

Effect of phosphate buffer on the complexation and photochemical interaction of riboflavin and caffeine in aqueous solution: A kinetic study



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ABSTRACT

A study of the photodegradation of 5×10^{-5} M riboflavin (RF) in 0.2–1.0 M phosphate buffer in the presence and absence of 2.50×10^{-4} M caffeine at pH 6.0–8.0 has been carried out. RF in phosphate buffer is photodegraded simultaneously by normal photolysis (photoreduction) and photoaddition reactions giving rise to lumichrome (LC) and cyclodehydroriboflavin (CDRF) as the main final products, respectively. RF and its photoproducts, formylmethylflavin (FMF), lumiflavin (LF), LC and CDRF in degraded solution have been determined by a specific multicomponent spectrophotometric method with an accuracy of $\pm 5\%$. The apparent first-order rate constants for the photodegradation of RF and for the formation of LC and CDRF are $5.47\text{--}15.05 \times 10^{-3} \text{ min}^{-1}$, $1.06\text{--}8.30 \times 10^{-3} \text{ min}^{-1}$ and $4.31\text{--}8.05 \times 10^{-3} \text{ min}^{-1}$, respectively. An increase in phosphate concentration leads to an increase in the rate of formation of CDRF and alters the photodegradation of RF in favor of the photoaddition reaction. This photoaddition reaction is further enhanced in the presence of caffeine which results in a further decrease of the fluorescence of RF in phosphate buffer. Caffeine may facilitate the photoaddition reaction by suppression of the photoreduction pathway of RF.

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1. Introduction

Buffers are considered as an important component of chemical and pharmaceutical systems to achieve optimum stability of a compound or to perform a reaction under controlled condition of pH, buffer concentration and ionic strength. In many cases, the buffers affect the stability of pharmaceutical compounds in aqueous solution [1–6]. Several studies have been carried out to evaluate the effect of buffer species on the photolysis of riboflavin (RF). These studies include the catalytic effect of acetate [7] and phosphate buffers [8–14] and the inhibitory effect of borate [15] and citrate buffers [16]. It has been shown that the monovalent phosphate ions (H_2PO_4^-) exert a catalytic effect on the normal photolysis or photoreduction (side-chain cleavage) of RF [11,17] and the divalent phosphate ions (HPO_4^{2-}) on the photoaddition (side chain

cyclization) reactions of RF [9–14]. These two major pathways of the photodegradation of RF occur simultaneously depending on the pH, buffer concentration and light intensity and wavelengths [9–14]. The rate of photoaddition reaction of RF at pH 7.0 is more than twice of the photolysis reaction [11].

RF is known to form molecular complexes with caffeine [18–24] which causes the stabilization of RF and thus inhibits its rate of chemical [25] and photodegradation reactions [26,27] in aqueous solution. However, the previous studies deal only with the individual effect of caffeine (CF) [27], or the phosphate [11–14] on the photodegradation of RF. These studies do not provide information on the combined effect of CF and phosphate on the reaction.

CF molecules may enter the human body from various food sources in large amounts and, thereby, may influence RF function in organism. Hence, the knowledge of photochemical interaction of CF–RF is important for understanding their interaction in a biological system. The knowledge of the set of factors altering RF photodegradation in solution is also important in view of the positive synergistic effect of blue light and RF with respect to suppression of tumor cells growth in vivo [28] and the blue

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light-mediated DNA repair by photolysate enzymes containing flavin adenine dinucleotide (FAD) as cofactor [29].

The present work is based on the evaluation of the effects of CF and phosphate together on the photodegradation of RF and the role of CF vis-a-vis phosphate in altering the rates of the reactions. The study provides kinetic evidence to support the view that CF is involved in modifying the role of phosphate to enhance the photodegradation of RF. This would facilitate the understanding of the interaction of CF–RF in phosphate buffer and its influence on structural orientation to cause a particular change in the mode of degradation reactions.

2. Materials and methods

RF, LC, LF and CF were obtained from Sigma–Aldrich Chemicals Co. (St. Louis, MD, USA). FMF, CMF and CDRF were prepared by the previously described methods, respectively [30,31]. All the reagents and solvents were of analytical grade or of the purest form available from Merck & Co. (Whitestone Station, NJ, USA).

2.1. Photolysis of RF

The photolysis of 5×10^{-5} M RF solutions, containing 2.5×10^{-4} M CF was carried out at $25 \pm 1^\circ\text{C}$ in the presence and absence of 0.2–1.0 M Na_2HPO_4 at pH 6.0–8.0. The pH of the solution was adjusted with 1.0–5.0 M HCl/NaOH solution. The solution (100 ml) was irradiated in a dark chamber using a Philips 125 W high pressure mercury vapor fluorescent lamp (emission at 405 and 435 nm), fixed horizontally at a distance of 30 cm from the center of the flask. The 435 nm band of the radiation source overlaps the 445 nm absorption maximum of RF [11] while CF (λ_{max} 273 nm) [32] does not absorb in the visible region. The solution was stirred by bubbling a stream of air into the flask. Samples were withdrawn at appropriate intervals for chromatographic examination and spectrophotometric determination. The photolysis of RF was also carried out under the same conditions in the presence of 1 M phosphate and $0.625\text{--}2.50 \times 10^{-4}$ M CF at pH 7.0.

2.2. Thin layer chromatography

The identification of the photodegradation products of RF was carried out by thin-layer chromatography (TLC) using 250- μm silica gel G plates (Merck) and the solvent systems: (a) 1-butanol–acetic acid/water (40:10:50 (v/v), organic phase) [33], and (b) chloroform–methanol (9:2 (v/v)) [9]. The detection of RF and its photodegradation products was performed by their characteristic fluorescence emission under UV (365 nm) excitation (RF, FMF, CMF – yellow green; LC, sky blue) using a Uvitech lamp (Cambridge, UK) or by visual examination (CDRF – red color). The progress of the photolysis reactions was monitored by observing the intensity variations of the spots of different products.

2.3. Spectrophotometric assay

The assay of RF and its side-chain cleavage photoproducts (FMF, LC, LF) and cycloaddition photoproduct (CDRF) was performed by a five component spectrophotometric method of Ahmad et al. [11]. This method has previously been used in a number of photodegradation studies of RF in the presence of phosphate buffer [11–14]. It involves the pre-adjustment of the photodegraded solutions to pH 2.0 (0.2 M HCl/KCl buffer), followed by the extraction of LC and LF with chloroform and determination of the chloroform residue at pH 4.5 (0.2 M acetate buffer) by a two-component assay

at 356 and 445 nm, the respective absorption maxima of LC and LF [11]. The other three compounds (RF, FMF, CDRF), present in the aqueous phase, are determined by a three-component assay at 385, 410, 445 nm, corresponding to the absorption maxima of FMF, CDRF and RF, respectively [11]. The absorption maxima of RF and FMF (445 nm, pH 7.0) can be distinguished at pH 2.0 where FMF exists in a protonated form (pK_a 3.5) [34] and thus can be assayed by this method. CMF is a minor product of the oxidation of FMF [13,17,35] and has not been considered in the assay scheme.

2.4. Spectral measurements

The spectral and absorbance measurements on RF and its photolyzed solutions were performed on a Shimadzu UV-1601 recording spectrophotometer using quartz cells of 10-mm path length.

2.5. Fluorescence measurements

The fluorescence of RF solutions was measured 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 wavelength [36]. The fluorescence was measured in relative fluorescence units using a pure 0.05 mM RF solution as the standard.

2.6. Light intensity measurement

The measurement of the intensity of Philips HPL-N 125 W high pressure mercury vapor fluorescent lamp was performed using potassium ferrioxalate actinometry [37]. The value of the intensity of the lamp was determined as $1.16 \pm 0.098 \times 10^{17}$ quanta s^{-1} .

3. Result and discussion

3.1. Composition of photodegraded solution of RF

It is necessary to ascertain the nature of products formed in the photodegraded solutions of RF in the presence of phosphate buffer and CF at pH 6.0–8.0. TLC of the solutions using solvent system (a) showed the presence of the compounds (R_f values in parentheses): undergraded RF (0.36), FMF (0.61), LC (0.67), LF (0.42) and CMF (0.26), with their characteristic fluorescence emission (mentioned in Section 2 under thin layer chromatography), and solvent system (b): undergraded RF (0.37), LC (0.82) and CDRF (0.46) (red color). These products have previously been identified in the photodegradation of RF under similar conditions and arise from the photoreduction and photoaddition pathways [9,17,27,38]. The formation (FMF, LC, LF, CMF, CDRF) and loss (RF, FMF, CMF) of these compounds was monitored by changes in the intensity of their spots. The formation of LF in the reaction takes place at pH 7.0–8.0.

3.2. Spectral characteristics of photodegraded solutions

The spectral variations in the photodegraded solutions of RF in the presence of CF [27] and the phosphate buffer [9,11,14] have been studied. In the presence of CF, RF shows a loss of absorbance at 374 and 445 nm and a slight increase around 385 nm indicating the formation of FMF with time in the aqueous phase (pH 2.0) after chloroform extraction to remove LC and LF. The mixture of these products in the aqueous solution (pH 4.5) exhibits a gradual increase in absorbance around 356 and 445 nm, the respective absorption maxima of LC and LF. The absorption spectrum of the photodegraded solutions of RF in the presence of phosphate buffer

Table 1

Apparent first-order rate constants (k_{obs}) for the photodegradation of RF and for the formation of CDRF (k_1) and LC (k_2') in the presence of CF (2.5×10^4 M) and phosphate buffer (0.2–1.0 M) at different pH values^a.

pH	Phosphate conc. (M)	$k_{\text{obs}} \times 10^3$ (min ⁻¹) $\pm$ SD	$k_1 \times 10^3$ (min ⁻¹) $\pm$ SD	$k_2' \times 10^3$ (min ⁻¹) $\pm$ SD	k_1/k_2'
6.0	0.2	5.69 $\pm$ 0.28	–	–	–
	0.4	7.49 $\pm$ 0.45	–	–	–
	0.6	8.76 $\pm$ 0.46	–	–	–
	0.8	9.94 $\pm$ 0.62	–	–	–
	1.0	11.17 $\pm$ 0.60 (5.14 $\pm$ 0.21)	–	–	–
6.5	0.2	5.43 $\pm$ 0.24	1.12 $\pm$ 0.07	4.31 $\pm$ 0.26	0.26
	0.4	6.76 $\pm$ 0.34	2.19 $\pm$ 0.11	4.57 $\pm$ 0.25	0.48
	0.6	8.83 $\pm$ 0.40	3.70 $\pm$ 0.22	5.13 $\pm$ 0.32	0.72
	0.8	10.40 $\pm$ 0.69	4.99 $\pm$ 0.32	5.41 $\pm$ 0.33	0.92
	1.0	11.70 $\pm$ 0.70 (6.15 $\pm$ 0.29)	6.05 $\pm$ 0.35 (1.35 $\pm$ 0.05)	5.65 $\pm$ 0.28 (4.80 $\pm$ 0.21)	1.07 (0.28)
7.0	0.2	5.87 $\pm$ 0.32	1.53 $\pm$ 0.08	4.32 $\pm$ 0.28	0.36
	0.4	7.92 $\pm$ 0.51	2.81 $\pm$ 0.14	5.11 $\pm$ 0.31	0.55
	0.6	10.99 $\pm$ 0.55	4.85 $\pm$ 0.29	6.14 $\pm$ 0.36	0.79
	0.8	13.18 $\pm$ 0.71	6.68 $\pm$ 0.30	6.50 $\pm$ 0.26	1.03
	1.0	15.05 $\pm$ 0.90 (8.13 $\pm$ 0.43)	8.30 $\pm$ 0.42 (3.23 $\pm$ 0.19)	6.75 $\pm$ 0.39 (4.90 $\pm$ 0.28)	1.23 (0.66)
7.5	0.2	8.85 $\pm$ 0.57	2.15 $\pm$ 0.15	6.70 $\pm$ 0.40	0.32
	0.4	10.73 $\pm$ 0.60	3.43 $\pm$ 0.24	7.30 $\pm$ 0.37	0.47
	0.6	11.85 $\pm$ 0.53	4.49 $\pm$ 0.27	7.36 $\pm$ 0.38	0.61
	0.8	13.12 $\pm$ 0.79	5.52 $\pm$ 0.33	7.60 $\pm$ 0.34	0.73
	1.0	14.98 $\pm$ 0.82 (5.81 $\pm$ 0.33)	6.92 $\pm$ 0.38 (1.88 $\pm$ 0.12)	8.05 $\pm$ 0.55 (3.93 $\pm$ 0.26)	0.86 (0.48)
8.0	0.2	5.47 $\pm$ 0.33	1.06 $\pm$ 0.04	4.41 $\pm$ 0.29	0.24
	0.4	6.57 $\pm$ 0.43	1.70 $\pm$ 0.10	4.87 $\pm$ 0.29	0.35
	0.6	7.59 $\pm$ 0.44	2.31 $\pm$ 0.12	5.28 $\pm$ 0.26	0.44
	0.8	9.31 $\pm$ 0.47	3.26 $\pm$ 0.19	6.05 $\pm$ 0.39	0.54
	1.0	10.25 $\pm$ 0.62 (4.88 $\pm$ 0.32)	4.03 $\pm$ 0.22 (1.32 $\pm$ 0.07)	6.21 $\pm$ 0.41 (3.56 $\pm$ 0.15)	0.65 (0.37)

^a The values of the rate constants in parentheses are in the absence of CF in 1.0 M phosphate buffer.

(pH 7.0) show the loss of RF around 445 nm and the formation of LC and CDRF around 356 and 410 nm, respectively [14]. The absorption spectra of RF solution (aqueous phase), photodegraded in the presence and absence of CF in phosphate buffer (pH 7.0) exhibit similar spectral variations indicating the loss of RF and the formation of FMF and CDRF by different pathways. It is interesting to note that the 410/445 nm (CDRF/RF) ratio in the presence and absence of CF in 1 M phosphate buffer on photodegradation in 2 h changes from 0.63 to 3.90 and 0.63 to 2.70, respectively (Fig. 1). This indicates a two-fold increase in the absorbance ratio leading to the formation of CDRF in the presence of CF. A three-component analysis of the spectral data in the presence and absence of CF and their kinetic treatment in terms of the rate constants (Table 1) indicates the effect of CF on the rates of the reactions. The magnitude of spectral variations depends on the concentration of phosphate buffer which alters the normal photolysis (photoreduction) pathway (leading to the formation of FMF, LC and LF by cleavage of the ribityl side-chain) in favor of the photoaddition pathway (leading to the formation of CDRF by cyclophotoaddition of the ribityl side-chain).

3.3. Assay of RF and photoproducts

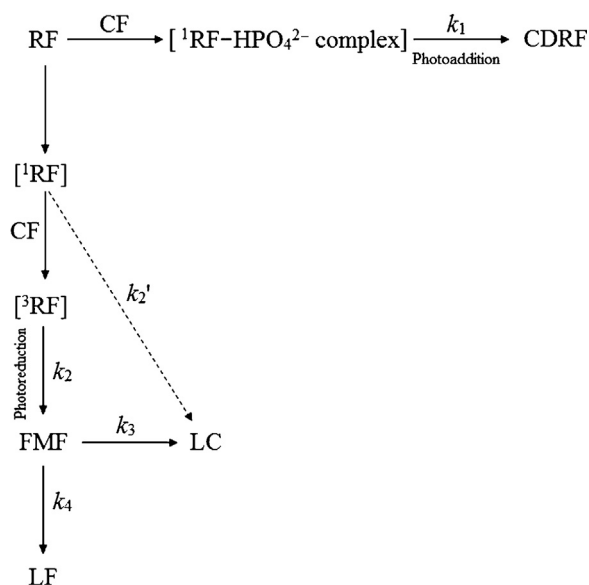
The photodegraded solutions of RF represent a complex mixture containing RF, FMF, LC, LF and CDRF in a variable composition and need a specific, convenient and reliable method for the assay of RF and its photodegradation products. In a previous study [9], no quantitative and kinetic study of these reactions has been carried out. Ahmad et al. [11] developed a multicomponent spectrophotometric method for the assay of RF, FMF, LC, LF and CDRF in

the photodegradation of RF in the presence of phosphate buffer and applied it to the kinetic studies of the reaction [11–14]. Ahmad and Rapson [39] had previously reported a similar spectrophotometric method for the assay of RF, FMF, LC and LF in the photodegraded solutions of RF. This method has extensively been applied to study the kinetics of normal photolysis of RF in aqueous solution in the absence [15–17] and presence of CF [27].

The assay method used in this study [11] takes into consideration the simultaneous determination of RF and its photodegradation products of normal photolysis (FMF, LC and LF) and photoaddition (CDRF). This method would throw light on the relative composition of the photodegradation product of the two reaction pathways and has previously been used for similar studies in the absence of CF [11–14]. CF (λ_{max} 273 nm) does not interfere in the region of wavelengths used for the assay of RF and its photoproducts [27].

3.4. Kinetics of photodegradation reactions

The photodegradation of RF takes place by several pathways including the photoreduction, photoaddition and photodealkylation [10,14,40–42]. In the presence of divalent phosphate ions (HPO_4^{2-}), RF undergoes simultaneous photoreduction and photoaddition reactions in aqueous solution to form LC and CDRF, respectively, as the main final photoproducts [9,11–14]. The rates of these reactions depend on the condition such as pH, buffer concentration and light intensity. The photodegradation of RF in the presence of phosphate buffer can be expressed by the following reactions:



Since the amount of LF formed at pH 7.0–8.0 is less than 5% of the reaction mixture at 50% photodegradation of RF, it could be omitted in the above scheme. LC is formed through FMF as an intermediate in this reaction, it could be assumed that CDRF and LC are formed by parallel first-order reactions. Therefore, the values of k_1 and k_2' can be determined from the values of k_{obs} ,

the overall first-order rate constant for the photodegradation of RF. The mathematical treatment of differential equations for the reactant and the products, the determination of the values of k_1 and k_2' (considering direct formation of LC from RF) and the determination of k_3' and k_4' for the H_2PO_4^- and HPO_4^{2-} ions catalyzed reactions has been dealt in an earlier communication [5,11,14]. The values of k_{obs} , k_1 and k_2' in the presence of CF along with the values in the absence of CF in 1.0 M phosphate buffer are reported in Table 1. The values of k_4' have been determined from the plots of k_{obs} versus $\text{H}_2\text{PO}_4^-/\text{Total phosphate concentration}$. This value in the presence of CF ($2.29 \times 10^{-2} \text{ M}^{-1} \text{ min}^{-1}$) is nearly twice of the value ($1.39 \times 10^{-2} \text{ M}^{-1} \text{ min}^{-1}$) in the absence of CF in 1.0 M phosphate buffer. This indicates that CF accelerates the photoaddition pathway of RF. This is further substantiated by the values of k_1/k_2' in the presence and absence of CF as discussed under Section 3.6.

3.5. Effect of phosphate buffer

Previous studies of the effect of phosphate buffer on the photodegradation of RF have indicated the simultaneous formation of CDRF and LC by different pathways. The rates of these reactions depend on the concentration of phosphate species present at a particular pH [11–14]. In the present study the photodegradation reactions of RF in phosphate buffer have been carried out in the presence or absence of CF. A consideration of the reaction in 1.0 M phosphate buffer alone at pH 7.0 shows that the ratio of the first-order rate constants for the formation of CDRF (photoaddition) and LC (photoreduction) (k_1/k_2') is 0.66, i.e., one-third of the photodegradation reaction leads to the formation of CDRF and two-third to the formation of LC. This is the same pattern as observed in the earlier studies of the effect of phosphate buffer on the photodegradation of RF [11,12,14]. This ratio has been found to increase with an increase in buffer concentration in the presence of $2.5 \times 10^{-4} \text{ M}$ CF (0.36–1.23) indicating an enhancement in the rate of photodegradation pathway of RF in favor of the photoaddition reaction (Table 1).

3.6. Effect of CF and phosphate buffer

An increase in CF concentration at a fixed concentration of phosphate buffer (1.0 M) also resulted in an increase in the ratios of k_1/k_2' (0.70–1.23) (Table 2), suggesting that CF in the presence of phosphate buffer influences the photoaddition pathway. This could be due to the suppression of the photoreduction pathway as observed in a previous study [27]. In the presence of CF, the k_1/k_2' ratio is twice (1.23) compared to that observed in the presence of phosphate buffer alone (0.66) at pH 7.0. Thus, in addition to the suppression of the photoreduction pathway of RF, CF may be involved in some interaction with phosphate buffer to accelerate the photoaddition reaction. CF forms molecular complexes with RF (see Section 1) and thus inhibits its rate of chemical [25] and photodegradation reactions [26,27] in aqueous solutions. It has been suggested that CF forms a hetero association complex with flavin mononucleotide (FMN) as a result of vertical stacking interaction between the aromatic chromophores of the two molecules [24,43]. In view of the formation of such an association complex between CF and RF and the fact that an increase in CF concentration would cause greater suppression of the photoreduction pathway [27], an acceleration in the photoaddition pathway is expected as evident from the values of k_1 and k_2' . It is interesting to know that the values of k_1/k_2' in the presence of CF are higher than these obtained in the absence of CF. Therefore, CF appears to exert an influence in determining the magnitude of the two degradation pathways of RF.

The observed ability of CF molecule to discriminate between different degradation pathways of RF, found in the present work and linked with the stacking-type hetero-complex formation, is

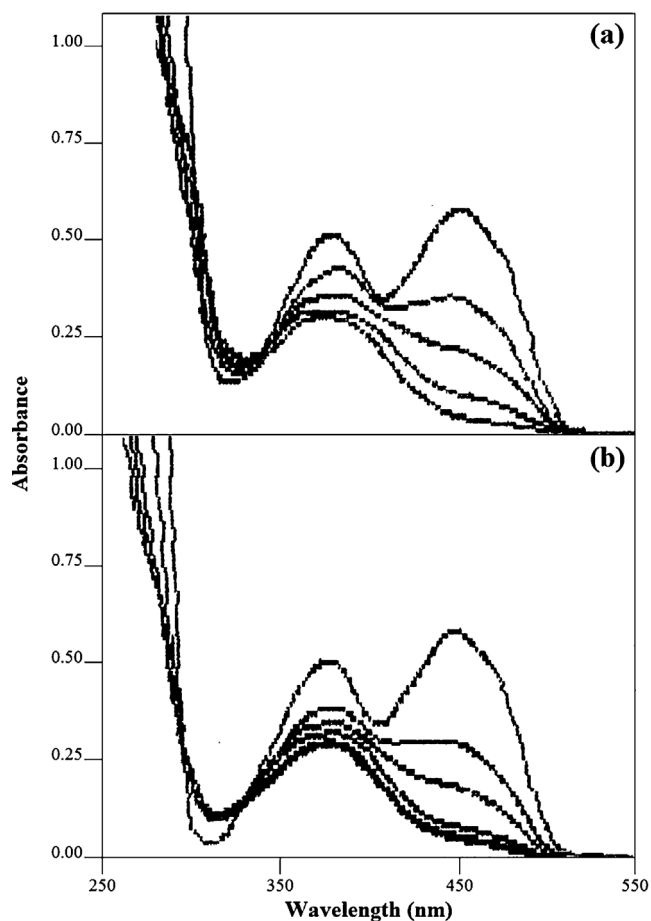


Fig. 1. UV and visible absorption spectra of photodegraded solutions of RF at pH 7.0 (1.0 M phosphate buffer). (a) In the presence of CF and (b) in the absence of CF at 0, 30, 60, 90 and 120 min.

Table 2

Apparent first-order rate constants (k_{obs}) for the photodegradation of RF and for the formation of CDRF (k_1) and LC (k_2') in the presence of CF ($0.625\text{--}2.50 \times 10^{-4}$ M) in phosphate buffer (1.0 M) at pH 7.0.

Caffeine concentration ($\text{M} \times 10^4$)	$k_{\text{obs}} \times 10^3$ (min^{-1}) $\pm$ SD	$k_1 \times 10^3$ (min^{-1}) $\pm$ SD	$k_2' \times 10^3$ (min^{-1}) $\pm$ SD	k_1/k_2'
0.625	8.42 ± 0.51	3.47 ± 0.19	4.95 ± 0.35	0.70
1.250	11.11 ± 0.67	5.10 ± 0.33	6.01 ± 0.36	0.85
1.875	12.86 ± 0.75	6.53 ± 0.39	6.33 ± 0.41	1.03
2.500	15.05 ± 0.90	8.30 ± 0.42	6.75 ± 0.39	1.23

very similar to well-known regulatory action of CF with respect to aromatic drugs binding with DNA [44,45] and antibiotic–vitamin interaction [46], which is also considered as a consequence of the hetero-association between CF and other molecular species in solution. The likely reason for the influence of the hetero-complex formation on the kinetics of RF photodegradation is the formation of low dielectric environment within the volume of hetero-complex as a consequence of water exclusion, altering the energetic barriers for chemical transformation of RF. This effect, being specific to stacking type of interactions, is also known to result in a variety of biochemical effects such as, for instance, the lowering of pK_a of certain atomic groups within the complexes [47], changing the drugs' pharmacokinetics [48] and solubility [49].

3.7. Effect of pH

The present study of the photodegradation of RF has been carried out at pH 6.0–8.0. The rate of HPO_4^{2-} ion catalyzed reaction is highest around pH 7 as observed earlier [9,11,14]. An essential requirement for the intramolecular photoaddition of RF is that it occurs at pH value >6 [9]. This is in accordance with the name assigned to the reaction as the anion-catalyzed neutral photochemistry by the discoverers [9] and has been found in this study. The rate of reaction increases with pH up to a maximum (pH 7) and then decreases as observed in a previous study [14]. The same pattern of reaction is observed in the presence of CF, however, the value of k_{obs} is nearly twice than that obtained in the absence of CF (Fig. 2). This shows that CF plays a significant role in the photodegradation of RF in phosphate buffer. This would further be explained in the next section.

3.8. Fluorescence studies

Fluorescence studies on RF solutions in the presence of CF and phosphate buffer have been carried out to observe their effect on the intensity of fluorescence emission (Table 3). RF exhibits intense yellow green fluorescence in aqueous solution [41,50,51]. The fluorescence of a 5×10^{-5} M RF solution (pH 7.0) is not affected by $1.25\text{--}2.50 \times 10^{-4}$ M CF. However, it is decreased by about 19% in

Table 3

Fluorescence intensity of 5×10^{-5} M RF solution at pH 7.0 in the presence of CF and phosphate buffer^a.

Caffeine concentration ($\text{M} \times 10^4$)	Phosphate concentration (M)	Relative fluorescence intensity at 525 nm
0.00	0.005	100.0
1.25	0.005	100.0
2.50	0.005	100.0
0.00	1.00	81.1
1.25	1.00	78.0
2.50	1.00	74.6

^a Measurements were made at ambient temperature ($\sim 25^\circ\text{C}$) under aerobic conditions.

the presence of 1.0 M phosphate buffer as observed in a previous study [12]. The addition of 2.50×10^{-4} M CF to this solution further decreases the fluorescence of RF to the extent of about 6%. Since CF alone does not affect the fluorescence of RF and its presence in RF solutions containing phosphate buffer leads to a further decrease in the fluorescence intensity, some interaction between CF and phosphate buffer may be stipulated. The fact that the first-order rate constants for the formation of CDRF are faster in phosphate buffer containing CF compared to those of the phosphate buffer alone (Table 1) and the values increase with an increase in phosphate concentration suggests that some interaction takes place between CF and phosphate ions to facilitate the reaction (photoaddition pathway). The loss of fluorescence of RF in the presence of phosphate buffer alone indicates an interaction between RF and phosphate ions (Table 3). This interaction could result from a RF-HPO_4^{2-} complex in the excited singlet state (^1RF) as suggested by Schuman Jorns et al. [9], to promote the photoaddition pathway. A further decrease in RF fluorescence in phosphate buffer in the presence of CF (6%) suggests that CF plays a role in the enhancement of the reaction leading to the photoaddition pathway.

In addition to the formation of a molecular complex between RF and CF to inhibit the degradation reaction, there is a possibility of the suppression of the photoreduction pathway by deactivation of the excited triplet state (^3RF). CF could quench ^3RF by an electron transfer in phosphate buffer and thus facilitate the photoaddition pathway. This is substantiated by a relatively little increase in k_2' with an increase in CF concentration compared to that of k_1 in the overall photodegradation (k_{obs}) of RF (Table 2). CF may influence the other pathways of photodegradation of RF. It has been suggested that the formation of LC also takes place by the participation of ^1RF in the intramolecular photoreduction [38] and the intramolecular photodealkylation reactions [40,42] of flavins. However, the predominant reaction in phosphate buffer involves the photoaddition of RF giving rise to CDRF. Further structural studies are required to ascertain the exact role of CF in the kinetics of degradation of RF.

4. Conclusion

The photodegradation of riboflavin in phosphate buffer at pH 6.0–8.0 occurs by simultaneous photoreduction (side chain cleavage) and photoaddition (side chain cyclization) pathways. The rates of these reactions depend on the concentration of phosphate buffer. An increase in phosphate concentration alters the

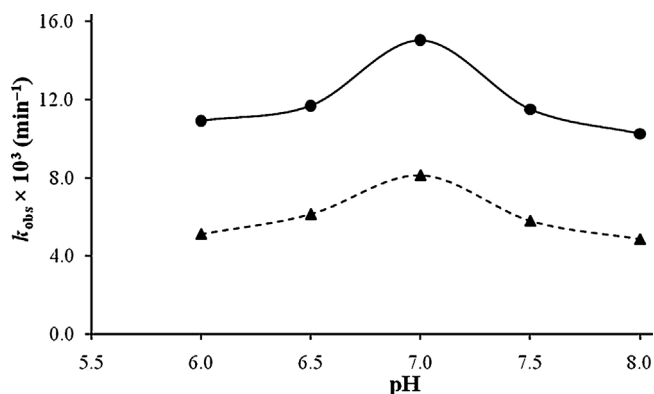


Fig. 2. Plots of k_{obs} versus pH for the photodegradation of RF in 1.0 M phosphate buffer solutions in the presence (—●—) and absence (—▲—) of CF (2.50×10^{-4} M).

photodegradation of riboflavin in favor of the photoaddition pathway and the highest rate of the reaction occurs around pH 7. Both H_2PO_4^- and HPO_4^{2-} ions catalyze the photodegradation of RF by photoreduction (LC) and photoaddition (CDRF) pathways, respectively. The addition of caffeine to riboflavin solutions in phosphate buffer has been found to influence the photodegradation of riboflavin by inhibiting the photoreduction pathway and enhancing the photoaddition pathway. Caffeine could interact with ^3RF in phosphate to cause suppression of the photoreduction pathway and thus facilitate the formation of CDRF as observed during these studies, and/or directly interact with RF within the RF–CF stacking-type hetero-complex altering the energetic barriers for chemical transformation of RF.

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