A Study of the Molar Absorptivity and Structure of Vitamin B_2 Relationship

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(Received 12th September, 2000, revised 5th November, 2001)

Summary: Molar absorptivity (ε) and E(1%, 1 cm) values have been obtained at differret pH of the medium containing vitamin B_2 in the solution. ε-values ranged from 12000-26000 when the measurement was made at 265 nm at different pH. E(1%, 1 cm) values ranged from 323-710, increasing from lower to higher values as the pH of the medium was changed from 2.6-7.8

Introduction

The molecular absorption spectroscopy usually deals with wavelengths ranged from 190-750 nm. Many useful information can be derived from these regions of the spectrum. This information when combined with the detail provided by infrared and nuclear magnetic resonance spectra can lead to variable structural proposals.

UV-visible spectroscopy without any doubt has provided many approaches for analysis of organic compounds. Determination of B-vitamins based on modern analytical techniques has been the area of much interest these days. Of the 13 vitamins, vitamin B_2, (riboflavin) is the topic of discussion in this manuscript. This vitamin is unique like other vitamins to play its role in the regulation of biochemical reactions [1] in which the body converts food into energy in living tissues.

The determination of vitamin B_2 in multicomponent preparations [2] tablets and injectables [3] based on the absorption property of the molecule has been very popular when coupled with other analytical techniques. These are HPLC [4-5], TLC [6], First derivative spectrophotometry [7] and an ion-pair reversed phase HPLC method [8] which have been used for rapid simultaneous determination of the water soluble vitamins. Most of these techniques make UV-visible spectroscopy as the basis for detection and quantification of vitamins including the riboflavin. The versatility of the method depends upon the structural property of the vitamin. At this stage, greater emphasis has been placed on the determination of absorbing property of the molecule and not on its quantitative determination.

Molar extinction coefficient (presently known as molar absorptivity) is one of the analytical measurements which helps in the assessment of ability of a structure for light absorption. This is a characteristic property of a molecule undergoing in electronic transition and is not a function of the variable parameters involved in preparing a solution.

The absorptivity is controlled by the size of the absorbing system and the probability that the electronic transition will take place. The absorptivity may range in numerical size from zero to 10^6, values above 10^4 are termed high intensity absorption. while values below 10^3 are termed low intensity absorptions. Forbidden transitions have absorptivities in the range from 100-1000.

The empirical expression, $\log I_o/I_t = \varepsilon.c.l$, is known as Beer-Lambert law. I_o-intensily of light incident upon the sample; It = intensity of light leaving the sample cell; (c) = molar concentration of solute; l = length of sample cell (cm); ε; = molar absorptivity. The term $\log I_o/I_t$, is known as absorbance.

In the present study it is aimed that the structural behaviour of the riboflavin becomes the main focus rather than its determination. The absorption spectrum of riboflavin exhibits four peaks [9] at 223, 267, 375 and 444 nm, respectively, each of which shows variations with change in pH. The peaks, however, show stability between pH 2 and 7. The study will include investigation of the effect of pH on the absorbing property of riboflavin, being determined by the values of molar absorptivity (ε)

and (1% 1 cm) of the vitamin, E(1%, 1 cm) will also be determined because this value at a particular pH may be used to establish the purity of the vitamin.

Results and Discussion

The wavelength of maximum absorption (λ_{max}) of the riboflavin solution at buffered pH 5.4 was determined. This necessarily requires one solution of vitamin of any concentration but in this experiment at the above pH condition a number of solutions of varying concentrations from 0.0008 to 0.001% were scanned for absorbance at different wavelengths. The absorbance versus wavelength trace is shown in Fig. 1, Absorbance at wavelength 266.8 nm was maximum. The peak height gradually increased as the concentration of the riboflavin was increased. It means the absorbance increased directly to the concentration change as per Beer-Lambert law.

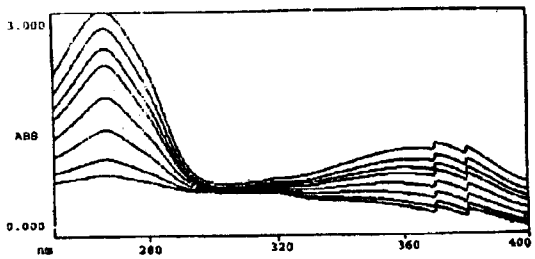

Fig. 1: Absorption spectra showing absorption at different wavelengths.

The effect of pH on λ_{max} was also investigated. λ_{max} did not change considerably as the pH of the medium was changed. The spectral data showed that at all the pH values, e.g., 2.6, 3.6, 4.2, 5.4 and 6.-6, the absorbance by the riboflavin solution was maximally observed at 266.8 nm or very close to it.

However, the absorbance vs. concentration relationship was followed nicely for a solution buffered at pH 5.4 in the range 0.0002 to 0.001% (w/v). This linearity suffered as the pH of the medium changed to 3.6, as shown in Fig. 2. The absorbance was sensitive to change in pH is shown also in an another experiment with and without buffer correction, for measurement of absorbance of riboflavin solutions (0.0002 to 0.005%w/v) at a little higher pH 4.2 than 3.6, Fig. 3. The linearity of the result was, however, maintained at the above noted pH values. In this figure, this was also observed that

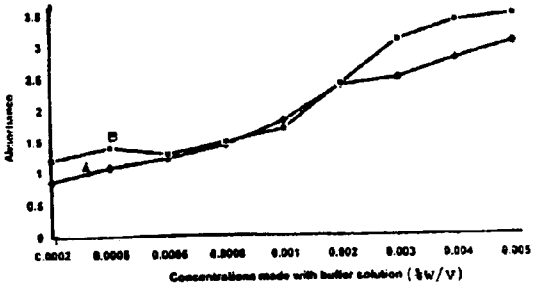

Fig. 2: Absorption spectra Riboflavin at 266 nm. Curve A. pH 5.4 and curve B pH 3.6

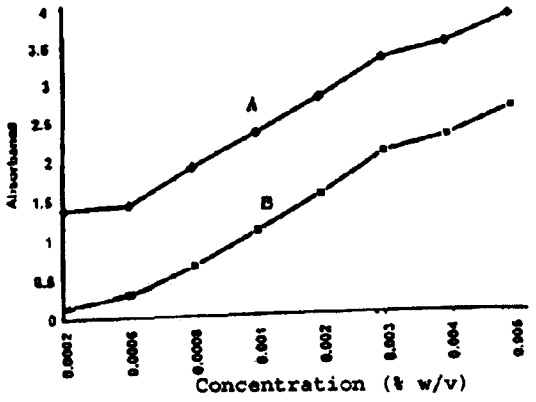

Fig. 3: Absorption versus concentration relationship Curve A (without buffer correction) – water as reference and Curve B (with buffer correction) – buffer as reference

this buffer too had its contribution towards the absorbance. This was true for all pH conditions. In order to get reliability of the results all the measurements were corrected for buffer blank.

Absorbance by the riboflavin solutions (0.0002 to 0.005% w/v) was measured at wavelength other than 266.8 nm. The other wavelength where the solutions absorbed maximally was 440 nm, same as reported in the literature cited. Fig. 4 showed the absorption spectra of vitamin B_2 solutions, indicating the absorption to take place at 266.5 and 440 nm. These two wavelengths were considered suitable for analytical measurements of the vitamin under study. At pH 7.8, absorbance vs. concentration plot (0.001-0.01%w/v) gave the curve, Fig. 5. This consisted of curve A (absorbance measured at 266.5 nm) and curve B (absorbance measured at 440 nm). The

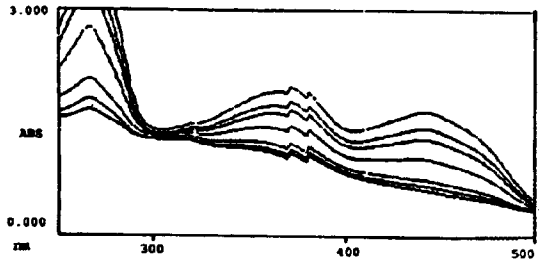

Fig. 4: Absorption spectra of riboflavin at pH 2.6.

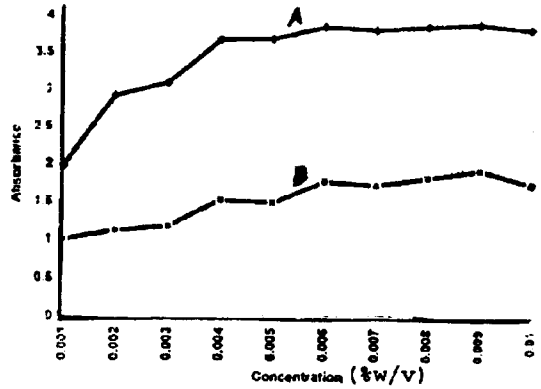

Fig. 5: Absorption spectra of riboflavin at pH 7.8
Curve A = Absorbance at λ_{max}=266 nm
Curve B + Absorbance at λ_{max}=440 nm

linearity of result appeared to fail at both the wavelengths due to high concentration of the vitamin solutions

Molar absorptivity (ε) and E(l%, 1 cm) of riboflavin has been presented in Table 1. Bonds of medium intensity (ε = 1,000 - 10,000) with λ_{max} above 200 nm, generally indicated the presence of an aromatic system. For an aromatic system, a good deal of fine structure in the longer wavelength band is expected but in non-polar solvents only. However, substitution of an aromatic rings, as in the case of vitamin B_2 had increased the molar absorptivity above 10,000; which may be due to the substitution causing increase in the length of the conjugated system.

The characteristic E (l%,l cm) values, being present in Table 1, further showed that the molecule has the chromophore which can absorb radiation efficiently. Compounds which are highly coloured have absorption in the visible region. This is true for vitamin B_2 which showed absorption to take place at 440nm too. Fig 4. This reflected obviously a polycyclic aromatic chromophore in the molecule. It is said that benzenoid compounds may be coloured if they have enough conjugating substituents.

Table-1: Molar absorptivity (ε) and E(l%, 1cm) of riboflavin: pH = 4.2, λ_{max} 266.6 nm.

Concentration		Absor-	ε (l%,1cm)	ε(mole^{-1}dm^{-1} cm^{-1})
% w/v	Mole/L	bance		
0.0004	1.06X10^{-5}	0.305	762-5	0.26 xl0-^{5}
0.0005	1.32x10^{-5}	0.381	762.0	29461.5
0.0006	1.59 xl0-^{5}	0.460	766.6	29259.2
0.0007	1.85 xl0^{-5}	0.539	770.0	30384.6
0.0008	2.12 xl0^{-5}	0.620	775.0	30000.0

The study of the effect of pH on the values of molar absorptivity (ε) gave results which have been presented in Table 2. The values of 'ε' at different pH appeared lo be affected greatly. It varied from 12,000-26,000 which definitely seemed to be the result of structural change taking place as expected. The molecule behaved as a cation in the acidic medium and an anion in the basic medium. The pH of the medium was changed in between 2.6 and 7.8 and the results obtained indicated that the absorbing property of the molecule changed too. The ε values rising above 10,000, indicated $\pi \rightarrow \pi^*$ transition with conjugation in the system, obscuring or burying the weaker $n \rightarrow \pi^*$ transition.

Table 2. Molar absorptivity (ε) of riboflavin alongwilh E(l%, 1cm) at difrcrent pH values of the medium. Numerical figures in the data were rounded off.

pH of the medium	Concentration		Absorbance at 266.5 nm	E(l%, lcm)	ε (mole^{-1} dm^3 cm^{-1})
	%w /v	Mole/L			
2.6	0-0002	0.53x10^{-5}	0.067	335	
	0.0004	1-06xl0^{-5}	0.131	327	12075.6
	0.0005	1,32 xl0^{-5}	0.167	334	13846.1
	0.0006	1.59 xl0^{-5}	0.200	333	12222.2
	0.0007	1.85xl0^{-5}	0.231	330	11923,0
	0.0008	2.12 xlO^{-5}	0-259	323	10370.0
5.4	0.0001	0.26 xl0^{-5}	0,040	400	
	0.0002	0.53x10^{-5}	0.086	430	17087.0
	0.0004	1.06 xl0^{-5}	0.170	425	15849.0
	0.0006	1.59 xl0^{-5}	0.254	423	15849.0
6.6	0.0002	0.53 xl0^{-5}	0.139	695	
	0.0004	1.06 xl0^{-5}	0.278	695	26226:4
	0.0005	1.32 xl0^{-5}	0.328	656	19230.7
	0.0006	1-59 xl0^{-5}	0.399	691	26296.2
	0.0008	2.12 xl0^{-5}	0.550	691	28490.5
7.8	0.0001	0.26 xl0^{-5}	0.071	710	21851.8
	0-0002	0.53 xl0^{-5}	0.130	650	22641.7
	0.0004	1.06 xl0^{-5}	0.250	600	23584.9
	0.0006	1.59 xl0^{-5}	0.375	683	22641,7

Molar absorptivity values have direct relationship with the structure of the molecule. The higher the values the more is the number of absorbing molecule. Secondly, molar absorptivity values may be exploited to determine the quantity of riboflavin in the sample. It is also apprehended that molar absorptivity coupled with differential uv-spectroscopic method [10] could determine riboflavin in presence of other vitamin such as vitamin C.

E(1%, 1cm) values have also been obtained as presented in Table 2. Its values varied from 232-710, depending upon the pH of the medium. The values being low at the lower pH but increased as the pH changed to the higher values.

Experimental

1. Materials

Riboflavin, acetic acid, citric acid, sodium acetate and dibasic sodium phosphate were used and obtained from BDH. All other chemicals used were of analytical grade.

2. Instruments

Spectrophotometer, Hitachi U-2000, pH meter Jenway, model-8020 were used for measurements.

3. Reagents

Riboflavin

(M.wt. 376.37) solution (0.02% w/v) was prepared by dissolving 0.1g of the vitamin in water and volume made upto 500 ml. This was used as stock solution which was later diluted with suitable buffers of different pH values to obtain solutions of different percentages. For molar concentrations, the percentage concentrations needs to be changed as follows: %w/v x 10/M.wt. of riboflavin.

Buffer solution

(i) Acetate buffer (pH 5.-4): 8.80 ml of 0.2 M acetic acid + 41.2 ml of 0.2 M. sodium acetate diluted to 100 ml with distilled water.

(ii) (a). Citrate-phosphate buffer (pH 2.6); 44.60 ml 0.1 M citric acid + 5.40 ml of 0.2 M dibasic sodium phosphate, diluted with water to 100 ml.

(b). Citrate-phosphate buffer (pH 6.6): 13.60 ml of 0.1M citric acid + 36.40 ml of 0.2 M dibasic sodium phosphate, diluted with water to 100ml

(iii) (a) Acetate buffer (pH 3.6): 46.30 ml of 0.2 M acetic acid + 3.70 ml of 0.2 M sodium acetate, diluted with water to 100 ml.

(b) Acetate buffer (pH 4.2): 36.80 ml of 0.2 M acetic acid + 13.20 ml of sodium acetate, diluted with water to 100 ml.

(iv) Sodium phosphate buffer (pH 7.8): 8.50 ml of 0.2 M monobasic sodium phosphate + 91.50 ml of 0.2 M dibasic sodium phosphate diluted with water to 200 ml.

General Procedure

A varying known aliquots (0.1ml - 2.5ml) of the stock riboflavin solution (0.1% w/v) were taken separately into different 50 ml volumetric flasks. To each of these was then added either water or the buffer solutions of suitable desired pH to makeup the volume upto the mark. The flasks were shaken thoroughly and the absorbance of the solution was measured. These solutions were also used to obtain spectra by scanning at different wavelengths.

For the determination of wavelength of maximum absorption (λ_{max}) by the solution (s) of interest, any one solution of a particular concentration and pH was used for scanning at different wavelengths. The wavelength at which the absorption was maximum, was selected as λ_{max} for all other determinations.

For all the absorbance measurements, a buffer correction was made by subtracting the absorbance of the blank buffer from the absorbance of the sample solution. This provided results obeying linearity to wide range of concentrations from 0.0005 to 0,001% (w/v).

5. Model Calculations

I. Molar extinction coefficient (ε) is obtained from the following relationships.

$\varepsilon = A/c.l$ = Absorbance/g.moleL^{-1} x light path length (cm)

ε. = Slope of the line in a graph

Now, slope of the line is obtained from the difference in absorbances (A_2-A_1) between any two corresponding molar concentrations (C_2-C_1) of the

same sample present on graph constructed by plotting absorbance against concentration. Thus,

$$\varepsilon = [(A_2-A_1)/(C_2-C_1)]/l$$

If the concentration is in %(w/v), this needs to be changed into its molar concentration (g.mole/L) of the solution.

II. E(1%,1 cm) is obtained as the absorbance due to a solution which is %(w/v). Thus,

E(1%,1 cm) = A/c = Absorbance/concentration (%w/v)

Conclusions

The molar absorptivity values bear direct relationship with the structure of the molecule. The values ranged from 12,000-26,000, giving clear indication that the molecule has the absorbing property. The E(1%,1cm) values have been obtained which have different values at different pH of the medium. The values changed to higher figure as the pH of the medium increased.

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