RESEARCH ARTICLE

PHOTODEGRADATION OF RIBOFLAVIN IN THE PRESENCE OF ACETATE BUFFER

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ABSTRACT

In the present work the photodegradation of riboflavin in the presence of acetate buffer has been studied. The assay of riboflavin and its photoproducts in degraded solutions has been carried out simultaneously using a multicomponent spectrometric method. Acetate buffer has been found to catalyze the photodegradation of riboflavin and the apparent first-order rate constants for the reaction at pH 4.2 in the presence of 0.125-0.625 M acetate buffer range from 0.45 (pH 5.6) to 2.05×10^{-2} min^{-1}. The *k*-pH profile for these reactions shows a maximum around pH 4.2 indicating the participation of different species of the molecule in the reaction. The decrease in the rate of the reaction after the maximum is due to a change in the redox bahviour of riboflavin. The results indicate that acetate buffer at a low concentration is suitable for maintaining the pH of vitamin preparations.

Keywords: Riboflavin, photolysis, kinetics, acetate buffer, spectrometry.

1. INTRODUCTION

Liquid pharmaceutical preparations are generally buffered to maintain the pH of the medium. The buffer salts may exert a catalytic effect on the degradation of drugs present in the preparations. The effect of buffer catalysis on the stability of drug substances has been described by several authors[1-3]. Vitamin B complex and multivitamin preparations are extensively used for prevention and cure of vitamin deficiency diseases. One of the important components of these preparations is riboflavin (RF) which is sensitive to light and undergoes photodegradation in liquid media. The majority of drug substances undergo general acid-base catalysis[3]. Several studies of the effect of buffers such as phosphate, borate and citrate on the photodegradation of RF have been conducted[4-8]. The divalent phosphate ions have been shown to alter the photodegradation pathway of RF from photoreduction to photoaddition[4-6]. Several examples of the catalysis of acetate buffer on the degradation of drugs such as carbencillin, cefotaxime, methotrexate, mitomycin C and thiamine hydrochloride have been reported[1]. In the present study the photodegradation of RF in the presence of acetate buffer has been carried out to evaluate the effect of buffer on the kinetics of the system. The work involves the application of a multicomponent spectrometric method for the assay of RF and its photoproducts for the degradation reactions carried out in the pH range 3.8-5.6. The method is specific and can determine the individual components in a mixture without separation. The information on the photodegradation of RF in acetate buffer may be helpful to the pharmacists in the formulation of vitamin preparations in industry and to create an awareness of the effect of buffers on the stability of the vitamins.

2. MATERIALS AND METHODS

Riboflavin (RF) and lumichrome (LC) were obtained from Sigma Chemical Co. Formylmethylflavin (FMF) and carboxymethylflavin (CMF) were prepared by the method of Fall and Petering[9] and Fukumachi and Sakurai[10], respectively. The purity of these compounds was confirmed by thin-layer chromatography. The buffer system used was acetic acid-sodium acetate, pH range 3.8-5.6. The ionic strength of the solutions was kept constant.

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2.1. Photodegradation

A 5×10^{-5} M RF solution was prepared at various pH values in a volumetric flask in the presence of acetate buffer (0.125-0.625 M). The solution was irradiated with a Philips HPLN 125 W high pressure mercury vapor fluorescent lamp (emission bands at 405 and 435 nm) fixed at a distance of 25 cm from the center of the flask in a dark chamber. Samples were removed at appropriate intervals for chromatography and assay.

2.2. Thin-Layer Chromatography

The photodegraded solutions of RF were examined for the photoproducts by thin-layer chromatography (TLC) on 250 µm cellulose plates (Whatman CC 40) and the solvent systems: (a) 1-butanol-acetic acid-water (40:10:50, v/v. organic phase), and (b) 1-butanol-1-propanol-acetic acid-water (50:30:2:18, v/v)[11]. The photoproducts were located by their fluorescence emission under UV light (366 nm), RF, FMF (yellow green) and LC (sky blue).

2.3. Measurements of Absorption Spectra

The measurements of absorbance and UV/visible spectra of the photodegraded solutions of RF were made on Shimadzu UV-1601 recording spectrophotometer with silica cells of 10 mm path length.

2.4. Assay of RF and Photoproducts

The assay of RF, FMF and LC in the photodegraded solutions were carried out by a multicomponent spectrometric method reported by Ahmad and Rapson[12]. The method involves the adjustment of the pH of photodegraded solutions to 2.0 (0.2 HCl-KCl buffer), followed by extraction of LC with chloroform, evaporation of chloroform and dissolution of the residue in 0.2 M acetate buffer (pH 4.5). The concentration of LC is determined at 356 nm. The determination FMF and RF in the aqueous phase is carried out by a two-component assay using the wavelength of 385 and 445 nm. FMF (pK_a 3.5)[13] exhibits an absorption maximum (385 nm) which is quite distinct from the maximum (445nm) of RF. FMF is oxidized to a minor extent to CMF[14,15] which does not interfere in the assay.

3. RESULTS AND DISCUSSION

3.1. Identification of Photoproducts of RF

The photodegraded solutions of RF in the acid pH range in acetate buffer were examined by TLC to identify the degradation products. RF and the degradation products exhibit their characteristic fluorescence on detection. The R_f values were compared with those of the reference standards. Two major products were detected in the solutions as FMF and LC. CMF showed weak spots on detection in the degraded solutions. The intensity of the spots of FMF indicates it as a major intermediate in the photodegradation reactions of RF. This intensity became weaker with time to show its transformation to LC in the solution[11]. On increasing the buffer concentration it was observed that the formation of LC is enhanced as a result of the catalytic effect of acetate buffer on RF photodegradation.

3.2. Assay of RF and Photoproducts

TLC studies of the photolysed solution showed the presence of RF, FMF and LC as the major photoproducts in acidic solutions. Therefore, an analytical method was required to determine RF and these photoproducts in photolysed solutions. The multicomponent spectrophotometric method of Ahmad and Rapson[12] was found suitable for the determination of these compounds. Therefore, it was applied for the simultaneous assay of RF and photoproducts in degraded solutions as mentioned under the methods. For the assay of these compounds the absorbance measurements on the solutions were carried out at 445, 385 (aqueous phase) and at 356 nm (chloroform extract). These wavelengths correspond to the absorption maxima of these compounds and, therefore, are most suitable for analytical purpose. The reproducibility of the method has been found to be within ±5% as originally

reported by the above authors[12]. The method is rapid, convenient and reliable for kinetic studies involving RF and its photoproducts and has previously been applied in several studies on RF photodegradation[4-7,11,12,14,16].

3.3. Effect of Acetate Buffer on the Kinetics of RF Photolysis

The photolysis of RF in aqueous solution is known to follow an apparent first-order kinetics[11]. This reaction involves FMF as an intermediate to which is further degraded to LC[17,18]. The photodegradation of RF in acid solution can be described by a consecutive first-order reaction[16].

$$RF \xrightarrow{k_1} FMF \xrightarrow{k_2} LC \qquad (1)$$

The differential equation for the reactant and he products are:

$$\frac{-d[RF]}{dt} = k_1[RF] \qquad (2)$$

$$\frac{d[FMF]}{dt} = k_1[RF] - k_2[FMF] \qquad (3)$$

$$\frac{-d[LC]}{dt} = k_2[FMF] \qquad (4)$$

The solution of these differential equations[7] gives the concentrations of RF, FMF and LC at various time intervals to calculate the rate constants for these reactions.

The apparent first-order rate constants (k_{obs}) for the photodegradation of RF at various pH values in acetate buffer (0.425-0.625 M) have been determined. These rate constants show that the acetate buffer has catalytic effect on the photodegradation of RF in this pH range. The values of apparent first-order rate constants for a typical photodegradation reaction performed at pH 4.2 are given in Table 1. A graph of k_{obs} versus buffer concentration shows a linear relation indicating the dependence of k_{obs} on buffer concentration (Fig. 1).

Table 1. Apparent first-order rate constants (k_{obs}) for the photodegradation of Riboflavin at pH 4.2.

Buffer Concentration (M)	$k_{obs} \times 10^2$ min^{-1} ± SD
0.125	0.45 ± 0.04
0.250	0.82 ± 0.03
0.375	1.20 ± 0.05
0.500	1.62 ± 0.06
0.625	2.05 ± 0.05

The effect of acetate species on the k_{obs} can be expressed as[7]:

$$k_{obs} = k_0 + k_1[H^+] + k_2[OH^-] + k_3[CH_3COO^-] \qquad (5)$$

where k_0 is the rate constants for the photoproducts of RF in the absence of the buffer.,

The value of k_3 (eq. 5) represents the rate constant for the acetate catalyzed photolysis of RF in the presence of acetate buffer.

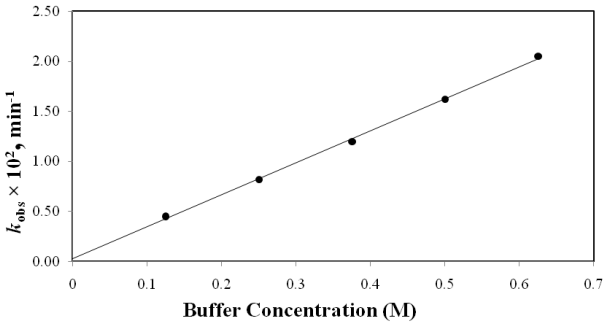

Fig. 1. A plot of k_{obs} versus buffer concentration.

3.4. Influence of pH on the kinetics of RF Photodegradation

This study has shown that the acetate ions exert a catalytic effect on the photodegradation of RF in the pH range 3.8-5.6, The reaction shows an increase in the rate constant up to pH 4.2 and then a decrease up to pH up to 5.6. This is due to deprotonation of RF (pK_a 1.7)[11] with an increase in pH. The decrease

in the rate above pH 4.2 could be due to a difference in redox characteristics of RF (E^0 pH 5.0 = 0.17 V)[3].

4. CONCLUSION

Riboflavin is sensitive to light and undergoes photodegradation to form a number of products in acid solutions including formylmethylflavin, lumichrome and carboxymethylflavin. The kinetics of photodegradation reactions in the presence of acetate buffer has been studied. The results indicate that the buffer species catalyze the photodegradation of RF and the reaction is enhanced with an increase in buffer concentration. The rate of photolysis reaction is pH dependent and is highest around pH 4.2. The optimum stability of RF is exhibited in the pH range of 5-6 and acetate buffer is suitable for maintaining the pH of vitamin preparations.

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