

**An advance chemical analysis technique for determination and kinetic study
of Ascorbic acid in various samples**

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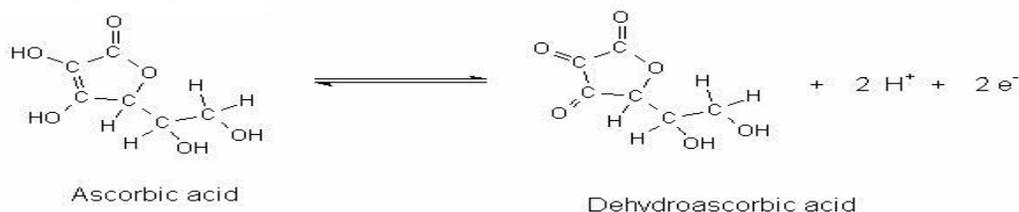
Abstract

Ascorbic acid is one of the important water soluble vitamin and is a powerful antioxidant naturally present in many foods especially in fruits and vegetables, which play an important role in the prevention of infectious diseases. In the present work an attempt has been done to develop simple indirect spectrophotometric method for determination of L- Ascorbic Acid in Various samples like in vegetables and milk. Results from the present study indicate that the developed method is robust, simple and reproducible.

Keywords: Ascorbic Acid, fruits, spectrophotometer, kinetic Study.

Cite this article as: Kalamkar DG, Thorat PV, Zamre GS, Meshram M . An advance chemical analysis technique for determination and kinetic study of Ascorbic Acid in various samples. Annalen der Chemischen Forschung. 2013; 1(3):41-46.

The best understood function of ascorbic acid is its role in synthesizing the protein collagen. Ascorbic acid is extremely important for the formation of intracellular material. It also influences the formation of hemoglobin and the maturation of erythrocytes. It is very important for wound healing. Ascorbic acid increases the cross-connections between amino acids in collagen, greatly strengthening the tissues it helps form[1]. It enhances iron absorption by keeping iron in its most absorbable form. It is vital for the function of the immune system, especially for the activity of certain cells in the immune system. Finally it is also necessary for the synthesis of a number of hormones, neurotransmitters, and other compounds, such as bile acids and DNA. Vitamin C is an essential nutrient for our organism. It is necessary for our body's development. Humans, as well as other primates and other species, cannot synthesize this nutrient due to the absence of the enzyme L-gulonolactone oxidase in their organisms. This enzyme is capable of catalyzing the conversion of glucose in vitamin C (without enzymatic action). Therefore, it is extremely important to maintain the intake of vitamin C in order to develop a healthy organism. Ascorbic acid is also useful in organic synthesis [2]. Ascorbic acid is present in various vegetables like tomato and lemon and in dairy products like milk. Even though, many methods are available method for determination of Ascorbic acid, many of reported methods [3- 9] suffer from one or the other disadvantages. Hence, in the present work an attempt has been done to estimate Ascorbic acid in vegetable and to study the degradation of vitamin C in various samples .An attempt will also be done to determine the order of reaction followed by samples and reaction rate as well as activation energy of the kinetic study will be carried out.



Chemicals used

Auramine, Sodium acetate, Oxalic acid, Sodium salt of EDTA, Potassium iodide. Potassium iodate and HCl were purchased from commercial source and used for the present study

Methodology

a) Stock Solution

Stock solution of Ascorbic acid (in double distilled water) was prepared by dissolving 100mg of Ascorbic Acid in 100ml in water. Working solutions were freshly prepared by appropriate dilution of stock solutions with water. Potassium iodide -potassium iodate (KI-KIA) Mixture was prepared by mixing 0.1 mol l⁻¹ potassium iodide and 0.2 mol l⁻¹ potassium iodate in 5:1 ratio. This solution was prepared daily and kept in amber colored bottle. A UV-Visible spectrophotometer SL-210 double beam was used for all spectral measurements. pH A centrifuge having a maximum centrifugal force of 1850 g with fixed swing out rotors was used for centrifugation.

b) Determination of Ascorbic Acid in fruit juices and Vegetables

Fruit samples were weighed; juice was separated from fruits with a mechanical press and centrifuged. A 1.0 ml aliquot of juice was diluted to 100ml with 0.2 mol l⁻¹ oxalic acid to avoid losses of Ascorbic acid due to air oxidation, to this mixture 5% EDTA (1 ml) was added and the solution was centrifuged for 5min. Supernatant liquid was further diluted to a suitable volume with deionized water on the basis of Ascorbic Acid concentration in fruits. In aliquot, auramine dye (2 ml, 0.01%) was added, followed by sodium hydroxide (2 ml, 0.01 N) and then analyzed.

Various samples of vegetables were cut into small pieces and 4-5 g were homogenized with 100-150ml of 0.2 mol l⁻¹ oxalic acid as soon as possible to avoid any oxidation of Ascorbic acid. To this, added 1 ml of EDTA(5%) and centrifuged. Supernatant liquid was diluted to a suitable volume and 1 ml aliquot was analyzed.

C) Determination of Ascorbic Acid in pharmaceuticals and Biological samples:

All drug samples tested were fresh and purchased from local pharmacy. An Ascorbic acid tablet or content of a capsule was weighed, ground to a fine powder and stirred for 2-3 min with 50ml of deionized water. Then EDTA (1 ml, 5%) was added and filtered through filter paper. Insoluble mass was washed with three successive 5 ml portions of water and filtrate plus washings were diluted to 250 ml calibrated flask. A known volume was further diluted depending on Ascorbic acid content and color of the sample. Since presence of Ascorbic Acid has been reported in samples, prior to determination of Ascorbic acid 5% EDTA (1 ml) and TCA (Trichloroacetic acid) (2 ml, 1%) were added, centrifuged, diluted to a suitable volume and aliquot (1 ml) was analyzed.

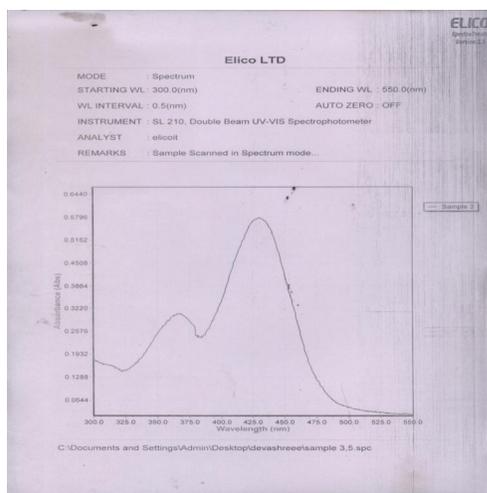


Figure-1: Analysis of ascorbic acid

Results obtained during the study are represented in figure 1-5. Further the data obtained from the experiments were substituted in kinetic equation as described in the following section.

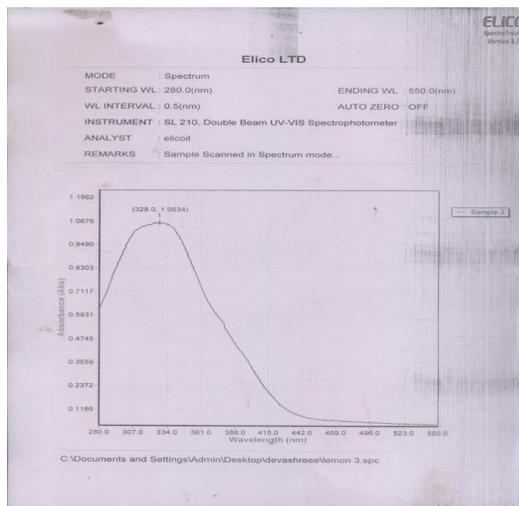


Figure-2: Analysis of ascorbic acid in lemon

D) Kinetic study

Loss of Ascorbic acid in various samples concentrates was calculated by using the Standard equation for first order reaction given below.

First –Order Reaction is given by:

$$\ln C = \ln C_0 - kt \quad \text{or} \quad \ln(C/C_0) = - kt$$

Where, C= concentration at time t, Co= concentration at time zero, K= first –order rate constant, t= time

Temperature Dependence of Ascorbic Acid was determined by using Arrhenius Equation

$$K=K_0 e^{-E_a/RT}$$

Where, K= rate constant ,K₀= pre-exponential factor, E_a= activation energy(K mol l⁻¹) R= gas constant ,T= absolute temperature in K

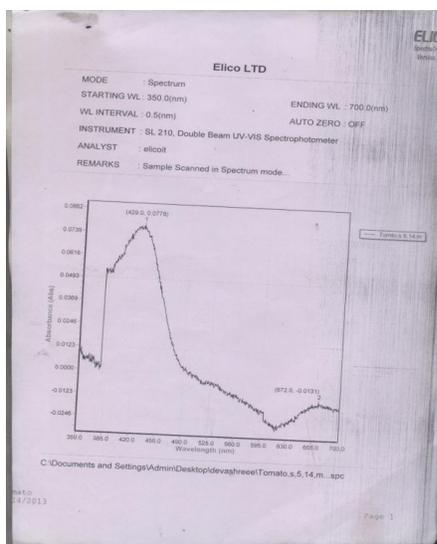


Figure-3: Analysis of ascorbic acid in tomato

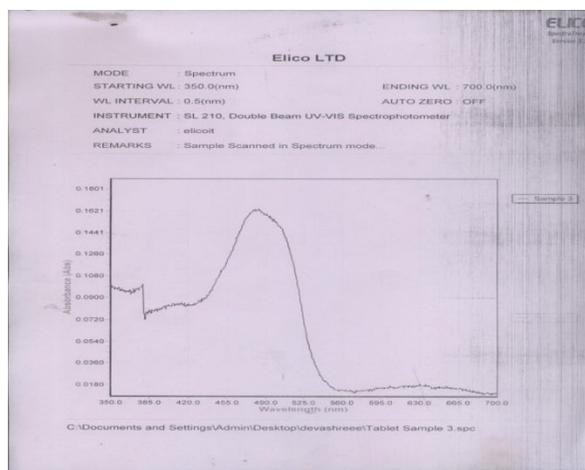


Figure-4: Analysis of ascorbic acid in tablet

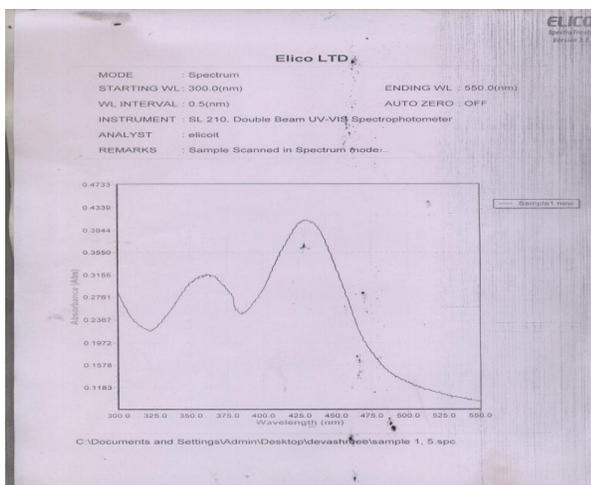


Figure-5: Analysis of ascorbic acid in milk

Diagrammatic representation of the results obtained by the kinetic study of ascorbic acid in Lemon and Tomato is represented in Figure-6 and Figure-7.

a) First-order Reaction

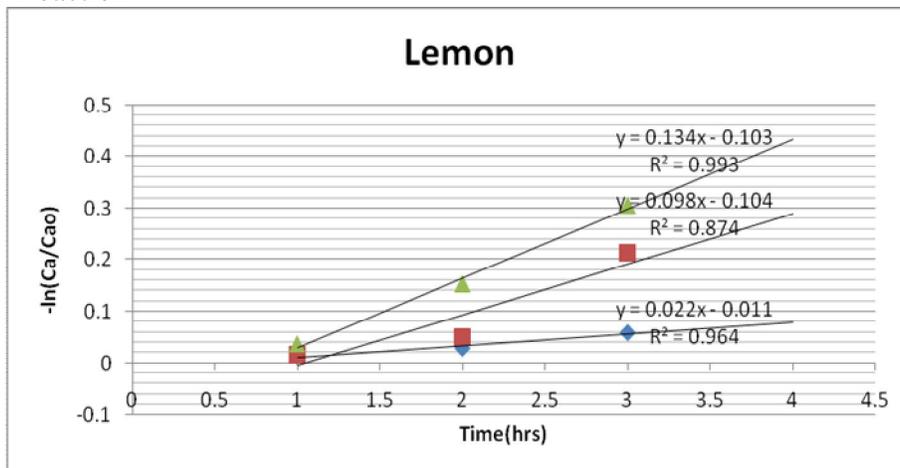


Figure-6: Kinetic study of ascorbic acid in Lemon

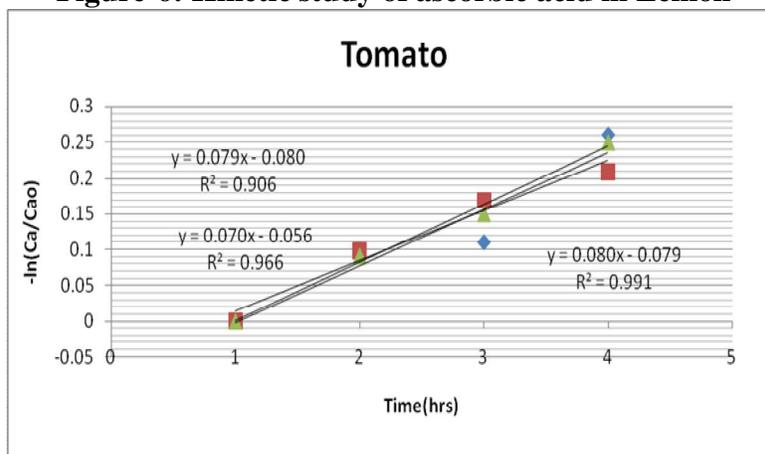


Figure-7: Kinetic study of ascorbic acid in Tomato

Results of kinetic study was used for calculating activation energy and the data is given in Table-1.

Sample	R ²
Tomato	0.9921
Lemon	0.9986

Table-1: Activation energy

In the present work, an attempt has been done to estimate ascorbic acid in various samples like in vegetables and milk. Outcome of study provided a new, simple and highly sensitive method for determination of Ascorbic Acid in Various samples. The stability of formed Auramine O dye is an added advantage of the method. The proposed mathematical models permit a simulation of the vitamin C degradation rate in samples.

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