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Research Article

Effect of Acetate and Carbonate Buffers on the Photolysis of Riboflavin in Aqueous Solution: A Kinetic Study

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Abstract. The photolysis of riboflavin (RF) in the presence of acetate buffer (pH 3.8–5.6) and carbonate buffer (pH 9.2–10.8) has been studied using a multicomponent spectrophotometric method for the simultaneous assay of RF and its photoproducts. Acetate and carbonate buffers have been found to catalyze the photolysis reaction of RF. The apparent first-order rate constants for the acetate-catalyzed reaction range from 0.20 to 2.86×10^{-4} s^{-1} and for the carbonate-catalyzed reaction from 3.33 to 15.89×10^{-4} s^{-1}. The second-order rate constants for the interaction of RF with the acetate and the carbonate ions range from 2.04 to 4.33×10^{-4} M^{-1} s^{-1} and from 3.71 to 11.80×10^{-4} M^{-1} s^{-1}, respectively. The *k*-pH profile for the acetate-catalyzed reaction is bell shaped and for the carbonate-catalyzed reaction a steep curve. Both HCO_3^- and CO_3^{2-} ions are involved in the catalysis of the photolysis reaction in alkaline solution. The rate constants for the HCO_3^- and CO_3^{2-} ions catalyzed reactions are 0.72 and 1.38×10^{-3} M^{-1} s^{-1}, respectively, indicating a major role of CO_3^{2-} ions in the catalysis reaction. The loss of RF fluorescence in acetate buffer suggests an interaction between RF and acetate ions to promote the photolysis reaction. The optimum stability of RF solutions is observed in the pH range 5–6, which is suitable for pharmaceutical preparations.

KEY WORDS: acetate effect; carbonate effect; photolysis; riboflavin; spectrophotometric assay.

INTRODUCTION

Buffer salts are commonly used in liquid pharmaceutical preparations to maintain the pH of the solutions. These salts may catalyze the degradation of drugs present in the medium. The theoretical concept of buffer catalysis and its effect on the stability of various drugs have been discussed in detail (1–7). Riboflavin (RF) is sensitive to light (8–10), and its photolysis in aqueous solution is influenced by general acid–base catalysis. The acetate buffer has been shown to exhibit a higher catalytic activity than the formate buffer (11). The rate of photolysis of RF is proportional to the concentration of the phosphate buffer (12). Extensive studies of the effect of phosphate (12–18), borate (19), and citrate species (20) on the kinetics of RF photolysis have been conducted. Phosphate ions promote the degradation of RF, whereas borate and citrate ions inhibit the reaction. Divalent phosphate ions also alter the normal photolysis pathway of RF in favor of the photoaddition pathway (13–18). So far, no work has been carried out to study the catalytic effect of acetate and carbonate buffers on the photodegradation of RF.

Acetate buffer catalyzes the degradation of several drugs including benzyl penicillin (21), carbenicillin (22), cefotaxime (23), epinephrine (24), methotrexate (25), mitomycin C (26), prostaglandin E_2 (27), and thiamine hydrochloride (28). Carbonate buffer is also known to catalyze the degradation of methotrexate (25), cefotaxime (23), cisplatin (29), cyclophosphamide (30), and doxorubicin (31). In view of the importance of the buffers in pharmaceutical systems and their catalytic effects on the degradation of active ingredients, the present study has been undertaken to evaluate the effects of acetate and carbonate ions in the pH range of their buffer activity on the photolysis of RF in aqueous solution. It has been conducted using a specific spectrophotometric method for the simultaneous determination of RF and its photoproducts, formylmethylflavin, lumichrome, and lumiflavin. The rate constants for the acetate and carbonate catalyzed reactions have been determined and correlated with the pH and buffer concentration. The individual effects of bicarbonate and carbonate species have also been determined. The study would facilitate the formulator of liquid vitamin preparations to make a judicious decision in the choice of buffer for these preparations. The pH range of acetate buffer is most suitable for a majority of vitamin preparations, and this buffer at 0.1–0.2 M concentration exhibits minimum catalytic activity on the degradation of RF.

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MATERIALS AND METHODS

RF, lumichrome (LC), and lumiflavin (LF) were obtained from Sigma Chemical Co. ST. Louis, MD, USA. Formylmethylflavin (FMF) and carboxymethylflavin (CMF) were prepared by the methods of Fall and Petering (32) and Fukumachi and Sakurai (33), respectively. These compounds were checked for purity using thin-layer chromatography. All reagents and solvents were of the purest form available from Merck & Co., Whitehouse Station, NJ. The following buffer systems were used: acetic acid-sodium acetate, pH 3.8–5.6 and sodium carbonate-sodium bicarbonate, pH 9.2–10.8. The ionic strength of the solutions was kept constant (0.6 M).

pH Measurements

The pH measurements of solutions were performed on an Elmetron LCD display pH meter (Model-CP501, sensitivity ±0.01 pH units, Poland) using a combination pH electrode. The electrode was automatically calibrated using phthalate (pH 4.008), phosphate (pH 6.865), and disodium tetraborate (pH 9.180) buffer solutions.

Photolysis

A 5×10^{-5} M solution of RF (100 ml) was prepared at the appropriate pH in a volumetric flask (Pyrex) using acetate (0.1–0.6 M) or carbonate (0.1–0.6 M) buffer and immersed in a water bath maintained at 25±1°C. The solution was exposed in a dark chamber to Philips HPLN 125 W high pressure mercury vapor fluorescent lamp (emission bands at 405 and 435 nm; the 435 nm bands overlap the 445 nm band of RF) (14), placed at a distance of 30 cm from the center of the flask. The exposure time varied from 1 to 3 h, depending upon the pH of the solution. Samples of the photolysed solutions were withdrawn at various intervals to carry out thin-layer chromatography and spectrophotometric assay.

Thin-Layer Chromatography

The photolysed solutions of RF were subjected to thin-layer chromatography (TLC) using 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*) (34). The compounds were detected by their characteristic fluorescence on exposure to UV (365 nm) light: RF, LF, FMF, CMF (yellow green), and LC (sky blue).

Spectral Measurements

The spectral and absorbance measurements on pure and photolysed solutions of RF were performed on a Shimadzu UV–1601 recording spectrophotometer using quartz cell of 10-mm path length.

Determination of Light Intensity

The intensity of the Philips HPLN 125 W lamp was determined using potassium ferrioxalate actinometry (35) as $1.21\pm0.10\times10^{17}$ quanta s^{-1}.

Spectrophotometric Assay

RF and its major photoproducts, FMF and LC in the presence of acetate buffer (pH 3.8–5.6) and FMF, LC and LF in the presence of carbonate buffer (pH 9.2–10.8), in photolysed solutions were assayed using a multicomponent spectrophotometric method developed by Ahmad *et al.* (36). It is based on the adjustment of the pH of photolysed solutions to 2.0 (0.2 HCl–KCl buffer), followed by extraction with 3×10 ml chloroform to remove LC and LF. The chloroform is evaporated and the residue is dissolved in 0.2 M acetate buffer (pH 4.5). The concentration of LC and LF is determined by a two-component assay at 356 and 445 nm. FMF and RF in the aqueous phase are determined by a two-component assay at 385 and 445 nm. FMF (p*K*a 3.5) (37) is protonated at pH 2.0, giving a distinct absorption maximum (385 nm) from that of RF (445 nm) and thus making the two-component assay feasible. CMF is a minor photoproduct obtained when FMF is slightly oxidized in the presence of air (38,39). The amount of CMF formed in acidic and alkaline solutions has been estimated to be ~0.2–0.4% of the degraded RF using TLC. This amount would not interfere with the assay method employed for RF and the photoproducts in this study. The assay method has been validated with respect to accuracy, precision, and linearity under the present experimental conditions.

Fluorescence Measurements

The fluorescence measurements on RF solutions were carried out at room temperature (~25°C) with a Spectromax 5 fluorimeter (Molecular Devices, Sunnyvale, CA, USA) in the end point mode, using 374 nm as the excitation wavelength and 525 nm as the fluorescence emission wavelength (40). The fluorescence was measured in relative fluorescence units using a pure 0.05 mM RF solution as standard.

RESULTS AND DISCUSSION

Photoproducts of RF

In order to accurately determine RF and its photoproducts formed in degraded solutions in the presence of acetate or carbonate buffer, it was necessary to carry out TLC studies of the photolysed solutions during the reactions. At around 50% photolysis of RF in the presence of acetate buffer (pH 3.8–5.6) and carbonate buffer (pH 9.2–10.8), the compounds identified by their characteristic fluorescence on comparison with the standards included undegraded RF, FMF, LC, LF (all major), and CMF (minor) with R_f values of 0.47, 0.70, 0.66, 0.53 and 0.38, and 0.28 in solvent system (a) and 0.27, 0.70, 0.63, 0.41, and 0.20 in solvent system (b), respectively. The intensity of the spots of FMF, a major intermediate product in the degradation sequence of RF, decreased with the progress of the reactions indicating its conversion to LC in the acid medium and to LC and LF in the alkaline medium (10,36). An increase in the buffer concentration leads to an increase in the intensity of the spots of LC and LF, suggesting a greater yield as a result of the buffer effect. The formation of LC in the thermal (41) and bacterial degradation of RF (42) has also been reported.

Assay of RF and Photoproducts

In view of the TLC results indicating the presence of undegraded RF and its photoproducts, FMF and LC in acid media and FMF, LC, and LF in alkaline media as major component of the degraded solutions, the assay of RF, FMF, LC, and LF has been carried out according to the multicomponent spectophotometric method of Ahmad and Rapson (36), described under the methods section. The assay method was validated under the present experimental conditions, and the various validation data for RF, FMF, LC, and LF are given in Table I. The method is specific since the absorbance measurements on the mixtures of degraded solutions are carried out at the wavelengths corresponding to the absorption maxima of the four compounds reported previously (36). The amount of CMF formed in the photolysis reactions is too small (~0.2–0.4%) for any accurate determination, and, therefore, its presence has not been considered in the assay method. Moreover, the accuracy of the method is within ±5%, which takes into consideration any minor impurities present in the solution. This assay method has extensively been employed to study the kinetics of RF photolysis under various conditions (10,14–20,36,43) and is appropriate for this present work.

Kinetics of Photolysis

Effect of Acetate Buffer

It has been reported that the photolysis of RF in aqueous solution follows an apparent first-order kinetics (10). This reaction occurs through the formation of FMF as an intermediate to give LC as the final product (10,36,39,44,45). The photolysis of RF in acid solution can be represented by a reaction scheme involving a consecutive first-order reaction as follows:

$$\mathrm{RF} \xrightarrow{k_1} \mathrm{FMF} \xrightarrow{k_2} \mathrm{LC} \tag{1}$$

where k_1 and k_2 are the rate constants for the formation of FMF and LC, respectively.

The differential equations for the reactant and the products are

$$\frac{-d[\mathrm{RF}]}{dt} = k_1[\mathrm{RF}] \tag{2}$$

$$\frac{d[\mathrm{FMF}]}{dt} = k_1[\mathrm{RF}] - k_2[\mathrm{FMF}] \tag{3}$$

$$\frac{-\mathrm{d}[\mathrm{LC}]}{\mathrm{dt}} = k_2[\mathrm{FMF}] \tag{4}$$

The differential equations can be solved (3,7,19) to obtain the molar concentrations of RF, FMF, and LC at time, t

$$[\mathrm{RF}] = [\mathrm{RF}]_0 e^{-k_1 t} \tag{5}$$

$$[\mathrm{FMF}] = \frac{[\mathrm{RF}]_0 k_1}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right) \tag{6}$$

and

$$[\mathrm{LC}] = [\mathrm{RF}]_0\left(1 + \frac{1}{k_1 - k_2}\left(k_2 e^{-k_1 t} - k_1 e^{-k_2 t}\right)\right) \tag{7}$$

The rate constants k_1 and k_2 have been determined by solving the above equations (3,7,46). The value of the apparent first-order rate constant (k_{obs}) (correlation coefficients, 0.996–0.999) for the photolysis of RF at pH 3.8–5.6 and the rate constants for the formation of FMF (k_1) and LC (k_2) in the presence of acetate buffer (0.1–0.6 M) are reported in Table I. The values of the rate constants indicate that the acetate buffer is exerting a catalytic effect on

Table I. Validation Data for Multicomponent Spectrophotometric Assay of RF, FMF, LC, and LF

Compound	RF	FMF	LC	LF
λ_{max} nm (pH 2.0)	445	385	356	445
Molar absorptivity (ϵ) M^{-1} cm^{-1}	12,530	16,380	10,800	10,400
Linearity				
Concentration range ($M\times10^5$)	0.5–5.0	0.5–5.0	0.5–5.0	0.5–5.0
Slope$\times10^{-4}$	1.253±0.003	1.638±0.005	1.080±0.004	1.040±0.004
Intercept	0.0032	0.0040	0.0042	0.0036
SE of slope	0.968	1.202	1.415	1.025
Recovery range (%)	98.6–101.1	98.4–101.0	98.2–100.6	98.0–101.8
Accuracy (%)±SD	100.2 ±1.21	100.8 ±1.42	99.8±1.58	99.7±1.50
RSD (%)	1.21	1.41	1.51	1.59
LOD ($M\times10^6$)	1.197	1.025	1.257	1.264
LOQ ($M\times10^6$)	4.189	3.587	4.399	4.425

Values are mean of five determinations
RSD relative standard deviation, *LOD* limit of detection, *LOQ* limit of quantification, *SE* standard error

the photolysis of RF in the pH range studied. The effect of acetate species on the k_1 can be expressed as

$$k_1 = k_1'[H^+] + k_2'[OH^-] + k_3[CH_3COO^-] \quad (8)$$

where k_1', k_2', and k_3 are the rate constants for the H^+ and OH^- and CH_3COO^- catalyzed reactions and k_0 is the rate constant for the photolysis of RF in the absence of the buffer. Under constant pH conditions, the terms k_1' $[H^+]$ and k_2' $[OH^-]$ are constant and Eq. (8) may be expressed as

$$k_1 = K + k_3[CH_3COO^-] \quad (9)$$

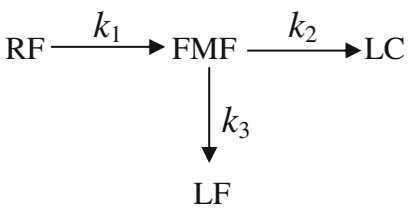
where

$$K = k_1'[H^+] + k_2'(OH^-)$$

In Eq. (9), k_3 represents the second-order rate constant for the acetate catalyzed photolysis of RF in the acid solution. In order to determine the effect of acetate ions on the photolysis of RF, plots of k_1 *versus* acetate concentrations at various pH values have been constructed. The values of the second-order rate constants, k' (correlation coefficients, 0.997–0.999), determined from the slopes of the linear curves, along with the values of k_0 determined by extrapolation of the second-order plots to the vertical axis, are reported in Table II. The values of k_0 at pH 3.8–5.6 are about 10–20 times slower that those of the highest buffer concentration (0.6 M), showing considerable effect of the buffer species on the rate of RF photolysis.

Effect of Carbonate Buffer

The photolysis of RF in the presence of carbonate buffer (9.2–10.8) is much faster than that of the acetate buffer. The photolysis of RF in alkaline solution (10,19,34,36,44,45) leads to the formation of LC and LF through FMF as an intermediate product and may be represented by the following reaction scheme:



(10)

where k_1, k_2'' and k_3' are the rate constants for the formation of FMF, LC, and LF, respectively.

This would require an additional differential equation in the reaction scheme involving Eqs. (2) to (4)

$$\frac{-d[LF]}{dt} = k_3'[FMF] \quad (11)$$

In order to determine the value of k_2'' and k_3' (k_2 in Eq. (1)) for the formation of LC and LF, respectively, the ratios of the concentrations of the two products have been considered, which depend upon the ratios of the corresponding rate constants.

$$\frac{[LC]}{LF} = \frac{k_2''}{k_3'} \quad (12)$$

The rate constant k_2'' and k_3' have been determined using the Eq. (12) as in the case of the photolysis reactions of RF in the presence of phosphate buffer (18). The same method for the determination of these rate constants has been described by some authors (6,7). The values of the apparent first-order rate constants (k_{obs}) (correlation coefficients 0.996–0.999) for the photolysis of RF at pH 9.2–10.8 in the presence of carbonate buffer and the rate constants for the formation of FMF (k_1), LC (k_2''), and k_3' (LF) are reported in Table II. Similar to the effect of acetate ions, the carbonate ions also exert a catalytic effect on the photolysis of RF at pH 9.2–10.8. The effect of carbonate ions and the bicarbonate ions (depending upon the pH of the solution) on the values of k_1 can be written as

$$k_1 = k_1'[H^+] + k_2'[OH^-] + k_3''[HCO_3^-] + k_4'[CO_3^{2-}] \quad (13)$$

where k_3'' and k_4' are the rate constants for the HCO_3^- and CO_3^{2-} catalyzed reactions, respectively, k_0 is the rate constant in the absence of the buffer species. At a constant pH, the k_1 could be expressed as

$$k_1 = k_0 + k_3''[HCO_3^-] + k_4'[CO_3^{2-}] \quad (14)$$

where

$$k_0 = k_1'[H^+] + k_2'[OH^-]$$

In Eq. (14), the values of k_3'' and k_4' indicate the second-order rate constants for the catalytic effect of HCO_3^- and CO_3^{2-} ions on the photolysis of RF in alkaline solution. The second-order rate constants (correlation coefficients 0.997–0.999), derived from the slopes of the straight lines of the plots of k_1 *versus* carbonate concentrations at various pH values, along with the values of k_0 are reported in Table III. The values of k_0 at pH 9.2–10.8 are about 1.3–1.4 times slower than those of the 0.6 M buffer concentration, indicating a little effect of the buffer on the photolysis of RF. This catalytic effect is much smaller than that observed in the presence of acetate buffer.

The values of the rate constants reported for these reactions have been determined under constant irradiation conditions (i.e., light intensity and wavelengths) and temperature to

Table II. Apparent First-Order Rate Constants (k_{obs}) for the Photolysis of Riboflavin (k_{obs}) in the Presence of Acetate Buffer (pH 3.8–5.6), for the Formation of Formylmethyflavin (k_1) and Lumichrome (k_2) and Second-Order Rate Constants for the Interaction of Riboflavin and Acetate Buffer (k')

pH	Concentration (M)	$k_{obs}\times10^4$ s^{-1}±SD	$k_0\times10^4$ s^{-1a}±SD	$k_1\times10^{-4}$ s^{-1}±SD	$k_2\times10^4$ s^{-1}±SD	$k'\times10^4$ (M^{-1} s^{-1})±SD
3.8	0.1	0.60±0.03	0.24±0.01	0.44±0.02	0.16±0.01	4.10±0.20
	0.2	1.01±0.05		0.71±0.03	0.17±0.02	
	0.3	1.42±0.08		0.95±0.05	0.23±0.02	
	0.4	1.85±0.07		1.22±0.06	0.30±0.04	
	0.6	2.40±0.16		1.60±0.08	0.40±0.02	
4.1	0.1	0.65±0.03	0.25±0.02	0.50±0.03	0.22±0.01	4.33±0.22
	0.2	1.12±0.06		0.76±0.04	0.37±0.02	
	0.3	1.51±0.08		1.05±0.06	0.50±0.03	
	0.4	1.93±0.08		1.33±0.07	0.63±0.04	
	0.6	2.85±0.10		1.98±0.20	0.88±0.06	
4.3	0.1	0.66±0.03	0.24±0.01	0.51±0.02	0.15±0.01	4.31±0.26
	0.2	1.14±0.06		0.80±0.04	0.35±0.02	
	0.3	1.70±0.09		1.10±0.05	0.47±0.03	
	0.4	2.23±0.11		1.40±0.06	0.63±0.03	
	0.6	2.88±0.15		2.24±0.20	0.98±0.05	
4.6	0.1	0.43±0.02	0.22±0.02	0.48±0.02	0.15±0.01	3.27±0.18
	0.2	0.63±0.03		0.76±0.04	0.26±0.02	
	0.3	1.02±0.05		1.00±0.05	0.37±0.02	
	0.4	1.10±0.06		1.20±0.07	0.48±0.03	
	0.6	2.20±0.11		1.87±0.12	0.71±0.03	
4.9	0.1	0.39±0.02	0.12±0.01	0.37±0.02	0.04±0.03	3.10±0.15
	0.2	0.44±0.03		0.56±0.03	0.14±0.05	
	0.3	0.97±0.05		0.73±0.04	0.32±0.02	
	0.4	0.83±0.05		0.95±0.05	0.35±0.02	
	0.6	1.62±0.10		1.26±0.08	0.59±0.03	
5.1	0.1	0.24±0.02	0.10±0.01	0.27±0.01	0.17±0.01	2.58±0.13
	0.2	0.44±0.03		0.41±0.02	0.22±0.02	
	0.3	0.83±0.05		0.54±0.03	0.37±0.02	
	0.4	0.83±0.05		0.66±0.03	0.41±0.03	
	0.6	1.20±0.07		0.89±0.04	0.48±0.03	
5.3	0.1	0.20±0.02	0.09±0.04	0.14±0.06	0.11±0.05	2.04±0.10
	0.2	0.38±0.02		0.16±0.07	0.14±0.06	
	0.3	0.67±0.03		0.39±0.02	0.27±0.02	
	0.4	0.78±0.04		0.49±0.04	0.45±0.03	
	0.6	0.95±0.06		0.67±0.06	0.79±0.04	
5.6	0.1	0.18±0.01	0.08±0.02	0.06±0.03	0.03±0.02	1.75±0.08
	0.2	0.34±0.02		0.16±0.07	0.14±0.01	
	0.3	0.62±0.03		0.34±0.02	0.23±0.02	
	0.4	0.76±0.04		0.42±0.03	0.33±0.02	
	0.6	0.60±0.04		0.64±0.04	0.53±0.03	

[a] k_0 is the first-order rate constant for the photolysis of riboflavin in the absence of acetate buffer

avoid any variations in the data. The values of the rate constants are relative and could be used for comparative evaluation.

Effect of pH

The effect of pH on the rates of photodegradation of RF has been shown by plotting k_1 values (for the formation of FMF) in the absence of acetate and carbonate buffers as a function of pH and discussed accordingly.

Acid Range (pH 3.8–5.6)

The *k*-pH profile (Fig. 1) for the reactions carried out in the pH range 3.8–5.6 is somewhat similar to those observed for the photolysis of RF (10,44) and FMF (39) in this range. The rate of photolysis is gradually decreasing and is about 2–3 times lower at pH 5.6 than that of pH 3.8. RF (pK_{a1}, 1.7) (47) exists 99% in the neutral form at pH 3.8–5.6 and, therefore, the decrease in the rate is not due to any change in the ionized state of the molecule. The photolysis of RF in aqueous solution basically involves intramolecular photoreduction of the isoalloxazine ring followed by cleavage of the ribityl side chain to produce FMF and subsequent photoproducts (LC, LF) (17,18,44,48,49). The decrease in the rate of RF photolysis could be ascribed to the redox behavior of RF in the pH range 3.8–5.6. The dependency of the redox potentials of RF on pH (7,50,51) would determine the rate of the reaction in a particular pH range. The redox potentials of RF are lowest in the pH range 5–6 (E° pH 5.0=–0.117 V) (7,52), and hence its

Table III. Apparent First-Order Rate Constants (k_{obs}) for the Photolysis of Riboflavin (k_{obs}) in the Presence of Carbonate Buffer (pH 9.2–10.8), for the Formation of Formylmethylflavin (k_1), Lumichrome (k_2''), and Lumiflavin (k_3') and Second-Order Rate Constants for the Interaction of Riboflavin and Carbonate Buffer (k'')

pH	Concentration (M)	$k_{obs}\times10^4$ s^{-1} ±SD	$k_0\times10^4$ s^{-1a} ±SD	$k_1\times10^4$ s^{-1} ±SD	$k_2''\times10^4$ s^{-1} ±SD	$k_3''\times10^4$ s^{-1} ±SD	$k''\times10^4$ (M^{-1} s^{-1}) ±SD
9.2	0.1	2.90±0.14	2.60±0.13	1.70±0.08	0.73±0.04	0.42±0.02	3.71±0.18
	0.2	3.33±0.16		2.22±0.11	0.91±0.05	0.51±0.02	
	0.3	3.71±0.18		2.90±0.14	1.01±0.05	0.66±0.03	
	0.4	4.02±0.20		3.42±0.18	1.22±0.06	0.92±0.05	
	0.6	4.71±0.24		4.64±0.25	1.51±0.07	1.31±0.07	
9.4	0.1	4.44±0.22	3.70±0.18	2.90±0.14	1.30±0.08	0.22±0.01	7.40±0.39
	0.2	5.10±0.24		3.42±0.17	1.42±0.07	0.43±0.02	
	0.3	5.92±0.27		3.94±0.11	1.51±0.06	0.51±0.03	
	0.4	6.77±0.26		4.44±0.26	1.63±0.09	0.62±0.03	
	0.6	8.11±0.46		6.32±0.28	1.71±0.09	0.84±0.05	
9.8	0.1	7.60±0.43	6.80±0.36	4.62±0.22	1.60±0.08	0.39±0.02	8.51±0.41
	0.2	8.33±0.53		5.11±0.27	1.94±0.09	0.53±0.02	
	0.3	9.01±0.56		5.73±0.30	2.32±0.12	0.82±0.05	
	0.4	9.72±0.52		6.31±0.31	2.61±0.13	1.03±0.05	
	0.6	11.12±0.66		9.44±0.37	3.22±0.17	1.44±0.08	
10.2	0.1	8.92±0.42	8.20±0.40	7.83±0.42	0.33±0.02	0.21±0.01	9.65±0.46
	0.2	9.90±0.55		8.88±0.41	0.64±0.03	0.43±0.02	
	0.3	10.75±0.56		9.92±0.52	1.02±0.05	0.66±0.04	
	0.4	11.58±0.67		10.91±0.45	1.33±0.06	0.85±0.04	
	0.6	13.21±0.66		12.93±0.62	2.14±0.10	1.32±0.06	
10.6	0.1	9.84±0.06	8.70±0.46	8.02±0.33	1.33±0.06	0.36±0.02	11.21±0.64
	0.2	10.80±0.54		9.32±0.34	1.84±0.09	0.53±0.03	
	0.3	12.02±0.65		10.74±0.46	2.50±0.13	0.88±0.04	
	0.4	13.01±0.63		12.12±0.58	3.01±0.15	1.05±0.06	
	0.6	15.24±0.86		14.44±0.79	4.32±0.24	1.52±0.08	
10.8	0.1	10.02±0.59	8.80±0.42	8.32±0.31	0.66±0.03	0.52±0.02	11.80±0.51
	0.2	11.14±0.51		9.71±0.45	1.22±0.07	0.64±0.03	
	0.3	12.32±0.63		10.89±0.41	2.01±0.11	0.86±0.04	
	0.4	13.54±0.66		12.31±0.62	2.63±0.15	1.02±0.06	
	0.6	15.89±0.87		15.13±0.81	3.91±0.19	1.38±0.07	

[a] k_0 is the first-order rate constant for the photolysis of riboflavin in the absence of carbonate buffer

rate of photolysis is expected to be the lowest in this range. The *k*-pH profile (Fig. 1) for the photolysis of RF is in agreement with the decrease in the redox potentials of RF, and it exhibits maximum stability to photolysis in this range. The increase in the rate at pH 7.0 (E°=–0.207 V) (51,52) and above is in accordance with the increase in the redox potentials of RF (10).

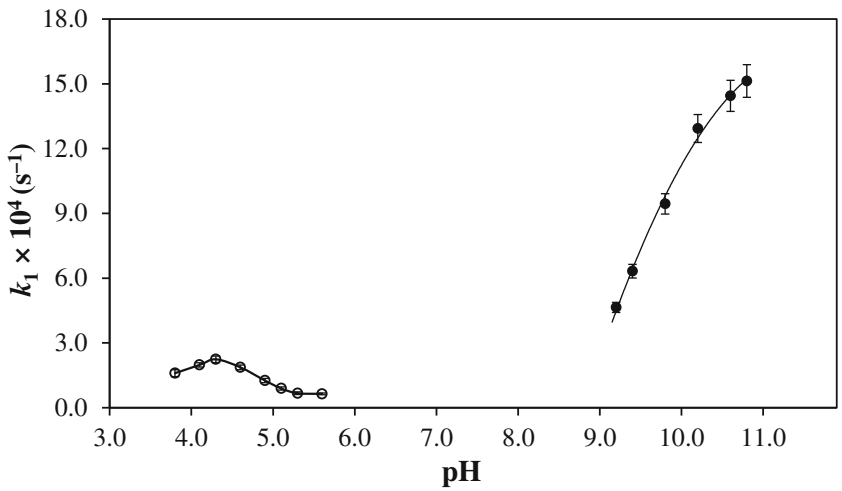


Fig. 1. *k*-pH profiles for the photolysis of riboflavin at pH 3.8–5.6 and pH 9.2–10.8

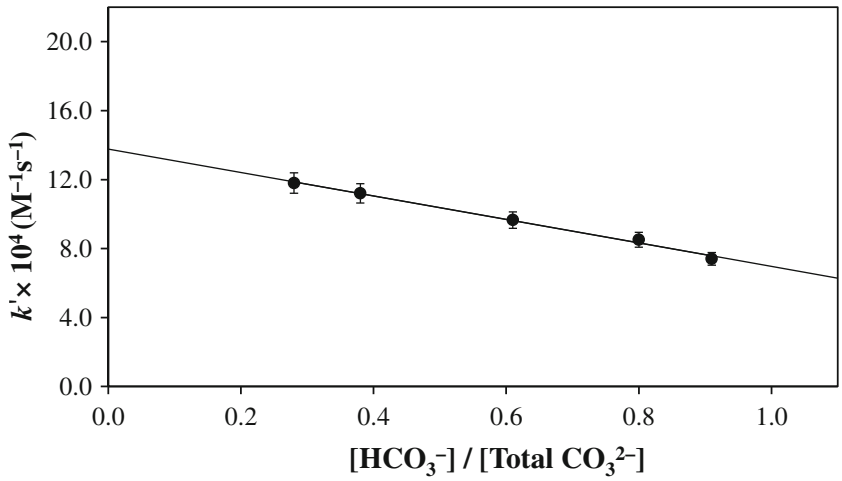


Fig. 2. Plot of k' for the photolysis of riboflavin *versus* $[HCO_3^-]/[\text{Total } CO_3^{2-}]$

Alkaline Range (pH 9.2–10.8)

The *k*-pH profile (Fig. 1) for the photolysis of RF in the pH range 9.2–10.8 shows an initial sharp increase in the rate followed by a reduced enhancement above pH 10.0. This may result from the ionization of RF (pK_{a2} N-3, 10.2) (47) and the low susceptibility of the RF anion to photolysis as observed in a previous study (10). The more than threefold increase in the rate constant from pH 9.2 to 10.8 is probably due to the higher reactivity of the flavin excited triplet state as suggested by Carins and Metzler (44). According to these authors, the high pK_a (6.5) and the increased reactivity of the excited triplet state at higher pH is due to its existence in a bent, diradical form. Carbonic acid (pK_{a1} 6.4, pK_{a2} 10.4) (53) gives rise to two species i.e., HCO_3^- and CO_3^{2-} ions in the alkaline solution. The catalytic effects of these ions on the k_{obs} for the photolysis of RF in the pH range 9.2–10.8 may be described by Eq. (14). In the presence of carbonate buffer, k_{obs} may be expressed as

$$k_1 = k_0 + k' C_B \tag{15}$$

where k' is the overall rate constant for the photolysis of RF in the presence of carbonate ions and C_B is the total concentration of carbonate buffer. A plot of k_1 *versus* C_B gives an intercept k_0 and a gradient k'. The two rate constants, k_3' and k_4' (Eq. (13), may be obtained by rearranging the Eq. (13) into a linear form (16).

$$k' = \frac{(k_1 - k_0)}{C_B} = \frac{k_3''[HCO_3]}{C_B} + \frac{k_4'\left[C_B - \left[HCO_3^-\right]\right]}{C_B} \tag{16}$$

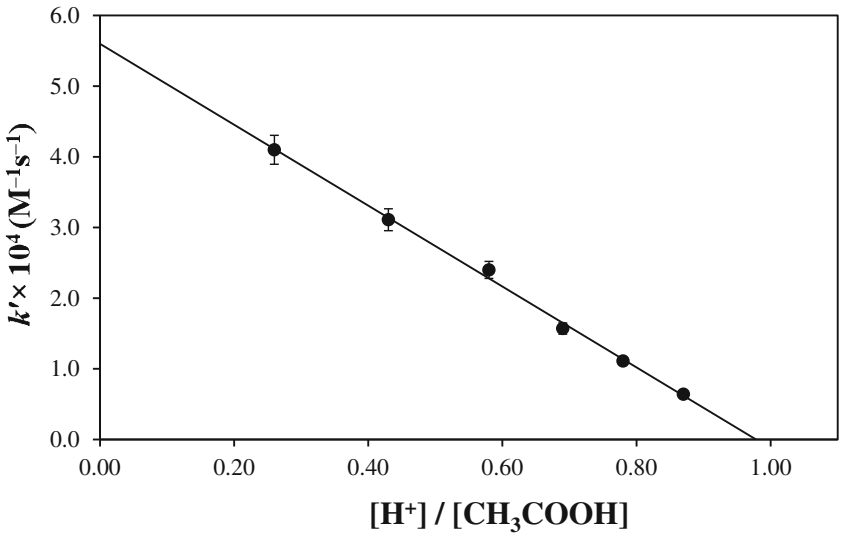


Fig. 3. Plot of k' for the photolysis of riboflavin *versus* $[H^+]/[CH_3COOH]$

A plot of *k′ versus* the fraction of HCO_3^-, i.e., $[HCO_3^-]/C_B$ would give the values of k_3'' and k_4'. The intercept at $[HCO_3^-]/C_B=0$ is equal to the rate constant k_4' (Fig. 2). The *k* value at $[HCO_3^-]$ $C_B=1$ gives the rate constant k_3''. In the present study, the values k_3'' and k_4' for the HCO_3^- and CO_3^{2-} anion-catalyzed photolysis of RF are 0.72 and 1.38×10^{-3} M^{-1} s^{-1}, respectively. The value of k_4' being greater than k_3'' indicates that the CO_3^{2-} ions have a greater catalytic effect on the photolysis of RF in the pH range studied.

Effect of Acetic Acid

The effect of acetic acid (pK_a 4.76) concentration on the rate of photolysis of RF has also been examined by constructing a plot of k_{obs} *versus* the fraction of $[H^+]/[CH_3COOH]$ in the presence of acetate buffer (pH 3.8–5.6) (Fig. 3), similar to that reported above in the presence of carbonate buffer (Fig. 2). It has been observed that the *k* value for the general acid catalyzed reaction ($[H^+]/[CH_3COOH]=1$) is around zero and the acetate anion-catalyzed rate constant ($[H^+]/[CH_3COOH]=0$), 5.61×10^{-4} M^{-1} s^{-1}. This indicates the absence of any general acid catalysis by acetic acid on the photolysis of RF and the enhancement in the rate of the reaction is due to the catalytic effect of acetate ions only.

Fluorescence Studies

Aqueous RF solutions exhibit intense yellow green fluorescence which is destroyed by mineral acids or alkalis (54–56). In the present study, fluorescence measurements on RF have been carried out in acetate buffer (pH 4.3) and in carbonate buffer (pH 9.8). Acetate ions (0.6 M) have been found to decrease the fluorescence intensity of RF to the extent of about 4% (Table IV). Since the acetate ions enhance the photodegradation of RF in the pH range 3.8–5.6 (Table II), the loss of fluorescence could not be attributed to the quenching of the excited singlet state (^{1}RF), which would inhibit the reaction. There is a possibility of the formation of a complex between ^{1}RF state and the acetate ions, as suggested in the case of ^{1}RF state and the monovalent or divalent phosphate ions to cause the photodegradation of RF (13–18). This is substantiated by a decrease in fluorescence emission of RF solutions in the presence of these species. The participation of the ^{1}RF state in the intramolecular photodegradation of RF leading to the direct formation of LC has been reported (44,57–60). Thus LC may also originate from the ^{1}RF state, in addition to that formed from the excited triplet state [^{3}RF] through FMF (62,63).

There is no significant decrease in the fluorescence of RF solutions in the presence of carbonate ions (Table II). This indicates that these ions do not quench the ^{1}RF state and probably interact with the ^{3}RF state to facilitate the photodegradation of RF in alkaline range.

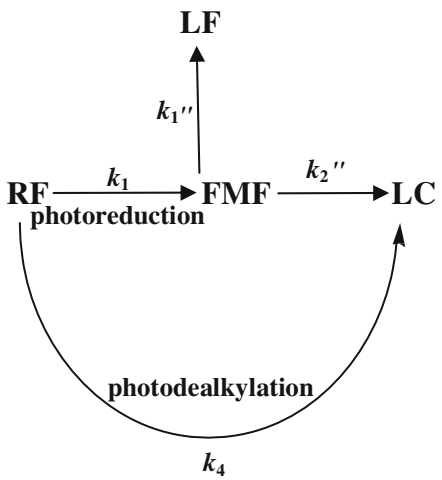
Table IV. Fluorescence Intensity of 5×10^{-5} M Riboflavin Solution in the Presence of Acetate and Carbonate Buffers

pH	Acetate concentration (M)	Carbonate concentration (M)	Relative fluorescence intensity at 525 nm
4.3	0.00	0.00	100.0
	0.20	0.00	98.3
	0.40	0.00	96.8
	0.60	0.00	95.1
9.8	0.00	0.00	100.0
	0.00	0.20	99.7
	0.00	0.40	99.4
	0.00	0.60	99.1

Measurements were made at ambient temperature (~25°C) under aerobic conditions

Mode of Photodegradation

The photodegradation of RF in the presence of acetate/carbonate buffers may be represented by the following overall reaction scheme.



According to the proposed reaction scheme, RF on the absorption of light undergoes photoreduction (k_1) through the [^{3}RF] state to form FMF (17,40,58). It may also be converted directly to LC (k_4) by photodealkylation through the [^{1}RF] state (44,57,61). FMF undergoes hydrolysis to LC (k_2) and LF (k_3) in aqueous media (34,38,39). The equilibria of RF, ^{1}RF, and ^{3}RF are not the rate determined steps in the reaction. Although the photoreduction is the main reaction involved in the photolysis of RF, the overall loss of RF (k_{obs}) could be considered as a combination of the rate constants, $k_1 + k_4$.

CONCLUSION

The photolysis of riboflavin in aqueous solution follows first-order kinetics and is catalyzed by the acetate and carbonate buffers. The rate constants for the reactions in the alkaline range (9.2–10.8) are ~5–15 times greater than those of the acid range (3.8–5.6). The flavin excited triplet state shows higher reactivity in the alkaline solution, resulting in an increase in the rate of the reaction, with pH. The lower rates in the pH range 5–6, compared to that of pH 4.3 (pH_{max}), are due to the lowest redox potentials of riboflavin in that range. The *k*-pH profiles indicate that the highest rate of RF photolysis is at ~pH

4.3 and 10.8. Both HCO_3^- and CO_3^{2-} ions participate in the catalytic activity of the photolysis of riboflavin in alkaline solution. The loss of fluorescence of riboflavin in the acid range probably is due to quenching of the excited singlet state, indicating an interaction between riboflavin and the buffer species. The participation of both the excited singlet and the excited triplet stated in the photolysis of riboflavin has been reported. The study suggests that a careful approach should be adopted in the selection of a buffer to minimize its catalytic effect on the degradation of the drug molecule in a particular pH range.

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