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Study the UV Spectra of Riboflavin (B_2) Aqueous Solution under Different Stress Conditions

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Abstract: ***Riboflavin (B_2) is a water-soluble vitamin that can be found in a variety of pharmaceutical and dietary products. In this the riboflavin standard aqueous solution was subjected to various conditions, including UV irradiation for 5, 10, 15, 20, 30, and 40 minutes, Temperature degrees at 30, 35, 40, 45, and 50°C, and 1 %, 5%, and 10% hydrochloric acid and sodium hydroxide. Its degradation is being studied using UV spectrophotometer. Riboflavin is UV sensitive after 20 minutes, thermostable up to 50°C, and unstable at varying acid and base concentrations. These methods can be used to investigate the stress degradation factors of riboflavin. An optimal pH with the most appropriate buffers would provide better vitamin stabilization in aqueous solutions.***

Keywords: Riboflavin, UV irradiation, temperature, acid, base

1.Introduction

Riboflavin (RF), generally known as vitamin B2, is a water soluble vitamin that can be found in a wide variety of foods. RF was isolated from a yellow enzyme and found as a yellow green fluorescent compound [1]. It can be found bound to proteins in practically all green, leafy, fast-growing vegetables. While RF is found in significant amounts in dairy products, meats, and fruits [2, 3], it is also present in varying amounts in all natural unprocessed foods.

RF which was isolated from whey more than a century ago is known to be the primary component of the cofactors flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which were isolated from brewers' yeast in 1932 and are required for all flavoproteins [1, 4]. As a result, RF is required for a wide range of cellular functions [4, 5].]. In 1934, the first pure riboflavin was produced [6]. Chemical synthesis or microbial fermentation is used to make RF commercially. Microbial fermentation produces 2500 tons, 3000 tons of RF annually [6, 7].

A deficiency of RF has been linked to impaired iron absorption, as well as an etiologic relationship to anemia and a risk of cancer [4]. As part of metabolism, RF plays a crucial role in a variety of internal redox reactions, and a deficiency in this vitamin could lead to problems with intermediate metabolism [4].

One of the most concerning issues in the field of medication development and formulation is investigation of thermal and heat effect in drug stability [8, 9]. Pharmaceutically, information about a drug's stability and degradation is important in determining its therapeutic outcomes, adverse effects, handling, packaging, and labeling practice [10].

RF is a photosensitive chemical that is commonly found in liquid vitamin preparations and parenteral nutrition solutions. The understanding of its photochemical behavior in aqueous solution has important pharmaceutical implications and is required to predict shelf-life [11].

Various RF containing drugs can be thermally treated for sterilizing purposes during production, and as a result, changes in RF properties might occur as a side effect [12]. As a result, these factors must be considered for its stability in food products and medicinal preparations [8]. The pH of the solution has a significant effect on the stability of RF. Under acidic and basic conditions, RF is degraded [8].

In this study, ultraviolet-visible (UV-Vis) spectroscopy is employed to investigate the forced degradation of RF. the spectrophotometric method for determining riboflavin in the ultraviolet and visible regions was found to be fast and precise in absence of interfering chemicals [12]. The aim of this study is to subject RF to various stress conditions such as UV irradiation, heat, acid, and base in order to investigate its degradation and stability under these conditions.

2.Materials and Methods

Materials

All chemicals used were of analytical reagent grade. Distilled water was used to prepare solutions wherever required, hydrochloric acid was obtained from laboratory supplies poole, england, sodium hydroxide was obtained from Searle company, and RF standard (purity 99 %) was supplied by laboratory supplies poole, England.

Standard Stock solution

Stock solution of RF is prepared by dissolving 10 mg of RF in 100 ml of distilled water in 100 ml volumetric flask to get 100 ppm drug solutions.

Reagents

1%, 5%, and 10% solutions of hydrochloric acid were prepared by diluting suitable volume of the commercially available HCl (36.5%) to 100 ml with distilled water in a volumetric flask.1%, 5%, and 10% solutions of sodium hydroxide were prepared by dissolving required amount of the pellets in distilled water. All of the reagents used were of analytical grade.

3.Procedures

Preparation of solutions for UV scanning

Into a series of 10 ml calibration flasks, aliquots of standard RF solution (0.5ml, 1ml, 2 ml, 3 ml, and 4ml) were accurately transferred and the volume was made up to the mark with the distilled water to produce (5, 10, 20, 30, and 40 ppm) respectively. These solutions were scanned in DU 800 UV/Visible Spectrophotometer in the range 200-500 nm using distilled water as a blank.

The effect of exposure to UVirradiation

Aqueous solutions of RF (5, 10, 20, 30, and 40 ppm) were exposed to UV radiation for 5, 10, 15, 20, 25, 30, 35, and 40 minutes in UV lamp chamber. After each time the solutions were scanned in DU 800 UV/Visible Spectrophotometer in the range 200-500 nm using distilled water as a blank.

The effect of Temperature

Aqueous solutions of RF (5, 10, 20, 30, and 40 ppm) were exposed to heat at 25, 30, 35, 40, and 45°C in heater. At these temperatures the solutions were scanned in DU 800 UV/Visible Spectrophotometer in the range 200-500 nm using distilled water as a blank.

The effect of acid solutions:

1 ml of 1% hydrochloric acid solution was transferred into series of 10 ml volumetric flask and the volume was made up to the mark with the aqueous solutions of RF (5, 10, 20, 30, and 40 ppm) Also the solutions with 5% and 10% hydrochloric acid solution are prepared and these solutions scanned in DU 800 UV/Visible Spectrophotometer in the range 200-500 nm using distilled water as a blank.

The effect of basesolutions:

1 ml of 1% sodium hydroxide solution was transferred into series of 10 ml volumetric flask and the volume was made up to the mark with the aqueous solutions of RF (5, 10, 20, 30, and 40 ppm) Also the solutions with 5% and 10% sodium hydroxide solution were prepared and these solutions scanned in DU 800 UV/Visible Spectrophotometer in the range 200-500 nm using distilled water as a blank.

4.Results and Discussion

Absorption spectrum of RF aqueous solution as shown in Figure 1 is characterized by maximum of absorbance in UV range (220, 263, 368 nm) and in visible light range (443 nm).

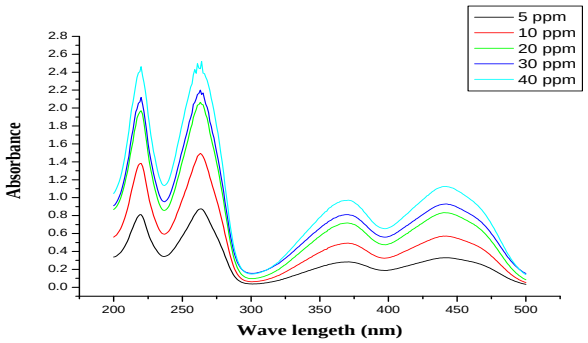

Figure 1: Absorption spectra of RF in aqueous solution

Effect of exposure time t o UV irradiation:

RF is a photosensitive compound [13]; in the dry form, RFis not much affected by light while in the solution formit is rapidly degraded to various photoproducts [8], its photolysis leads to formation offormylmethylflavin (FMF), lumichrome (LC), lumiflavin (LF), carboxymethylflavin (CMF), 2, 3-butanedione, aβ-keto acid and a diketo compound [8, 13].

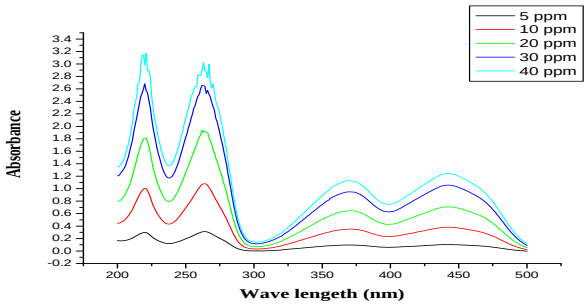

Figure 2: Absorption spectra of RF aqueous solution after 5 min of exposure to UV irradiation

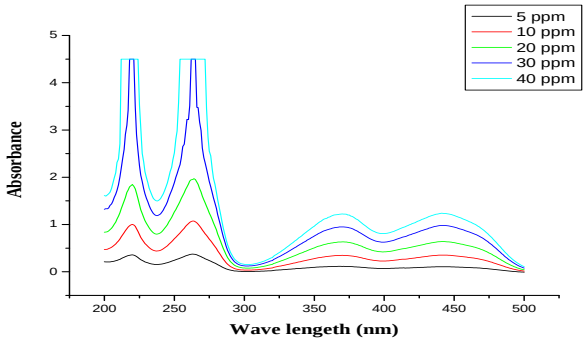

Figure 3: Absorption spectra of RF aqueous solution after 10 min of exposure to UV irradiation

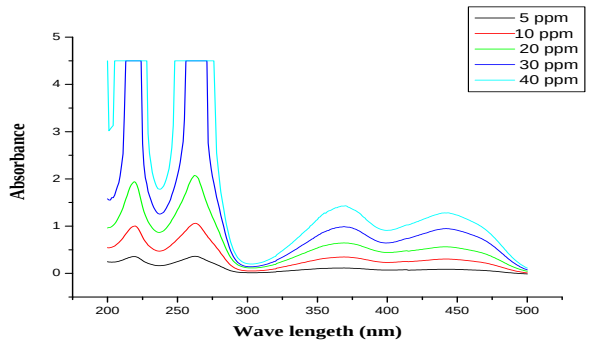

Figure 4: Absorption spectra of RF aqueous solution after 15 min of exposure to UV irradiation

The UV irradiation of RF aqueous solution for 5, 10, and 15 min, represented in Figure 2, 3, and 4, respectively. The shift in the peaks of the absorption spectrum of RF at 220, 263, 269, and 442 nm are not noticeable.

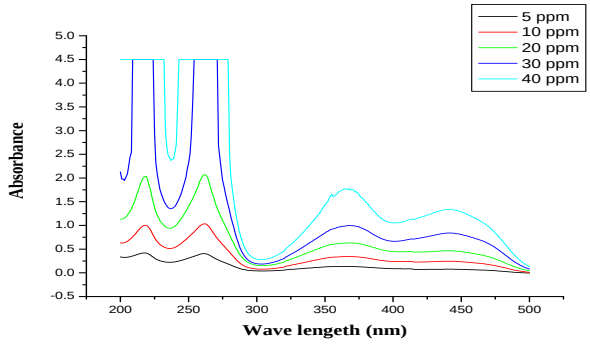

Figure 5: Absorption spectra of RF aqueous solution after 20 min of exposure to UV irradiation

Upon exposure to UV irradiation for 20 min as shown in Figure 5, the peak at 220 was found to shift to 218, the peak at 263 has shifted to 261, the shift at 268 nm is not noticeable but the peak at 443 nm has shifted to 441 nm.

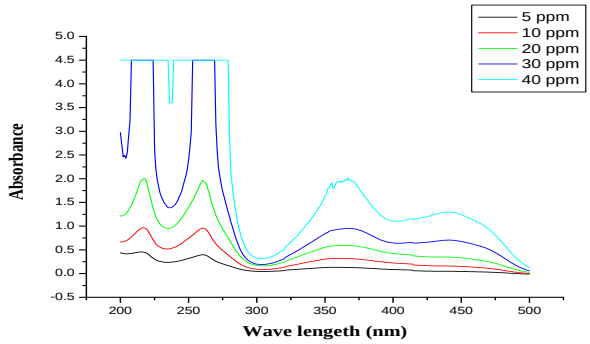

Figure 6: Absorption spectra of RF aqueous solution after 25 min of exposure to UV irradiation

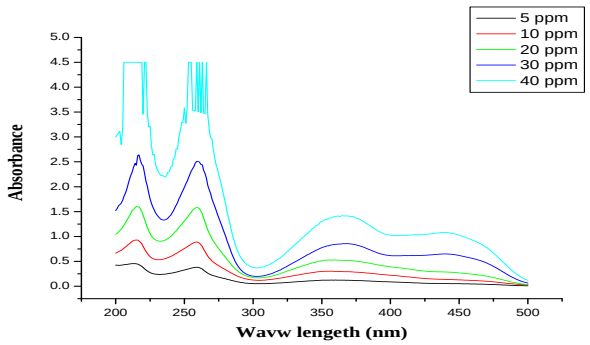

Figure 7: Absorption spectra of RF aqueous solution after 30 min of exposure to UV irradiation

In Figure 6 and Figure 7, the pronounced shift in the peaks of the absorption spectrum of RF is observed, the peak at 220nm was found to shift to 218 nm, the peak at 263 has shifted to 260nm, and the peak at 368 has shifted to 355nm, and the peak at 443 nm shift to 440 nm at high concentration, but there is loss of this peak at low concentrations.

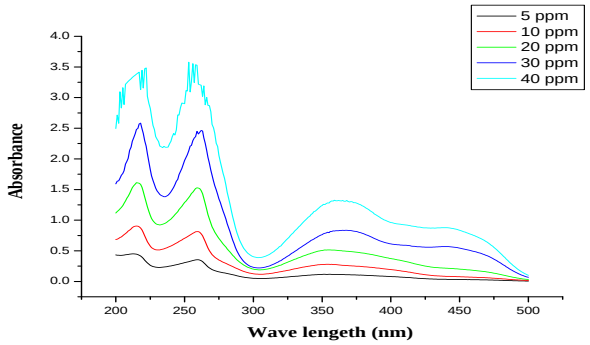

Figure 8: Absorption spectra of RF aqueous solution after 35 min of exposure to UV irradiation

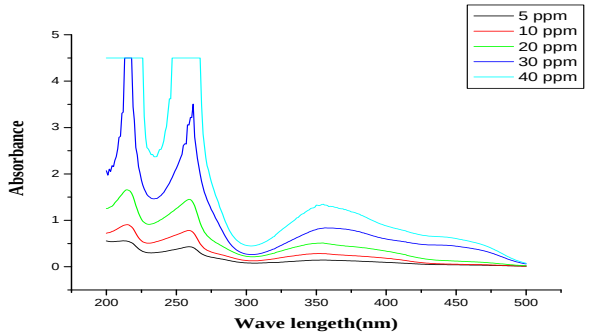

Figure 9: Absorption spectra of RF aqueous solution after 40 min of exposure to UV irradiation

Figure 8 and Figure 9 Shown that the peak at 220nm was found to shift to 215 nm, the peak at 263 has shifted to 259 nm, and the peak at 368 has shifted to 353nm, and there is loss of peak in visible light at 443nm.

Effect of temperature

As seen in Figures below the absorption spectrum of RF, the λ max values of RF in aqueous solution were stable with increasing temperature values. RF is a thermo-stable

substance and little information is available regarding its thermal decomposition in aqueous solution. RF crystalline melts in the range of 278–282 °C with decomposition [14, 15]. It is stable to heat and is not affected by heating processes like hot air convection, infrared, high-pressure steam, or microwave during cooking as well as to milk pasteurization [15, 16]. It is known that RF thermal degradation occurs with high temperature and exposure time [11, 17].

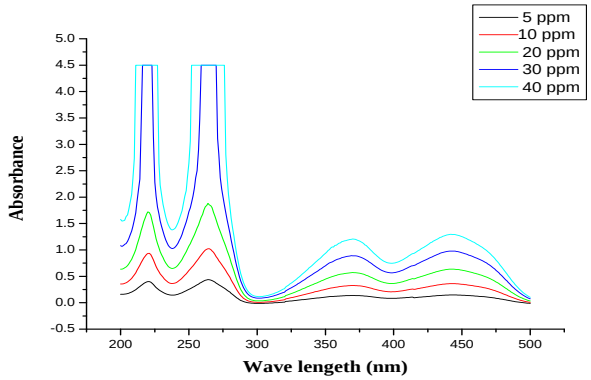

Figure 10: Absorption spectra of RF aqueous solution at 30°C

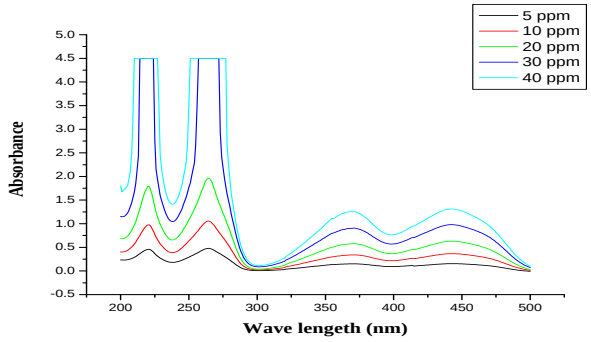

Figure 11: Absorption spectra of RF aqueous solution at 35°C

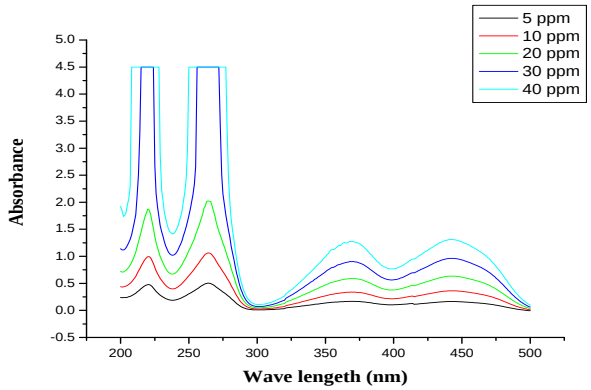

Figure 12: Absorption spectra of RF aqueous solution at 40°C

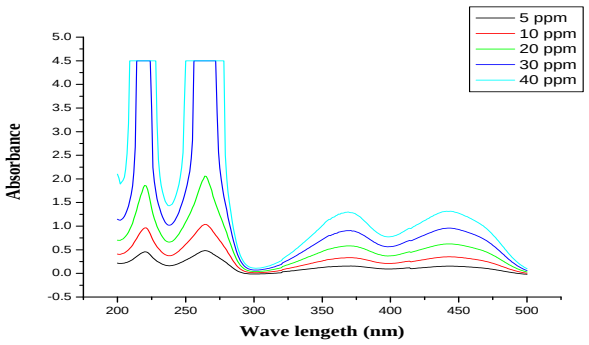

Figure 13: Absorption spectra of RF aqueous solution at 45°C

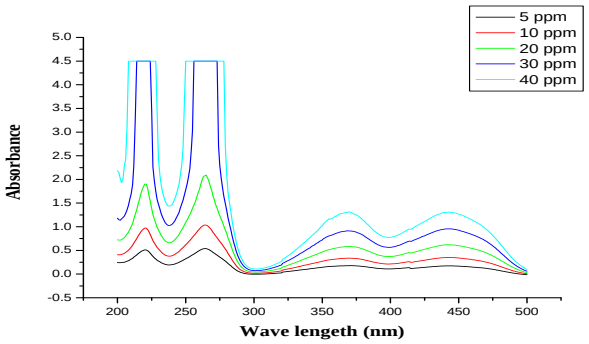

Figure 14: Absorption spectra of RF aqueous solution at 50°C Effect of Base solutions

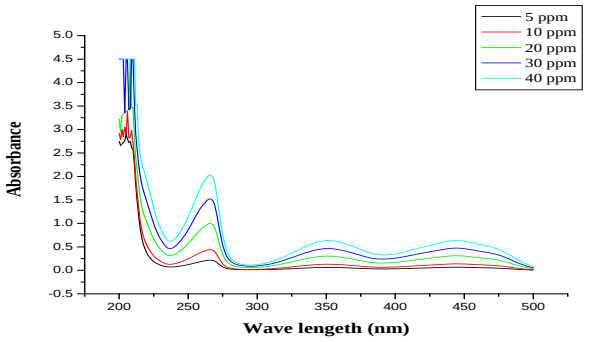

Figure 15: Absorption spectra of RF aqueous solution after addition of 1% NOH

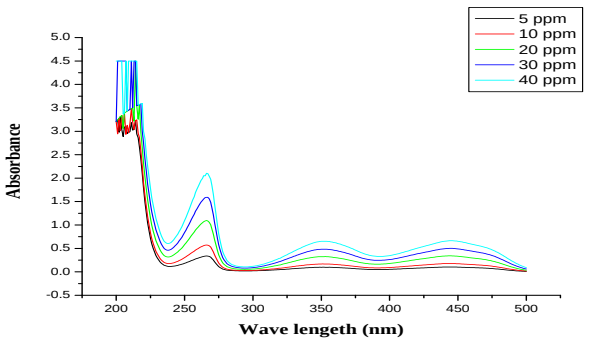

Figure 16: Absorption spectra of RF aqueous solution after addition of 5 % NOH

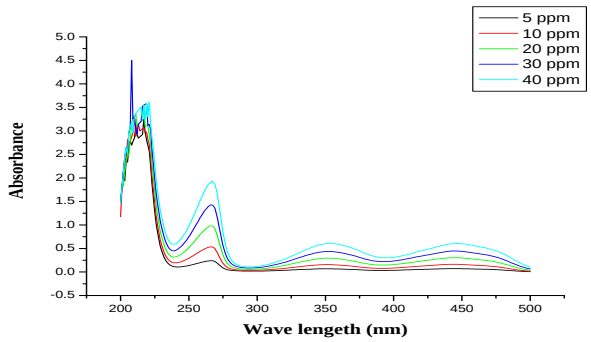

Figure 17: Absorption spectra of RF aqueous solution after addition of 10 % NOH

Figure 15, Figure 16, and Figure 17, respectively, show that the peak at 220nm has shifted to 205nm, 214, and 217. The peak at 368 has shifted to 266 nm, and there is loss of peaks around 368 and 443 nm at low concentration can be showed in these figures, while at high concentration the peak at 368 nm was found to shift to 351 nm and the peak at 443 has shifted to 345nm.

Effect of acid solutions

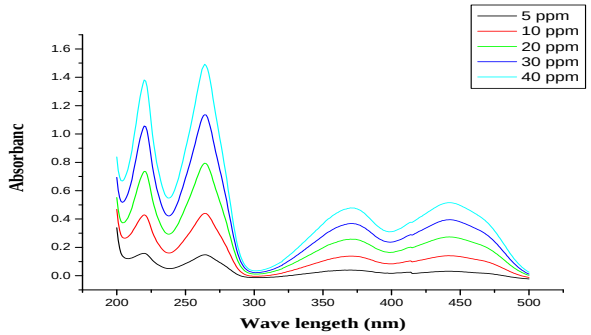

Figure 18: Absorption spectra of RF aqueous solution after addition of 1% HCL

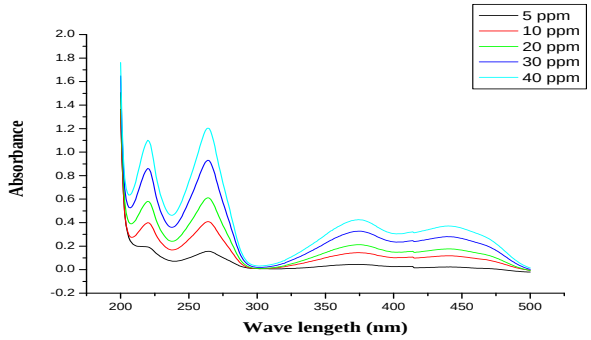

Figure 19: Absorption spectra of RF aqueous solution after addition of 5% HCl

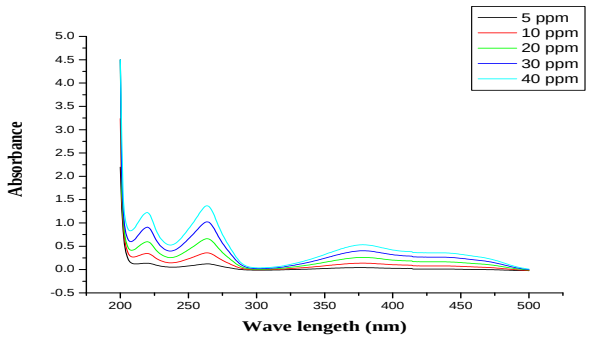

Figure 20: Absorption spectra of RF aqueous solution after addition of 10% HCl

Figure 18, Figure 19, and Figure 20 shown that the shift in the peak at 220nm is not noticeable, the peak at 263 nm has shifted to 264 nm, the peak at 368 nm has shifted to 370, 371, 373 nm respectively, and there is loss of peak in visible light at 443nm at low concentration and at high concentration the peak at 443 nm was found to shift to 442 nm.

5.Conclusion

Vitamin preparations often include RF as a component. It is a highly sensitive vitamin to UV irradiation and degrades in aqueous solution to produce a variety of photoproducts. Thermal degradation of RF occurs at temperatures greater than 50 °C. In the presence of hydrochloric acid and sodium hydroxide, RF is chemically degraded. The factors impacting the stability of riboflavin products must be carefully considered. For RF preparations to be stable, they must be packaged in a suitable material that provides light protection and stored at the correct temperature. An optimal pH with the most appropriate buffers would provide better vitamin stabilization in aqueous solutions.

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