Green, Rapid, Reproducible, and Economical (GRRECO) Method for Estimation of Ascorbic Acid: Iodine Clock Method

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ABSTRACT

Ascorbic acid is an essential micronutrient and a potent antioxidant for humans which performs pleiotropic functions related to the donation of electrons. Over the years, ascorbic acid is estimated by numerous methods like titration, and spectrophotometric, which are slightly more time-consuming, complex, and requires more chemicals. However, determination nowadays is becoming important because of its diversified roles in a simple, economical method and causing less harm to the environment. The Iodine clock method in this paper requires minimalistic chemicals, no spectrophotometer, and is easy to perform. It is a simple time-based, oxidation-reduction reaction in which colourless reagents are mixed and following a short pause, the mixture turns blue–black in the presence of iodine. The chemical determination of ascorbic acid is based upon its highly reducing capacity by which oxidation of iodide ions to iodine takes place. This conversion is reported in time taken, which is generally a few seconds. This paper covers the estimation of ascorbic acid in a few organic samples by the iodine clock method and a comparative analysis with other conventional methods. Thus, the iodine clock method holds as a potential method for the estimation of ascorbic acid in the coming future.

Keywords : *Ascorbic acid, oxidation-reduction, iodine-clock, anti-oxidant, green analytical chemistry*

1. Introduction

Vitamins are group of organic compounds that are important for critical functions in the body. They are categorized into fat-soluble and water-soluble based on their solubility and stability. Vitamin C is a six-carbon keto-lactone that is naturally synthesized from glucose by most animals in the liver or kidney and exists in different redox forms (Holford et.al, 2020). This is an essential water-soluble vitamin that is helpful in some fundamental pathways in the body which includes hydroxylation of proline and lysine in collagen, antioxidant defense (López-Pastor et.al, 2020), co-factor to mono-oxygenase and di-oxygenase enzymes (Carr et.al, 2017) and even against COVID, where its virucidal activity and effector mechanisms against innate and adaptive immune systems showed a great response in affected patients (Holford et.al, 2020). Thus, consuming vitamin C in adequate amounts in our diet is important. Epidemiology studies in India state the prevalence of vitamin C deficiency, where 73.9% (95% confidence Interval, CI 70.4,77.5) in 2668 people in north India and 45.7% (95% CI 42.5,48.9) in 2970 from south India. Merely, 10.8% in the north and 25.9% in the south met the criteria for adequate levels (Ravindran et.al, 2011).

Now, it becomes important to estimate vitamin C in different natural sources for designing a proper diet intake by an individual. There are many different methods for measuring ascorbic acid like 2, 6-Dichlorophenol indophenol (DCPIP) titration (Shrestha et.al, 2016), a spectrophotometric method using 2, 4- Dinitrophenyl hydrazine (Al-Ani et.al, 2015), etc. All these methods require more resources and are time-consuming, whereas the Iodine clock method developed is a green and economical method for the estimation of ascorbic acid. It is a redox reaction based on the oxidation rate of iodine to iodide ion by hydrogen peroxide. The detection of iodide ion is correlated with the time in which iodide ion reacts with ascorbic acid to produce iodine, eventually forming the iodine- starch complex which is blue-black in color, hence the chromaticity is time dependent. The more time required for color development by the sample, the higher the amount of vitamin C in it.

Principle

$$H_2O_2\,(aq) + 2I^-\,(aq) \longrightarrow I_2\,(aq)$$

The liberated iodine reacts with Ascorbic acid and reduces to iodide ions.

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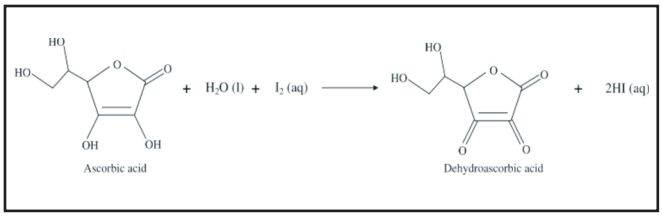


Due to this reaction, the iodine reduces to iodide as long as the ascorbic acid is present in the solution. Once all ascorbic acid has been completely consumed, excess iodine reacts with the starch solution forming a blue-black color. The time elapsed before the appearance gives an idea of the ascorbic acid concentration in the sample. Thus, the higher the concentration of ascorbic acid present in the solution, the more time it will take to develop the color.

2. Materials and Methods

Chemicals

Hydrogen peroxide (3%), starch (1.5%), Tincture of Iodine (2%), oxalic acid (4%)

Samples

Lemon pulp, lemon peel, tomato, orange pulp, and orange peel.

Standard ascorbic acid solution and calibration curve

The standard ascorbic acid solution was prepared and using this stock solution calibration curve of different amounts of ascorbic acid i.e., 1mg, 2mg, 3mg, 4mg, and 5mg was plotted.

Sample extract preparation

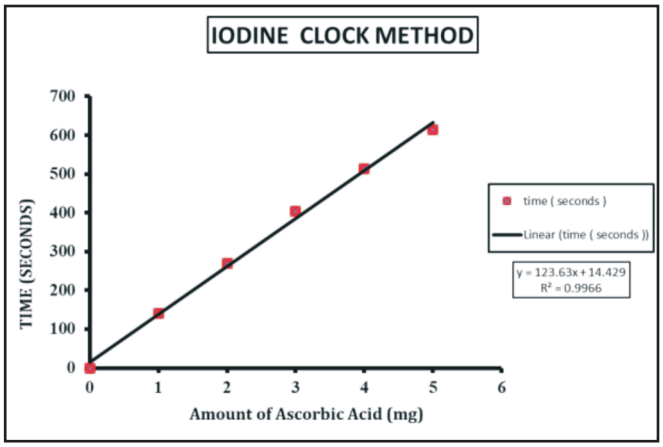
Standard was made using the stock solution of ascorbic acid ranging from 1mg to 5 mg. Samples taken for this study were orange, tomato, and lemon (pulp and peel both). These were cut into pieces and ground to a fine paste with 4% oxalic acid. The extracts were filtered with muslin cloth and aliquots of the filtrate were collected in a beaker. Similarly, all the samples were prepared.

3. Procedure for Estimation of Vitamin C : Iodine Clock Assay

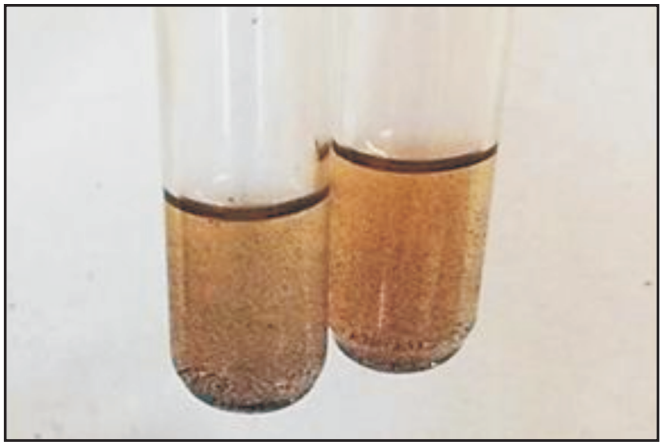
1ml of solution (standard samples or test samples) was taken into a test tube to which 1ml of solution A (containing Tincture of Iodine (2%), distilled water) and 10 ml of solution B (hydrogen peroxide (3%), starch (1.5%)) were added in 1:5 ratio and stirred properly. The time was noted when the color was changed from clear to blue-black. Similarly, the assay was performed for all the standards and test samples.

4. Results

The calibration curve (Figure 1a) was plotted as time (seconds) vs the amount of ascorbic acid (mg) and from which the amount of ascorbic acid in experimental samples was estimated.



(a)



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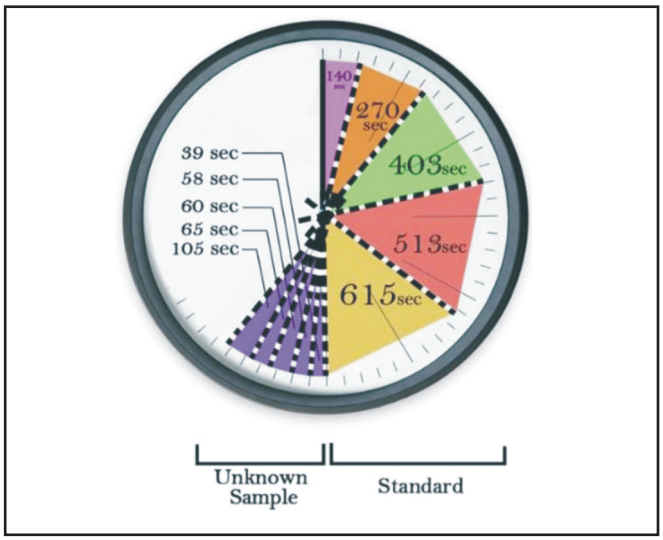

(c)

Figure 1 : *(a) Calibration curve plotted as time (seconds) vs the amount of ascorbic acid (mg) (b) Illustration of color change from blue to black upon initiation of reaction (c) Graphical abstract of iodine clock method, representing different time points for the samples and standard.*

Table 1 : *Amount of ascorbic acid present in different experimental samples per 100g*

Sample	Amount of Ascorbic Acid (Per 100g)	Time (Seconds)
Orange (Pulp)	45.8 mg	65
Lemon (Pulp)	52.5 mg	105
Tomato (Pulp)	27.46 mg	58
Orange (Peel)	138.02 mg	60
Lemon (Peel)	123.4 mg	39

The equation of the standard curve was found to be y = 123.63x + 14.429 and coefficient of determination (R2) = 0.9966. Table 1 above summarizes the amount of ascorbic acid per 100g of the sample weight for test samples. Other common methods were also performed such as redox and DCPIP titration and 2,4-DNPH spectrophotometric method to estimate the amount of ascorbic acid (data not shown) to validate the results, which were comparable to the iodine clock assay.

5. Discussion

Ascorbic acid is an essential micronutrient and a powerful dietary antioxidant with pleiotropic functions. Several methods have already been proposed to estimate the amount of ascorbic acid; however, none to date have been suggested to be rapid, green, and economical. This study can be referred to as a preferable alternative for the precise determination of ascorbic acid concentration.

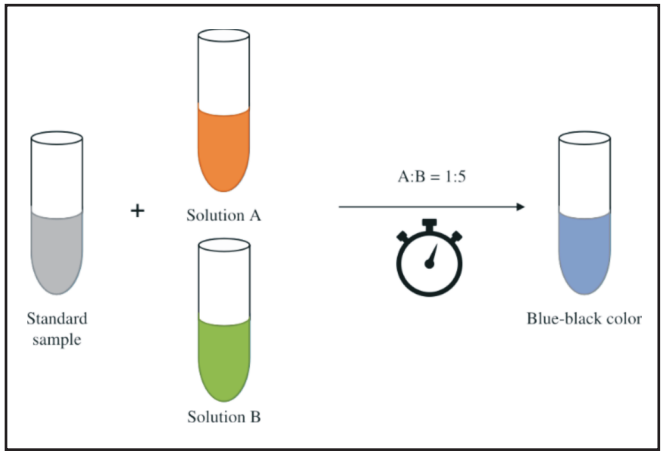

Figure 2 : *A proposed working model of the study*

The most common and exact methods for ascorbic acid evaluation involve redox titration, HPLC techniques with UV and electrochemical detection, and electrochemical methods (EM) which are time taking and involve many chemicals making the method costly (Škrovánková et.al, 2015). On the other hand, we see that the iodine clock method (GRRECO) was found to be sensitive, inexpensive, and economical.

Previously similar investigations (López-Pastor et.al, 2020) have been done to estimate ascorbic acid but using different solvent systems with varying ratios. In this study, besides determining the concentrations of ascorbic acid in fruit pulp extract, we have also estimated the levels of ascorbic acid in non-edible parts which were found to be more significant than in pulp extract. Thus, peels should not be considered waste instead they can be of industrial and biochemical importance.

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Annexure 1 : *Titre volume (mL) required for estimation of the amount of ascorbic acid in the mentioned samples*

SAMPLES	Titre volumes(ml)	Titre volume(ml) (Average)	Amount of Unknown (per 10ml)	Amount of Unknown (per 10ml) Average	Amount of Ascorbic Acid (per 100g)
LEMON	6.1	6.16	7.59375	7.59375	54.77
	6.2		7.64375		
	6.2		7.64375		
TOMATO	3.1	3.1	3.76875	3.76875	29.35
	3.1		3.76875		
	3.1		3.76875		
ORANGE	7.0	7.1	8.64375	8.64375	59.4
	7.1		8.76875		
	7.2		8.89375		
LEMON PEEL	0.5	0.5	0.51875	0.51875	180.6
	0.5		0.51875		
	0.5		0.51875		
KIWI	10.7	10.8	13.26875	13.26875	97.06
	10.8		13.39375		
	10.9		13.51875		
ORANGE PEEL	1.0	1	1.14375	1.14375	161.9
	1.0		1.14375		
	1.0		1.14375		

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