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# Spectrophotometric Methods for the Determination of Ascorbic acid

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Ascorbic acid can be determined in trace quantities based on its reactions with vanadium(V), iron(III)-thiocyanate complex, alkaline potassium ferricyanide and potassium permanganate. Probable explanations are given.

## INTRODUCTION

Ascorbic acid is largely present in citrus fruits and tomatoes. It is essential for the formation of collagen and intercellular material. It also influences the formation of haemoglobin. Prolonged deficiency of ascorbic acid causes scurvy and loss of disease resistance. Its highest concentrations are in the adrenal cortex and in the eye, hence it is concerned in bodily reactions to stress.

A number of methods have been used for the determination of ascorbic acid. Ascorbic acid was assayed colorimetrically using phenylhydrazine[1] and dimethoxy diquinone[2] by redox titration with NBS[3]. It is also analysed using titrimetric method with 2,6-dichloroindophenol[4] as the titrant. Though the reaction is rapid, the reagent is unstable and must be standardised before use. Ascorbic acid can also be determined by micro fluorimetric method[5] which is very time consuming. The differential pulse polarographic method[6,7] suffers from interferences from electroactive impurities present in the sample. Ascorbic acid can be determined by complex formation involving iron(II)-1,10-phenanthroline, iron(III)-reacetophenone oxime[9], reaction with *o*-iodosobenzoate[10], kinetic methods based on bromine oxidation[11], derivative spectrophotometry[12] and direct ultraviolet spectrophotometry[13].

In spite of their simplicity some of these methods are elaborate, of low sensitivity and are not specific for ascorbic acid determination.

We propose simple, fast, sensitive and selective spectrophotometric methods for the determination of ascorbic acid and describe them in the present communication.

The methods are based on the following reactions involving ascorbic acid.

1. Reduction of vanadium(V) to vanadium(IV),
2. Reaction with iron(III)-ammonium thiocyanate complex,
3. Reaction with alkaline potassium ferricyanide,
4. Reaction with potassium permanganate.

## EXPERIMENTAL

The absorbance measurements were made on a UV visible spectrophotometer, Shimadzu Model 160–A, Japan.

Analytical reagent grade chemicals were used in these investigations and distilled water was used to prepare solutions. Ascorbic acid was prepared from the pharmaceutical grade tablets by following usual procedure[14]

### Procedures

#### 1. Reduction of vanadium(V) in acid solutions

5.0 Ml of conc. sulphuric acid and 8 ml of 0.1 M vanadium(V) solution and known amount of ascorbic acid solution were taken in a 25 ml standard flask. The contents were made up to the mark with distilled water. The absorbance for the resulting blue solution was measured at 760 nm against the blank solution. Similar measurements were made on different solutions each containing a known aliquot of ascorbic acid. A calibration curve was plotted between the absorbance and the amount of ascorbic acid.

#### 2. Reaction with iron(III)-ammonium thiocyanate complex

8 Ml of 0.01 M ammonium thiocyanate, 1.6 ml of iron(III) and 0.1 ml of perchloric acid were taken in a series of 25 ml standard flasks. Blood red coloured solution due to iron(III)-thiocyanate complex was formed. Various aliquots of ascorbic acid (1 to 7 ml of 0.05 M) solution were added to each of the standard flasks. The contents were made up to the mark with distilled water, shaken well for homogeneity. The absorbance was measured at 460 nm against the blank solution. The experiment was repeated with different known aliquots of ascorbic acid and a linear plot was obtained between the absorbance and the amount of ascorbic acid.

#### 3. Reaction with alkaline potassium ferricyanide

4.0 Ml of 1 M sodium hydroxide and 4 ml of 0.01 M potassium ferricyanide were taken in a 25 ml standard flask and known amount of ascorbic acid was added to the resulting yellow coloured solution. The contents were made up to the mark with distilled water and shaken well for uniform concentration. The procedure was repeated with different known aliquots of ascorbic acid and the absorbance was measured at 418 nm (Fig. 4) against the reagent blank. A linear calibration plot was obtained between absorbance and the amount of ascorbic acid.

#### 4. Reaction with potassium permanganate

Known aliquots of ascorbic acid were pipetted out into a series of 25 ml standard flasks containing 1 ml of 0.01 M $KMnO_4$ and 2 ml of conc. $H_3PO_4$. The solutions are made up to the mark with distilled water and shaken well for

homogeneous concentration. Absorbance of each solution was measured against the blank solution at 545 nm and a calibration curve was constructed as usual.

## RESULTS AND DISCUSSION

### Reduction of vanadium(V) with ascorbic acid

Transition metals in their higher oxidation state get reduced to lower oxidation state by organic compounds. In the present case the authors noticed an instantaneous blue colour formation probably due to the reduction of vanadium(V) to vanadium(IV) on the addition of ascorbic acid. It has maximum absorbace at 760 nm (Fig. 3). The requisite concentrations of vanadium(V) and $H_2SO_4$ for the instantaneous development of blue colour were established as 0.1 M and 3.0 M respectively. Studies with different known aliquots of ascorbic acid show linearity between the amount of ascorbic acid and the absorbance in the range 0.352–2.464 μg/ml (Fig. 1). Vanadium can also be determined in the range ($8–32 \times 10^{-3}$ M) employing this method, as linear relationship exists between vanadium concentration and absorbance as well.

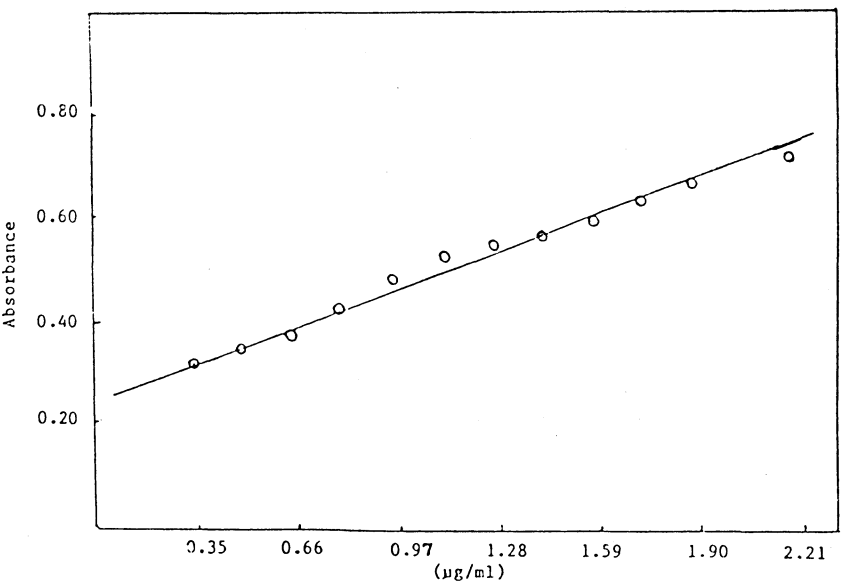


Fig. 1. Dependence of absorbance on concentration of ascorbic acid: [Vanadium(V)] = $3.2 \times 10^{-2}$ M, [$H_2SO_4$] = 7.2 M [Ascorbic acid] = $2–14 \times 10^{-3}$ M

### Reaction with ammonium thiocyanate-Fe(III) complex

It is known that ammonium thiocyanate forms a blood red coloured complex with iron(III). Ascorbic acid bleaches this solution in perchloric acid medium. This fact has been successfully employed for the indirect determination of ascorbic

acid. With the successive addition of known aliquots of ascorbic acid, the absorbance values decrease. Therefore ascorbic acid can be determined employing this indirect method. The range of determination is 35.2–246 μg/ml (Fig. 2A).

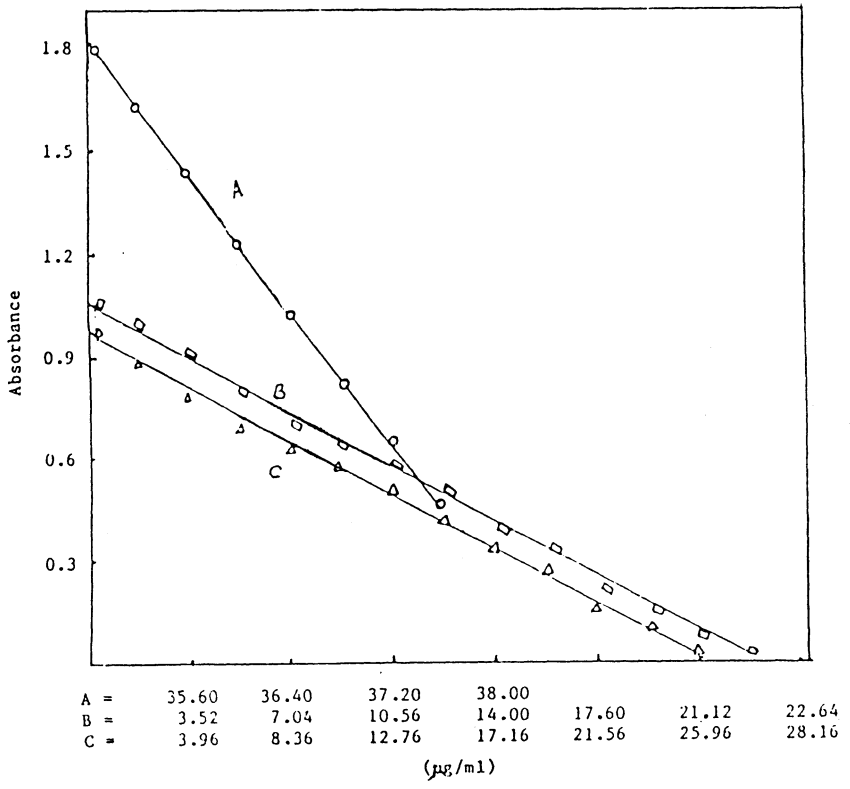


Fig. 2. Dependence of absorbance on concentration of ascorbic acid: (A) [NaOH] = $1.6 \times 10^{-2}$ M, [$K_3Fe(CN)_6$] = $1.6 \times 10^{-3}$ M, [Ascorbic acid] = $1-14 \times 10^{-4}$ M; (B) [$NH_4SCN$]= $3.2 \times 10^{-2}$ M, [$HClO_4$] = $4.8 \times 10^{-3}$ M, [Iron(III)]= $6.4 \times 10^{-4}$ M, [Ascorbic acid]= $2-14 \times 10^{-3}$ M; (C) [$H_3PO_4$]= 1.12 M, [$KMnO_4$]= $4 \times 10^{-4}$ M, [Ascorbic acid] = $1-16 \times 10^{-4}$ M

**Reaction with alkaline potassium ferricyanide**

Alkaline potassium ferricyanide has absorbance maximum at 418 nm (Fig. 4). On the addition of ascorbic acid, the yellow colour of the ferricyanide decreases progressively. This fact can be suitably employed for the determiation of ascorbic acid. The optimum concentrations of sodium hydroxide and ferricyanide for the formation of stable yellow coloured species were found to be 0.1 M, 0.01 M respectively. The absorbance should be measured within 15 minutes after the

addition of ascorbic acid. The linear plot (Fig. 2B) obtained between absorbance and amount of ascorbic acid indicates suitability of the method for the determination of ascorbic acid in the range 1.76–24.64 μg/ml.

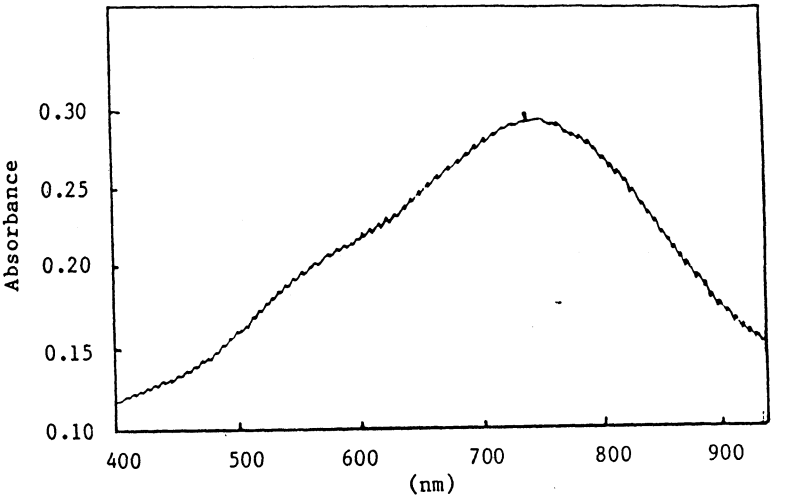


Fig. 3. Reduction of vanadium(V) with ascorbic acid: [Vanadium(V)] = 12.5 M, [$H_2SO_4$] = 3.6 M, [Ascorbic acid] = $4 \times 10^{-3}$ M

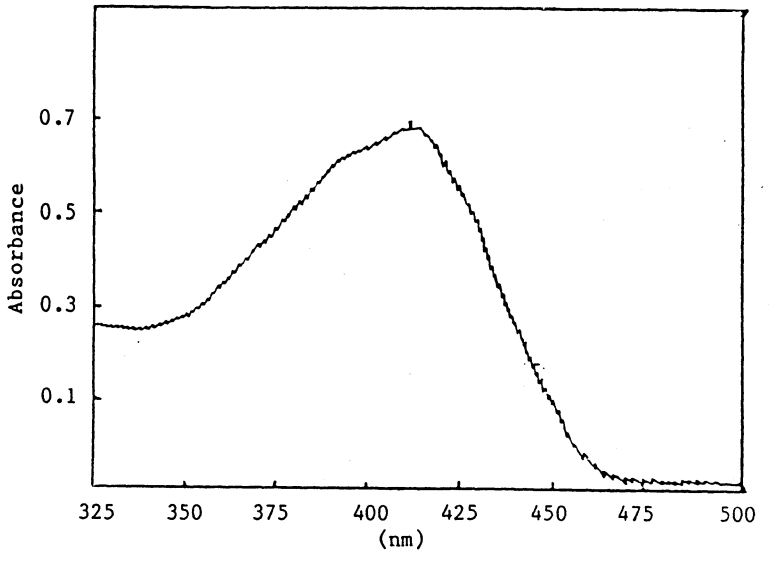


Fig. 4 Reaction with alkaline potassium ferricyanide: [NaOH] = 0.16 M, [$K_3Fe(CN)_6$] = $1.6 \times 10^{-2}$ M, [Ascorbic acid] = $2 \times 10^{-4}$ M

**Reaction with potassium permanganate**

Ascorbic acid also bleaches the pink colour of the potassium permanganate in acid medium. This fact has been utilised to develop a method for the determination of ascorbic acid. 1 Ml of 0.01 M permanganate and 2 ml of concentrated phosphoric acid were found to be suitable. Since the bleaching action is fast, absorbance measurements were made immediately after the addition of ascorbic acid. The fall in intensity is proportional to the concentration of the added ascorbic acid in the range 1.76–28.16 μg/ml. (Fig. 2C).

The applicability of the methods is tested with real samples (tablets) containing ascorbic acid. They are Sukcee, Celin, Citrarite, Cobadex Forte. The results are satisfactory.

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