Trade Science Inc.

April 2008 Volume 7 Issue 6

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ 7(6) 2008 [423-428]

Determination of ascorbic acid in human's blood serum using electrochemically activated pencil graphite electrode

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Received: 3rd March, 2008 ; Accepted: 8th March, 2008

ABSTRACT

The intake of sufficient amount of vitamin C (ascorbic acid) in the daily nutrition of human being is necessary to have a healthy life. Therefore, determining the exact amount of this supplement in the Human's blood samples is one of the important measures for the assessment of healthy life. After improving the catalytic activity, the pencil graphite electrode was used to determine the amount of vitamin C in the human's blood plasma samples. The obtained results show that the anodic peak current for ascorbic acid is linear for the concentration range of 10^{-6} to 10^{-4}M having the correlation coefficient of 0.9998. The obtained detection limit for AA determination using amperometry technique was 1.18×10^{-6}M.

KEYWORDS

Ascorbic acid;
Pencil graphite electrode;
Electrochemically activated electrode;
Amperometry detection.

INTRODUCTION

Ascorbic acid (AA) or vitamin C is an essential nutrient for higher primates and a small number of other species. The presence of vitamin C is required for a range of essential metabolic reactions in all animals and plants. It is made naturally by almost all organisms, human being one notable exception. It is widely known as the vitamin whose efficiency causes scurvy in humans. It is also widely used as a food additive[1].

Vitamin C deficiency leads to scurvy, a disease characterized by weakness, small hemorrhages throughout the body that cause gums and skin to bleed, and loosening of the teeth. Sailors that were out 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 species attack the body from many directions. Smog and cigarette smoke both contain high levels of oxidizing molecules that cause tissue damage. The body makes oxidizing molecules in response to an infection, and these molecules both kill the infecting organism and cause tissue damage[1,3,20].

The pharmacopoeia of vitamin C is the ascorbate ion. In living organisms, ascorbate ion is an antioxidant as it protects the body against oxidative stress and is a cofactor in several vital enzymatic reactions[1,4].

Ascorbic acid is found as both L and D enantiomers where only L enantiomer exists in nature and D enantiomer is produced synthetically. The later enantiomer has no vitamin value or property[3]. The significant role of AA in some chemical and biochemical reactions of live tissues was proved and the suitable amount of daily intake of vitamin C for human is 80 mg[5].

The method of determination of AA is based on its redox property, where it is easily is reduced to dehy

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droascorbic acid via oxidation reaction. It is important to know that the amount of vitamin C in fruit juices is decreased by time due to the oxidation of AA to dehydroascorbic acid. Therefore, in determining the amount of AA in fruit juice samples, the prompt measurement of vitamin C in the juice is needed to obtain the correct amount of AA. In order to prevent the occurrence of the oxidation reaction of vitamin C during the measurement period, 0.1N sulfuric acid solution is added to the vitamin C samples[6,17].

United States of America pharmacopoeia issued the standard method of determination of vitamin C in medicines[4] where a 0.15g amount of the medicine is solved in 80 ml distilled water and 10ml of 1.0M sulfuric acid is added to it. The obtained solution was tittered using a 0.05M of I_2 solution after adding 1ml of 1% starch glue solution. The titration end point is determined when the violet blue color is appeared. One ml 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 is Titrimetric using 2,6-dichlorophenol (DCIP) solution[3,18]. In this method, 2 ml of the standard solution of AA is tittered by an indophenols-sodium bicarbonate solution, where the end point is determined upon the appearance of pink color.

Recent techniques of AA determination include Titrimetric[3,7], chemiluminscence[8], fluorometery[9,19] and electrochemical methods[10,11.20,21]. The electrochemical method of determination of AA is the preferred technique for such measurements in biological tissues. The only disadvantage of this method is the decrease in the response level of the electrode in multiple application runs which is due to the contamination of the electrode surface by oxidation reaction products after some repeated runs[11,16].

Different electrochemical techniques such as polarography analysis, square wave voltametery, differential pulse polarography, amperometric sensor, potantiometric titration, colorimtry and improved electrodes were applied for such a measurements using conductive polymers[12-15].

The presence of some components such as uric acid, dopamine and proteins cause some interference in AA determinations. The application of the GC electrode coated by polypyrol and dopped by ferocyanide in a mixed solution of AA and uric acid, for AA determination[1] as well as some newly developed techniques for selective determination and measurements of AA samples are cited in the literature[20,21].

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

EXPERIMENTAL

TABLE 1 Shows the used materials that are analytical grade and are purchased from Merck company (Germany).

All chemical materials has used without subsequent purification. Deionized water 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 has prepared and utilized on time. Human's blood serum (SA X1 model) purchased from RAZI pharmacy (Iran)

Apparatus and set up

Autolab apparatus (PGSTAT20 model, Netherlands) was used in voltametery measurements as a source of potential supply and its stabilizing. The apparatus was connected to a Pentium S computer (200MHz) equipped with a GPES software (level 4 and 5). Metrohm pH-meter (691 model, Switzerland) was used to measure pH values.

TABLE 1: The used materials

Usage	Chemical formulation	The name of stuff
For buffer preparation	$C_8 H_5 K O_4$	Potassium dihydrogen phthalate
For buffer preparation	KH_2PO_4	Potassium dihydrogen phosphate
For buffer preparation	K_2HPO_4	Dipotassium phosphate
For buffer preparation	$NaHCO_3$	Sodium bicarbonate
For buffer preparation	NH_3	ammoniac
For buffer preparation	HCl	Hydrochloride
For buffer preparation	NaOH	Sodium hydroxide
For assaying electrocatalytic property	C6H8O6 C6H8O6	Uric acid ascorbic acid
For polishing the surface of electrode	Al2O3	Alumina powder

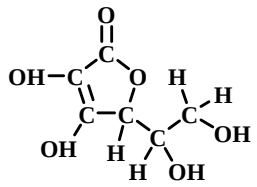

Figure 1: The molecular structure of ascorbic acid

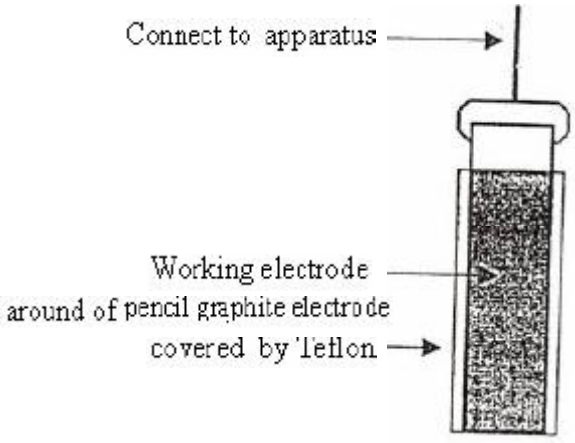

Figure 2: The schematic structure of pencil graphite electrode

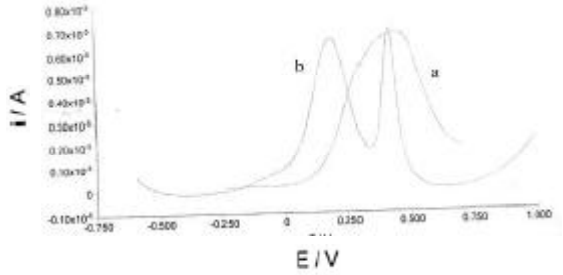

Figure 3: Differential pulse voltamograms of solution contain 2mM AA and 0.2mM UA, in phthalate buffer solution (pH=5) on: a- inactivated pencil graphite electrode and b- activated pencil graphite electrode

Applied electrodes

In order to carry out the voltametery technique, a standard three electrodes system was used to decrease the level of ohmic drop. The used cell volume is 20 ml. A pencil graphite electrode and a platinum bar electrode were used as the working and counter electrodes, respectively. A saturated calomel electrode was used as the reference electrode, as well. Both reference and counter electrodes, are made by Azar Electrode Company (Tabriz, Iran).

Preparation of pencil graphite electrode

The preparation of pencil graphite electrode In order to obtain a constant cross sectional area of the pencil graphite electrode, the side area of the pencil is completely covered by wrapping a teflon tape on such a way that only the cross sectional area of the pencil in one end is uncovered and this side is immersed inside the solution while the other end is connected to the electrode wire.

Using differential pulse voltametry technique, the response of AA on the surface of all the mentioned electrodes are studied.

RESULTS AND DISCUSSION

The effect of the following parameters on the feasibility and accuracy of AA determination were investigated:

Activation of pencil graphite electrode

Due to the co-existence of ascorbic acid and uric acid in real samples, it occurs the peaks overlapping of these two acids. Therefore, the applied electrode should have the potential of giving two distinct peaks for AA and uric acid. The pencil graphite electrode was activated for such a purpose.

The experimental results show that the activation produce by a cyclic voltametry technique yields a linear calibration curve. In this method 10 cycles at the range of -0.6 to 2.0V with scanning rate of 100 mV/s in the sodium bicarbonate buffer solution (pH=10) was applied to activate the electrode. At these conditions, the severance between two peak potentials (?E_p) was 232 mV as shown in figure 3.

The effect of scanning rate

The cyclic voltamograms of AA for different scan rates including 5, 10, 20, 50, 100, 200 and 300 mV/s are obtained for 2mM AA in a potassium dihydrogen phthalate buffer solution (pH=5) to analyze the effect of the scanning rate on the voltamograms. The results are shown in figure 4.

As shown in figure 4, by increasing the scanning rate, the anodic peak current (Ip) of AA is increased and the current is displaced to the more positive peak potential (Ep) values.

It can be founded in figure 4, that the anodic peak

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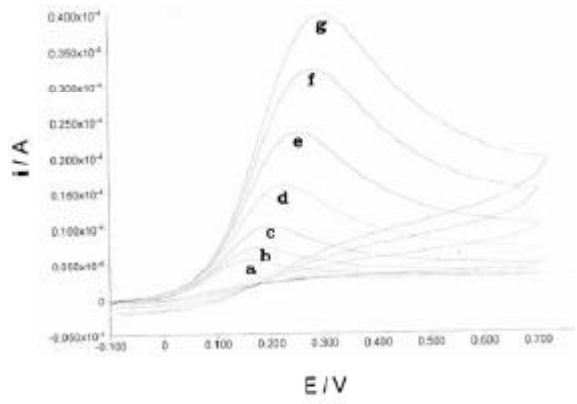

Figure 4: The cyclic voltamograms of 2mM AA solution on activated electrode with applying different scanning rates: (a) 5, (b) 10, (c) 20, (d) 50, (e) 100, (f) 200 and (g) 300mV/s

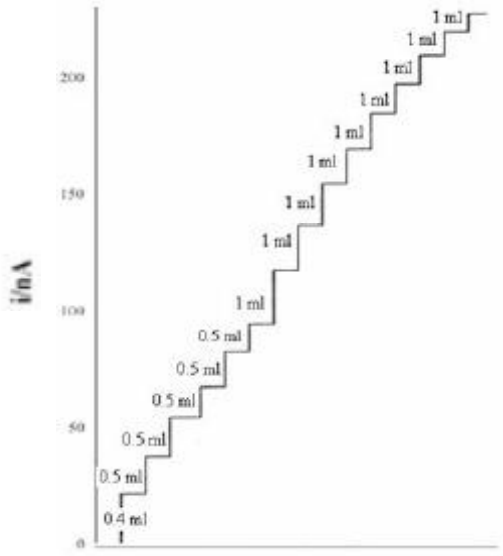

Figure 5: The amperometric response due to addition of solutions with different AA concentration to electrochemically cell containing mother solution(0.2mM of AA) in phthalate buffer (pH=5). Other conditions are: E=0.13V / SCE , by applying constant potential of working electrode

current of AA has a linear relationship with the scanning rates ($R^2 = 0.9986$), which proves the diffusive mechanism of the transfer of AA[2].

The anodic peak potential values for AA has a linear relationship versus the logarithm of scanning rate. Such a linear relationship proves that the electrochemical reaction is an irreversible and diffusion controlled reaction[2].

The electrocatalytic oxidation of ascorbic acid on the activated electrode

The concentration of AA in the human's blood serum is at the range of 0.5 to 1.5mg/dl (28 to 85μM). However, the detection concentration threshold of AA determination by differential pulse voltametry technique is 7.5μM. So, the more sensitive amperometry technique was applied for AA concentration determination.

In this method the potential of the working electrode is fixed versus the reference electrode and the real sample or a standard AA sample is added to the electrochemical cell while the electrolyte is mixing at constant rate. Since the potential value is stabilized near the peak potential value and the intermediate is immediately oxidized at the electrode surface, the background current approaches to zero. In such a condition, by adding AA, the obtained current has a quite electrocatalytic character and is proportional to AA concentration[2].

Analytical parameters

Ascorbic acid concentration standard curve

The electroactivated electrode was prepared under the optimum condition as mentioned at section 3.1.

For achieve to the range of concentration that the current changing be linear, amperometry responses are plotted versus AA concentration values at the fixed potential (0.13 V/SCE) of the working electrode. The obtained linear plot was used as the standard curve.

Figure 5 Represents the amperometry responses upon increasing AA concentration at the range of 10^{-6} to 10^{-4} M of AA concentration.

L.O.D appointment

The results obtained from standard curve portrait are presented in TABLE 2. From some easy statically calculation, the gained detection limit for AA was 1.18μM Using amperometric method.

TABLE 2: The statically date related to AA

X_i(μM)	6.89	14.8	22.2	29.0	35.3	41.1	51.6	60.8	69	76	82.7	88.5	98.6	103
Y_i(nM)	21	37	54	67	82	94	117	136	154	169	184	197	219	227
$\hat{Y}$(nA)	19.3	36.5	52.6	67.3	81.0	93.6	116.5	136.5	154.3	169.5	184.1	196.7	218.7	228.2
$(Y_i - \hat{Y})^2$	2.99	0.28	2.07	0.11	0.92	0.12	0.27	0.22	0.09	0.27	0.01	0.09	0.11	1.48

$Y = a + bx$ $\sum (y_i - \hat{y})^2 = 9.63$ n-2=13

$a = 4.2935$ $X_{L.O.D=} 1.18 \Rightarrow b = 2.1741$

$S_{y/x} = 0.86$ $Y_{L.O.D} = 6.87 \times 10^{-6} M$

Determination of AA in human's blood serum

The normal range of the amount of vitamin C in the human's blood serum is 0.5 to 1.5 mg/dl[2,21]. The used real sample for AA determination, was Humans' blood serum (SA X1 model) purchased from RAZI pharmacy (IRAN).

In this real sample, some proteins and both AA and UA are present, so at first stage these disturbances must be considered.

For plasma's protein elimination, it used protein's coagulator solution. The plasma protein coagulant solution was prepared by solving 3g glacial metaphosphoric acid in 10 ml of 80% acetic acid and consequent diluted by distilled water to achieve 100ml[22].

After preparation of protein's coagulator solution, a volume of 0.8 ml of solution was mixed with 0.8 ml of fresh blood plasma and was centrifuged for 15-20 min at 800 rpm. After centrifugation, the supernatant is used for analysis of AA. This solution is free proteins but contain uric acid (UA) as disturbance.

Using electroactivated electrode as proposed method and with applying deferential pulse voltametry (DP), it cause to segregation of AA and UA peak potential with appropriate distance as shown in figure 3. So it is possible to simultaneous determination of AA and UA in the real samples.

In order to determine the amount of AA in the Human's blood serum, some serum samples, which are previously tested for uric acid content (by differential pulse voltametry method), are selected and treated by the following method.

The measurement of AA was carried out using 0.4 ml of the prepared sample and poured in the electrochemical cell. The amperometry response after mixing the solution was graphed in the constant potential of the working electrode.

For applying standard addition method, it was used four steps to AA standard addition (with concentration of 0.2mM) to unknown amount of AA at phthalate buffer solution (pH=5), with E=0.13 V/SCE and diffusion controlled steady state condition. The four-step additions were: 0.5, 0.5, 1 and 1ml.

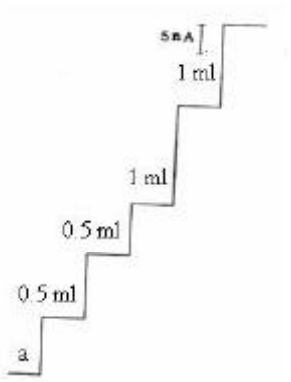

Figure 6: The amperometric response related to four steps of AA standard addition: 0.5, 0.5, 1 and 1 ml. (a) is related to 0.4 ml of free protein human's blood's plasma with no standard addition

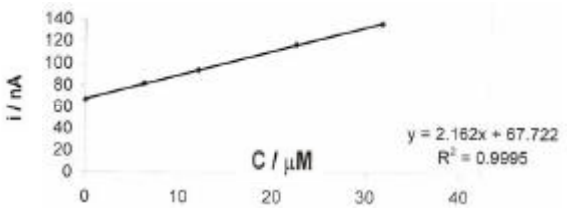

Figure 7: AA amperometric response changes versus added standard solution concentration

After each addition, it portrayed amperometric response of mixed solution. The result has shown in figure 6.

The unknown concentration of AA in the real samples can be found on extrapolation of AA amper ometric response's changing versus added standard solution concentration. The result is shown in figure 7.

The calculated concentration of AA in this real sample was achieved: 0.516mg/dl (2.93×10^{-5}M) using this method.

CONCLUSION

Pencil graphite electrode could be used as the working electrode for vitamin C determinations in the real samples. The electrode precision order is comparable to common inert electrodes such as platinum and gold electrodes. The activation of pencil graphite electrode is very simple and cheap. After carrying out electrochemical activation and optimizing the working electrode condition, the anodic peaks of ascorbic acid and uric acid are quite distinct. After improving the catalytic

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activity, the pencil graphite electrode was used to determine the amount of vitamin C in the human's blood plasma samples. The obtained results show that the anodic peak current for ascorbic acid is linear for the concentration range of 10^{-6} to 10^{-4}M having the correlation coefficient of 0.9998. The obtained detection limit using differential pulse voltametery technique was 7.54×10^{-6}M, where amperometry method was used to improve the detection precision. The obtained detection limit for AA determination is 1.18×10^{-6}M.

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