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Notes

Ascorbic acid in aqueous solution: Bathochromic shift in dilution and degradation

E. Touitou [1], D. Gilhar [1], F. Alhaique [2], A. Memoli [3], F.M. Riccieri [3] and E. Santucci [3]

[1] *Department of Pharmacy, School of Pharmacy, The Hebrew University of Jerusalem, Jerusalem (Israel),* [2] *Instituto di Chimica Farmaceutica, Tossicologica ed Applicata, Facolta di Farmacia, Universita di Cagliari, Cagliari (Italy)* and [3] *Dipartimento di Studi di Chimica e Tecnologia delle Sostanze Biologicamente Attive, Universita di Roma, Roma (Italy)*

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Summary

Spectrophotometric and conductivity determinations were performed for aqueous solutions of ascorbic acid in the concentration range of 5.7×10^{-5} to 10^{-3} M. The results show a significant bathochromic shift of up to 30 nm of λ_{max} with a decrease in ascorbic acid concentration. The discrepancy between the absorbance values at λ_{max} and λ_{fix} was found to reach up to three absorbance units. These results have important implications for ascorbic acid stability tests and other systems in which ascorbic acid degradation occurs. When absorbance is directly read for ascorbic acid solutions of various concentrations, λ_{max} should be determined for each individual solution.

Although L-ascorbic acid (vitamin C) has been extensively studied for several decades, interest in this vitamin has never waned and further aspects are currently being investigated (Burns et al., 1986).

Several methods are used for quantitative evaluation of ascorbic acid (AA) in different fluids and under various environmental conditions (Clarke's Isolation and Identification of Drugs, 1986). Among the different techniques, the classic UV spectrophotometric determination, when applicable, remains to the present day the easiest and most rapid.

Information on UV absorption spectra, obtained from natural and synthetic samples of AA in different media, has been appearing since 1932 (Bowden and Snow, 1932; Mohler and Lohr, 1938; DMS UV Atlas of Organic Compounds, 1966). Nevertheless, discrepancies among the λ_{max} and absorbance values for various concentrations of AA in aqueous solutions and possible artifacts related to this method of analysis were not taken into serious consideration. In order to shed more light on the UV absorption behavior of this molecule in aqueous solutions, spectrophotomet-

Correspondence: E. Touitou, Dept of Pharmacy, School of Pharmacy, The Hebrew University of Jerusalem, P.O. Box 12065, Jerusalem 91120, Israel.

ric and conductivity determinations were performed for aqueous solutions of AA in the concentration range of 5.7×10^{-5} to 10^{-3} M.

The UV spectrophotometric measurements of AA solutions were carried out using a Lambda 3A UV/Vis Perkin Elmer spectrophotometer, and 0.1 cm quartz cells, the spectra being obtained for each AA concentration studied. The absorbance was read at two λ values: λ_{max} and λ_{fix}.

Conductivity determinations were carried out by means of a CDN_3 Radiometer, equipped with a CDC 304 cell in a thermostatic bath at 20 ± 0.5 °C.

The results summarized in Table 1 show a significant bathochromic shift – up to 30 nm of λ_{max} with decrease in AA concentration. The discrepancy between the absorbance values at λ_{max} and λ_{fix} may reach close to 3 absorbance units. In fact, this is a considerable deviation from the Lambert-Beer Law. It is also interesting to observe that although the change of pH within the concentration range studied is only about 0.3 units, the bathochromic shift is considerable, probably due to the chromophore group, which is very sensitive to dissociation; consequently, even small variations of environmental pH cause a dramatic shift in the value of λ_{max} of UV absorption. At the same time, since the degree of dissociation (α) depends upon the concentration, λ_{max} is directly related to the amount of AA in solution. This behavior is shown in Fig. 1, where the trends of α, as determined by conductivity and λ_{max}, are directly compared.

TABLE 1

UV spectrophotometric parameters, degree of dissociation and pH of ascorbic acid in aqueous solution

Solution concentration (M)($\times 10^4$)	α	λ_{max} (nm)	Absorbance		pH
			λ_{max}	λ_{fix} [a]	
0.57	0.647	263.5	0.67	0.67	4.7
1.42	0.491	260.0	1.42	1.39	
2.83	0.383	250.0	2.71	2.31	
4.25	0.327	247.5	3.84	3.08	
5.67	0.291	245.8	5.19	3.95	
7.10	0.264	244.5	6.79	4.69	
8.50	0.245	243.5	8.85	5.93	4.4
10.00	0.216	242.5	10.12	7.81	

Absorbance value obtained by multiplication by 10 (cell 0.1 cm).

[a] $\lambda_{fix} = 263$ nm.

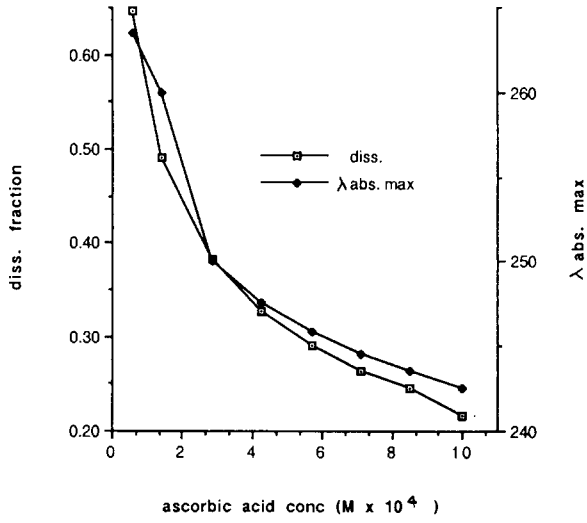

Fig. 1. Bathochromic shift of UV absorption λ_{max} (right ordinate) as ascorbic acid concentration decreases compared with the trend of dissociation (left ordinate).

From the above results it is clear that when absorbance is directly read for undiluted AA solutions of various concentrations, λ_{max} should be determined for each solution. Clarke (Clarke's Isolation and Identification of Drugs, 1986) recommends a λ_{max} value of 243 nm for aqueous acidic solutions of AA; since all the AA solutions tested in this work are aqueous acid (Table 1), spectrophotometric reading at this recommended wavelength gives imprecise data.

A further implication of the sharp change in λ_{max} with variation in AA concentration may be in the AA stability tests or in solutions in which AA degradation occurred.

Aqueous solutions of AA within the concentration range of 0.57×10^{-4}–2.83×10^{-4} M were stored at room temperature for 27 h and their UV absorbance was measured periodically. Table 2 lists the differences between the absorbance values read at λ_{max} and λ_{fix} (243 nm). These results clearly indicate that the rapid degradation of AA in aqueous solutions leads to a change in concentration and consequently to a bathochrom-

TABLE 2

Absorbance values of ascorbic acid in aqueous solutions stored at room temperature

Solution concentration (M)($\times 10^{-4}$)	Absorbance							
	0 h		7 h		23 h		27 h	
	λ_{max}	λ_{243}	λ_{max}	λ_{243}	λ_{max}	λ_{243}	λ_{max}	λ_{243}
2.83	2.70	2.30	2.30	2.16	1.94	1.79	1.88	1.73
1.14	2.11	0.87	0.98	0.74	0.67	0.48	0.62	0.45
0.57	0.67	0.44	0.52	0.31	0.20	0.12	–	–

ic shift. For these systems, the determinations of the entire spectrum should be performed for each sample and the actual λ_{max} determined in order to obtain the real absorbance values.

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