Spectrophotometric Determination of Ascorbic Acid in Aqueous Solutions

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Abstract

Anew spectrophotometeric method for determination of ascorbic acid(A.A) in aqueous solutions depending on its ability to reduce the colors of permanganate at (530nm) and dichromate at (350 nm). The optimum conditions such as best concentration of reactant and order of addition were studied to get the highest sensitivity (ε=2355.70 and 3094.75) $L.mol^{-1}.cm^{-1}$ with wide range of calibration curves (1-16) and (2-90) ppm good repeatability (RSD% 0.73 and 1.49), the recovery %(100.20 and 99.42), E_{rel}% (-0.20 and 0.58) for gave, Pharmaceutically prepared tablets were measured with the new technique using Standard Addition Method given a very good recovery % (101.25 and 98.75) with a small E_{rel}% (-1.25 and 1.25).

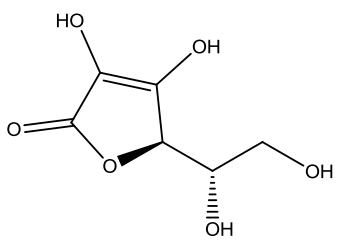
Keywords: ascorbic acid, UV/VIS spectrophotometry, pharmaceutical analysis.

Introduction

Ascorbic acid (also called Vitamin C) is a naturally occurring organic compound with antioxidant properties. It is a white solid but impure samples can appear yellowish. Ascorbic acid is one form ("vitamer") of vitamin C. The name is derived from *a-* (meaning "no") and *scorbutus* (scurvy), the disease caused by a deficiency of vitamin C. Being derived from glucose, many animals are able to produce it, but humans require it as a nutritional supplement [1]**.**

The chemical formula is (R)-5-((S)-1,2-dihydroxyethyl)-3,4-dihydroxyfuran-2(5H)-one, molar mass 176.12 g mol−1, Density 1.65 g/cm^3, melting point 190-192 °C, with solubility in water about 33 g/100 mL.

It is a water-soluble vitamin which can be found in many biological systems and foodstuffs (fresh vegetables and fruits, namely, citrus). Ascorbic acid plays an important role in collagen biosynthesis, iron absorption, and immune response activation and is involved in wound healing and osteogenesis. It also acts as a powerful antioxidant which fights against free-radical induced diseases [2-6].



ascorbic acid

Most importantly, ascorbic acid is a mild reducing agent. For this reason, it degrades upon exposure to oxygen, especially in the presence of metal ions and light. It can be oxidized by one electron to a radical state or doubly oxidized to the stable form called dehydroascorbic acid.

Many analytical methods can be used for ascorbic acid determination. Classic (conventional) techniques are represented by volumetric methods—titration with an oxidant solution such as dichlorophenol indophenol (DCPIP) [7,8], potassium iodate[9], or bromate [10]. Volumetric techniques can suffer from lack of specificity [11] which limits their use to samples not containing other reducing agents.

Güçlü et al. [12] have proposed a spectrophotometric method based on ascorbic acid oxidation to dehydroascorbic acid, by using the Cu(II)-neocuproine complex, which is reduced to Cu(I)-bis(neocuproine), the absorbance of the latter being determined at 450 nm. Other optical methods for vitamin C estimation include spectrophotometrical determination of iodine reacted with ascorbic acid [13] and chemiluminescence [14].

Liquid chromatography is a successful method for vitamin C determination when selectivity and specificity are concerned [15-17]. HPLC with electrochemical detection has turned out to be a selective and sensitive

method for ascorbic acid assessment in foodstuffs and biological fluids [18-20].

A potentiometric biosensor [21] for ascorbic acid was made by ascorbate oxidase immobilization in a polymeric matrix, fixed on a graphite-epoxy composite electrode.

Amperometric biosensors were obtained by ascorbate oxidase immobilization on a nylon net [22] or on a collagen membrane, using a Clark oxygen electrode as transducer [23]. Vitamin C analysis was also performed by using a glassy carbon working electrode as transducer incorporated in a flow system [24]. Ascorbic and uric acids were determined by coupling an amperometric technique with flow analysis [25]. Voltammetric and amperometric measurements were performed in a flow cell, using gold microelectrodes on which Pd was electrochemically deposited.

Experimental

Apparatuses, chemicals and reagents:

- UV/VIS spectrophotomitric (Shimadzo, 1650PC, Japan) with 1cm matched quartz cell
- Chemicals: Ascorbic Acid (analytical grade), potassium dichromate (99.9% purity) and potassium permanganate (99.5% purity) was obtained from BDH.
- Vitamin C tablets was obtained from pharmaceutically prepared tablets
  - German Vitamin C, T and D Pharma GmbH, GERMANY.
  - Cetavit 500mg Vitamine C, ALSHAHBA LABS. ALEPPO SYREIA.

Preparation of standard solutions

A standard solution of 100 ppm ascorbic acid was freshly prepared by dissolving 0.01g of ascorbic acid and diluted to 100 mL with distilled water.

A 100 ml of $KMnO_4$ Standard solution (100 ppm) was prepared by dissolving 0.01g $KMnO_4$ in 5M H_2SO_4 and completed with distilled water, and in a dark place.

A 500 ppm $K_2Cr_2O_7$ in 100 mL Standard solution, was prepared by dissolving 0.05g $K_2Cr_2O_7$ in 5M H_2SO_4 and completed with distilled water.

Preparation of Mixtures

For 5 different (25 mL) volumetric flask a 40ppm $KMnO_4$ was added, then 1, 4, 8, 12 and 16 ppm ascorbic acid was added for each respectively. The absorbency of mixture were measured using UV-Vis spectrophotometer at 530 nm.

For 10 different (25mL) volumetric flask a 100 ppm $K_2Cr_2O_7$ was added, then 2, 10, 20, 30, 40, 50, 60, 70, 80, 90 ppmascorbic acid was added for each respectively. The mixtures were measured at (350nm) using UV-Vis spectrophotometer.

Results and Discussion

Procedure of appropriate wave lengths:

The absorbance of each solution was measured from (450-600nm) for permanganate and from (300-600nm) for dichromate in presence of different concentrations of A.A, the spectra obtained were represented in Fig.(1) and Fig. (2).

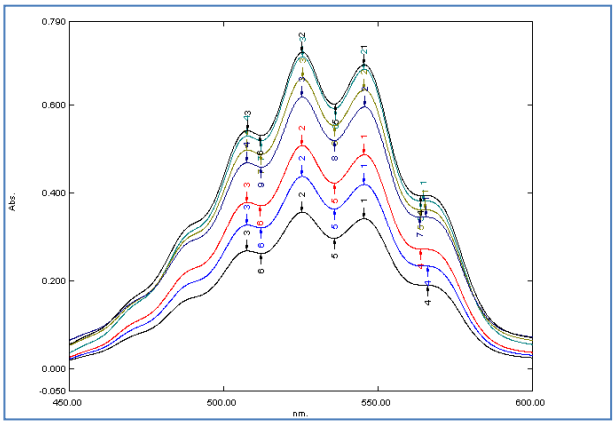


Fig. (1) Spectra of $KMnO_4$ in the presence of (1- 16 ppm) A.A.

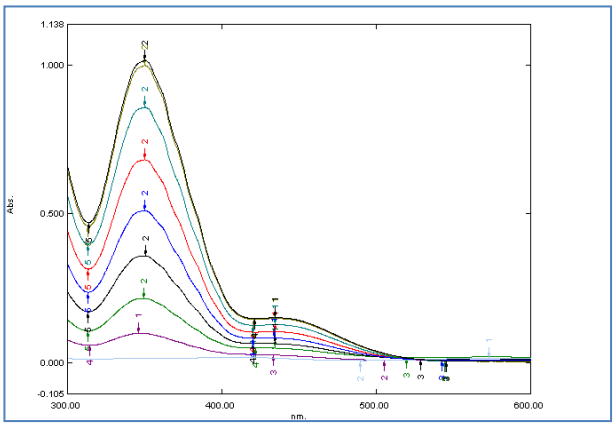


Fig. (2) Spectra of $K_2Cr_2O_7$ in the presence of (2- 90 ppm) A.A.

Procedure of the optimum conditions

The best absorption of $KMnO_4$ was in the concentration of 40ppm while for the $K_2Cr_2O_7$ it was 100ppm. See Fig. (3) (the absorption of different concentrations of $KMnO_4$ shows that the 40ppm is the highest peak), see also Fig.(4) (the absorption of different concentrations of $K_2Cr_2O_7$ shows that the 100ppm is the highest peak).

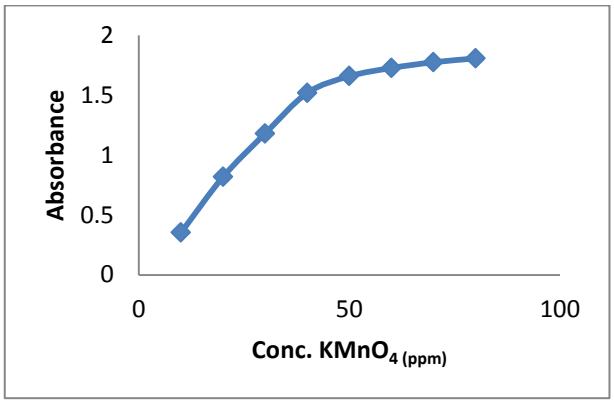


Fig. (3) The best absorbance at different concentrations of $KMnO_4$.

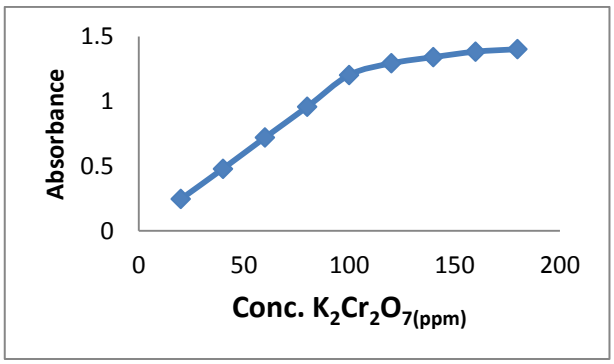


Fig. (4) The best absorbance at different concentrations of $K_2Cr_2O_7$.

The effect of order of addition

In order to have high sensitivity and a linear range for the calibration curve, the effect of the order of addition of A.A. to $KMnO_4$ and $K_2Cr_2O_7$ respectively was studied as shown in Table (1) :

Table (1)
Shows the order of addition of A.A. and reagents.

Order no.	*Reaction component*	*absorbance*
I	$KMnO_4$+A.A.	0.418
II	A.A.+$KMnO_4$	0.337
I	$K_2Cr_2O_7$ +A.A.	0.523
II	A.A.+$K_2Cr_2O_7$	0.438

Employing the conditions described in the procedure, a linear calibration graph of ascorbic acid is obtained from difference between absorbance of zero conc. (blank) of $KMnO_4$ and $K_2Cr_2O_7$ respectively and the absorbance of different concentrations from pure ascorbic acid combined with the same mixture. Fig.(5) and (6) which shows that beers law is obeyed over the concentration range (1-16) and (2-90) ppm for ($KMnO_4$+A.A.) & ($K_2Cr_2O_7$+A.A.) respectively. With correlation coefficient 0.9975 and 0.9980 while the molar absorptivies 2355.7 and 3094.8 $L.mol^{-1}cm^{-1}$ for $KMnO_4$ and $K_2Cr_2O_7$ respectively.

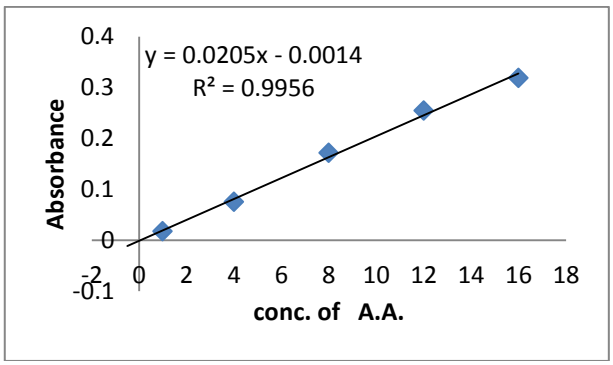


Fig.(5) Calibration curve of permanganate with A.A.

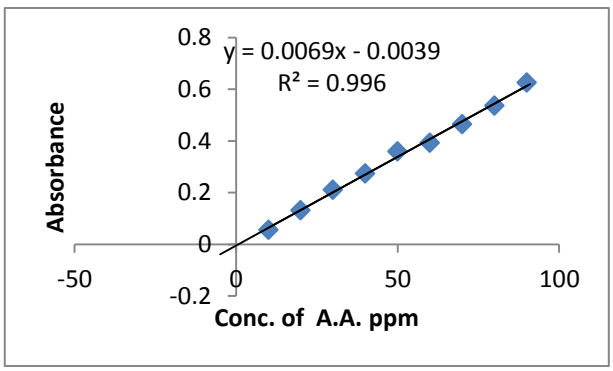


Fig. (6) Calibration curve of dichromate with A.A.

The absorbance concentration plots for the proposed methods were found to be linear over the range of (1-16) ppm & (2-90) ppm for A.A. with and A.A. withdichromate respectively.

Table (2)
Comparism of linear range for the two methods.

Parameters	*Value ($KMnO_4$)*	*Value ($K_2Cr_2O_7$)*
Linearity range (ppm)	1-16	2-90
Regression equation	y = 0.020x - 0.001	y = 0.006x - 0.003
Slop	0.02	`0.006
intercept	-0.001	- 0.003
Correlation coefficient (r)	0.9975	0.9980
Linearity (%r^2)	0.9950	0.9960
Molar absorpitivity ($L.mol^{-1}.cm^{-1}$)	2355.70	3094.75

Determination of the pure A.A. was carried out through replicate analysis of 5 times for 10 ppm manganese +A.A. & 80 ppm dichromate +A.A. the results of recovery % , RSD% (Relative Standard Deviation), E_{rel} % (error relative) and C.L. (Confidence Limit at 95% confidence level and 4 degrees of freedom (t=2.78)) are shown in Table (3) below:

Table (3)
Accuracy and precision results for the proposed methods*.

compounds	*Amount (ppm) of A.A*		*%RSD*	*%Rec.*	*% $E_{rel.}$*	*C.L.*
	taken	*found*				
$KMnO_4$+ A.A.	10	10.02±0.0032	0.73	100.2	-0.2	10.02±0.00399
$K_2Cr_2O_7$+ A.A.	80	79.53±0.0087	1.49	99.42	0.58	79.53±0.01086

mean foe 5 independent analysis

Drug Analysis

Two pharmaceuticallyprepared drugs were tested using Standard Addition Method (SAM), the results of the method are shown in Fig.(7& 8) and summary of results is shown in Table (4) below:

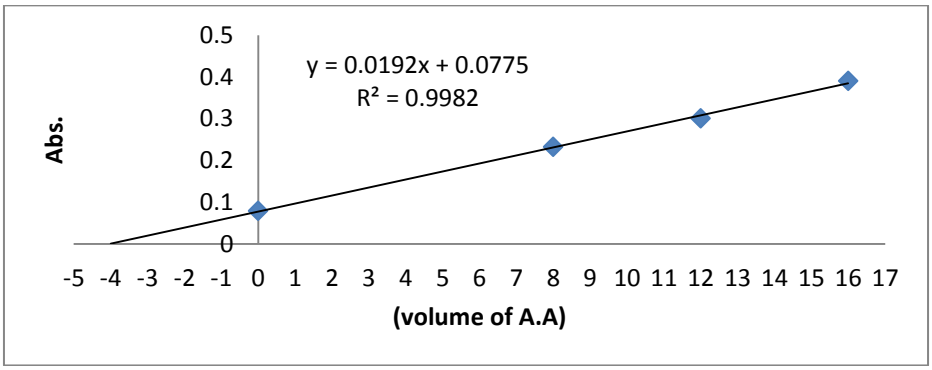


Fig. (7) Standard Addition curve for the determination of (cetavit (Vitamin C)) using permanganate.

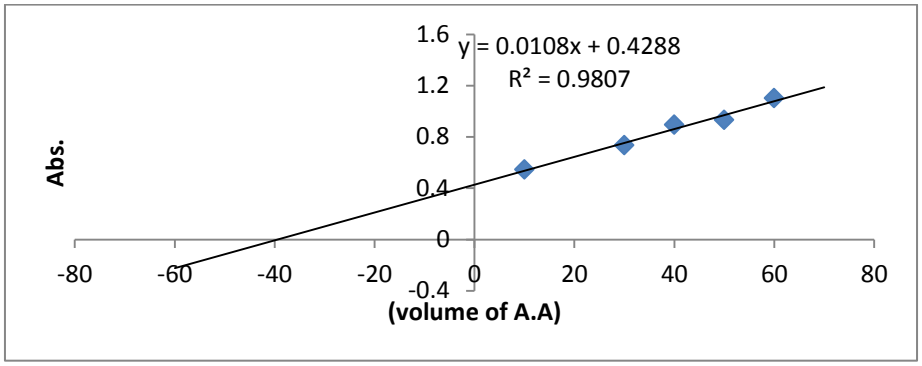


Fig. (8) Standard Addition curve for the determination of (German Vitamin C) using dichromate.

Table (4)
Summary of linearity studies of A.A. in pharmaceutically prepared tablets using SAM.

Drugs	*Regression equation*	*Slop*	*Intercept*	*Len.*	*Correlation coefficient (r)*
CetavitVit C	y = 0.019x + 0.077	0.019	0.077	99.80	0.9989
German Vit C	y = 0.010x + 0.428	0.010	0.428	98.00	0.9899

The results are reported in Table (5) as can be seen from it that the recovery % and E_{rel} %. The results were reliable and accurate.

Drugs	*Amount added ppm*	*Amount recovered ppm*	*Recovery %*	*E_{rel}%*
CetavitVit C	4	4.05	101.25	1.25
German Vit C	40	39.50	98.75%	1.25

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الخلاصة

تم استخدام طريقة طيفية لتقدير حامض الاسكوربيك في المحاليل المائية اعتماداً على قابليته لاختزال المحلول المائي لبرمنكنات البوتاسيوم والذي له اعظم طول موجي عند (٥٣٠ نانو متر) والمحلول المائي لدايكرمات البوتاسيوم والذي له اعظم طول الموجي عند (٣٥٠ نانومتر) ولقد درست الظروف العملية الفضلى لاخماد هذا التفاعل والمتضمنه تأثير تراكيز المواد المتفاعله وتأثير ترتيب الأضافة للحصول على حساسية عالية حيث بلغت قيمة معامل الممتصية المولارية (2355.7 و3094.75) لتر. مول$^{-1}$. سم$^{-1}$ على التوالي ووجد ان المدى التركيزي الذي يخضع لقانون بير من (1-16) ومن (2-90) مايكروغرام.مل$^{-1}$. اما التكرارية للنتائج المحصلة فكانت جيدة تقدر بـ(0.73 و1.49) على التوالي اما قيم الاسترداد المئوي فكانت (100.20و99.8) مع نسبة خطأ (0.2- و0.58) لمحلول البرمنغنات مع حامض الأسكوربيك ومحلول الدايكرومات مع حامض الأسكوربيك على التوالي. ولقد وجدت أقراص مُحَضّرة بشكل صيدلي قيست بالتقنية الجديدة وقد اعطت قيم جيده جدا للأسترداد المئوي (101.25 و98.75) مع نسبة خطأ قليلة جداً (-1.25 و1.25).