

# Optimization of Electrochemical Detection of L-Ascorbic Acid from Plant Food Supplements Using Screen Printed Transducers

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**Abstract** The importance of Ascorbic acid (vitamin C) in several biological processes, and during the last years as active compounds used in several pesticides (L-Ascorbic acid is used as a fungicide on glasshouse tomato, potato, and field and glasshouse flower bulbs) is a challenge for groups of researchers to develop new, rapid and sensitive method for its detection. Methods for the detection of Ascorbic acid (AA) were developed and optimised using differential pulse voltammetry (DPV) analysis with modified carbon-printed electrodes with cobalt phthalocyanine. There was studied detection of Ascorbic acid in hydrochloric acid 2% as supporting media. Results showed that the method developed for Ascorbic acid analyses can be used to assay different samples as teas and plant food supplements containing different botanicals.

**Keywords** Ascorbic acid, Differential pulse voltammetry, Botanical extracts

## 1. Introduction

### 1.1. Ascorbic Acid in Biological Processes

Widespread in green plants, especially fruit and vegetables, the highest concentrations are found in citrus fruits, underbrush, strawberries, kiwi, tomatoes, peppers, broccoli [1]. Sea-buckthorn fruit among the most enriched plant sources of vitamin C - in a range of 114 to 1550 mg per 100 grams with an average content (695 mg per 100 grams) about 15 times greater than oranges (45 mg per 100 grams) [2]. During processing and storage, vitamin C losses may occur [3-4].

Studies indicated that quercetin (antioxidant belongs to the broad flavonoids group) and vitamin C supplementation may not be improve lipid profile, although it may reduce induce muscle damage and the proportion of body fat [6,7].

Vitamin C helps to synthesize the amino acid L-carnitine and the catecholamines [8] that occur in the regulation of the nervous system, promotes iron absorption by reducing, at stomach level, from Fe<sup>2+</sup> to Fe<sup>3+</sup> [9], lowering histamine ingested and if an allergic reaction occurs.

Involved in the metabolism of tyrosine, and tryptophan, folic acid, the presence of Ascorbic acid is essential for the hydroxylation of dopamine to norepinephrine and in the reaction of tryptophan in order to obtain [10].

Ascorbic acid is an important antioxidant, it has the property of being water soluble, may act as both intracellular and extracellular in order to protect the body against free radicals and pollutants [5].

An amount below the lower limit of vitamin C in the body can cause anemia [11], scurvy [12], various infections [13], mental disorders [14].

Mega doses of vitamin C are used in the treatment and prevention of large number of disorders like diabetes, cataracts, glaucoma, macular degeneration, atherosclerosis, stroke, heart diseases and cancer [5].

An amount above the upper limit, normally not cause toxicity due to water solubility, the excess is excreted. A normal amount of vitamin C in plasma is between 4.6 and 14.9 mg/L [15, 16].

Several controversies were related to the action of vitamin C and the dosage [17]. The topic is also now very interesting for investigators and EFSA (European Food Safety Association) that launched open consultations on dietary reference values for vitamin C. The document proposed dietary reference values for Ascorbic acid for different ages (adults, infants and children), and different status of women (pregnant and lactating women) [18].

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Under the European law, manufacturers of food supplements are responsible for the safety of their products before they are commercialized. They are also responsible for determining that the claims on their labels are accurate and truthful.

### 1.2. Ascorbic Acid as Pesticide

L-Ascorbic acid (IUPAC name-((5*R*)-[(1*S*)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one) is a new active substance for which received an application from Citrex Europe B.V. for approval (in accordance with Article 6(2) of Council Directive 91/414/EEC the Netherlands). The competent authority from Netherlands prepared a review concerning the initial risk assessments of the pesticide active substance L-Ascorbic acid. There have been evaluated the representative uses of L-Ascorbic acid as a fungicide on glasshouse tomato, potato, and flower bulbs (field and glasshouse) [19].

The context of this peer review was that required by Commission Regulation (EU) No 188/2011. Based on those conclusions, European Food Safety Authority (EFSA) identified that:

Data gaps were found for the studies concerning physical and chemical properties and analytical methods.

Toxicological issues and metabolism sections were completed and no data gaps or critical concerns were identified.

In the fields of residues no data gaps were identified and the risk assessment studies can be done.

A low risk was observed for honey bees, non-target plants, birds, mammals, and sewage treatment organisms.

On the basis of the available scientific information, a high risk to non-target arthropod was identified for their use on potatoes.

The Ascorbic acid can have a double action. There were also studies reflecting the protective effect of Ascorbic acid on liver enzyme activity in rats exposed to diazinon (organophosphate pesticide). The results revealed that vitamin C have a potent beneficial (protective) effect against diazinon-induced hepatotoxicity in rats. These could be explained be due to the scavenging effect of free radicals and increased the total antioxidant status [20].

Animal studies (micronucleus test in Swiss albino mice) indicated the modulatory effect of higher doses of vitamin C on the genotoxicity of the some pesticides (endosulfan, mancozeb, and phosphamidon) [21].

### 1.3. Detection Methods of Ascorbic Acid

Classic method recommended for Ascorbic acid detection was presented as titrimetric method (iodometric titration in presence of amidon [22].

Szalai and colab. [23] developed a simple, selective, economic, precise and stable HPLC method for the detection of ascorbate in plant tissue.

Electrocatalytic study for detection of Ascorbic acid using

single-walled carbon nanotube/tungsten oxide (SWCNT/WO<sub>3</sub>) modified glassy carbon electrode (GCE) prepared by mechanical attachment was conducted. The SWCNT/WO<sub>3</sub>-modified glassy carbon electrodes possess better sensitivity when compared to WO<sub>3</sub>-modified electrode and bare GCE [24].

Murthy [25] proposed a modified electrode with TCNQ for Ascorbic acid oxidation in phosphate buffer solutions (pH 7) using cyclic voltammetry. The method is suitable for a quantitative determination of Ascorbic acid (linear variation of current with concentration of Ascorbic acid in the range 1–10 mM).

Differential pulse voltammetry (DPV) has been used to simultaneously detect of dopamine, serotonin (5-HT), and Ascorbic acid [26]. Linear calibration curve were obtained. The linearity range for Ascorbic acid was 300.0-2,000.0 μM, with detection limits (S/N = 3) of 0.6 μM. The proposed electrode has been applied to dopamine, serotonin (5-HT), and Ascorbic acid in real samples using standard addition method. The obtained results were satisfactory.

Electrochemical studies (voltammetric and amperometric methods) were done in the past by our group for detection of vitamin C, using cobalt phthalocyanine as redox mediator [27].

This work proposes an alternative to titrimetric methods, usually used for detection of Ascorbic acid from food samples. The new proposed method also is innovative because use the new type of transducers prepared in the BIOMEM laboratory from University of Perpignan Via Domitia, France [28].

## 2. Materials and Equipment

Analytical grade reagents were used. L-Ascorbic acid was purchased from Merck, Darmstadt, Germany. Solutions of HCl 2% were used for real sample preparation and as supporting electrolyte.

Three plant food supplements (PSF) were used as real samples.

Sample 1 is a PFS containing *Hippophae fructus*, *cuticle of chicken gizzard*, *Chamomillae flos*, *Hyperici herba*, *Amaranthus caudatus herba*, *Thymi vulgaris aetheroleum*, *Lavandulae aetheroleum* and excipients. Sample 2 is a PFS containing *Hippophae fructus*, *Spirulina platensis*, *Foeniculi aetheroleum*, *Lavandulae aetheroleum*, *Menthae piperita aetheroleum* and excipients. Sample 3 is a PFS containing *Hippophae rhamnoides*, *Liquiritiae radix*, *Violae herba*, *Bardanae radix*, *Taraxaci herba* cum radices and excipients.

As electrochemical transducers there were used screen printed electrodes (SPE), obtained on laboratories of University de Perpignan Via Domitia, France [29]. Working electrodes were made from carbon (SPE) and carbon - modified with cobalt phthalocyanine (SPE-Co), as redox mediator (Fig.1). Palm Sense Potentiostat with Soft PS Trace software was used for analysis.



Figure 1. Design of SPE electrodes

### 3. Working Procedure

The measurements were performed at room temperature.

Titrimetric measurements of Ascorbic acid were done according with procedure proposed by Cioroi [22].

To measure Ascorbic acid in hydrochloric acid medium there was used DPV method with SPE-Co sensor. The working parameters were maintained constants during the experiments, according with other previous studies [22, 30, 31]. Differential pulse voltammetry (DPV) parameters were maintained as: start potential-0.6V; final potential 1V; scan rate 0.01 V/s; t pulse 0.2s; deposition time 240s.

When HCl solution 2% was used as electrolyte, the final concentration of Ascorbic acid in the media was between 0.05mM and 0.25mM respectively.

Calibration curves for L-Ascorbic acid in hydrochloric acid 2% were obtained (each standard sample was tested three times). Data interpolation for detection of Ascorbic acid content from real samples (plant food supplements in acidic media) was done.

A quantity of 5 g plant food supplement was grind with approx. 10 cm<sup>3</sup> solution 2% HCl and 2.5 g of quartz sand for 10 minutes. The resulting mixture was transferred into a 50 mL volumetric flask and brings to volume with HCl 2%. It is filtered through a fluted filter into a dry flask. 10 cm<sup>3</sup> filtrate was pipetted into a 100 ml Erlenmeyer flask.

### 4. Results and Discussions

The obtained signals using Differential Pulse Voltammetry (DPV) were proportional with the concentration of Ascorbic acid from the standard solutions. Analysis of Ascorbic acid in hydrochloric acid 2% indicated the calibration line from Fig.2.

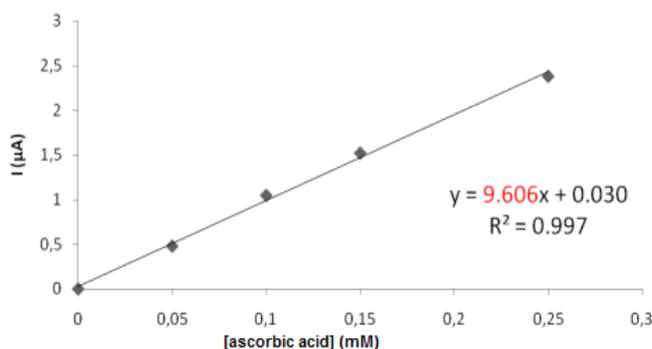


Figure 2. The calibration curve for measurement of L-Ascorbic acid (vitamin C) in a solution HCl 2% using DPV method (sensor SPE-Co)

The calibration curve showed a linearity range between 0.05-0.25 mM Ascorbic acid. The sensitivity of the method was 9.606 µA/mM.

Real samples were extracted and tested in HCl 2%. The results are presented in Table 1.

Table 1. Concentration of Ascorbic acid in acidic media coming from real samples tested electrochemical (DPV method) in solution HCl 2%

Samples	I (µA)	Concentration Ascorbic acid (µM)
Sample 1	2.176	229.98
Sample 2	1.366	141.73
Sample 3	1.576	164.61

The results obtained using DPV were compared with the results using iodometric titrimetry and the correspondence between two methods are good, as presented in Table 2.

Table 2. Concentration of Ascorbic acid in initial real samples using DPV method and titrimetric method in solution HCl 2%

Samples	Concentration of Ascorbic acid using DPV method (mg AA /100g product)	Concentration of Ascorbic acid using titrimetric method (mg AA /100g product)	Correspondence between methods (%)
Sample 1	202.38	211.2	104.36
Sample 2	124.72	84.48	67.74
Sample 3	144.86	126.72	87.48

Using DPV method with SPE-Co electrodes had good results in acidic media (HCl 2%) which is recommended in the determination of Ascorbic acid in products food by titrimetric method. Taking advantage of rapid techniques, this electrochemical method can be recommended for replacement of iodometric titration methods which are more laborious and subjective (colour changes during the titration is subjectively appreciated).

### 5. Conclusions

Methods for the detection of Ascorbic acid (AA) were developed and optimised using differential pulse voltammetry (DVP) analysis with a screen printed modified carbon-printed electrodes with cobalt phthalocyanine.

Results showed that the method developed for Ascorbic acid analyses can be used to assay different samples as plant food supplements as well as possible pharmaceutical samples and teas. This detection method of Ascorbic acid from real samples was possible and it was more sensitive than the recommended titration for detection of Ascorbic acid from food samples.

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## REFERENCES

- [1] M.C. Vissers, A.C. Carr, J.M. Pullar, S.M. Bozonet, *The bioavailability of vitamin C from kiwifruit*, *Adv Food Nutr Res.* 2013;68:125-47.
- [2] A. Zeb, *Chemical and nutritional constituents of sea buckthorn juice*, *Pakistan Journal of Nutrition* 3 (2), 2004, 99-106.
- [3] P. Khandpur, P.R. Gogate, *Effect of novel ultrasound based processing on the nutrition quality of different fruit and vegetable juices.*, *Ultrason Sonochem.*, 2015 Nov; 27: 125-36.
- [4] V. Andrés, M.J. Villanueva, M.D. Tenorio, *The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage*, *Food Chem.* 2016 Feb 1;192:328-35.
- [5] K. Iqbal, A. Khan, M. Muzaffar Ali Khan Khattak, *Biological Significance of Ascorbic Acid (Vitamin C) in Human Health – A Review*, *Pakistan Journal of Nutrition*, 2004, 3 (1), 5-13.
- [6] Gh. Coman, C. Drăghici, M. Badea, N. Bîgiu, C. Ciurea, *Biochimie Descriptivă*, Ed. Univ. Transilvania, Braşov, 2009.
- [7] G. Askari, M. Hajishafiee, R. Ghiasvand, M. Hariri, L. Darvishi, S. Ghassemi, B. Iraj, V. Hovsepian, *Quercetin and vitamin C supplementation: effects on lipid profile and muscle damage in male athletes.*, *Int J Prev Med.*, 2013 Apr; 4 (Suppl 1): S58-62.
- [8] M. Bürzle, M.A. Hediger, *Functional and physiological role of vitamin C transporters*, *Curr Top Membr.*, 2012; 70:357-75.
- [9] L. Hallberg, M. Brune, L. Rossander, *The role of vitamin C in iron absorption*. *Int J Vitam Nutr Res Suppl.*, 1989; 30:103-8.
- [10] Z. Harel, F. Biro, L. Kollar, S. Riggs, P. Flanagan, R. Vaz, *Supplementation with vitamin C and/or vitamin B(6) in the prevention of Depo-Provera side effects in adolescents.*, *J Pediatr Adolesc Gynecol*, 2002 Jun;15(3):153-8.
- [11] S. Rocha Dda, F.D. Capanema, M.P. Netto, C.A. de Almeida, C. Franceschini Sdo, J.A. Lamounier, *Effectiveness of fortification of drinking water with iron and vitamin C in the reduction of anemia and improvement of nutritional status in children attending day-care centers in Belo Horizonte, Brazil.*, *Food Nutr Bull.*, 2011 Dec; 32(4): 340-6.
- [12] E. Zito, H.G. Hansen, G.S. Yeo, J. Fujii, D. Ron, *Endoplasmic reticulum thiol oxidase deficiency leads to Ascorbic acid depletion and noncanonical scurvy in mice*, *Mol Cell.*, 2012 Oct 12; 48(1): 39-51.
- [13] Y. Kim, H. Kim, S. Bae, J. Choi, S.Y. Lim, N. Lee, J.M. Kong, Y.I. Hwang, J.S. Kang, W.J. Lee, *Vitamin C Is an Essential Factor on the Anti-viral Immune Responses through the Production of Interferon- $\alpha/\beta$  at the Initial Stage of Influenza A Virus (H3N2) Infection.*, *Immune Netw.* 2013 Apr; 13(2): 70-4.
- [14] M. Gautam, M. Agrawal, M. Gautam, P. Sharma, A.S. Gautam, S. Gautam, *Role of antioxidants in generalised anxiety disorder and depression*, *Indian J Psychiatry.*, 2012 Jul; 54(3): 244-7.
- [15] R.K. Chaganti, I. Tolstykh, M.K. Javaid, T. Neogi, J. Torner, J. Curtis, P. Jacques, D. Felson, N.E. Lane, M.C. Nevitt; Multicenter Osteoarthritis Study Group (MOST). *High plasma levels of vitamin C and E are associated with incident radiographic knee osteoarthritis.*, *Osteoarthritis Cartilage.* 2014 Feb; 22(2): 190-6.
- [16] M. Dehghan, N. Akhtar-Danesh, C.R. McMillan, L. Thabane, *Is plasma vitamin C an appropriate biomarker of vitamin C intake? A systematic review and meta-analysis*, *Nutrition Journal*, 2007, 6:41.
- [17] J.N. Hathcock, *Council for Responsible Nutrition (CRN), Vitamin and Mineral Safety*, 2nd Edition, Washington, 2004.
- [18] C.E. West, J. Dunstan, S. McCarthy, J. Metcalfe, N. D'Vaz, S. Meldrum, W.H. Oddy, M.K. Tulic, S.L. Prescott, *Associations between maternal antioxidant intakes in pregnancy and infant allergic outcomes.*, *Nutrients.* 2012 Nov 14; 4(11): 1747-58.
- [19] European Food Safety Authority; *Conclusion on the peer review of the pesticide risk assessment of the active substance L-ascorbic acid*. *EFSA Journal* 2013;11(4):3197, available at <http://www.efsa.europa.eu/en/efsajournal/pub/3197.htm>
- [20] M. Shokrzadeh, S. Shobi, H. Attar, S. Shayegan, S.S. Payam, F. Ghorbani, *Effect of vitamins A, E and C on liver enzyme activity in rats exposed to organophosphate pesticide diazinon*. *Pak J Biol Sci.* 2012 Oct 1;15(19):936-41.
- [21] P.K. Khan, S.P. Sinha, *Impact of higher doses of vitamin C in modulating pesticide genotoxicity*. *Teratog Carcinog Mutagen.* 1994; 14(4): 175-81.
- [22] M. Cioroi, *Study on L-Ascorbic Acid Contents from Exotic Fruits*, *Cercetări Agronomice în Moldova*, 2007, 1 (129).
- [23] G. Szalai, T. Janda, M. Pál, *Routine sample preparation and HPLC analysis for Ascorbic acid (vitamin C) determination in wheat plants and Arabidopsis leaf tissues*, *Acta Biol Hung.* 2014 Jun; 65(2): 205-17.
- [24] K.S. Ngai, W.T. Tan, Z. Zainal, R. Mohd Zawawi, M. Zidan, *Electrochemical Oxidation of Ascorbic Acid Mediated by Single-Walled Carbon Nanotube/Tungsten Oxide Nanoparticles Modified Glassy Carbon Electrode*, *Int. J. Electrochem. Sci.*, 2012, 7:4210 – 4222.
- [25] A.S.N. Murthy, *Electrochemical oxidation of L-Ascorbic acid on 7,7,8,8-tetracyanoquinodimethane*, *Biosensors and Bioelectronics*, 1994, 9, 439-444.
- [26] J. Zhou, M. Sheng, X. Jiang, G. Wu, F. Gao, *Simultaneous determination of dopamine, serotonin and Ascorbic acid at a glassy carbon electrode modified with carbon-spheres*. *Sensors (Basel).*, 2013 Oct 16;13(10):14029-40.
- [27] M. Badea, M. Florescu, Gh. Coman, N. Taus, J.L. Marty, *Characterization of electrochemical sensors and enzymatic methods used for Ascorbic acid detection, in Sensing in*

*Electroanalysis*, vol.4, Karel Vytras, Kurt Kalcher, Ivan Svancara (Eds.), Ed. University of Pardubice, Pardubice, 2009, 155-164.

- [28] M. Badea, L. Rogoza, M. Idomir, N. Taus, D.P. Balaban, J.L. Marty, T. Noguer, G.S. Nunes, *Biosensors for Life Sciences, in "Environmental Technologies. New Developments"*, Edited by Burcu Ozkaraova Gungor, I-Tech Education and Publishing, Viena, 2007,. 1-24.
- [29] M. Badea, N. Taus, M. Potrovita, A. Nicolau, L. Dumitrescu, G. Sechel, M. Moarcas, *Heavy metals influence in living bodies using enzymatic, immunohistochemical and chromatographic detections*, In: Environmental Science and Technology, 2008 (vol.1). G. Sorial, J. Hong, Eds., *American Science Press*, Houston, USA, 2008, page 389-396.
- [30] L. Yongxin, H. Xue, C. Yuanli, W. Lun, L. Xiangqin, *Simultaneous determination of dopamine and serotonin by use of covalent modification of 5-hydroxytryptophan on glassy carbon electrode*, *Microchim Acta*, 2009, 164(1-2): 107-112.
- [31] S. Yongxiang, F. Junjie, H. Jie et all., *Simultaneous determination of dopamine and serotonin using a carbon nanotubes-ionic liquid gel modified glassy carbon electrode*, *Microchim Acta*, 2009, 165(3-4), 373-379.