A. Shokuhi Rad

Vitamin C Determination in Human Plasma Using an Electro-Activated Pencil Graphite Electrode

Abstract The intake of sufficient amounts of vitamin C (ascorbic acid) is necessary for good human health. Therefore, simple, rapid methods for determination of the exact amount of vitamin C in human plasma are important for assessment purposes. A pencil graphite electrode was used to quantitate the amount of vitamin C in human plasma samples. The results indicated that the anodic peak current for ascorbic acid was linear in the concentration range of $10^{-6}$ to $10^{-4}$ M with a correlation coefficient of 0.9998. The obtained detection limit for AA using this amperometry technique was $1.18 \times 10^{-6}$ M.

Keywords Ascorbic acid · Pencil graphite electrode · Electrochemically activated electrode · Amperometry detection

1 Introduction

Ascorbic acid (AA), (Fig. 1) is an essential nutrient for higher primates and a small number of other species. The presence of AA or vitamin C is required for a range of essential metabolic reactions in all animals and plants. It is made naturally by almost all organisms, with humans being a notable exception. For this reason, vitamin C is widely used as a food additive [1]. A vitamin C deficiency leads to scurvy, a disease characterized by weakness, small hemorrhages that cause gums and skin to bleed, and loosening of the teeth. Sailors at sea for months on end would often develop scurvy unless the captain had the foresight to pack limes and other citrus fruits. Vitamin C is a water-soluble antioxidant, and plays a vital role in protecting the body. Oxidizing agents such as smog and cigarette smoke both contain high levels of oxidizing molecules that cause tissue damage. The body also makes oxidizing molecules in response to an infection, and these molecules kill both the infecting organism and cause tissue damage [1–3].

The pharmacophore of vitamin C is the ascorbate ion. In living organisms, ascorbate ions are an antioxidant as they protect the body against oxidative stress and are a cofactor in several vital enzymatic reactions.
Ascorbic acid can exist as the L or D enantiomer; whereas only the L enantiomer exists in nature, the D enantiomer, which has no biological functions, is produced synthetically. For humans, the amount of vitamin C suitable for daily intake is 80 mg.

The method for the determination of AA is based on its redox property as it is easily reduced to dehydroascorbic acid via an oxidation reaction. Indeed, the amount of vitamin C in fruit juice is decreased over time because of the oxidation of AA to dehydroascorbic acid. Therefore, for determining the amount of AA in fruit juice samples, prompt measurement of vitamin C in the juice is needed to obtain the correct amount of AA. To prevent the occurrence of the oxidation reaction of vitamin C during the measurement period, 0.1 N sulfuric acid solution is added to vitamin C samples. The US pharmacopoeia describes a standard method for determination of vitamin C in medicines where a 0.15 g amount of the medicine is dissolved in 80 ml of distilled water and 10 ml of 1.0 M sulfuric acid. The obtained solution is titrated using a 0.05 M I2 solution after adding 1 ml of a 1% starch solution. The titration end point is determined when a violet blue color appears. One millilitre of the consumed iodine corresponds to 8.805 mg of AA in the sample.

The standard method of determination of vitamin C in vegetable and fruit juices uses a 2,6-dichlorophenol (DCIP) solution. In this method, 2 ml of the AA standard solution is titrated with an indophenol-sodium bicarbonate solution, where the end point is determined upon the appearance of pink color. Other techniques of AA determination include titrimetric, chemiluminescence, fluorometry and electrochemical methods. The electrochemical method of determination of AA is the preferred technique for measurements in biological tissues. The only disadvantage of this method is the decrease in the response level of the electrode in multiple application runs because of the contamination of the electrode surface by oxidation reaction products after repeated runs. Other electrochemical techniques such as polarography analysis, square wave voltammetry, differential pulse polarography, amperometric sensing, potentiometric titration, colorimetry and modified electrodes have also been applied for measurements of AA. The presence of some components such as uric acid, dopamine and proteins cause some interference in AA determinations. A Glaccy Carbon electrode (GC) coated with polypyrrole and dipped in ferrocyanide can be used in a mixed solution of AA and uric acid for AA determination. Recently some new techniques have been reported for selective determination and measurement of AA.

In the present paper, a pencil graphite electrode was used as the measuring electrode for AA determination. This electrode is cheap, easily prepared and has a wide electrochemical activity range with a high AA peak current.

2 Material and Methods

Table 1 shows the analytical grade materials that were purchased from Merck (Germany). All chemicals were used without further purification. Deionized water was produced in the water treatment unit of Shahid Qazi Pharmaceutical Company (Tabriz, Iran) and was used in the preparation of solutions. The UA and AA solutions were prepared immediately before use. Human plasma was purchased from RAZI pharmacy (Iran).

2.1 Apparatus and Set up

An auto lab apparatus (PGSTAT20 model, The Netherlands) was used for voltammetry measurements as a source of potential supply. The apparatus was connected to a Pentium S computer (200MHz) equipped with...

Fig. 1 The molecular structure of ascorbic acid