

## Vitamin C as Contributor to the Total Antioxidant Capacity - Importance, Occurrence, Methods of Determination

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Vitamin C is a water-soluble antioxidant vitamin which can be found in many biological systems and foodstuffs (fresh vegetables and fruits). It is important in synthesizing collagen, in the absorption of iron, and is also involved in wound healing and osteogenesis. It represents the L-enantiomer of ascorbic acid and is recognized as one of the most common electroactive biocompound. Significant sources include blackcurrant, citrus fruit, leafy vegetables, tomatoes, green and red peppers [1-4]. Humans, some other primates, and guinea pigs lacking L-gulonolactone oxidase due to a genetic mutation, are unable to synthesize ascorbic acid and diet is the unique source [5].

Ascorbic acid is easily degraded by enzymes and atmospheric oxygen. Its oxidation can be accelerated by heat, light, and heavy metal cations, especially in alkaline media [6]. Its content variation determined by this lability implies the use of performant methods of analysis in complex media during the stages of manufacturing and storage, as vitamin C represents a quality indicator and contributor to the antioxidant properties of foodstuffs [7,8].

Ascorbic acid behaves as a free-radical scavenger, can quench singlet oxygen, or act as chelating agent [6]. Excessive ascorbic acid amounts may result in taste or aroma alteration, caused by the inhibition of natural processes occurring in food [8]; an ascorbic acid excess can lead to gastric irritation, and its metabolic product (oxalic acid) can cause renal problems [8]. Another drawback of its excess is the ability to act as a prooxidant in the presence of heavy metal cations [9]. Traditional methods for ascorbic acid assessment involve titration with an oxidant solution: Dichlorophenol Indophenol (DCPIP) [6], potassium iodate [10] or bromate [11].

Fluorimetric methods based on dehydroascorbic acid reaction with o-phenylene diamine were also used for ascorbic acid estimation [12]. UV-VIS absorbance was applied to ascorbic acid quantification in horticultural products [13]. Other optical methods for vitamin C estimation involve spectrophotometrical determination of iodine reacted with ascorbic acid [14], spectrophotometrical determination after the analyte reaction with hexacyanoferrate III [15], with complexes such as Fe(III)-1,10 phenantroline [16] or Cu(II)-neocuproine [17] or with dinitrophenylhydrazine [18], and chemiluminescence [19]. Ascorbic acid is able to quench DPPH, yielding the absorbance diminution of the free radical solution at 515 nm, with linearity obtained up to 50  $\mu$ M, which constitutes a premise for the future use of vitamin C as a reference for total antioxidant capacity assessment [20]. Vitamin C has been successfully determined by HPLC with electrochemical detection [21,22].

Electrochemical methods allow rapid, simple, selective and sensitive determination of low molecular weight antioxidants, including ascorbic acid and drugs, without the necessity of laborious sample pretreatment or time consuming separation. Voltammetry is based on imposing a controlled potential variation in time, with the recording of the intensity-potential dependence-the voltammogram [8]. In cyclic voltammetry, the potential varies as a triangular wave form and the analytical signal is represented by the intensity of the anodic peak,

corresponding to ascorbic acid oxidation to dehydroascorbic acid. In differential pulse voltammetry regular voltage pulses are superimposed on potential linear sweep or stair steps. The current is measured immediately before each potential change, and the current difference is plotted as a function of potential. In differential pulse techniques, a differential value is actually measured, aiming to minimize the interference of the capacitive current and to enhance the contribution of the faradaic one [23].

A cyclic voltammetric method at bare Pt disc working electrode allows viable vitamin C determination in fruit juices, with linearity obtained between 0.1 and 10 mM. The richest sample in ascorbic acid concentration was lemon juice, showing a 1.93 mM ascorbic acid content. The degrees of recovery were comprised between 94.35% and 104%. Comparative voltammetric assessment of vitamin C at unmodified carbon paste and Pt strip working electrodes proved better sensitivity of carbon paste electrode: the limit of detection and the limit of quantification obtained by differential pulse voltammetry were 0.087 mM and 0.29 mM respectively, when a Pt electrode was used, vs. the values of 0.02 mM and 0.068 mM obtained with carbon paste [24].

In media where interference occurrence is expected, the use of modifiers enhances the catalytic peak current of the analyte of interest and reduces overvoltage, allowing better peak separation from interferent compounds. The electrooxidation and determination of ascorbic acid were studied, at unmodified carbon paste and tetrabromo-p-benzoquinone modified carbon paste electrodes, pH 7.0, in phosphate buffer. Thus, a decrease in the overvoltage of approximately 430 mV and an enhancement of the peak current is achieved with the modified electrode [25]. A carbon paste electrode modified with a 2,2'-(1,8-octanediy)bisnitriloethylidene)-bis-hydroquinone exhibited high electrocatalytic activity vs ascorbic acid; the current was enhanced, in comparison to the unmodified electrode. Differential pulse voltammetric calibration curves for ascorbic acid were obtained over the range of 5-30  $\mu$ M and 40-1,500  $\mu$ M, respectively allowing viable assessment in pharmaceuticals [26].

Potentiometric biosensors based on ionic species concentration change following a biocatalytical reaction or, more often amperometric biosensors relying on electron transfer reactions, were also applied

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in specific ascorbic acid quantification in real time. The reduction of  $\text{Cu}^{2+}$  in the ascorbate oxidase structure to  $\text{Cu}^+$  by ascorbate, determines changes in the electronic density on the surface of a potentiometric transducer based on a poly(ethylene-co-vinyl acetate) matrix, fixed on a graphite-epoxy composite [27]. Amperometric ascorbic acid biosensors were obtained by ascorbate oxidase immobilization on a nylon net or on a collagen membrane, using a Clark oxygen electrode as transducer [28,29].

More recently, Carbon Nanotubes (CNTs) have been used in biosensor development due to their enhanced electroactivity and large surface area, as well as to their good biocompatibility [30,31]. Ascorbate oxidase was covalently immobilized on carboxylated multiwalled carbon nanotubes - polyaniline layer, electrochemically deposited on an Au electrode [31]. The amperometric response was given by the reduction of molecular oxygen non-consumed in the enzyme reaction, and vitamin C was assessed in sera, fruit juices and tablets, with results consistent with those provided by the dichlorophenolindophenol method. The different instrumental or titrimetric methods applied to vitamin C quantitation should be tuned to the nature of the sample analysed.

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