Article

Simple and Sensitivity Spectrophotometric Method for Ascorbic Acid Determination in Vegetables, Fruits, and Different Pharmacological Models

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Abstract

Within the present study, ascorbic acid was estimated based on a simple and fast reaction with a distinctive sensitivity based on an oxidation and reduction reaction in the presence of a mixture of iron(III) ions and 1,10-phenanthroline reagent in an acidic medium. The measurements were made at the wavelength of the greatest absorption of 510 nm, and the reaction included two basic stages: The first stage was the oxidation of ascorbic acid in an acidic medium in the presence of iron (III) ions. When the iron (III)ion reduces to an iron (II) ion, 1,10-phenanthroline reacts with the iron (II) ion in a second stage to form a red complex. In the study, the optimal conditions for the reaction were determined, including the acidity function, the type of buffer solution, the concentration of iron(III) ions, the concentration of 1,10-phenanthroline, citric solution as a buffer solution, 10^{-6} and 10^{-4} mol/L, respectively, and the volume of iron(III) ions, 1,10 reagent-phenanthroline, hydrochloric acid, and ascorbic acid was 4, 2.5, 1 and 0.5 ml, respectively. A calibration curve was prepared in the optimal conditions and had a linear range ranging from 0.5 to 10 mg/L and a correlation coefficient of 0.9969, and then a negative deviation occurred. The analytical method under study was applied to determine ascorbic acid in different models, including vegetables and fruits, and different pharmacological models to determine the method's accuracy by calculating the recovery ratio.

Keywords:Ascorbic acid,Vitamin C, 1, 10-phenanthroline, hydrochloric acid.

1. Introduction

Vitamin C, scientifically referred to as L-ascorbic acid, is a water-soluble vitamin. It has a potent capability to reduce substances and performs a significant role as a co-enzyme in internal hydroxylation processes. Vitamin C is present in two distinct forms: the oxidized form, referred to as dehydroascorbic acid, and the reduced form, known as ascorbic acid. This is a commonly used food ingredient that serves several functional purposes. Most of these functions rely on its oxidation-reduction capabilities. This substance's functional functions include serving as an antioxidant, a nutritious food additive, a reducing agent, a stabilizer, a color stabilizer, and a modifier.[1-3]

Investigation into the cause and treatment of scurvy led to the discovery of L-ascorbic acid. It plays an important function in human physiology. The redox activity of ascorbic acid is primarily responsible for its biochemical action. In simpler terms, ascorbic acid may function as a soluble antioxidant by releasing electrons in a water-based solution, which helps to remove reactive oxygen species (ROS) and free radicals. Additionally, lowering metal ions in the active region of the enzyme functions as a cofactor to support catalytic activity. Carries out other crucial human health and cell physiology tasks, such as maintaining significant biomolecules in a decreased state[4-6].Vegetables and fruits are good sources of vitamin C, one of the most essential vitamins for human nutrition [7]. Ascorbic acid, a vital water-soluble vitamin for humans, plays a crucial role in constructing the body's framework. This nutrient actively participates in the development of blood vessel walls, dental structures, connective tissues, and skeletal system. It further aids the body to absorb amino acids and iron[8].

Due to the significant and extensive A.A use, several analytical methods have been suggested for its quantification in various matrices and concentrations. The methods used in determination of A.A include voltammetry [9, 10], titrimetry[8], fluorometry [11], potentiometry [12, 13], spectrophotometry [14, 15], flow injection (FI) analysis [16, 17], and chromatography [18, 19].
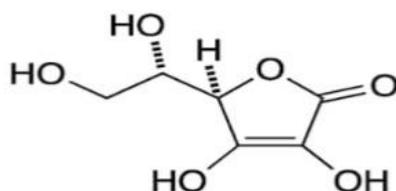
Schame 1. Chemical structure of ascorbic acid M.wt. 176.12 g/mol .

1.1 Spectroscopic Techniques

Spectrophotometry has gained popularity due to its significant advantages over other analytical techniques. This method is particularly favored in developing countries because of its precision,simplicity, accuracy,and speed[20,21].

2. Materials and Methods

2.1 Apparatus

An Oakton 2100 Series with pH/mV/Ion/0C/0F Meter was employed to measure pH levels. An Ohaus PA214 Pioneer Analytical Balance determined the weight of all samples. Lastly, a Shimadzu UV-1700 spectrophotometer identified the peak wavelength.

2.2 Preparation of Standard Solutions

A 100 mg/L concentration of ascorbic acid solution was obtained by dissolving 0.0100 g of the acid in 100 mL of distilled water.The 0.01 mol/L hydrochloric acid solution was prepared by taking 1 mL from 1mol/L of acid and diluting it to the mark in a 100 ml volumetric flask . 1×10^{-2} mol/L of 1,10-phenonphthalein solution was produced by dissolving 0.1803 g of it in 5 mL of alcohol, then diluting to the mark using a suitable volumetric flask(100 ml) with distilled water. 1×10^{-3} mol/L of $FeCl_3.6H_2O$ prepared by dissolved 0.0271 g of its and dissolved in 100 mL of distilled water after add 2 drops from HCl concentrated.

3. Results and Discussion

### *3.1 Selected Wavelength of Maximum Complex Absorbance*

The complex's highest sensitivity and maximum absorbance in various media were measured using a Shimadzu UV1700 spectrophotometer. The best spectra were found in the acidic medium, according to the results. Figure 1 illustrates the complex's maximum absorption at 510 nm, and these findings led to the setting of 510 nm as the maximum wavelength for this study.
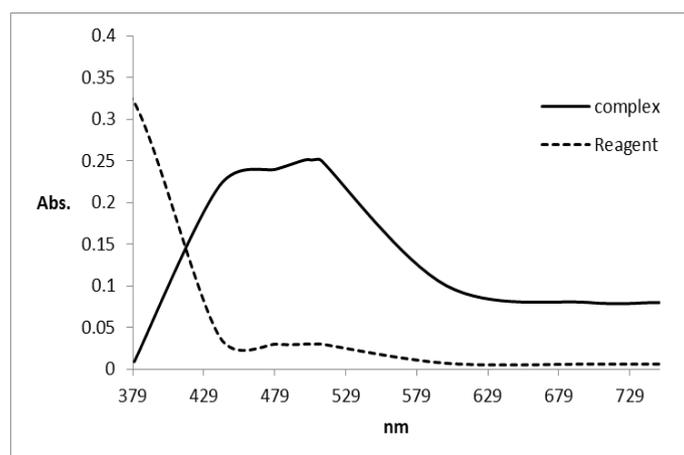
Figure1. The absorption spectrum of the complex and the reagent

3.2 Mechanisms of Chemical Reactions of Redox and Complex Formation

The reaction included two basic stages: The first stage is the oxidation of ascorbic acid in an acidic medium in the presence of iron(III) ion. If the ion is reduced to iron(II) ion, the iron(II) ion reacts with 1,10-phenanthroline as a second stage to be a red complex, asshown inFigure 2.
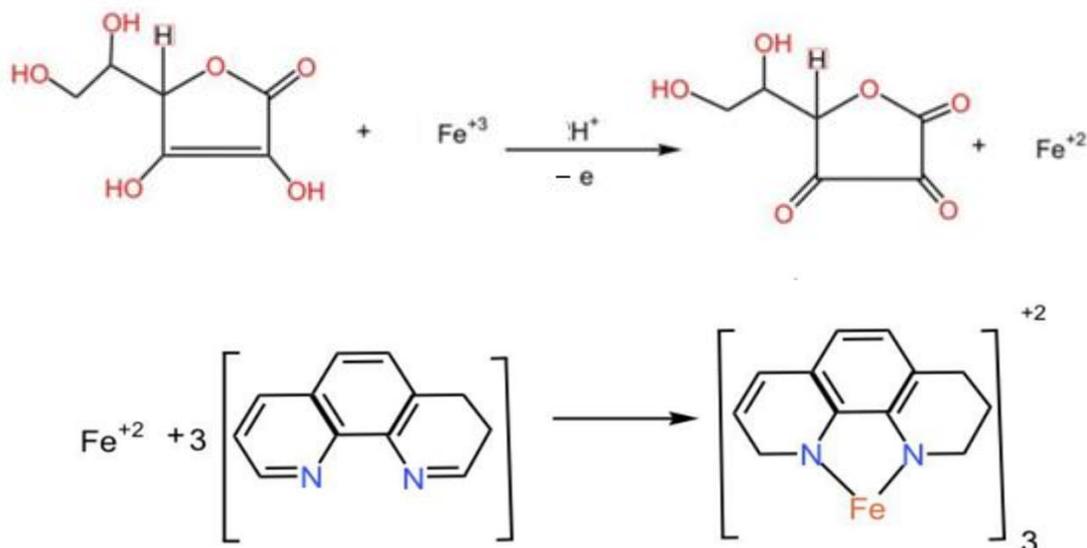

Figure 2.Suggested mechanisms of complex formation chemical reaction

3.3 Effect of pH on Complex Reaction

Different acid function changed from 3 to 12, were used to prepare the complex. This was done to investigate the effect of acid function on the chemical

reaction. The ideal reaction pH was determined to be 5, as shown in Figure 2. This pH was also used for the test shown in Figure 3. The test conditions were fixed: 10^{-6} mol/L (2 mL) Fe(III), 10^{-4} mol/L (2.5 mL) 1,10-phenanthroline, 0.01 mol/L (1 mL) hydrochloric acid, and 10 mg/L (2 mL) ascorbic acid.
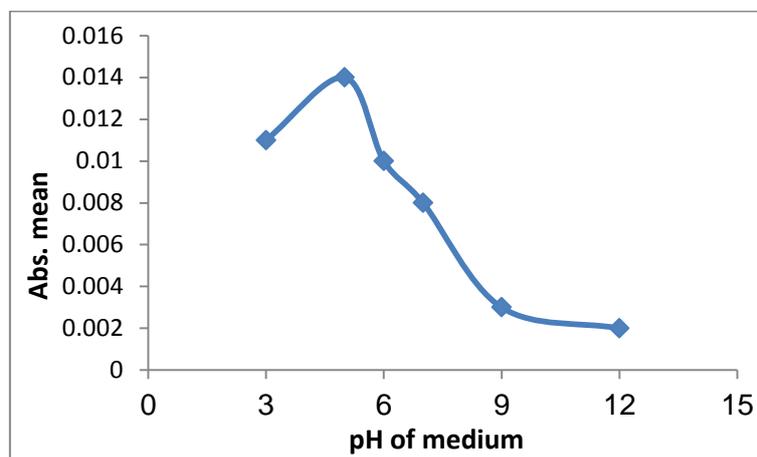
Figure 3: Effects of pH on the complex formation

3.4 Effect of Buffer Solution

Several buffer solutions were tested to identify the ideal buffer for the current reaction. The results of these tests are presented in Figure 4. The test conditions were fixed: 10 mg/L ascorbic acid (2 mL), 10^{-6} mol/L Fe(III) (2 mL), 10^{-4} mol/L 1,10-phenanthroline (2.5 mL), 0.01 mol/L hydrochloric acid (1 mL), and pH 5. Based on the results, phosphate citrate buffer solution was the best option. Three types of solutions were evaluated:
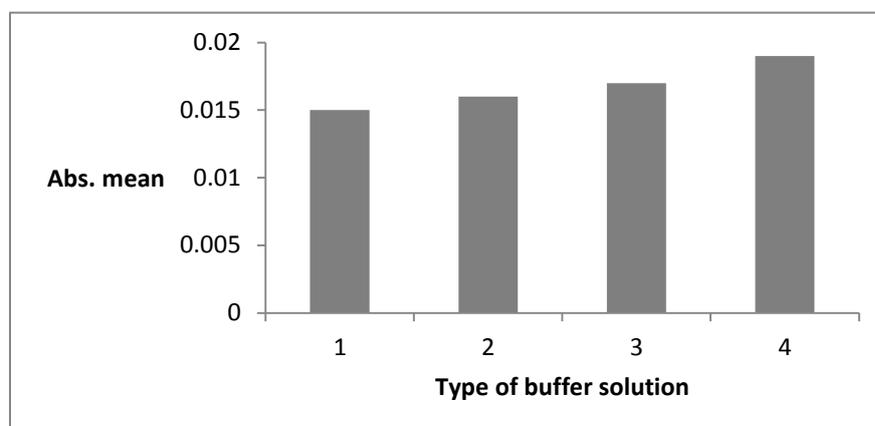

Figure 4.Effects of buffer solution on complex formation

1. Distilled water at pH 5(without buffer solution)
2. . CH$_3$COOH +CH$_3$COONa → acetate buffer
3. . Sodium citrate + Citric acid → citrate buffer
4. Na$_2$HPO$_4$ + Citric acid → phosphate citrate buffer.

3.5 Effect of 1, 10-Phenanthroline Concentration on the Reaction

The complex was made with different amounts of 1,10-phenanthroline ranging from $1x10^{-5}$ to $1x10^{-2}$ mol/L to study how the concentration of 1,10-phenanthroline affects the creation of the complex. Each amount used was 2.5 ml. The results are shown in Figure 6. It seems that using $1x10^{-4}$ mol/L of 1,10-phenanthroline gives the best results. This test was done while keeping the amount and concentration of Fe(III) at 10^{-6} mol/L (2 mL), hydrochloric acid at 0.01 mol/L (1 mL), ascorbic acid at 10 mg/L (2 mL), and the pH at 5.
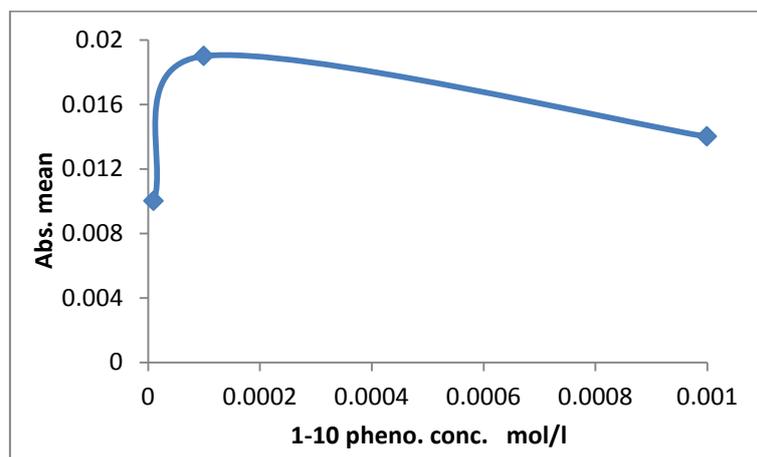
Figure 5. Influence of 1,10-phenanthroline concentration on reaction behavior
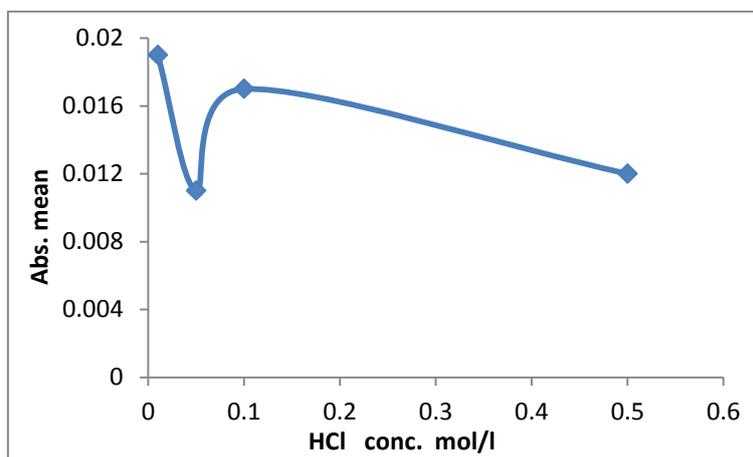
3.6 Effect of Fe (III) Concentration on the Reaction

Iron was prepared at different concentrations, ranging from $1x10^{-6}$ to $1x10^{-4}$ mol/L with a volume of 2 ml, to investigate how changes in iron concentration affect reaction sensitivity. The data shows that the highest absorption occurs at a concentration of $1x10^{-6}$ mol/L, as illustrated in Figure 6. This experiment was conducted while maintaining a constant concentration and volume of 1,10-phenanthroline (10^{-4} mol/L, 2.5 ml), hydrochloric acid (0.01 mol/L, 1 ml), ascorbic acid (10 mg/L, 2 ml), and a pH of 5.
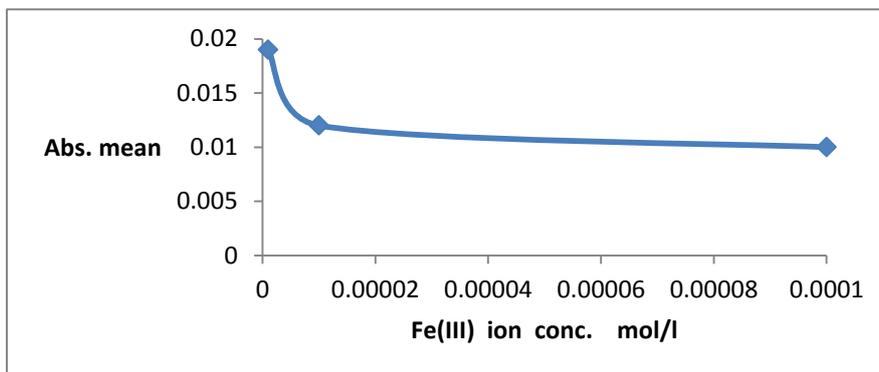
Figure 6.Influence of Fe(III) conc. on reaction behavior

## 3.7 Effect of Hydrochloric Acid Concentration on the Reaction

The impact of HCl concentration was evaluated across a range of 1×10^{-2} to 1 mol/L, with a volume of 1 ml. The results indicated a rapid increase in absorbance without any precipitate formation in the solution. Consequently, a concentration of 0.01 mol/L was selected as optimal. These findings are presented in Figure 7. This experiment was conducted while maintaining a constant concentration and volume of Fe(III) (10^{-6} mol/L, 2 mL), 1,10-phenanthroline (10^{-4} mol/L, 2.5 ml), ascorbic acid (10 mg/L, 2 ml), and a pH of 5 using phosphate citrate buffer as buffer solution .

Figure 7.Influence of HCl concentration on complex formation

3.8 Effect of Fe(III) Volume on the Reaction

Different volumes of Fe(III), from 1 to 5 ml, were used Fe(III) (10^{-6} mol/L) to study of the Fe(III) volume affects on the redox reaction. Figure 8 shows that 4 mL of Fe(III) gives the best results without making any solid stuff in the solution. This test was done with the same amount and concentration of 1,10-phenonthroline (10^{-4} mol/L, 2.5 ml), hydrochloric acid (0.01 mol/L, 1 ml), ascorbic acid (10 mg/L, 2 ml), and pH of the medium= 5.
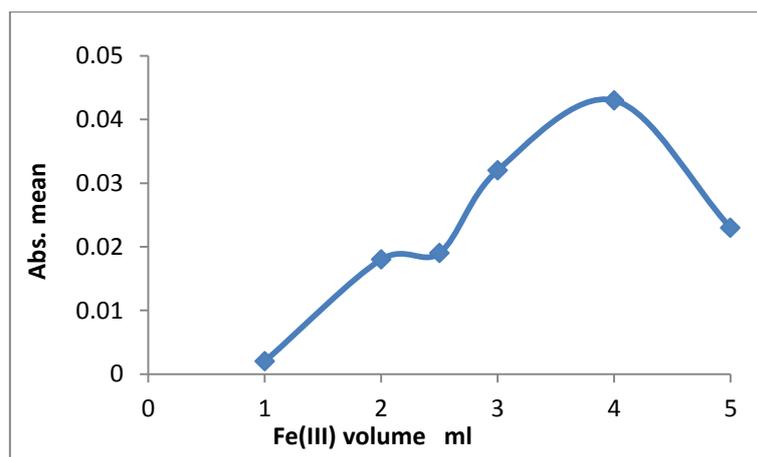
Figure 8.Influence of Fe(III) volume on the reaction behavior

3.9 Effect of the 1,10-Phenanthroline Volume

To investigate the optimal volume of 1,10-phenanthroline, solutions with varying volumes of 1,10-phenanthroline (from 1 to 4 ml) were prepared. The results revealed that the ideal volume of 1,10-phenanthroline is 2.5 ml, as depicted in Figure 9. This experiment was conducted using a fixed concentration and volume of Fe(III) (10^{-6} mol/L, 4 mL), 1,10-phenanthroline concentration (10^{-4} mol/L), hydrochloric acid (0.01 mol/L, 1 ml), ascorbic acid (10 mg/L, 2 ml), and pH of the medium= 5.
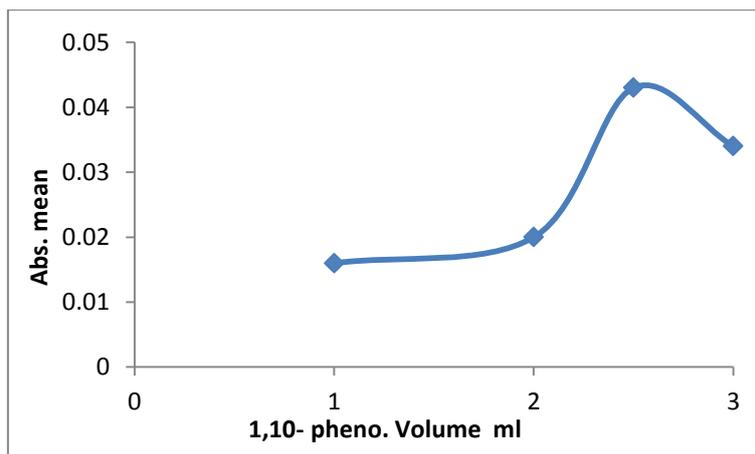
Figure 9. Effects of 1,10 phenanthroline volume on the reaction

3.10 Influence of the Volume of Hydrochloric Acid on the Reaction

To determine the optimal volume of HCl, solutions with different volumes (from 0.5 to 2.5 ml) were prepared. The results showed that the ideal volume of HCl is 1 ml, as presented in Figure 10. This experiment was conducted using a fixed concentration and volume of Fe(III) (10^{-6} mol/L, 4 mL), 1,10-phenanthroline concentration (10^{-4} mol/L, 2.5 ml), 0.01 mol/L hydrochloric acid, ascorbic acid (10 mg/L, 2 ml), and pH of the medium= 5.
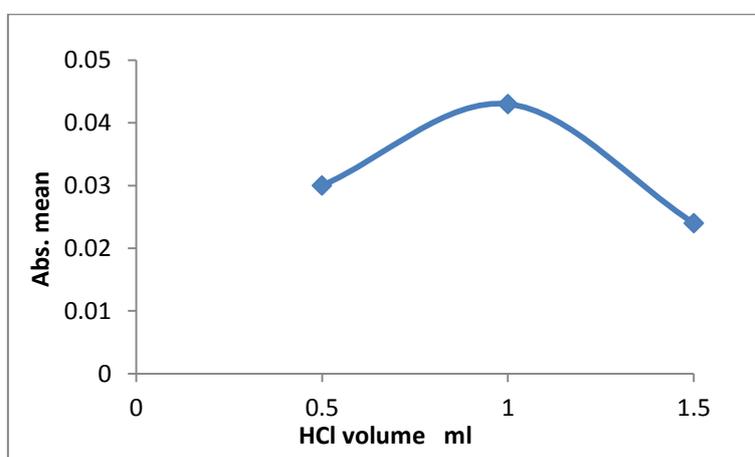
Figure 10.Influence of HCl volume on the reaction
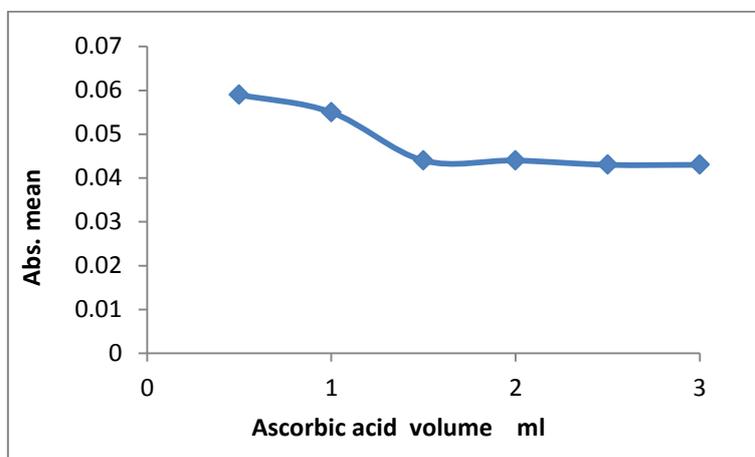
3.11 Effect of Ascorbic Acid Volume on Redox reaction

To examine the impact of ascorbic acid, solutions with varying volumes (from 0.5 to 3 ml) were prepared. Based on the results, the optimal ascorbic acid volume was determined to be 0.5 ml, as illustrated in Figure 11. This is because the acid

within these volumes achieves maximum absorption into the solution without forming any precipitate. The experiment was conducted using a fixed concentration and volume of Fe(III) (10^{-6} mol/L, 4 mL), 1,10-phenanthroline concentration (10^{-4} mol/L, 2.5 ml), hydrochloric acid (0.01 mol/L, 1 ml), ascorbic acid (10 mg/L), and a pH of 5.

Figure 11. The effects of Ascorbic volume on complex formation

3.12 Calibration Curve for Redox Reaction

To find the right amounts for Lambert-Beer's law, we made different amounts of ascorbic acid (from 0.1 to 50 mg/L) in the best conditions. We found that the amounts from 0.5 to 10 mg/L make a straight line (correlation coefficient of 0.9969). From these results, we figured out that the smallest amount we can find (LOD) is 0.285 mg/L, and the smallest amount we can measure (LOQ) is 0.95 mg/L. The results are displyed in Figure 12.

$y = 0.006x - 0.001$
$R^2 = 0.9969$
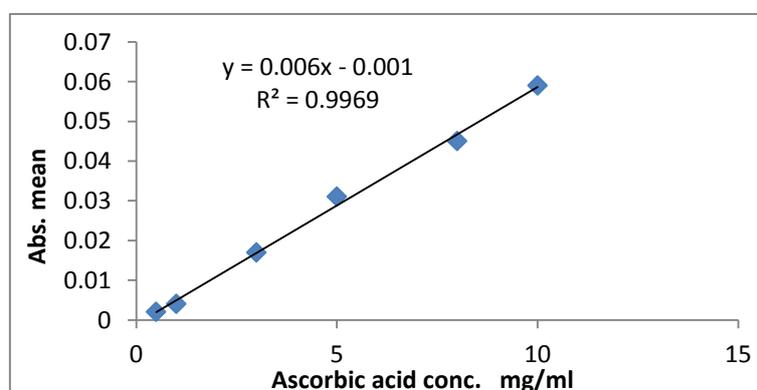
Figure12. Calibration Curve of reaction

4. Applications

Several samples (pepper, celery, spinach, potato, lemon, strawberry, orange, vit_c, KivoZinc+C, CRANBeRRyPlus) . The vegetables and fruits prepared by take 10g of sample was weighed and then transferred to a 200ml beaker.20 mL from concentrated nitric acid HNO_3 was added and the mixture was heated up by using a boiling water bath until the total remnant volume of the mixture became 2-3mL.The mixture cooled down.20mL from concentrated HNO_3,10mL from concentrated H_2SO_4 and 8mL from H_2O_2 were added and the mixture was heated up for another time. After completing the digestion process, 20 mL of distilled water is added and the mixture is heated up to discard all extra acids. (Repeat this step as needed until remove all extra acids).Finally the mixture cooled down, then filtered in a volumetric flask100 mL, and the solution was diluted to the mark with distilled water. [20,22] and were tested for the detection of ascorbic acid using the proposed methods. The usual procedure is to use the pure value. The table below illustrates that comparingthe proposed approach and the pure value yields high accuracy with % recovery.

Table 1. Tested samples detection of ascorbic acid

No	Sample	Company	found mg. L^{-1}	Measured value mg. L^{-1}	E%	Recovey%
1	Pepper	....	10	10	0	100
2	Celery	....	10	10	0	100
3	Spinach	....	10	10.6	6	106
4	Potato	....	10	10.6	6	106
5	Lemon	....	10	7.77	-22.3	77.7
6	strawbery	....	10	10	0	100
7	Orange	....	10	7.77	-22.3	77.7
8	Vit-c	United Kingdom/ 1000 mg Per 1cap	10	10.6	6	106
9	CRAN BERRY	Germany/ 120mgPer1cap	10	10.5	5	105
10	Kivo zinc+c	UKrain/ 30mg Per5ml	10	10	0	100

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