Determination of Ascorbic acid Via Semi automated On –line merging zone technique

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Abstract

A Flow injection analysis method for determination of ascorbic acid as Molybdenum blue is described. Orthophosphate ion, Molybdenum and ascorbic acid were reacted to form molybdenum blue then the absorbance was measured at 660 nm.
The typical analytical working curve obtained under the optimized experimental conditions were rectilinear form (1 $x10^{-3}$ _1x 10^{-4} mM) and the limit of detection was 11.4ng .Measurement of one sample (with out measurement time loading) takes 1minute. The method setup offers a precise, sensitive, selective and high sample through put technique for determination of Ascorbic acid in standards sample .It compared well with conventional standard [1] spectrophotometer method .

الخلاصة

تم تقدير حامض الاسكوربيك بطريقة التحليل بالحقن الجرياني على هيئة مولبيـدات الفوسـفات الحامضية الزرقاء . ايون الفوسفات , محلول المولبيدات ومحلول حامض الاسـكوربيك تحـول الـى مولبيدات الفوسفات الحامضية الزرقاء خلال التفاعل ثم قياس الامتصاصية له عند الطول الموجي 660 نانوميتر . تم الحصول على منحي المعايرة تحت الظروف القياسية وبحدود(1*10^{-3}_1*10^{-4} ملـي مول) وحد الكشف 11.4 نغم / مايكروليتر . قياس النموذج الواحد(من دون حسب وقت التحميـل) يستغرق دقيقة واحدة . تميزت الطريقة بدقتها وحساسيتها وانتقائيتها لتقدير حامض الاسكوربيك وضمن مدى واسع للتركيز .استعملت هذه الطريقة لتقدير حامض الاسكوربيك في محاليل قياسـية وقورنـت النتائج بالطريقه الطيفية القياسية.[1].

Introduction

Vitamin C is a water soluble anti – oxidant present naturally in wide range of foods ,particularly fruit and vegetables and has a number of physiological roles . The principle form of Vitamin C is L-ascorbic acid (A.A), a though the first oxidation product, is the dehydrate ascorbic Acid (DHAA) which is also physiologically active. Ascorbic acid has limited Stability and may be lost during storage, preparation and cooking. For this reason, many foods are supplemented with the vitamin and nutritional supplements are available in which ascorbic acid is present alone or is formulated with other micronutrients at wide range of levels .The Eu recommend daily allowance of a 60 mg. [1, 2] ascorbic acid has been determined by a number of methods many of which are based on the oxidation of ascorbic acid to dehydrator ascorbic acid using a variety of reagents in which 2, 6 – dichlorophenol indophenol is the most commonly used [1, 3]. Previous work has shown that the oxidation of ascorbic acid with phosphors vanado tungsten acid at pH=1.5, [4] Iron (III) converted to iron (II) by ascorbic acid to give complex [5].(2-Oximinocyclo hexamine) with absorption at 516 nm [6] or by hexacyano ferrate [7]. Copper (II) converted to copper (I) by ascorbic acid (with neocuprion as reagent) to give complex which an absorption at450 nm. [8] A number of flow injection systems have been developed were Iodine [9] or bromine is reduced by ascorbic acid [10, 11]. Enzyme catalyses oxidation with ascorbate oxidase ([12] and photo oxidation with thionnine blue were used ([13]. The oxidation of ascorbic acid with permanganate has been used analytically with spectrophotometer [14], and chemiluminescence's method [15-18]

Experimental

All reagents used were of analytical grade reagent.

Orthophosphate ion standard solution (1000 ppm) was prepared by dissolving an appropriate amount of sodium orthophosphate in distilled water.

Molybdenum standard solution (10 mM) was freshly prepared by dissolving an appropriate amount of ammonium molybdate in distilled water.

Ascorbic acid (100 mM) was prepared by direct dissolving in distilled water.

The following equipments were used in the work:

Spectronic 21Milton 120 Y Ray Company.

Peristaltic pump model 37 SA.

Valve (homemade).

Quartz flow cell (1cm) and mixing coil (homemade).

Recorder model pm 8222, dual-pen Philips Holland.

Water-bath (Textron 30000543)

Mode of operation

Fig. (1) shows a carrier stream of Orthophosphate ion was pumpe. molybdenum ion solution was injected in the first loop and ascorbic acid was injected in the second loop .The carrier(phospho molybdic acid)and sample were mixed in the mixing at 60 c° by using water bath and blue complex was measured at 660 nm.

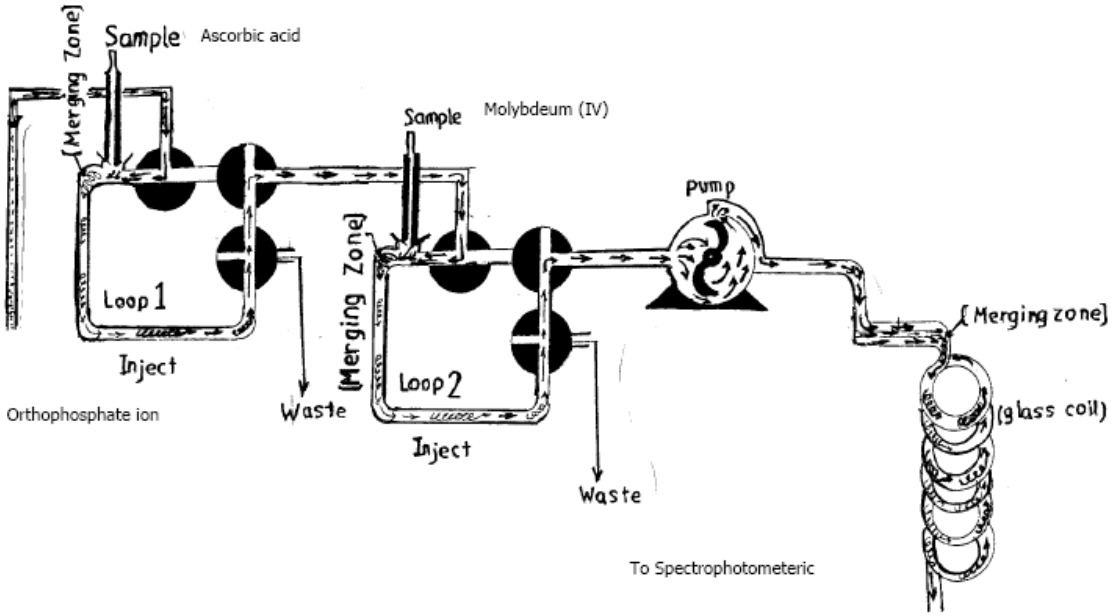

Fig (1) : The manifold used for determination of Ascorbic acid

Results and Discussion

The effect of Molybdenum and phosphate concentration were studied. Fig (2) shows the variation of phosphate ion on the response of ascorbic acid determination and ,found that 9 ppm was the most suitable concentration to be used through out this work .While fig (3) shows the Variation of molybdenum ion concentration and found that 7mM (40micro liter) was optimum for ascorbic acid determination . Fig (4) show that 2 meter coil length for determination of ascorbic acid dipped in water bath at 60 c˚was suitable. While fig(5) show effect of sample volume (50 micro liter was give height measured but 40 micro liter was suitable for this work) . Optimum flow rates (single line) were 24ml.min^{-1}.

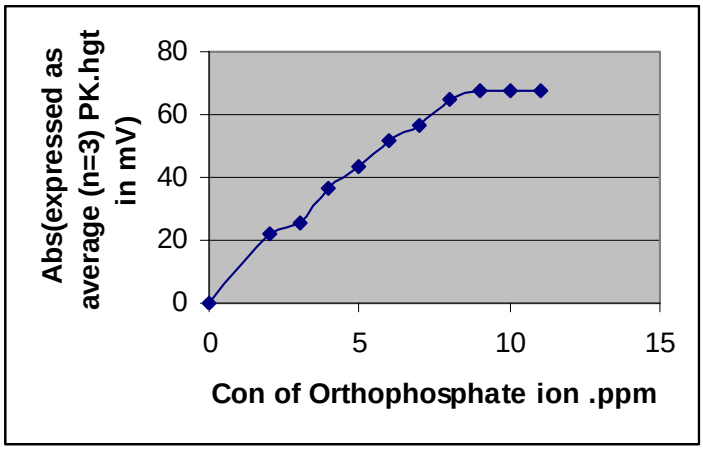

Fig (2) The effect of Orthophosphate ion concentration

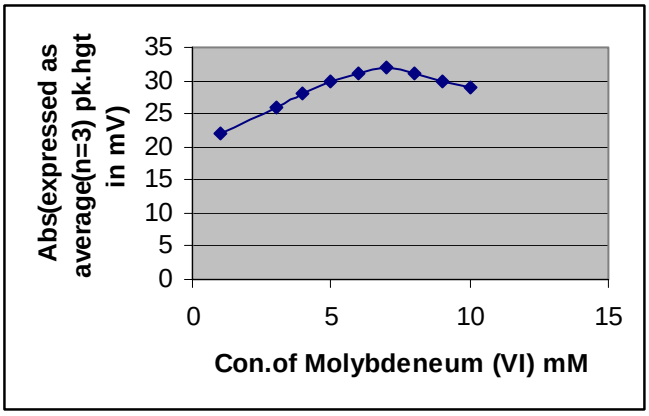

Fig (3). The effect of Molybdenum concentration

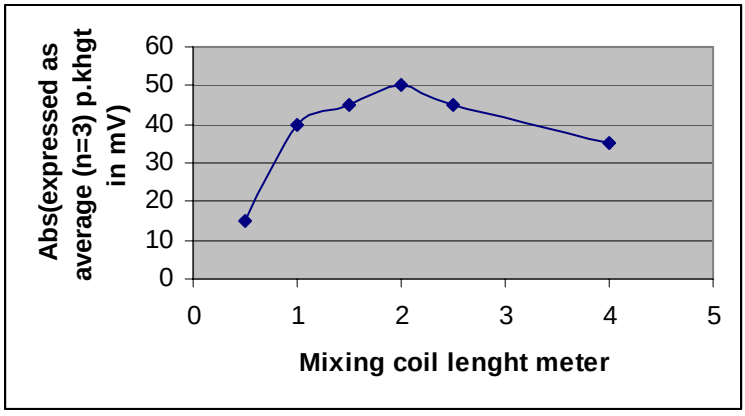

Fig (4): Effect of mixing coil length (meter)

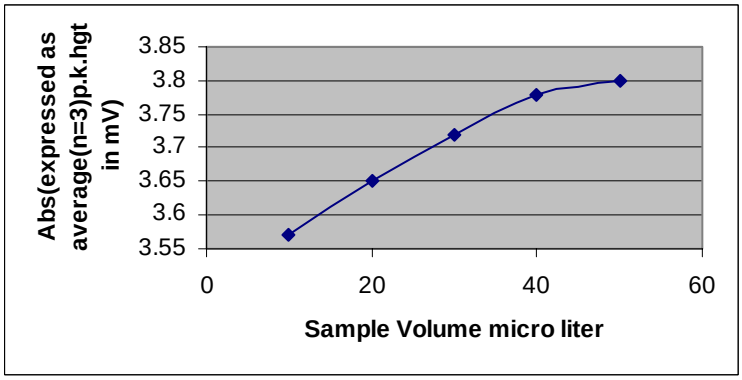

Fig (5): Effect of sample volume

Calibration Plot for Ascorbic acid

A (40 micro liter) sample volume containing various amount of ascorbic acid was treated according to the mentioned procedure. The typical detector response for ascorbic acid is show in fig (6) .The response peak appeared at about 1 minute after the injection of Ascorbic acid solution. Calibration graph for Ascorbic acid gave a straight line up to$1*10^{-3}$ mM With a correlation coefficient of 0.9736. The limit of detection was 11.4ng /40ul.The result obtained were treated by liner regression analysis show in Fig (7) while Fig(8) .shows the (σ_y),(2 σ_y),(3 σ_y) standard deviation of the liner regression graph . While Fig (9) shows

the percentage residual of the Calibration graph .Table no.(1) tabulate linear analysis data and the ANOVA analysis table for the linear Calibration graph

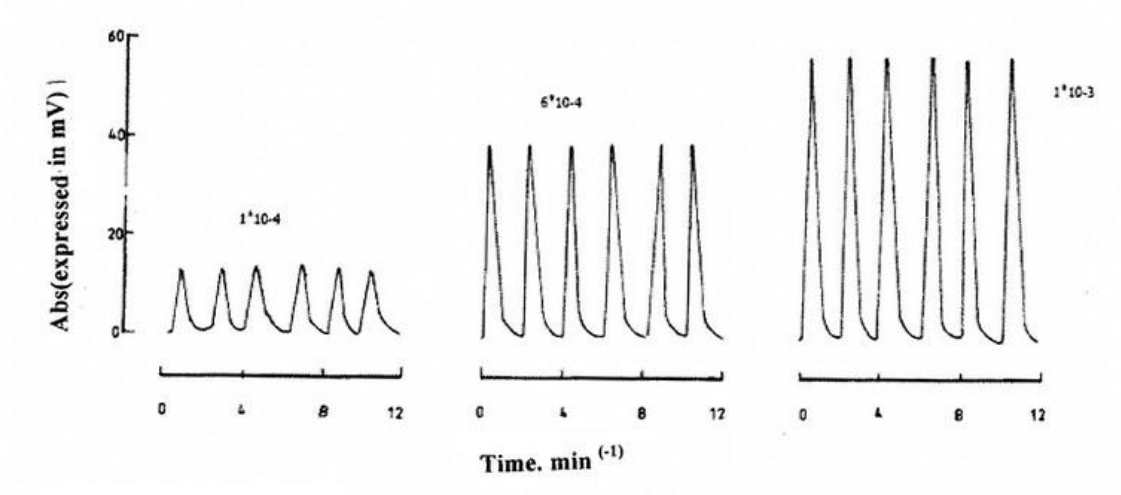

Fig (6) : Variation of absorbance of released Molybdenum blue for the determination of Ascorbic acid ($1*10^{-4}$,$6*10^{-4}$,$1*10^{-3}$ mM)

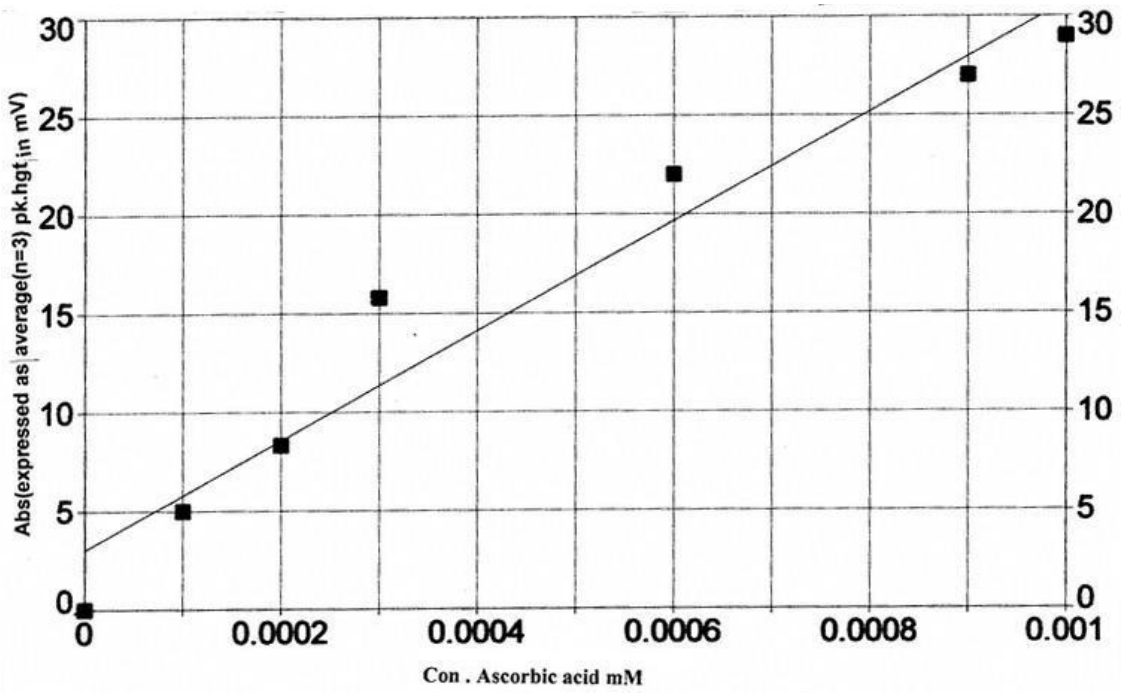

Fig (7): Calibration graph of ascorbic acid

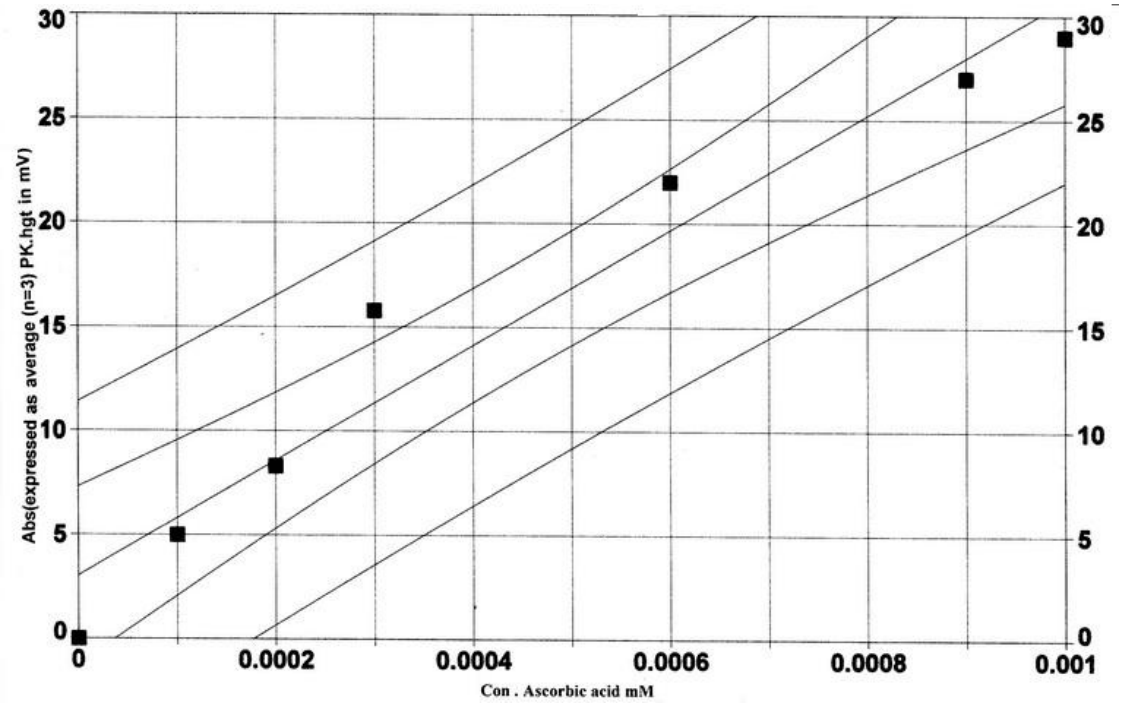

Fig (8): The (σ_y),(2 σ_y),(3 σ_y) standard deviation of the liner calibration graph

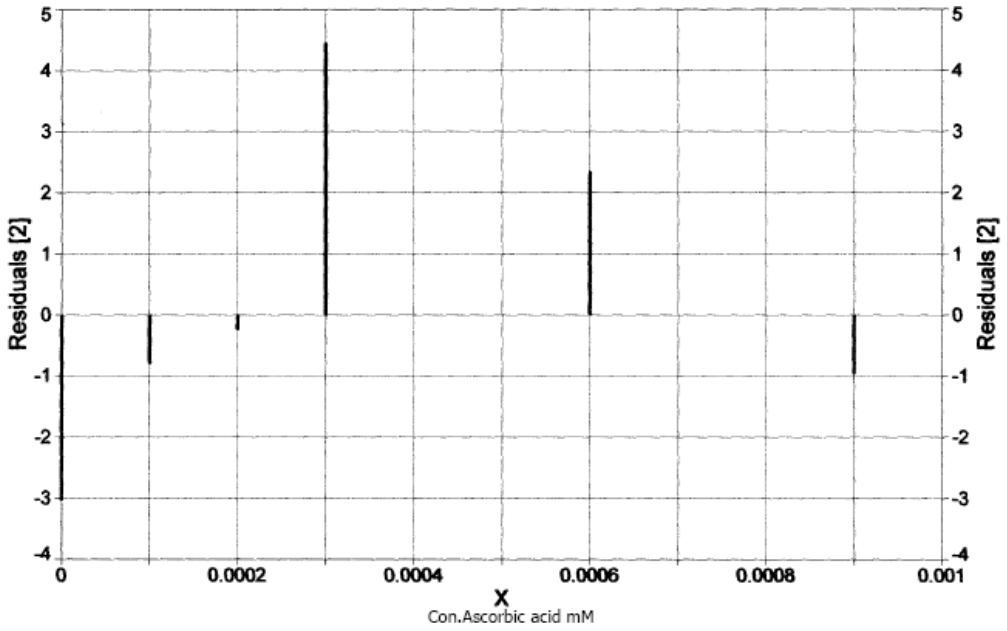

Fig (9): Percentage Residual of all Ascorbic acid concentration

used through calibration graph .

Table (1) : Linear regression analysis of ascorbic acid determination and the ANOVA analysis table

Para	Value	Std Error	t-Value	95% Confidence Limits
a	3.03532	1.66168	1.82664	-1.23618_7.3068
b	27704	27704	9.57749	20268.3_35139.8

source	Sum of squares	DF	Mean Square	F-Statistic
Regr	719.27404	1	719.27404	91.72839
Error	39.2067	5	7.84134	
Total	758.48077	6		

Determination of Ascorbic acid in solution

Table (2) shows the agreement between the results in both methods and the paired t-test illustrates that the FIA method has no significance difference when compared with standard spectrophotometer Method .there for it can regarded as alternative determination method from the many advantages that this method have .

Table (2): Comparison of results obtained for Ascorbic acid concentration in Standard solution utilizing the FIA method and standard method.

Sample	Concentration .of A.A		d (M)	Xd (M)	Sd (1) (M)	Paired t -test	T form table at 95% n-1
	FIA	standard					
(1)	$6*10^{-4}$	$6.5*10^{-4}$	$0.5*10^{-4}$	$6*10^{-5}$	$1.732*10^{-5}$	0.600>4.303	
(2)	$3*10^{-4}$	$3.5*10^{-4}$	$0.5*10^{-4}$				
(3)	$9.5*10^{-4}$	$10.3*10^{-4}$	$0.8*10^{-4}$				

Conclusion

The pre sent method allows semi-automated highly sensitive, micro determination of A.A. It takes 3min for a single complete measurement ,while it takes only 1mintes for each sample loading to determine A.A The reproducibility obtained for the individual sample Solutions (%RSD= zero, for n=3) was zero ,for A.A . The ease of application of the indirect spectrophotometer determination of A.A is clearly an advantage compared with other spectrophotometer methods [1].

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