

Spectral Study of the Photolysis of Aqueous Thiamine Hydrochloride and Ascorbic Acid Solutions in the Presence and Absence of Riboflavin

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Summary: The UV and visible absorption characteristics of thiamine hydrochloride, riboflavin and ascorbic acid have been studied in the pH range 1.0-11.0 in relation to the photochemical interaction of thiamine hydrochloride/ascorbic acid with riboflavin due to overlapping of their absorption bands in the UV region. The spectral variations in thiamine hydrochloride and ascorbic acid solutions on photolysis in the presence and absence of riboflavin have been monitored and the effect of pH on the nature of these variations has been discussed. A comparison of the magnitude of spectral variations in the presence and absence of riboflavin indicates that the photolysis of thiamine hydrochloride is inhibited by riboflavin whereas that of ascorbic acid is promoted by riboflavin. The non-ionised forms of thiamine hydrochloride and ascorbic acid appear to be less susceptible to photolysis than the ionised forms. Thiamine hydrochloride and ascorbic acid are more stable to photodegradation in the acid range in the presence and absence of riboflavin.

Introduction

Aqueous solutions of thiamine hydrochloride (vitamin B₁) and ascorbic acid (vitamin C) are sensitive to ultraviolet light and are degraded to various products [1-10]. The photolysis of ascorbic acid solutions is accelerated in the presence of riboflavin [11-20]. On UV irradiation thiamine hydrochloride and ascorbic acid solutions undergo spectral variations indicating the degradation of the respective molecules. The magnitude of these variations is affected by the presence of riboflavin [21-22]. Thiamine hydrochloride and ascorbic acid are components of liquid vitamin preparations and parenteral nutrition solutions and may be exposed to UV radiation during manufacture or sterilisation. The present work has been undertaken to study the nature and magnitude of spectral variations in thiamine ascorbic acid solutions photolysed at pH 1.0-11.0 in the presence and absence of riboflavin and to draw some conclusions on the effect of pH on the rate of these reactions.

Results and Discussion

Spectral characteristics of thiamine hydrochloride, riboflavin and ascorbic acid solutions:

In order to develop an understanding of the nature of light absorption of thiamine hydrochloride, riboflavin and ascorbic acid and its effect on the

photochemical interaction of these molecules, it is necessary to consider the spectral characteristics of these vitamins.

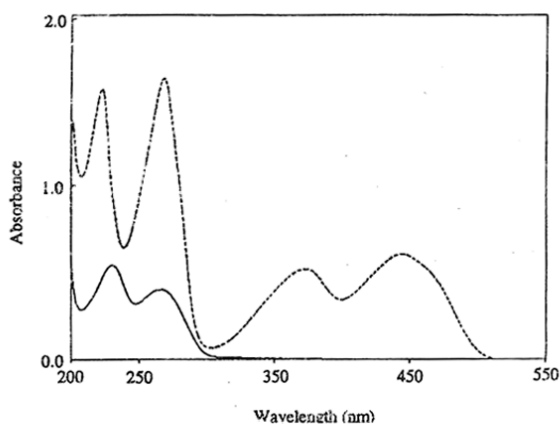
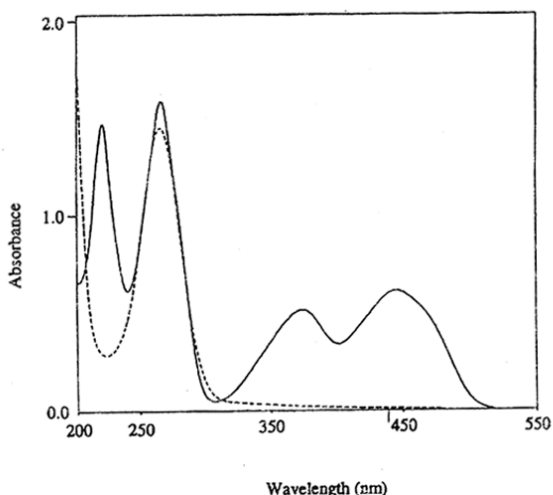
The UV and visible absorption spectra of thiamine hydrochloride, riboflavin and ascorbic acid were determined at pH 2.0, 7.0 and 10.0 to observe the absorption maxima in acid, neutral and alkaline media. A typical set of the absorption spectra of thiamine hydrochloride and riboflavin, and ascorbic acid and riboflavin at pH 7.0 are shown in Fig. 1 and 2, respectively and the absorption maxima at pH 1.0-2.0, 7.0 and 10.0-11.0 are reported in Table 1. The values of the absorption maxima of these vitamins are in agreement with those reported in the literature [10, 23-32].

Aqueous solutions of all the vitamins (B₁, B₂, C) exhibit strong absorption (ϵ_{\max} above 10,000) [33] in the UV region at pH 1.0-11.0 and the positions of the absorption maxima of thiamine hydrochloride (pK_a 4.8 and 9.0) [34] and ascorbic acid (pK_a 4.2 and 11.6) [34] are pH dependent. It is interesting to observe that the 265-266 nm absorption maxima of thiamine hydrochloride, riboflavin and ascorbic acid overlap indicating the possibility of mutual interaction (e.g. exciplex formation, energy transfer, deactivation/quenching of excited singlet state) [35]

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Table-1: Absorption maxima of thiamine hydrochloride, riboflavin and ascorbic acid*

Compound	pH	λ_1 max, nm	λ_2 max, nm	λ_3 max, nm	λ_4 max, nm
Thiamine hydrochloride	1.0-2.0	246			
	7.0	233	265		
	10.0-11.0	233			
Riboflavin	1.0-2.0	223	266	373	444
	7.0	223	266	373	444
	10.0-11.0	223	266	373	444
Ascorbic acid	1.0-2.0	243			
	7.0	265			
	10.0-11.0	265			

* ± 1 nmFig. 1: Absorption spectra of 5×10^{-5} M thiamine hydrochloride (—) and 5×10^{-5} M riboflavin (---) solutions at pH 7.0 (phosphate buffer).Fig. 2: Absorption spectra of 5×10^{-5} M ascorbic acid (---) and 5×10^{-5} M riboflavin (—) solutions at pH 7.0 (phosphate buffer).

on excitation by UV absorption. Thus thiamine hydrochloride and riboflavin, and ascorbic acid and riboflavin in aqueous solutions may interact on photolysis and thereby influence the spectral characteristics of the vitamins and hence the rate of reactions.

Spectral characteristics of photolysed solutions of thiamine hydrochloride, thiamine hydrochloride and riboflavin, ascorbic acid, and ascorbic acid and riboflavin:

The UV and visible absorption spectra of thiamine hydrochloride, thiamine hydrochloride and riboflavin, ascorbic acid, and ascorbic acid and riboflavin solutions, on photolysis at pH 1.0-11.0, were measured at various intervals to study the spectral variations, with time, in the individual vitamins and those exhibited on mutual interactions to draw some conclusions on the nature and rate of the photoreactions.

a. Thiamine hydrochloride solutions

A set of typical absorption spectra of thiamine hydrochloride solution, photolysed at pH 8.0, is shown in Fig. 3. There is a gradual decrease in absorbance at 233 and 265 nm with time, and appearance of an isobestic point around 240 nm, indicating the transformation of the molecule to species probably having molar absorptivities lower than thiamine hydrochloride in the region of these wavelengths. Thin-layer chromatographic examination of the photolysed solutions showed the presence of 2-methyl-4-amino-5-aminomethylpyrimidine as the major degradation product [22], which exhibits absorption maximum at 243 nm [36]. Slight bathochromic shift of the 233 nm absorption maximum, with time, indicates the gradual formation of this compound. However, the spectral variations observed are due to absorbance of the mixture of all the components present in the degraded solution (i.e.

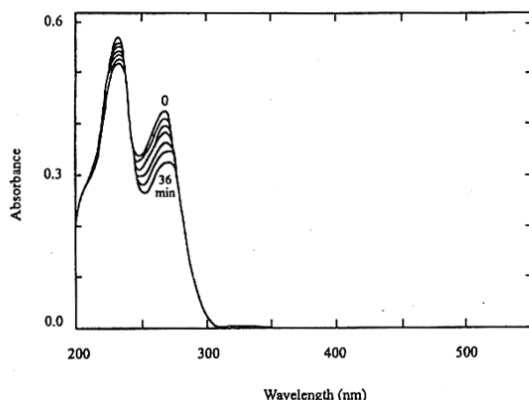


Fig. 3: Absorption spectra of 5×10^{-5} M thiamine hydrochloride solution photolysed at pH 8.0, measured at 6 minutes interval.

undegraded thiamine hydrochloride and its known/unknown photoproducts). Similar spectral changes, at a relatively slow rate, are observed in thiamine hydrochloride solution photolysed at pH 6.0. In the acid range at pH 1.0-2.0 and 4.0, the photolysed solutions exhibit absorbance loss at the maximum at 246 nm, with a gradual bathochromic shift, indicating the formation of the major degradation product mentioned above. The magnitude of absorbance loss at pH 4.0 is greater than that observed at pH 1.0-2.0 suggesting a relatively fast reaction rate at this pH. At pH 10.0-11.0 also, the photolysed solutions depict a decrease in absorbance at the absorption maximum (233 nm) leading to the formation of degradation product. The rate of change is slower at pH 11.0 than that at pH 10.0 probably due to ionisation of the molecule. It may be concluded from the spectral variations of thiamine hydrochloride solutions photolysed at pH 1.0-11.0 that the nature and magnitude of these variations depend upon the predominant species of thiamine hydrochloride (pK_a 4.8 and 9.0) present at a particular pH [37], undergoing photolysis, and the absorption characteristics/composition of the photoproducts in the degraded solutions. On the basis of spectral variation and chromatographic evidence 2-methyl-4-amino-5-aminomethylpyrimidine appears to be the major photoproduct of thiamine hydrochloride which is obtained by cleavage of the thiazole moiety.

b. Thiamine hydrochloride and riboflavin solutions

It would be worthwhile to make a comparison of the spectral variations of photolysed solutions of

thiamine hydrochloride with those of thiamine hydrochloride photolysed at pH 1.0-11.0 in the presence of riboflavin under the same conditions. The spectral variations in these solutions appear to be similar to those observed in thiamine hydrochloride solutions in the UV region along with those of riboflavin in the UV and visible region. Typical variations in a set of absorption spectra of an equimolar solution (5.0×10^{-5} M) of thiamine hydrochloride and riboflavin, photolysed at pH 8.0, are shown in Fig. 4. The loss of absorbance at 265-266 nm (λ_{max} of thiamine hydrochloride and riboflavin) is much less than that observed in thiamine hydrochloride solutions alone (Fig. 3) suggesting the inhibitory influence of riboflavin on the rate of photolysis of thiamine hydrochloride. In addition to this, loss of absorbance is also observed at 233 nm maximum of thiamine hydrochloride and 223 and 444 nm maxima of riboflavin, indicating that both molecules are not only interacting but also undergoing photolysis. The spectral variations in solutions photolysed at pH 6.0 are similar to those observed at pH 8.0 but occur at a relatively slow rate, as in the case of thiamine hydrochloride solutions alone, due to relatively greater stability of the molecules. Isosbestic points are observed near 256, 278, 298 and 340 nm, indicating the presence of more than one absorbing species in the solution.

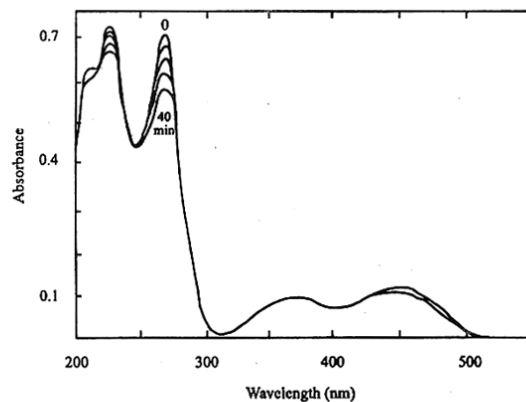


Fig. 4: Absorption spectra of 5×10^{-5} M thiamine hydrochloride solution photolysed at pH 8.0 in the presence of 5×10^{-5} M riboflavin, measured at 8 minutes interval.

The spectral variations in solutions of the two vitamins at pH 1.0-2.0 (protonated thiamine hydrochloride and protonated riboflavin, pK_a 's 4.8

and 1.7, respectively) [29] show absorbance loss at 246 nm (due to thiamine hydrochloride) and at 266 and 444 nm (due to riboflavin). The decrease in absorbance in the UV region (246 and 266 nm) is greater than that of the visible region (444 nm) suggesting that riboflavin, in the protonated form, is less susceptible to degradation than the non-ionised form and is mainly involved in interaction with thiamine hydrochloride. It is interesting to note that the absorbance loss in thiamine hydrochloride solutions at 246 nm in the presence of riboflavin is much less than that observed in its absence, suggesting that it may play an inhibitory role in the photolysis of thiamine hydrochloride. The absorbance changes observed in solutions photolysed at pH 4.0 are similar to those of pH 1.0-2.0 and occur at a relatively fast rate indicating that the photolysis of thiamine hydrochloride at pH 4.0 in the presence of riboflavin is faster than that at pH 2.0. In the alkaline medium at pH 10-11, the loss of absorbance at 266 and 444 nm (λ_{\max} of riboflavin) is much greater than that at 233 nm (λ_{\max} of thiamine hydrochloride) indicating a greater loss of riboflavin than that of thiamine hydrochloride in this region. Thus, under these conditions riboflavin would be able to exert little influence on the photolysis of thiamine hydrochloride due to its own greater susceptibility to degradation in alkaline solution. However, compared with the photolysed solutions of thiamine hydrochloride alone in this pH range, the spectral changes observed are of a lower magnitude, suggesting a low degree of interaction between the two molecules.

c. Ascorbic acid solutions

The absorption spectra of ascorbic acid solutions, photolysed at pH 1.0-11.0, show a gradual decrease in absorbance at 243 nm (pH 1.0-2.0, non-ionised molecule) and at 265 nm (pH 4.0-11.0, ionised molecule), with time. A typical set of absorption spectra of ascorbic acid solution, photolysed at pH 6.0, is shown in Fig. 5. The magnitude of change in absorbance appears to depend upon the pH and hence the ionised state of the molecule (pK_{a1} 4.2 and 11.6) [34], indicating that an increase in pH leads to an increase in the rate of reaction. The loss of absorbance at 243 nm or at 265 nm suggests the photooxidation of the molecule to dehydroascorbic acid [1] which does not significantly absorb in the 230-280 nm region [38,39]. Thus the ionised form of ascorbic acid is more susceptible to photolysis imparting greater variations to the

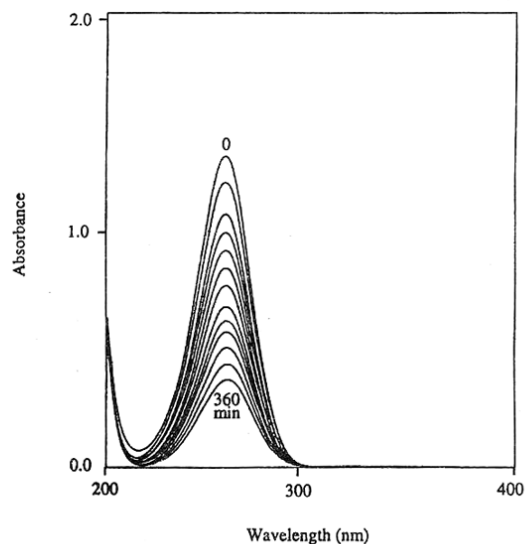


Fig. 5: Absorption spectra of 1×10^{-4} M ascorbic acid solution photolysed at pH 6.0 in the presence of 1×10^{-4} M riboflavin, measured at 15 minutes interval (1:1 dilution).

absorption spectra of the molecule during irradiation. In the alkaline region some air oxidation to dehydroascorbic acid may also take place [30].

d. Ascorbic acid and riboflavin solutions

The UV and visible absorption spectra of ascorbic acid solutions photolysed in the presence of riboflavin at pH 1.0-11.0 were determined. Typical variations in a set of absorption spectra of an equimolar solution (1.0×10^{-4} M) of ascorbic acid and riboflavin, photolysed at pH 6.0, are shown in Fig. 6. It has been observed that the loss of absorbance at 265-266 nm (λ_{\max} of ascorbic acid and riboflavin) is mainly due to ascorbic acid since riboflavin shows very little change in the UV (223 and 373 nm) and visible (444) region. A comparison of absorbance of ascorbic acid solution and ascorbic acid solution containing riboflavin at 265nm, on photolysis for 75 minutes, gives 22.2% and 34.5% loss, indicating that riboflavin is promoting the photolysis of ascorbic acid. The fact that riboflavin leads to the photosensitised degradation of ascorbic acid is well known [11, 12, 19-21] and is confirmed by the present study.

The absorption spectra of ascorbic acid solutions containing riboflavin, on photolysis at pH 4.0 and 8.0 show spectral variations similar to those

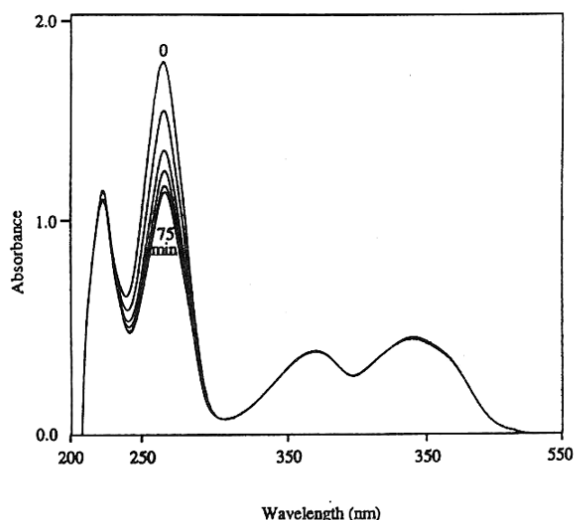


Fig. 6: Absorption spectra of 1×10^{-4} M ascorbic acid solution photolysed at pH 6.0 in the presence of 1×10^{-4} M riboflavin, measured at 15 minutes interval (1:1 dilution).

observed at pH 6.0. However, the absorbance loss at 265 nm is smaller at pH 4.0 and greater at pH 8.0, compared to that of pH 6.0, for a fixed period of time. Similar changes in loss of absorbance, with a very low magnitude, are observed at 223 nm and 444 nm due to the involvement of riboflavin as a photosensitizer in the degradation of ascorbic acid. At pH 1.0-2.0, the photolysed solutions exhibit little change in absorbance at 243 nm (λ_{\max} of ascorbic acid) and at 444 nm (λ_{\max} of riboflavin) indicating that both molecules in the protonated state are resistant to photolysis and riboflavin exerts little or no influence on the degradation of ascorbic acid in this range. On photolysis at pH 10.0-11.0, the solutions show extensive loss of absorbance at 265 nm and at 444 nm, indicating greater degradation of both ascorbic acid and riboflavin than that observed at lower pH values. This may be due to the alkaline hydrolysis of dehydroascorbic acid and formylmethylflavin, which are intermediates in the photolysis of ascorbic acid and riboflavin, respectively [40-44], and other photodegradation reactions of these vitamins.

Experimental

Thiamine hydrochloride, riboflavin and ascorbic acid were obtained from Sigma Chemical Co. All reagents were analytical grade or of the purest form available from BDH/Merck. The

following buffer systems were used. KCl-HCl, pH 1.0-2.0; citric acid - Na_2HPO_4 , pH 2.5-8.0; $\text{Na}_2\text{B}_4\text{O}_7$ -HCl, pH 8.5-9.0; $\text{Na}_2\text{B}_4\text{O}_7$ -NaOH, pH 9.5-10.5; Na_2HPO_4 -NaOH, pH 11.0; the ionic strength was 0.05 M in each case.

Photolysis

A $0.5-1.0 \times 10^{-4}$ M aqueous solution of thiamine hydrochloride or ascorbic acid containing equimolar concentration of riboflavin was prepared and the pH was adjusted in the range 1.0-11.0 with appropriate buffer. A 100 ml portion of the solution was placed in a beaker immersed in a water bath maintained at $25 \pm 1^\circ\text{C}$. It was subjected to UV irradiation using a Philips TUV 30 W mercury discharge UV tube (88.7% emission at 254 nm [45] fixed vertically at a distance of 30 cm from the centre of the vessel. Samples of the photolysed solution were withdrawn at appropriate intervals for measurement of UV and visible absorption spectra.

Spectral measurements

The UV and visible absorption spectra of freshly prepared thiamine hydrochloride, riboflavin and ascorbic acid solutions and the photolysed solutions were measured with a Shimadzu UV 240 recording spectrophotometer using silica cells of 10 mm pathlength.

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