Development of a Spectroscopic Method for Quantitative Determination of Pharmaceutical Preparations of Vitamin C

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Summary: Vitamin C in aqueous solution shows concentration v s absorbance a linear relationship for dilute solutions (0.004 % to 0.005 % w/v). The pH also affects the linearity of the curve such as pH 3 is found to give a linear relationship between concentration and absorbance up to a wide range (0.001 % to 0.009 % w/v). The E (1 %, 1 cm) values at pH 3 may be exploited to determine the % age purity of the vitamin C in separate vitamin C tablets or syrups. This method for the quantitative determination of vitamin C is more accurate, precise and applicable as compared to simple methods of UV spectrophotometry.

Introduction

Some names for Ascorbic acid [1] are Vitamin-C [2], Cevitamic acid [3] , L-ascorbic acid, L-threo-3-keto-hexuronic acid Lactone and L-threo-2-Carboxylic acid Lactone. Ascorbic acid, a white crystalline solid with molecular formula $C_6H_8O_6$ and molecular weight 176.1 g, shows λmax 274 nm and 447 nm in H_2O. It is insoluble in most organic solvent, benzene, ether, chloroform, fats and oils. Crystallographic and X-ray measurement of crystallized form show that the molecule is almost completely flat [4]. Ascorbic acid is present in all living plant cells, the largest amounts being usually in the leaves and flowers, i.e., in actively growing parts [5]. A deficiency of ascorbic acid causes scurvy, which is characterized by malformation of connective tissue. Large doses of vitamin-C have been recommended by some for the prevention and treatment of diseases ranging from common cold to cancer. Such large doses of vitamin C are not with out hazard [6].

UV-visible spectroscopy (wavelength 190-750 nm), a type of molecular spectroscopy provides many useful information as well as many approaches for quantitative determination of organic compounds. Of the 13 vitamins, vitamin C is the topic of discussion in this manuscript. Ascorbic acid may be determined by bioassay or by chemical procedures. Although the formers are the most specific and accurate, the latter for practical reasons has largely replaced them. The quantitative determination of the Ascorbic acid is based on the spectrophotometric method using different analysis techniques. i.e; Flow injection spectrophotometric methods that involve several parameters e.g., length of reaction coil, flow rate of carrier liquid, pH, and involve complexation reactions [7-10], (2) Derivative spectrophotometric methods are used to determine Ascorbic acid contents in vegetables, fruits and tablets. All 4 (Ist to 4th) derivatives of spectrometry were available for analysis [11-13], (3) Chromatographic spectrophotometric methods. All these methods are used for the determination of ascorbic acid in tablets but the third method is selective and highly sensitive. At this stage, greater emphasis will be given to the quantitative determination of this vitamin rather than qualitative and structural analysis.

Two fundamental laws are applied: that of a French scientist, Pierre Bouguer, which is also known as Lambert's law, relates the amount of light absorbed and the distance it travels through an absorbing medium; and Beer's law relates light absorption and the concentration of the absorbing substance. The two laws may be combined and expressed by the equation. $A = \alpha c l$

The reason, A is the most useful measure of light absorption, is that it is directly proportional to the concentration of the sample, c, as well as the length of the light path through the sample cell, l. The proportionality constant, α is called the extinction coefficient if c is in moles per liter, and l is in cm, α has the units liter $mole^{-1}$ cm^{-1} and is often referred to as the molar extinction coefficient [15].

In the present study a UV spectroscopic method has been developed for the quantitative determination of ascorbic acid in pharmaceutical preparations of ascorbic acid i.e., separate vitamin C tablets and syrups. This method can be applied equally to the micro levels (*i.e.,* ppm levels) determinations of ascorbic acid in analytical laboratories. The absorption spectrum of ascorbic acid exhibits two peaks *i.e.,* at 274 nm and 447 nm, each of which shows variations with change in pH. Under normal conditions i.e., vitamin C in aqueous solution at λ_{max} 274 nm gives a curve which is linear for dilute solutions (0.004 % to 0.005 % w/v). This range of linearity is very less and Beer's law is not fully applied. In order to obtain the maximum linearity of the curve and the Beer's law is obeyed, different buffer solutions having pH between 2-11 have been used in the first step. Then, by plotting the curves between conc. and absorbance, the buffer solution that gives the best curve (*i.e.,* the curve that obeys the Beer's law up to a wide range of concentrations) is sought. Then E (1 %, 1 cm) is calculated at this pH to determine the % age purity of pharmaceutical preparations of ascorbic acid.

Results and Discussion

The vitamin C, soluble in water, has been studied in aqueous system in the present work. E (1 %, 1 cm) values at λ_{max} 274 nm have been determined by varying the composition of buffer systems. λ_{max} 274 nm is considered suitable for the analytical measurements of the vitamin under study because λ_{max} 447 nm does not change considerably as the pH of the medium is changed. Buffers of different pH have been used with this view in mind that concentration versus absorbance gives a linear relationship. Further more the buffer systems are kept as reference for calculating the exact and accurate values of E (1 %, 1 cm). In the end, the % age purity of some commercially available tablets containing vitamin C has been studied by calculating the E (1 %, 1 cm) values of the samples. These tablet solutions which have been determined quantitatively, were buffered with the buffer solution which gives the linear curve and supports the Lambert Beer's Law.

When distilled water is used for making the solutions, vitamin C at λ_{max} 274 nm gives highly non-linear curve. Only small linearity at 0.004 % to 0.005 % is observed. At this small linear portion, the calculated E (1 %, 1 cm) is 54.5 and 54.8

Table-1 Ascorbic Acid at λ_{max} 274 nm pH 3.

Sr. No.	Conc.(Percentage)	Absorbance	E(1%, 1cm)
1	0.001	0.038	38
2	0.002	0.076	38
3	0.003	0.110	36.67
4	0.004	0.161	40.25
5	0.005	0.190	38
6	0.006	0.229	38.17
7	0.007	0.269	39.43
8	0.008	0.304	38
9	0.009	0.342	38
10	0.01	0.369	37

respectively. When, buffered with pH 3 buffer solution, the calibration curve, at λ_{max} 274 nm, is quite linear and obeys the Lambert-Beer's Law. This linearity ranges from concentration 0.001 % to 009 % so at these concentrations the E (1 %, 1 cm) are very close to each others i.e. 38, 38, 36.67, 40.25, 38, 38.17, 38.43, 38 and 38 respectively. Table-1. When buffer system of pH 3.5 is used, absorption curve increases gradually but the curve is linear from concentration 0.003 % to 0.007 %. At these concentration the E (1 %, 1 cm) are 97.67, 98, 95.6, 97.33 and 87.86 respectively. At pH 4 the absorbance increases with the concentration and at concentration 0.005 % to 0.009 % the curve is straight line. At these concentration the E (1 %, 1 cm) are 157.92, 157.56, 159.02, 158.71 and 157.13 respectively. At pH 4.5, according to fig. the absorbance increases rapidly by increasing concentration but at concentrations 0.004 % to 0.01%, the curve is linear. The E (1 %, 1 cm) at these concentrations are 309.5, 287.2, 270.17, 267.14, 252, 247 and 240.8 respectively. At pH 5, the concentration versus absorbance is linear from 0.002 % to 0.005 %, the E (1 %, 1 cm) are 362.3, 372.9, 391.15 and 378.36 respectively. At pH 5.5 although the absorbance increases with the conc., yet the linearity is not good at this pH. Only small linearity at 0.002 % to 0.004 % is observed and E (1 %, 1 cm) are 867.5, 747 and 708.5 respectively. The pH 6 gives linear curve at 0.001 % to 0.003 % only. Above these concen-trations, the absorbance increases with conc., but the curve is non-linear and E (1 %, 1 cm) values are 791.5, 829.33 and 728.5 respectively. When buffer system of pH 7 is used, linear portion at 0.001 % to 0.005 % concentrations is seen. Above these concentrations, although the absorbance gradually increases with the conc. yet the curve is non-linear. E (1 %, 1 cm) are 773, 703, 689.67, 647.75 and 643 respectively for linear readings. At pH 8, the linearity is found at concentrations 0.005 % to 0.007 %. The E (1 %, 1 cm) are 58.2, 78.33 and 95.86 respectively (increases

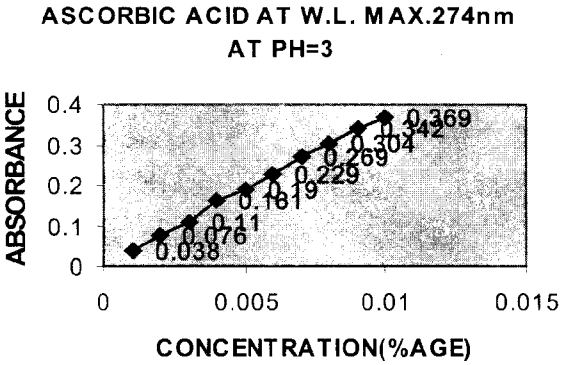

Fig. 1: Ascorbic acid at W. L. Max 274 nm and pH.

gradually). pH 9 gives very small linear portion at 0.006 % to 0.008 %. At these concentrations the E (1 %, 1 cm) are 44.17, 82.86 and 122.25 respectively. Ascorbic acid at pH 10, gives small linearity at 0.007 % to 0.009 %. E (1 %, 1 cm) at these concentrations are 47.51, 84.5 and 125.56 respectively.

The above results and calculations show that ascorbic acid at λ_{max} 274 nm with pH 3 gives outstanding calibration curve. This curve is quite linear and completely obeys the Lambert Beer's law. All the E (1 %, 1 cm) values are very stable and very close or equal to 38. PH 5 also gives linear curve but E (1 %, 1 cm) values are not stable as compared to that of pH 3. So, owing to the best results with buffer solution of pH 3 at λ_{max} 274 nm, pH 3 has been selected for the quantitative determination of pharmaceutical preparations of ascorbic acid.

So, all the sample tablets were buffered at pH 3 and studied at λ_{max} 274 nm. The two samples of Ascorvit (0.005 % and 0.006 %) have shown absorbance 0.128 and 0.170 and E (1 %, 1 cm) values 25.60 and 28.33 respectively while % age purity values are 67.3 % and 74.5 %. Thus these two samples contain 336.5 mg and 372 mg respectively rather than 500 mg which is written on the table leaflets. (Table-2) The four samples of Cecon show E (1 %, 1 cm) 26.00, 33.83, 34.00 and 25.66 and thus 68.42 %, 89.03 %, 89.94 % and 67.54 % purity, indicating 342.1 mg, 445.15 mg, 449.7 mg and 337.7 mg respectively rather than 500 mg as labeled. Also the Ascorbin samples (*i.e.*, 4) show E (1 %, 1 cm) values 29.40, 29.66, 31.00 and 31.00 pointing to 77.36 %, 78.07 %, 81.77 % and 81.57 % purity respectively Table-3.

Experimental

Materials

Ascorbic acid, citric acid, sodium citrate, tris acid maleate, maleic acid, sodium carbonate, and sodium bicarbonate, obtained from BDH, were used in the present work. All other chemicals were also of analytical grade.

Table-2: The bio-data of pharmaceutical preparations (tablets) is given .

Sr. No.	Tablet Name	Mfg. Company	Batch No.	Mfg. date	Exp. date	Mfg. Lic. No.	Reg. No.
1	Ascorvit (500 mg).	Epla Laboratories (Pvt.) Ltd. D-12,Estate Avenue, S.I.T.E., Karachi-75700	2T019	7- 2002	1-2004	000071	010848
2	*Cecon* (500 mg).	Abbot Laboratories (Pak) Ltd. Landhi Karachi	830540XV	2-2002	2-2003	000001	006119
3	Ascorbin (500 mg).	12-West. Wharf Karachi	222-29061	2-2002	2-2004	000026	---------

Table-3: The % age purity and amount of ascorbic acid present in different pharmaceutical preparation is given

Sr. No	Tablet Name	S. No.	% Age Conc.	Abs.	E(1 %, 1 cm) =Abs. / % age	% age Purity of sample	Amount of A.A.	Average Amount (mg)
1	Ascorvit (500 mg).	1	0.005 %	0.128	25.60	67.30 %	336.5 mg	354.5
		2	0.006 %	0.170	28.33	74.50 %	372.5 mg	
2	*Cecon* (500 mg).	1	0.005 %	0.130	26.00	68.42 %	342.1 mg	393.66
		2	0.006 %	0.203	33.83	89.03 %	445.15 mg	
		3	0.005 %	0.170	34.00	89.94 %	449.7 mg	
		4	0.006 %	0.154	25.66	67.54 %	337.7 mg	
3	Ascorbin (500 mg).	1	0.005 %	0.147	29.40	77.36 %	386.8 mg	398.21
		2	0.006 %	0.178	29.66	78.07 %	390.35 mg	
		3	0.005 %	0.155	31.00	81.57 %	407.85 mg	
		4	0.006 %	0.186	31.00	81.57 %	407.85 mg	

Instruments

UV Spectrophotometer, SHIMADZU, 1601 was used to measure the absorbance of the solutions while pH meter, Jenway, model-8020 was used to measure the pH of the buffer solutions.

Reagents

Ascorbic acid (stock solution)

0.1 % or 1000 ppm of ascorbic acid was prepared by dissolving 1.0 g of ascorbic acid in some deionised water and making the volume up to 1 liter with deionised water. This was used as stock solution that was later diluted with suitable buffer solutions of different pH values to make solutions of different percentages.

Buffer solutions

(a) Citrate buffer (pH 3).

46.5 ml of 0.1 M citric acid +3.5 ml of 0.1 M sodium citrate diluted to 100 ml with distilled water.

(b) Citrate buffer (pH 3.5).

38.5 ml of 0.1 M citric acid +11.5 ml of 0.1 M sodium citrate diluted to 100 ml with distilled water.

(c) Citrate buffer (pH 4).

33 ml of 0.1 M citric acid + 17 ml of 0.1 M sodium citrate diluted to 100 ml with distilled water.

(d) Citrate buffer (pH 4.5).

26.5 ml of 0.1 M citric acid + 23.5 ml of 0.1 M sodium citrate diluted to 100 ml with distilled water.

(e) Citrate buffer (pH 5).

20.5 ml of 0.1 M citric acid + 29.5 ml of 0.1 M sodium citrate diluted to 100 ml with distilled water.

(f) Citrate buffer (pH 5.5).

14.7 ml of 0.1 M citric acid + 35.5 ml of 0.1 M sodium citrate diluted to 100 ml with distilled water.

(g) Citrate buffer (pH 6).

9.5 ml of 0.1 M citric acid + 41.5 ml of 0.1 M sodium citrate diluted to 100 ml with distilled water.

(h) Maleate buffer (pH 7).

50 ml of 0.2 M tris acid maleate and maleic acid + 48 ml of 0.2 M NaOH diluted to 200 ml with distilled water.

(i) Maleate buffer (pH 8).

50 ml of 0.2 M tris acid maleate and maleic acid + 69 ml of 0.2 M NaOH diluted to 200 ml with distilled water.

(j). Carbonate-Bicarbonate buffer (pH 9).

4 ml of 0.2 M sodium carbonate + 46 ml of 0.2 M Sodium bicarbonate diluted to 200 ml with distilled water.

(k) Carbonate-Bicarbonate buffer (pH 10).

27.5 ml 0.2 M sodium carbonate + 22.5 ml of 0.2 M Sodium bicarbonate diluted to 200 ml with distilled water.

General Procedure

Different known aliquots (0.5 ml – 5 ml) of the stock ascorbic acid solution (0.1 % w/v) were taken separately into differently labeled 50 ml volumetric flasks. Then dist. H_2O was added to each flask to make the volume up to the mark in the first procedure. Then λ_{max} of ascorbic acid was measured by wavelength scanning. The selection of λ_{max} was done in this procedure (*i.e.* λ_{max} 274 nm). While in all the other procedures buffer solutions of suitable pH were used to make the volume up to the mark in order to study the ascorbic acid at λ_{max} 274 nm at different pH. All these procedures can be generalized in the form of Table-4.

Model Calculations

Calculations of E (1 %, 1 cm) are made by Formula

E (1 %, 1 cm) = Absorbance / % age Concentration (w/v)

While % Age purity of the samples is calculated by the formula,

$$\% \text{ Age purity} = \frac{E\ (1\ \%,\ 1\ cm)\ \text{Sample}}{E\ (1\ \%,\ 1\ cm)\ \text{Pure}} \times 100$$

Table- 4: Dilutions of different % ages.

Sr. No.	Amount of stock soln. taken	Dilution with buffer of specific pH	Total Vol.	% Age of soln. (w/v).
1	0.5 ml	49.5 ml	50 ml	0.001 %
2	1 ml	49 ml	50 ml	0.002 %
3	1.5 ml	48.5 ml	50 ml	0.003 %
4	2 ml	48 ml	50 ml	0.004 %
5	2.5 ml	47.5 ml	50 ml	0.005 %
6	3 ml	47 ml	50 ml	0.006 %
7	3.5 ml	46.5 ml	50 ml	0.007 %
8	4 ml	46 ml	50 ml	0.008 %
9	4.5 ml	45.5 ml	50 ml	0.009 %
10	5 ml	45 ml	50 ml	0.01 %

Conclusions

λ_{max} 274 nm and pH 3 is quite suitable for the study of ascorbic acid because of the linearity of the curve up to a wide range of concentration (0.001 % - 0.009 %). E (1 %, 1 cm) i.e., 38 is quite stable through out the curve. So, determination of % age purity of ascorbic acid in usual analytical laboratory experiments and pharmaceutical laboratory exercises can be done at λ_{max} 274 nm and pH 3.

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