Detection limit = $8.0\text{x}10^{-6}$ M, response time = 5 min, self life of 1 month when stored in refrigerator	[29]
6.	Ascorbate oxidase immobilized with gelatin using glutaraldehyde and fixed on pretreated teflon membrane served as an enzyme electrode	Response time = 45 s, response linear range = 5.0×10^{-5} - 1.2×10^{-3} M, self life = 2 months.	[30]
7.	Carbon paste electrode modified with peroxidase.	Detection limit = 2.2×10^{-5} mol L^{-1}, analytical recovery = 98.1 to 102.1 %.	[31]
8.	Ascorbate oxidase immobilized on cyanogen bromide activated-Sepharose 4B	Detection limit = 4.0 ng ml^{-1}, correlation coefficient = 0.9994.	[32]
9.	Enzyme-based biosensors involving three-electrode systems.	– – –	[33]
10.	Glassy carbon electrode modified with Ni(II)-macrocyclic complex, tetrabutylammonium perchlorate and acetonitrile solutions.	Detection limit of 2.5×10^{-4} mM.	[34]
11.	Ascorbate oxidase immobilized on nylon net and fixed over the polypropylene membrane of the oxygen electrode by an o-ring.	Linear range = 1.2×10^{-4} to 1.0×10^{-3} mol L^{-1}.	[35]
12.	Ascorbate oxidase based platinum microelectrode prepared by electrochemical etching	– – –	[36]
13.	Carbon fiber microelectrodes modified with multi-walled carbon nanotubes	– – –	[37]
14.	microplate calorimetric biosensor	Linear response range = 2.4 to 350 mM, detection limit = 0.8 mM.	[38]
15.	OMC/Nafion composite film-coated glassy carbon electrode	Concentration range = 40-800 μM, detection limit = 20 μM, correlation coefficient (r) = 0.998	[39]
16.	Ascorbate oxidase immobilized epoxy resin	Retention = 79.4 %, self life of 90 days when stored at 4 °C.	[40]
17.	Ascorbate oxidase immobilized on multiwalled carbon nanotubes/polyaniline modified Au electrode.	Linear range = 2–206 μM, response time = 2 s, detection limit = 0.9 μM, analytical recovery = 91 %, correlation coefficient (r) = 0.98, self life of 2 months when stored at 4 °C.	[41]
18.	Gold electrode modified with copper nanoparticles (CuNPs), carboxylated multi-walled carbon nanotubes (MWCNTs) and polyaniline (PANI) composite by electro-deposition.	Detection limit = 1.0 μM, response time = 2s, half-life time = 4 months.	[42]
19.	Ascorbate oxidase immobilized on ZnO nanorods.	Linear dynamic concentration range = 1×10^{-6} to 5×10^{-2} M.	[43]
20.	Ascorbate oxidase (AO) electrochemical biosensor based on the biocompatible poly(3, 4-ethylenedioxythiophene) (PEDOT) matrices	– – –	[44]

4. Conclusion

Due to its rapid and automated analysis, biosensors provide an early warning about the health of the peoples. Advances in application of conducting polymers to biosensors also bring revolutionary changes in the biosensor techniques and technologies. Miniaturization of these biosensors makes them easy to use for consumers. Thus, biosensors with all these properties are more reliable, simple, sensitive and inexpensive than many other conventional methods.

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References

[1]. R. A. Jacob, Vitamin C, In Modern Nutrition in Health and Disease, 9th ed. *William & Wilkins*, Balt., 1999, pp. 467-483.
[2]. J. L. Groff, S. S. Gropper and S. M. Hunt, The water Soluble Vitamins, In: Advanced Nutrition and Human Metabolism, *West Publishing Company*, 1995, pp. 222-237.
[3]. M. K. Douglas and A. L. Miller, Nutritional Support for Wound Healing. Alternative, *Medicine Review*, 8, Issue 4, 2003, pp. 359-377.
[4]. P. J. Nelson, R. E. Pruitt, L. R. L. Henderson, R. Jenness, L. M. Henderson, Effect of ascorbic acid deficiency on the in vivo synthesis of carnitine, *Biochim. Biophys. Acta*, 672, 1981, pp. 123-127.
[5]. J. A Simon, Ascorbic acid and cholesterol gallstones, *Medical Hypotheses*, 40, Issue 2, 1993, pp. 81-84.
[6]. M. N. Garcia-Casala, M. Layrisse, J. P. Pena-Rosas, J. Ramirez, I. Leets, P. Matus, Iron Absorption From Elemental Iron-Fortified Corn Flakes In Humans: Role of Vitamins A and C[1-3], *Nutrition Research*, 23, 2003, pp. 451–463.
[7]. Levine and Mark, New concepts in the biology and biochemisty of ascorbic acid, *New England Journal of Medicine*, 314, 1986, pp. 892- 902.
[8]. Hankinson and E. Susan, Nutrient intake and cataract extraction in women: A prospective study, *British Medical Journal*, 305, 1992, pp. 335-39.
[9]. G. Block, Epidemiologic evidence regarding vitamin C and cancer, *American Journal of Clinical Nutrition*, 54, 1991, pp. 1310S-14S.
[10].P. R. Woodhouse and K. T. Khaw, Seasonal variations in vitamin C status, infection, fibrinogen and cardiovascular disease - Are they linked ?, *Age and Ageing*, 23, 1994, pp. 5.
[11].S. R. Morcos, F. A. El-Shobaki, Z. El-Hawary and N. Saleh, Effect of vitamin C and carotene on the absorption of calcium from the intestine, *Chemistry and Materials Science*, 15, 1976, pp. 387-390.
[12].http://www.anyvitamins.com/
[13].http://whqlibdoc.who.int/hq/1999/WHO_NHD_99.11.pdf
[14].J. H. Roe and C. A. Kuether, Chemical determination of dehydroascorbic acid, *J Biol Chem.,* 147, 1943, p. 399.
[15].M. F. Oberbacher and H. M. Vines, Assay procedure for ascorbate oxidase by UV spectrophotometer, *Nature*, 197, 1963, pp. 1203.
[16].R. Stevanato, L. Avigliano, A. Finazzi-Agrò and A. Rigo, Determination of ascorbic acid with immobilized green zucchini ascorbate oxidase, *Anal Biochem.,* 149, 1985, pp. 537-542.
[17].J. Lykkesfeldt, S. Loft and H. E. Poulsen, Determination of ascorbic acid and dehydroascorbic acid in plasma by HPLC with colorimetric detection, *Anal. Biochem.,* 229, 1995, pp. 329-335.
[18].H. Ihara, Y. Shino, Y. Aoki, N. Hashizume and Minegishi, A simple and rapid method for the routine assay of total ascorbic acid in serum and plasma using ascorbate oxidase and o-phenylenediamine, *J Nutr Sci Vitaminol (Tokyo),* 46, 2000, pp. 321-324.
[19].M. Polásek, P. Skála, L. Opletal and L. Jahodár, Rapid automated assay of anti-oxidation/radical scavenging activity of natural substances by Sequential Injection Technique (SIA) using spectrophotometric detection, *Anal Bioanal Chem.,* 379, 2004, pp. 754-758.

[20].R. B. Frei, L. England and B. N. Ames, Ascorbate is an outstanding antioxidant in human blood plasma, *Proc. Nalt. Acad. Sci*., 86, 1989, pp. 6377-6381.
[21].M. Esaka, K. Suzuki and K. Kubota, Determination Method for L-Ascorbic acid in foods with immobilized ascorbate oxidase, *Agric Biol Chem*., 49, 1985, pp. 2955-2960.
[22].N. B. Saari, S. Fujita, R. Miyazoe and M. Okugawa, Distribution of ascorbate oxidase activities in the fruits of family cucurbitaceae and some if their properties, *J Food Biochem*., 19, 1995, pp. 321-327.
[23].Y. R. Kim, S. W. Yu, S. R. Lee, Y. Y. Hwang and S. O. Kang, SO, A heme-containing ascorbate oxidase from Pleurotus ostreatu, *J Biol Chem*., 271, 1996, pp. 3105-11.
[24].G. A. White and F. G. Smith, Ascorbic acid oxidase of Myrothecium verrucaria, *Plant Physiol*., 37, Issue 6, 1962, pp. 742-750.
[25].J. Hirose, T. Sakurai, K. Imamura, H. Watanabe, H. Iwamoto, K. Hiromi, H. Itoh, T. Shin and S. Murao, Characterization of ascorbate oxidase from Acremonium sp. HI-25, *J Biochem*., 115, 1994, pp. 811-3.
[26].K. Matsumoto, K. Yamada and Y. Osajima, Ascorbate electrode for determination of L-ascorbic acid in food, *Anal Chem.,* 53, 1981, pp. 1974-1979.
[27].L. Macholán and B. Chmelíková, Plant tissue-based membrane biosensor for L-ascorbic acid, *Anal Chim Acta*, 185, 1986, pp. 187-193.
[28].E. T. Marques and J. L. Lima-Filho, Ascorbic acid biosensor using ascorbate oxidase immobilized on alkylamine glass beads, *Appl Biochem Biotechnol*, 32, 1992, pp. 73-78.
[29].J. C. B. Fernandes, L. T. Kubota and G. D. O. Neto, Potentiometric biosensor for L-ascorbic acid based on ascorbate oxidase of natural source immobilized on ethylene-vinylacetate membrane, *Anal Chim Acta.,* 385, 1999, pp. 3-12.
[30].E. Akyilmaz and E. Dinçkaya, A new enzyme electrode based on ascorbate oxidase immobilized in gelatin for specific determination of L-ascorbic acid, *Talanta,* 50, 1999, pp. 87-93.
[31].O. Fatibello-Filho and C. Vieira Iolanda da, L-ascorbic acid determination in pharmaceutical formulations using a biosensor based on carbon paste modified with crude extract of zucchini (Cucurbita pepo), *J Braz Chem Soc.,* 11, 2000, pp. 412-418.
[32].G. M. Greenway and P. Ongomo, Determination of ascorbic acid in fruits and vegetables by flow injection with ascorbate oxidase, *Anaystl*, 115, 1990, pp. 1297-1299.
[33].S. Jawaheer, S. F. White, S. D. Rughooputh and D. C. Cullen, Development of a common biosensor format for an enzyme based biosensor array to monitor fruit quality, *Biosens. Bioelectron.,* 18, 2003, pp. 1429-37.
[34].M. Khorasai-Motlagh and M. Noroozifar, Electrocatalytical determination of ascorbic acid using glassy carbon modified with nickel (II) macro cycle containing dianionic tetraazaannulene ligand, *Turk J Chem.,* 28, 2004, pp. 369-378.
[35].I. N. Tomita, A. Manzoli, F. L. Fertonani and H. Yamanaka, Amperometric biosensor for ascorbic acid, *Eclet Quím*, 30, 2005, pp. 37-42.
[36].T. R. L. C. Paixão, D. Lowinsohn and M. Bertotti, Use of an Electrochemically Etched Platinum Microelectrode for Ascorbic Acid Mapping in Oranges, *J Agric. Food. Chem.,* 54, 2006, pp. 3072 -3077.
[37].M. Zhang, K. Liu, L. Xiang, Y. Lin, L. Su and L. Mao, Carbon nanotube-modified carbon fiber microelectrodes for in vivo voltammetric measurement of ascorbic acid in rat brain, *Anal Chem.,* 79, 2007, pp. 6559-6565.
[38].S. Vermeir, B. M. Nicolaï, P. Verboven, P. V. Gerwen, B. Baeten, L. Hoflack, V. Vulsteke and J. Lammertyn, Microplate differential calorimetric biosensor for ascorbic acid analysis in food and pharmaceuticals, *Anal Chem*., 79, 2007, pp. 6119-6127.
[39].D. Zheng, J. Ye, L. Zhou, Y. Zhang and C. Yu, Simultaneous determination of dopamine, Ascorbic acid and uric acid on ordered mesoporous carbon/Nafion composite film, *J. Electroanal Chem*., 625, 2009, pp. 82–87.
[40].C. S. Pundir, N. Chauhan and Jyoti, Construction of an amperometric ascorbate biosensor using epoxy resin bound Lagenaria sicrania fruit ascorbate oxidase, *Artifical Cells, Blood Subst. Biotechnol.,* 39, 2010, pp. 177-184.
[41].N. Chauhan, J. Narang and C. S. Pundir, Fabrication of MWCNT/PANI modified Au electrode for ascorbic acid determination, *Analyst*, 136, 2011, pp. 1938-1945.
[42].N. Chauhan, J. Narang, R. Rawal and C. S. Pundir, A highly sensitive non-enzymatic ascorbate sensor based on copper nanoparticles bound to multi walled carbon nanotubes and polyaniline composite, *Synthetic Metals*., 161, 2011, pp. 2427-2433.
[43].Z. H. Ibupoto, S. M. U. Ali, K. Khun and M. Willander, L-Ascorbic Acid Biosensor Based on Immobilized Enzyme on ZnO Nanorods, *J Biosens Bioelectron*., 2, Issue 3, 2011, pp. 1-7.

[44]. Y. P. Wen, L. M. Lu, D. Li, M. Liu, H. H. He and J. K. Xu, Ascorbate oxidase electrochemical biosensor based on the biocompatible poly(3, 4-ethylenedioxythiophene) matrices for agricultural application in crops, *Chinese Chemical Letters*, 23, Issue 2, 2012, pp. 221-224.

Sensors & Transducers Journal

Guide for Contributors

Aims and Scope

Sensors & Transducers Journal (ISSN 1726-5479) provides an advanced forum for the science and technology of physical, chemical sensors and biosensors. It publishes state-of-the-art reviews, regular research and application specific papers, short notes, letters to Editor and sensors related books reviews as well as academic, practical and commercial information of interest to its readership. Because of it is a peer reviewed international journal, papers rapidly published in *Sensors & Transducers Journal* will receive a very high publicity. The journal is published monthly as twelve issues per year by International Frequency Sensor Association (IFSA). In additional, some special sponsored and conference issues published annually. *Sensors & Transducers Journal* is indexed and abstracted very quickly by Chemical Abstracts, IndexCopernicus Journals Master List, Open J-Gate, Google Scholar, etc. Since 2011 the journal is covered and indexed (including a Scopus, Embase, Engineering Village and Reaxys) in Elsevier products.

Topics Covered

Contributions are invited on all aspects of research, development and application of the science and technology of sensors, transducers and sensor instrumentations. Topics include, but are not restricted to:

- Physical, chemical and biosensors;
- Digital, frequency, period, duty-cycle, time interval, PWM, pulse number output sensors and transducers;
- Theory, principles, effects, design, standardization and modeling;
- Smart sensors and systems;
- Sensor instrumentation;
- Virtual instruments;
- Sensors interfaces, buses and networks;
- Signal processing;
- Frequency (period, duty-cycle)-to-digital converters, ADC;
- Technologies and materials;
- Nanosensors;
- Microsystems;
- Applications.

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