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Effect of divalent anions on photodegradation kinetics and pathways of riboflavin in aqueous solution

Iqbal Ahmad [a], Sofia Ahmed [a], Muhammad Ali Sheraz [a,*], Faiyaz H.M. Vaid [b], Izhar A. Ansari [b]

[a] Institute of Pharmaceutical Sciences, Baqai Medical University, 51, Deh Tor, Toll Plaza, Super Highway, Gadap Road, Karachi 74600, Pakistan
[b] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi, Karachi 75270, Pakistan

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ABSTRACT

The present investigation is based on a study of the effect of buffer and non-buffer divalent anions (phosphate, sulphate, tartrate, succinate, malonate) on the kinetics, product distribution and photodegradation pathways of riboflavin (RF) at pH 6.0–8.0. RF solutions (5×10^{-5} M) were photodegraded in the presence of divalent anions (0.2–1.0 M) using a visible light source and the photoproducts, cyclodehydroriboflavin (CDRF), formylmethylflavin (FMF), lumichrome (LC) and lumiflavin (LF) were assayed by a specific multicomponent spectrophotometric method. RF degradation in the presence of divalent anions follows parallel first-order kinetics to give CDRF and LC as the final products through photoaddition and photoreduction reactions, respectively. The divalent anion-catalysed CDRF formation is affected in the order: phosphate > sulphate > tartrate > succinate > malonate, showing maximum activity of the anions around pH 7. The divalent anions cause deviation of the photoreduction pathway in favour of the photoaddition pathway to form CDRF. The first- and second-order rate constants for the reactions involved in the photodegradation of RF have been determined and the rate–pH profiles and pathway relationships discussed. The catalytic activity of the divalent anions appears to be a function of the relative strength and chemical reactivity of the RF–divalent anion complex acting as a mediator in the photoaddition reaction.

1. Introduction

In pharmaceutical systems the pH of a solution is maintained by the addition of buffers. The divalent anions in buffered and un-buffered solutions are known to catalyse the degradation of drug substances by participation in the breakdown of an activated complex (Yoshioka and Stella, 2000). These include the effect of phosphate anions ($H_2PO_4^-$, HPO_4^{2-}) on the degradation of cefadroxil (Tsuji et al., 1981), methotrexate (Hansen et al., 1983), mitomycin C (Underberg and Lingeman, 1983), triamcinolone (Das Gupta, 1983), heroin (Poochikian et al., 1983), phenytoin (Varia et al., 1984), codeine (Powell, 1986), ciclosidomine (Carney, 1987), spironolactone (Pramar and Das Gupta, 1991), gonadorelin (Hoitink et al., 1996) and riboflavin (vitamin B2) (Schuman Jorns et al., 1975; Ahmad and Vaid, 2006; Ahmad et al., 2004a, 2005, 2006a), sulphate anions on the degradation of riboflavin (Schuman Jorns et al., 1975) and succinate and tartrate anions on the degradation of amines and amine derivatives (DeLuca and Boylan, 1992). Riboflavin is an important compound in view of its biological function, photosensitivity and nutritional value (Powers, 2003; Ahmad and Vaid, 2006; Rivlin, 2007). Its deficiency may enhance carcinogenesis by increasing the activation of carcinogens, particularly nitrosamines (Rivlin, 1973; Webster et al., 1996). Riboflavin is a well known sensitizer and is involved in the photooxidation of a number of compounds including ascorbic acid (Görner, 2007), benzo[a]pyrene (Zhao et al., 2007), acaricide abamectin (Escalada et al., 2008), dopamine (Massad et al., 2008), 5-methyltetrahydrofolate (Juzeniene et al., 2009), tyrosine and tryptophan (Montaña et al., 2009), bisphenol A (Barbieri et al., 2008; Ha et al., 2009), lysozyme (Zhang and Görner, 2009) and guanine (Kino et al., 2009). Divalent phosphate and sulphate anions cause a change in the mode of photodegradation of riboflavin leading to different pathways including intramolecular photoreduction followed by oxidation of the side chain (normal photolysis) and intramolecular photoaddition via the riboflavin–divalent anion complex (Schuman Jorns et al., 1975; Ahmad and Vaid, 2006; Ahmad et al., 2004a, 2005, 2006a). Since some of these anions act as buffering agents in pharmaceutical preparations, they may affect the stability of riboflavin and cause photodegradation by the photoaddition pathway in addition to the normal photolysis pathway. Therefore, their role in the photodegradation of riboflavin is worth considering.

The aim of the present work is to conduct a kinetic study of the effect of divalent phosphate and sulphate anions on the change in photodegradation pathways of riboflavin and to observe whether other common divalent anions such as tartrate, succinate and malonate exert any such effect on the reaction. The presence of divalent

* Corresponding author. Tel.: +92 21 4410293; fax: +92 21 4410317.
E-mail address: ali_sheraz80@hotmail.com (M.A. Sheraz).

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anions may lead to an increase in the photodegradation of riboflavin and their contribution in the reaction and interrelationship of the photodegradation pathways need investigation. Therefore, the kinetics of these reactions has been studied using a specific multicomponent spectrophotometric method for the determination of riboflavin and photoproducts (Ahmad and Rapson, 1990; Ahmad et al., 2004a), and the influence of divalent anions on the mode of photodegradation has been evaluated.

2. Materials and methods

Riboflavin (RF), lumichrome (LC) and lumiflavin (LF) were obtained from Sigma Chemical Co. Formylmethylflavin (FMF), carboxymethylflavin (CMF) and cyclodehydroriboflavin (CDRF) were prepared by the methods of Fall and Petering (1956), Fukumachi and Sakurai (1954) and Schuman Jorns et al. (1975), respectively. The purity of all these compounds was confirmed by thin-layer chromatography. All reagents and solvents were of the purest form available from BDH/Merck.

2.1. Buffered and un-buffered solutions

The following buffered and un-buffered solutions containing divalent anions were used for the photodegradation studies of RF: pH 6.0–8.0, disodium phosphate (Na_2HPO_4); pH 7.0, ammonium sulphate [$(NH_4)_2SO_4$], sodium tartrate ($C_4H_4O_6Na_2{\cdot}2H_2O$), sodium succinate ($C_4H_4O_4Na_2{\cdot}6H_2O$) and sodium malonate ($C_3H_2O_4Na_2$). All the solutions were prepared at the experimental temperature ($25 \pm 1\,^{\circ}C$). The pH (±0.05 pH unit) of all the solutions was adjusted using an Elmetron pH meter CP-501(Poland) equipped with a combination electrode. The divalent ion concentrations were in the range of 0.2–1.0 M. All the solutions were maintained at a constant ionic strength by adjustment with potassium chloride.

2.2. Precautions

All experimental work was carried out in a dark chamber under subdued light. Aqueous solutions of RF were freshly prepared for each experiment and protected from light before irradiation.

2.3. Photolysis

A 5×10^{-5} M aqueous solution of RF, containing 0.2–1.0 M salts forming divalent anions, was prepared and the pH adjusted in the range of 6.0–8.0 with 1.0–5.0 M HCl/NaOH solution. The solution was placed in a 100 ml volumetric flask (Pyrex), immersed in a water bath maintained at $25 \pm 1\,^{\circ}C$ and irradiated in a dark chamber using a Philips HPLN 125 W high pressure mercury vapour fluorescent lamp (emission at 405 and 435 nm) (Ahmad et al., 2006a) fixed horizontally at a distance of 30 cm from the centre of the flask. The major emission wavelength of the irradiation source closely corresponds to the visible absorption maximum (445 nm) of RF (Ahmad et al., 2004a). The solution was continuously bubbled with a gentle stream of air. Thin-layer chromatographic examination and spectrophotometric determination of the irradiated solutions were carried out at appropriate intervals.

2.4. Thin-layer chromatography

The photodegraded solutions of RF were subjected to thin-layer chromatography (TLC) on 250 μm silica gel G plate (Merck). The following solvent systems were used: (A) 1-butanol–acetic acid–water (4:1:5, v/v, organic phase) (Ahmad et al., 1980) and (B) chloroform–methanol (9:2, v/v) (Schuman Jorns et al., 1975). The compounds were detected by their characteristic fluorescence emission under UV (365 nm) excitation (RF, FMF, CMF and LF – yellow green, LC – sky blue) using a Uvitech lamp (UK) or visually (CDRF – red colour).

2.5. Spectral determinations

All spectral determinations on RF and its photodegraded solutions were carried out on a Shimadzu UV-1601 recording spectrophotometer using silica cells of 10 mm path length.

2.6. Fluorescent measurements

Fluorescence measurements on RF solutions were performed on a Versa Fluor fluorimeter (Bio-Rad Laboratories, CA, USA) equipped with a 20 W quartz halogen lamp (350–390 nm) and a photodiode detector. The measurements were carried out at $25 \pm 1\,^{\circ}C$ using quartz cells of 10 mm path length. The calibration of the fluorescence intensity scale was carried out using pure 0.05 mM RF solutions at the desired pH. The excitation wavelength (374 nm) and the emission wavelength (525 nm) (Song and Metzler, 1967) were set by using appropriate filters and the relative fluorescence intensity of RF solutions containing divalent anions was measured.

2.7. Light intensity measurement

The measurement of the intensity of Philips HPLN 125 W high pressure mercury vapour fluorescent lamp was carried out by potassium ferrioxalate actinometry (Hatchard and Parker, 1956) and a value of $1.15 \pm 0.10 \times 10^{17}$ quanta s^{-1} was obtained.

2.8. Spectrophotometric assay

The assay of RF and its photoproducts (CDRF, FMF, LC and LF) in degraded solutions was carried out using a specific multicomponent spectrophotometric method developed by Ahmad et al. (2004a). The method was based on the preadjustment of degraded solutions to pH 2.0 (HCl–KCl buffer), extraction of LC and LF with chloroform and evaporation of the chloroform under reduced pressure. The chloroform residue was redissolved in aqueous solution (pH 4.5, acetate buffer) and the concentrations of LF and LC were determined by a two-component assay at 445 and 356 nm. The aqueous phase was used to determine the concentrations of RF, CDRF and FMF by a three-component assay at 445, 410 and 385 nm using a suitable software such as "multicomponent analysis by full spectrum quantitation (FSQ)". All the analytical wavelengths used correspond to the absorption maxima of RF (445 nm) and photoproducts (CDRF 410 nm, FMF 385 nm, LF 445 nm and LC 356 nm) (Schuman Jorns et al., 1975; Ahmad et al., 2004a) to achieve specificity of the method. A distinction between the absorption maxima of RF and FMF is achieved by measurement at pH 2.0 (HCl–KCl buffer) where the absorption maximum of FMF (pK_a 3.5) (Suelter and Metzler, 1960) is shifted from 445 to 385 nm due to protonation. CMF is a minor degradation product, obtained by the oxidation of FMF (Fall and Petering, 1956; Ahmad et al., 2006b) as indicated by the intensity of its fluorescence on TLC plates.

3. Results

3.1. Photodegradation products

The photodegradation products of RF formed on irradiation in the presence of divalent anions at pH 6.0–8.0 were detected by TLC and identified by comparison of their characteristic fluorescence emission and R_f values with those of the authentic compounds. The products present in photolysed solutions at 30–50% degradation were RF, CDRF, FMF, LC and LF (all major) and CMF (minor) and have

previously been reported (Schuman Jorns et al., 1975; Ahmad and Rapson, 1990; Ahmad et al., 2004a). The confirmation of the identity of photoproducts was necessary before they could be assayed in degraded solutions. Since CMF is a minor product of the reaction (Ahmad et al., 2008, 2009), it was not studied in this work.

3.2. Absorption spectra of photodegraded solutions

The photodegradation of RF at pH 6.0–8.0 in the presence of divalent anions leads to the simultaneous formation of CDRF by photoaddition, and FMF, LC, LF, and CMF by photoreduction reactions (Schuman Jorns et al., 1975; Ahmad and Rapson, 1990; Ahmad et al., 2004a). The rates of formation and spectral characteristics of the two major final photoproducts, CDRF and LC, depend on the pH, divalent ion concentration and light intensity/wavelengths (Schuman Jorns et al., 1975; Ahmad and Rapson, 1990; Ahmad et al., 2004a, 2005, 2006a). Two sets of typical absorption spectra of the photodegraded solution of RF (pH 7.0) in the presence of 1.0 M phosphate and sulphate are shown in Fig. 1. In both cases the loss of absorbance at 445 nm, with time, is indicative of the photodegradation of RF with a concomitant increase around 410 and 356 nm showing the formation of CDRF and LC, respectively. It is evident from the absorption spectra that the reaction at ~75% degradation of RF in the presence of sulphate (~22 min) is much faster than that in the presence of phosphate (225 min), however, the absorption around 410 nm is greater (0.161) in the case of phosphate compared to that of sulphate (0.106). The spectra also show that the absorption around 356 nm is greater in the case of sulphate compared to that of the phosphate indicating the formation of a greater amount of LC. The appearance of isosbestic points around 360 and 410 nm indicates the presence of more than one species in the solution.

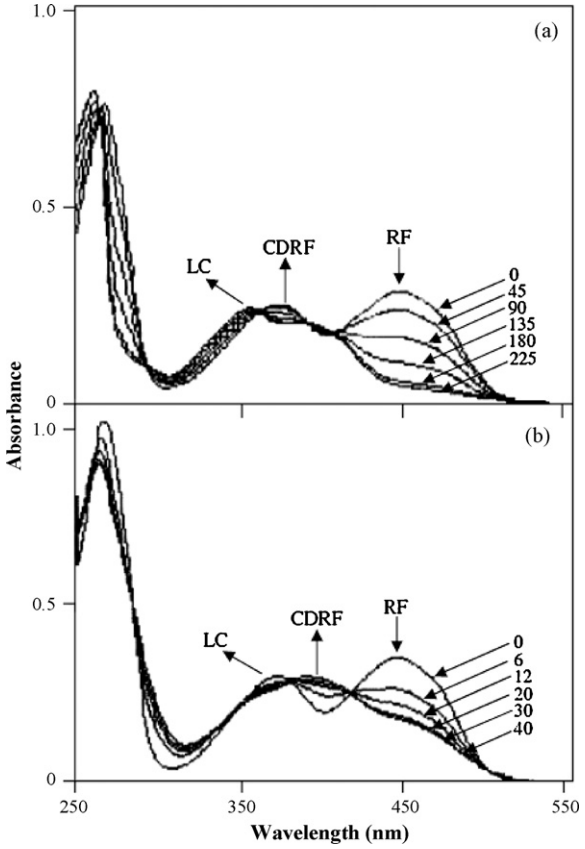


Fig. 1. UV and visible absorption spectra of photodegraded solutions of RF (5×10^{-5}) at pH 7.0. (a) In the presence of 1.0 M phosphate and (b) in the presence of 1.0 M sulphate. Times indicated are in minutes.

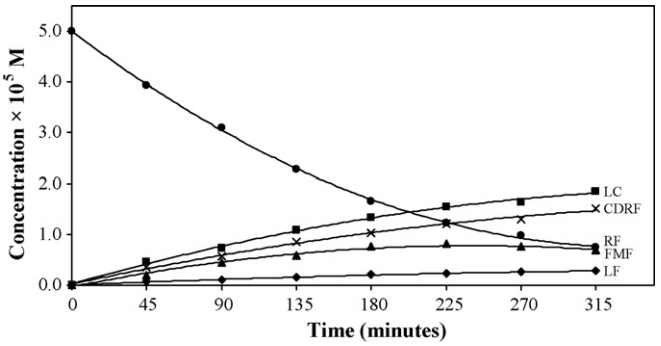


Fig. 2. Photodegradation of 5×10^{-5} M riboflavin solution (pH 7.0) in the presence of 1.0 M phosphate.

FMF and RF both exhibit the same absorption maxima (445 nm) at pH 7.0 and, therefore, cannot be distinguished in the spectra of photodegraded solutions. No significant difference in the absorption spectra of RF in the presence of the individual anions has been observed.

3.3. Assay of RF and photoproducts

An important aspect of the present study was to evaluate the kinetics of photodegradation reactions of RF in the presence of various divalent anions. In view of the complexity of photodegraded mixtures, a specific multicomponent spectrophotometric method (Ahmad et al., 2004a) was used for the simultaneous assay of five compounds (RF, CDRF, FMF, LC and LF), by chloroform extraction for partial separation of the mixtures. The details of the method are given under 'Spectrophotometric assay'. A typical assay result for the photodegradation reaction carried at pH 7.0 in the presence of 1.0 M phosphate shows uniformly decreasing values of RF with concomitantly increasing values of CDRF, FMF, LC, and LF during the reaction (Fig. 2). After 225 min the FMF concentration starts decreasing as a result of its conversion to LC and LF as reported earlier in its photolysis reactions at pH 7.0–9.0 (Heelis et al., 1980), whereas CDRF appears to be directly formed from RF (Schuman Jorns et al., 1975). The assay method was validated in the presence of divalent anions before its application to the study of the kinetics of RF photodegradation and the precision of the method was found to be within ±5%. It has previously been applied to the study of photodegradation of RF in aqueous solutions (Ahmad et al., 2004a, 2005, 2006a).

3.4. Distribution of photoproducts

The divalent anions cause variations in product distribution on the photodegradation of RF at pH 6.0–8.0. The product distribution (ratios) at 50% degradation of RF solutions (pH 7.0) in the presence of 1.0 M divalent anions is presented in Table 1. A comparison of the products indicates that the ratios of CDRF formed (20.3–30.8) decrease in the order of phosphate > sulphate > tartrate > succinate > malonate, while that of LC

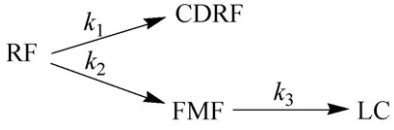
Table 1
Product distribution at 50% photodegradation of RF solutions (pH 7.0) in the presence and absence of 1.0 M divalent anions.

Divalent anion	CDRF	FMF	LC	LF	CDRF/LC
Un-buffered	0.0	21.6	70.4	8.0	–
Phosphate	30.8	22.7	41.4	5.1	0.74
Sulphate	29.3	10.3	56.6	4.0	0.52
Tartrate	25.5	9.2	62.2	3.1	0.41
Succinate	22.4	8.0	66.3	3.1	0.34
Malonate	20.3	7.1	69.4	3.1	0.29

(41.4–69.4) increase in the same order, suggesting that the normal photolysis pathway is increasingly deviated in favour of photoaddition as the divalent anions are changed in the reverse order (i.e., malonate to phosphate). The increased formation of LC (normal photolysis) in the above order is in accordance with the decreasing values of FMF in the reactions (Table 1). Thus the activity of divalent anions to catalyse the formation of CDRF (photoaddition) appears to be the highest in the case of phosphate anions. The values of product distribution in the presence of divalent phosphate anions indicate a higher ratio of CDRF/LC compared to that in the presence of other divalent anions. This appears to be due to the greater catalytic effect of phosphate anions on the photoaddition reaction. In the absence of divalent anions CDRF is not formed and LC is the major product that is formed about 30% more than that in the presence of phosphate anions.

3.5. Kinetics of photodegradation reactions of RF

The kinetic plot for the photodegradation reactions of RF at pH 7.0 in the presence of 1.0 M phosphate buffer (Fig. 2) shows the formation of CDRF by photoaddition and FMF by photoreduction, subsequently leading to LC and LF by hydrolysis (Schuman Jorns et al., 1975; Ahmad and Vaid, 2006; Ahmad et al., 1980, 2004a, 2005, 2006a). Thus FMF is an intermediate in the photoreduction reaction and CDRF and LC are the two major final products obtained by different pathways. Considering the formation of LF (~5% of the total mixture and formed at pH 7–8 only) through a minor reaction under the conditions employed, as observed in the case of the effect of borate anions on the reaction (Ahmad et al., 2008), it may be omitted in the following first-order reaction scheme:



The differential equations for the reactant and the products are:

$$\frac{-\mathrm{d}[\mathrm{RF}]}{\mathrm{d}t} = k_1[\mathrm{RF}] + k_2[\mathrm{RF}] \quad (1)$$

$$\frac{\mathrm{d}[\mathrm{CDRF}]}{\mathrm{d}t} = k_1[\mathrm{RF}] \quad (2)$$

$$\frac{\mathrm{d}[\mathrm{FME}]}{\mathrm{d}t} = k_2[\mathrm{RF}] - k_3[\mathrm{FMF}] \quad (3)$$

$$\frac{\mathrm{d}[\mathrm{LC}]}{\mathrm{d}t} = k_3[\mathrm{FMF}] \quad (4)$$

Assuming the formation of CDRF and LC by parallel first-order reactions, the rate equation is:

$$\frac{-\mathrm{d}[\mathrm{RF}]}{\mathrm{d}t} = (k_1 + k_2')[\mathrm{RF}] = k_{\mathrm{obs}}[\mathrm{RF}] \quad (5)$$

where k_1 and k_2' are the rate constants for the formation of CDRF and LC, respectively, and k_{obs} is the experimentally determined rate constant for the overall photodegradation of RF. The values of the rate constants, k_1 and k_2', may be evaluated by determining the ratio, R, of the concentrations of the products (CDRF and LC) formed by each reaction (Carstensen, 2000; Florence and Attwood, 2006):

$$R = \frac{[\mathrm{CDRF}]}{[\mathrm{LC}]} = \frac{k_1}{k_2'} \quad (6)$$

Since

$$k_{\mathrm{obs}} = k_1 + k_2' \quad (7)$$

$$k_{\mathrm{obs}} = k_1 + \frac{k_1}{R} \quad (8)$$

Solving for k_1 gives the equation:

$$k_1 = k_{\mathrm{obs}}\frac{R}{R+1} \quad (9)$$

Similarly,

$$k_2' = \frac{k_{\mathrm{obs}}}{R+1} \quad (10)$$

The values of k_{obs}, k_1 and k_2' for the photodegradation reactions of RF at pH 6.0–8.0 in the presence of phosphate and sulphate anions and at pH 7.0 in the presence of tartrate, succinate and malonate anions are reported in Table 2. A comparison of these values at pH 7.0 indicates a gradual increase in the rate with an increase in the divalent ion concentration. However, the increase in rate depends on the nature and interaction of the divalent anion with RF.

The effect of buffer species such as phosphate (monovalent and/or divalent anions) on the overall photodegradation rate constant, k_{obs}, has been discussed (Sinko, 2006; Florence and Attwood, 2006), and may be described as:

$$k_{\mathrm{obs}} = k_0 + k_1'[\mathrm{H}^+] + k_2''[\mathrm{OH}^-] + k_3'[\mathrm{H_2PO_4}^-] + k_4'[\mathrm{HPO_4}^{2-}] \quad (11)$$

where k_0 is the first-order rate constant for photodegradation of RF in water only, k_1' and k_2'' are the second-order rate constants for the H^+ and OH^- ion catalysed degradation. At neutral pH the H^+ and OH^- ions would have little effect on the reaction (Florence and Attwood, 2006) and the major buffer species ($H_2PO_4^-$, HPO_4^{2-}) may act as a catalyst for RF degradation. Therefore, Eq. (11) may be written as:

$$k_{\mathrm{obs}} = k_0 + k_3'[\mathrm{H_2PO_4}^-] + k_4'[\mathrm{HPO_4}^{2-}] \quad (12)$$

or

$$k_{\mathrm{obs}} = k_0 + k'P_{\mathrm{B}} \quad (13)$$

where k' is the overall rate constant in the presence of phosphate and P_B is the total concentration of phosphate species. The values of k_3' and k_4' represent the second-order rate constants for the photodegradation reactions catalysed by the two buffer species, $H_2PO_4^-$ and HPO_4^{2-}, respectively.

Considering Eq. (12), a graph of k_{obs} versus P_B, for example, for phosphate at pH 7.0, gives the intercept k_0 and slope of apparent second-order rate constant k'. The values of k_{obs} at pH 7.0 in the presence of 1.0 M phosphate (Table 2) are more than twice the values of k_0 (Table 3), indicating considerable influence of phosphate species on the rate of reaction. The graphs of k_{obs} versus sulphate or tartrate/succinate/malonate concentration follow the same pattern as that of the phosphate. A comparison of k' values at pH 7.0 (Table 3) shows that the rates of interaction of the divalent anions with RF are in the order of sulphate > phosphate > tartrate > succinate > malonate. Thus the sulphate anions appear to have the highest rate of interaction with RF to cause photoaddition.

The values of the buffer catalysed rate constants, k_3' and k_4', may be obtained by rearrangement of Eq. (12) into a linear form:

$$k' = \frac{k_{\mathrm{obs}} - k_0}{P_{\mathrm{B}}} = \frac{k_3'[\mathrm{H_2PO_4}]}{P_{\mathrm{B}}} + k_4'\left(\frac{P_{\mathrm{B}} - [\mathrm{H_2PO_4}^-]}{P_{\mathrm{B}}}\right) \quad (14)$$

A graph of k' against the fraction of the monovalent component of the buffer, $[H_2PO_4^-]/P_B$ (Fig. 3), gives an intercept at $[H_2PO_4^-]/P_B = 0$ equal to the rate constant k_4'. The k' value at $[H_2PO_4^-]/P_B = 1$ is the rate constant k_3' (Florence and Attwood, 2006). The values of k_3' and k_4' for the $H_2PO_4^-$ and HPO_4^{2-} anion-catalysed photodegradation of RF are 3.82×10^{-3} and $9.93 \times 10^{-3}\ \mathrm{M^{-1}\,min^{-1}}$, respectively. The k_4' value indicates a greater effect of HPO_4^{2-} anions on the reaction to form CDRF by photoaddition than the k_3' value for the effect of $H_2PO_4^-$ anions on the formation of LC by photoreduction. Thus both $H_2PO_4^-$ and

Table 2
Apparent first-order rate constants (k_{obs}) for photodegradation of RF and for the formation of CDRF (k_1) and LC (k_2') in aqueous solutions containing divalent anions.

pH	Divalent anion	Concentration (M)	$k_{obs} \times 10^2$ (min^{-1}) ± SD[a]	$k_1 \times 10^2$ (min^{-1})	$k_2' \times 10^2$ (min^{-1})	k_1/k_2'
6.0	Phosphate	0.2	0.35 ± 0.012	–	0.35 ± 0.019	–
		0.4	0.38 ± 0.018	–	0.38 ± 0.017	–
		0.6	0.40 ± 0.031	–	0.40 ± 0.026	–
		0.8	0.42 ± 0.025	–	0.42 ± 0.022	–
		1.0	0.43 ± 0.027	–	0.43 ± 0.025	–
6.3		0.2	0.35 ± 0.025	0.01 ± 0.001	0.34 ± 0.022	0.03
		0.4	0.38 ± 0.020	0.02 ± 0.001	0.36 ± 0.018	0.06
		0.6	0.41 ± 0.027	0.03 ± 0.003	0.38 ± 0.027	0.08
		0.8	0.43 ± 0.025	0.04 ± 0.002	0.39 ± 0.024	0.11
		1.0	0.46 ± 0.029	0.05 ± 0.004	0.41 ± 0.029	0.13
6.5		0.2	0.36 ± 0.017	0.02 ± 0.001	0.34 ± 0.020	0.06
		0.4	0.38 ± 0.027	0.03 ± 0.002	0.35 ± 0.018	0.09
		0.6	0.42 ± 0.033	0.05 ± 0.004	0.36 ± 0.024	0.14
		0.8	0.44 ± 0.021	0.06 ± 0.003	0.37 ± 0.022	0.16
		1.0	0.47 ± 0.019	0.08 ± 0.005	0.38 ± 0.024	0.21
6.7		0.2	0.36 ± 0.021	0.02 ± 0.002	0.34 ± 0.020	0.06
		0.4	0.39 ± 0.024	0.05 ± 0.004	0.35 ± 0.018	0.13
		0.6	0.43 ± 0.021	0.08 ± 0.003	0.36 ± 0.024	0.21
		0.8	0.47 ± 0.024	0.10 ± 0.010	0.36 ± 0.022	0.30
		1.0	0.51 ± 0.023	0.14 ± 0.009	0.36 ± 0.024	0.39
7.0		0.2	0.36 ± 0.018	0.05 ± 0.004	0.31 ± 0.016	0.17
		0.4	0.42 ± 0.020	0.10 ± 0.009	0.32 ± 0.018	0.32
		0.6	0.49 ± 0.022	0.16 ± 0.012	0.33 ± 0.015	0.49
		0.8	0.56 ± 0.025	0.22 ± 0.017	0.34 ± 0.022	0.66
		1.0	0.63 ± 0.029	0.28 ± 0.015	0.35 ± 0.025	0.78
7.3		0.2	0.34 ± 0.021	0.05 ± 0.004	0.29 ± 0.017	0.16
		0.4	0.37 ± 0.018	0.09 ± 0.005	0.29 ± 0.016	0.30
		0.6	0.41 ± 0.017	0.12 ± 0.009	0.29 ± 0.017	0.40
		0.8	0.45 ± 0.020	0.16 ± 0.008	0.30 ± 0.012	0.52
		1.0	0.49 ± 0.026	0.19 ± 0.014	0.30 ± 0.018	0.62
7.6		0.2	0.33 ± 0.018	0.05 ± 0.004	0.28 ± 0.015	0.18
		0.4	0.35 ± 0.019	0.07 ± 0.004	0.28 ± 0.014	0.26
		0.6	0.38 ± 0.021	0.10 ± 0.008	0.28 ± 0.016	0.35
		0.8	0.41 ± 0.020	0.12 ± 0.007	0.29 ± 0.012	0.43
		1.0	0.44 ± 0.021	0.15 ± 0.009	0.29 ± 0.015	0.51
8.0		0.2	0.31 ± 0.015	0.04 ± 0.003	0.28 ± 0.016	0.14
		0.4	0.34 ± 0.014	0.06 ± 0.004	0.28 ± 0.014	0.22
		0.6	0.36 ± 0.017	0.08 ± 0.004	0.28 ± 0.015	0.29
		0.8	0.38 ± 0.018	0.10 ± 0.009	0.28 ± 0.014	0.35
		1.0	0.41 ± 0.016	0.13 ± 0.008	0.29 ± 0.016	0.44
6.0	Sulphate	0.2	0.26 ± 0.015	–	0.26 ± 0.014	–
		0.4	0.34 ± 0.014	–	0.34 ± 0.015	–
		0.6	0.42 ± 0.017	–	0.42 ± 0.024	–
		0.8	0.53 ± 0.021	–	0.53 ± 0.021	–
		1.0	0.63 ± 0.030	–	0.63 ± 0.036	–
6.3		0.2	0.35 ± 0.018	0.01 ± 0.001	0.34 ± 0.018	0.04
		0.4	0.50 ± 0.025	0.03 ± 0.002	0.47 ± 0.025	0.06
		0.6	0.61 ± 0.029	0.04 ± 0.004	0.57 ± 0.031	0.07
		0.8	0.73 ± 0.035	0.05 ± 0.003	0.67 ± 0.035	0.08
		1.0	0.86 ± 0.040	0.07 ± 0.004	0.79 ± 0.032	0.09
6.5		0.2	0.39 ± 0.021	0.02 ± 0.002	0.38 ± 0.022	0.05
		0.4	0.55 ± 0.026	0.05 ± 0.004	0.50 ± 0.028	0.10
		0.6	0.68 ± 0.032	0.09 ± 0.008	0.59 ± 0.030	0.15
		0.8	0.82 ± 0.039	0.14 ± 0.007	0.68 ± 0.042	0.20
		1.0	0.96 ± 0.045	0.19 ± 0.014	0.77 ± 0.036	0.25
6.7		0.2	0.42 ± 0.020	0.02 ± 0.002	0.40 ± 0.025	0.06
		0.4	0.59 ± 0.025	0.06 ± 0.002	0.53 ± 0.023	0.12
		0.6	0.76 ± 0.031	0.13 ± 0.010	0.63 ± 0.033	0.20
		0.8	0.95 ± 0.042	0.19 ± 0.013	0.76 ± 0.030	0.25
		1.0	1.16 ± 0.050	0.27 ± 0.015	0.89 ± 0.046	0.30
7.0		0.2	1.46 ± 0.056	0.13 ± 0.011	1.33 ± 0.068	0.10
		0.4	2.65 ± 0.080	0.58 ± 0.025	2.07 ± 0.092	0.28
		0.6	3.99 ± 0.110	1.10 ± 0.082	2.89 ± 0.158	0.38
		0.8	5.40 ± 0.081	1.65 ± 0.077	3.75 ± 0.082	0.44
		1.0	6.58 ± 0.101	2.13 ± 0.096	4.45 ± 0.095	0.48
7.5		0.2	1.38 ± 0.071	0.08 ± 0.006	1.30 ± 0.067	0.06
		0.4	2.50 ± 0.075	0.48 ± 0.028	2.02 ± 0.080	0.24
		0.6	3.80 ± 0.066	0.99 ± 0.055	2.81 ± 0.065	0.35

Table 2 (*Continued*)

pH	Divalent anion	Concentration (M)	$k_{obs} \times 10^2$ (min^{-1}) ± SD[a]	$k_1 \times 10^2$ (min^{-1})	$k_2' \times 10^2$ (min^{-1})	k_1/k_2'
		0.8	5.17 ± 0.096	1.50 ± 0.085	3.67 ± 0.102	0.41
		1.0	6.30 ± 0.092	1.93 ± 0.077	4.38 ± 0.097	0.44
8.0		0.2	1.30 ± 0.062	0.06 ± 0.004	1.24 ± 0.046	0.05
		0.4	2.35 ± 0.081	0.41 ± 0.032	1.94 ± 0.085	0.21
		0.6	3.60 ± 0.075	0.81 ± 0.056	2.79 ± 0.110	0.29
		0.8	4.80 ± 0.060	1.22 ± 0.048	3.58 ± 0.090	0.34
		1.0	5.90 ± 0.105	1.62 ± 0.085	4.28 ± 0.099	0.38
7.0	Tartrate	0.2	0.21 ± 0.015	0.02 ± 0.002	0.19 ± 0.012	0.09
		0.4	0.25 ± 0.009	0.04 ± 0.002	0.21 ± 0.010	0.19
		0.6	0.30 ± 0.017	0.07 ± 0.004	0.23 ± 0.013	0.29
		0.8	0.36 ± 0.021	0.10 ± 0.008	0.26 ± 0.011	0.38
		1.0	0.41 ± 0.026	0.12 ± 0.007	0.29 ± 0.016	0.43
7.0	Succinate	0.2	0.19 ± 0.011	0.01 ± 0.001	0.18 ± 0.010	0.08
		0.4	0.23 ± 0.012	0.03 ± 0.003	0.20 ± 0.009	0.15
		0.6	0.27 ± 0.015	0.04 ± 0.003	0.23 ± 0.012	0.19
		0.8	0.31 ± 0.017	0.06 ± 0.005	0.25 ± 0.011	0.24
		1.0	0.35 ± 0.019	0.08 ± 0.006	0.27 ± 0.020	0.28
7.0	Malonate	0.2	0.16 ± 0.007	0.01 ± 0.001	0.14 ± 0.006	0.08
		0.4	0.18 ± 0.009	0.02 ± 0.002	0.16 ± 0.010	0.11
		0.6	0.20 ± 0.010	0.03 ± 0.003	0.17 ± 0.008	0.15
		0.8	0.22 ± 0.007	0.03 ± 0.002	0.19 ± 0.012	0.18
		1.0	0.24 ± 0.012	0.04 ± 0.003	0.20 ± 0.010	0.21

[a] $N = 3$.

Table 3
Apparent first-order rate constants (k_0) for photodegradation of RF in the absence of divalent anions and second-order rate constants (k') for the photochemical interaction of RF and divalent anions.

pH	Divalent anion	$k_0 \times 10^2$ (min^{-1})	$k' \times 10^2$ ($M^{-1}\ min^{-1}$)
6.0	Phosphate	0.33 ± 0.016	0.11 ± 0.008
6.3		0.33 ± 0.015	0.13 ± 0.006
6.5		0.33 ± 0.016	0.14 ± 0.008
6.7		0.33 ± 0.017	0.17 ± 0.010
7.0		0.28 ± 0.012	0.35 ± 0.018
7.3		0.30 ± 0.015	0.19 ± 0.010
7.6		0.30 ± 0.014	0.14 ± 0.006
8.0		0.29 ± 0.016	0.12 ± 0.007
6.0	Sulphate	0.16 ± 0.009	0.57 ± 0.025
6.3		0.24 ± 0.011	0.63 ± 0.038
6.5		0.26 ± 0.014	0.70 ± 0.027
6.7		0.23 ± 0.012	0.99 ± 0.062
7.0		0.15 ± 0.008	6.50 ± 0.310
7.5		0.10 ± 0.005	6.38 ± 0.281
8.0		0.10 ± 0.006	5.95 ± 0.356
7.0	Tartrate	0.15 ± 0.006	0.26 ± 0.012
7.0	Succinate	0.15 ± 0.007	0.20 ± 0.010
7.0	Malonate	0.14 ± 0.005	0.11 ± 0.062

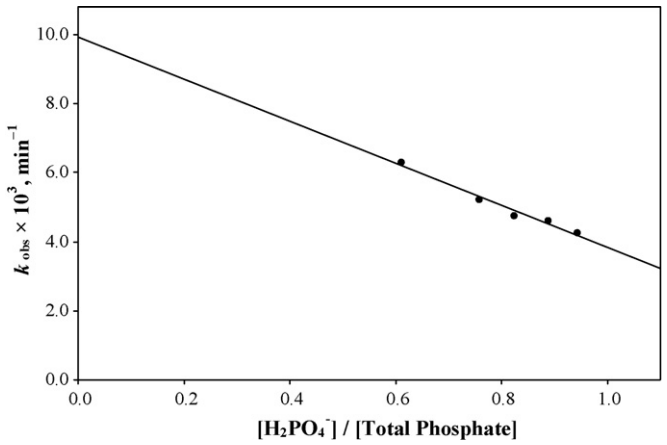


Fig. 3. Plot of k_{obs} for photodegradation of riboflavin versus [$H_2PO_4^-$]/[total phosphate].

HPO_4^{2-} anions appear to influence the photodegradation pathways of RF as suggested earlier (Schuman Jorns et al., 1975; Ahmad et al., 2004a).

All the rate constants determined in this study depend on the sample irradiation conditions which were kept constant during the reactions to avoid any variations. The values of the rates constants are relative and may be used for comparative purposes.

4. Discussion

4.1. Effect of divalent anions

A comparison of the catalytic activity of divalent anions on the formation of CDRF (k_1) and LC (k_2') at pH 7.0 (Table 2) shows that the rates of formation of CDRF and LC are much faster in the presence of sulphate compared to that of phosphate. The kinetic data in terms of the reaction rates imply that in the presence of sulphate CDRF is formed faster than that of the phosphate. However, the product distribution (Table 1) shows that the amount of CDRF formed is nearly the same as that of the phosphate. This is supported by the fact that the fluorescence quenching of RF excited singlet state is also nearly the same for both anions (Table 4). A consideration of the first-order rate constants shows that the k_1/k_2' value at pH 7.0 in the presence of 1.0 M sulphate (0.48) is considerably lower than that of 1.0 M phosphate (0.74) (Table 2), indicating that the selectivity for the formation of CDRF is lower in the presence of sulphate compared to that of the phosphate. This is also evident from the values of LC in product distribution at 50% degra-

Table 4
Fluorescence intensity of 5×10^{-5} M RF solutions in the presence of divalent anions at pH 7.0.

Divalent anion concentration (M)	Relative fluorescence intensity				
	Sulphate	Phosphate	Tartrate	Succinate	Malonate
0	100.0	100.0	100.0	100.0	100.0
0.2	96.0	95.5	95.6	96.5	97.1
0.4	92.2	92.8	93.7	94.8	95.0
0.6	88.9	89.0	91.0	92.3	94.2
0.8	86.7	86.2	90.2	91.7	93.5
1.0	85.1	84.5	89.5	90.5	92.2

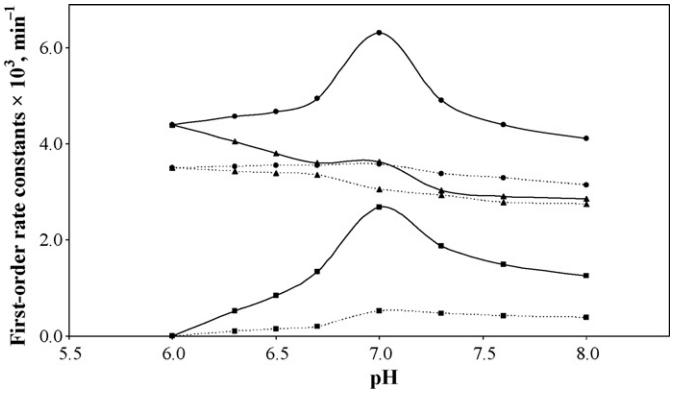


Fig. 4. Plots of k_{obs} (●), k_1 (■) and k_2' (▲) versus pH for photodegradation of riboflavin in the presence of 0.2 M (- - - -) and 1.0 M phosphate (—).

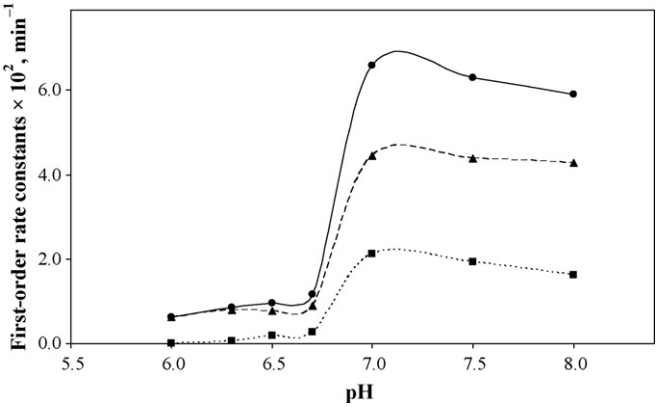


Fig. 5. Plots of k_{obs} (●), k_1 (■) and k_2' (▲) versus pH for photodegradation of riboflavin in the presence of 1.0 M sulphate.

dation of RF (Table 1) in the presence of both anions (41.4:56.6). The much higher rate of interaction between RF and sulphate anions compared to that of RF and phosphate anions (Table 3) suggests some difference in their mode of action. Since at 50% degradation of RF, the LC formation is relatively greater in the case of sulphate anions in spite of much higher rate of degradation, the possibility of LC formation directly through the excited singlet state (Song, 1971) cannot be ruled out. In view of the almost equal fluorescence quenching of RF in the presence of both sulphate and phosphate anions (~15%), the CDRF yield is expected to be nearly the same as evident from the values of product distribution. The normal photolysis pathway for the formation of LC is by photoreduction occurring through the excited triplet state (Schuman Jorns et al., 1975) which leads to the formation of FMF as an intermediate in this reaction (Ahmad and Vaid, 2006). The lower k' values for the tartrate, succinate and malonate catalysed reactions are probably due to their low ionisation and weak complex formation with RF. The variations in the rates of RF and divalent ion interactions (k') may result from the difference in the relative strength and chemical reactivity of the RF–divalent ion complexes to undergo photoaddition reaction. However, this needs further evidence and confirmation.

4.2. Effect of pH

In drug stability studies it is important to establish the effect of pH on degradation kinetics. The catalytic effects of ionic species on the reaction may be evaluated by the determination of rate–pH profiles in the presence and absence of these species. Several types of rate–pH profiles for the degradation of drug substance have been reported (Connors et al., 1986; Carstensen, 2000; Yoshioka and Stella, 2000). The graphs of k_{obs} and k_1 versus pH for the photodegradation of RF and the formation of CDRF, respectively, in the presence of 0.2 and 1.0 M phosphate (Fig. 4) show the maximum rate around pH 7 (neutral pH) for the phosphate catalysed reactions. These rates have been found to increase with the increasing buffer concentration (Table 2). The rate of formation of LC (k_2') is slightly higher at lower pH (6.0–6.75) at 1.0 M phosphate concentration probably due to the catalytic effect of monovalent phosphate anions as suggested earlier (Ahmad et al., 2004a, 2005). The k_1/k_2' ratios at 0.2 and 1.0 M phosphate are 0.17 and 0.78, respectively indicating a prominent catalytic effect of divalent phosphate anions on the formation of CDRF. This rate–pH relationship is in contrast to that observed for the photolysis of RF (photoreduction) in the absence of phosphate species showing a gradual increase in the rate, with pH, in this region (Ahmad et al., 2004b). A graph of k_{obs} for the sulphate catalysed degradation RF at 1.0 M concentration (Fig. 5) shows a slight increase in the rate at pH 6.0–6.7, a greater increase up to pH 7, followed by little change in rate up to pH 8.0. This indicates a greater activity of sulphate anions around pH 7. The graphs of k_1 and k_2' versus pH (Fig. 5) follow the same pattern as that of k_{obs}.

A graph of k' versus pH for the phosphate catalysed reaction (Fig. 6) represents a bell-shaped curve indicating the effect of pH on the rate of reaction. Since the photoaddition pathway leading to the formation of CDRF is catalysed by HPO_4^{2-} anions only (Schuman Jorns et al., 1975), an increase in the ratio of $HPO_4^{2-}/H_2PO_4^-$ (pK_a 7.2) with an increase in pH would cause an increase in the rate of photoaddition. The pH of the maximum rate of reaction appears to be around 7. This is in accordance with the conditions of photoaddition to occur at pH values >6 (Schuman Jorns et al., 1975). The decrease in the rate at pH values below and above 7 may be due to the lack or breakdown of the RF–divalent anion complex in the acid and alkaline regions in favour of the photoreduction reaction as indicated by the values of k_1/k_2' (Table 2).

A graph of k' versus pH for the sulphate catalysed reaction is a sigmoid curve (Fig. 6) and shows a great increase in the rate above pH 6.7 reaching a maximum around pH 7 and then a little decrease in the rate of reaction. This is probably due to the highest rate of interaction of sulphate anions with RF to form CDRF at the neutral pH. However, the rate–pH profile suggests a difference in the mode of action of sulphate compared to that of the phosphate as discussed in Section 4.1.

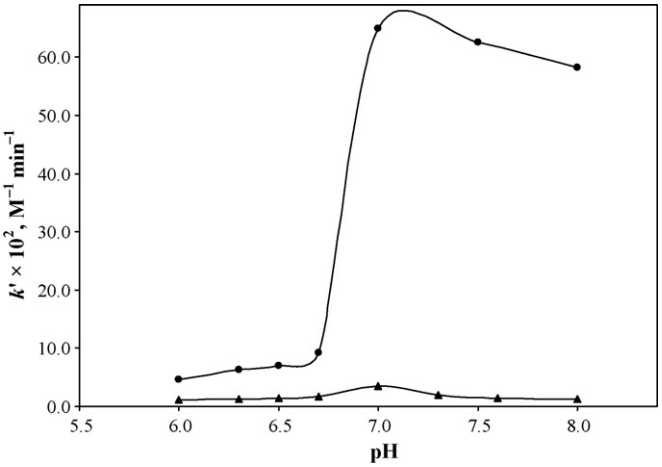


Fig. 6. Plots of k' versus pH for phosphate (▲) and sulphate (●) catalysed photodegradation of riboflavin.

4.3. Effect of fluorescence quenching

The fluorescence of RF is quenched by phosphate anions (Ahmad et al., 2005) as a result of the RF–HPO_4^{2-} complex formation in the ground state (Schuman Jorns et al., 1975). The extent of fluorescence quenching varies with the divalent anion and at 1.0 M concentration is greater (~15%) for phosphate and sulphate anions compared to that of succinate, tartrate and malonate anions (8–10%) (Table 4). The increase in the values of k_1/k_2' (Table 2) is in accordance with the degree of fluorescence quenching by a particular anion indicating the extent of RF–divalent anion interaction leading to photoaddition.

4.4. Interaction of RF and divalent anions

The values of product distribution (Table 1) and kinetic data (Tables 2 and 3) indicate a varying degree of interaction between RF and divalent anions to catalyse the photoaddition reaction. The reaction occurs in the presence of a nucleophile function in the ribityl side chain of RF to form a ground state complex that may have a structure such as >N(10)–CH2–C(OH)R′PO_3^{2-} or containing –SO_3^-. Schuman Jorns et al. (1975) have suggested that the complex in the excited singlet state creates sterically favourable conditions for C(9)/O(2′α) interaction to undergo photoaddition of RF.

The greater interaction of SO_4^{2-} anions appears to be due to the strong electronegative character and abundance of these anions (100%) compared to those of HPO_4^{2-} anions (38%) at pH 7 in 1.0 M solutions. The kinetic studies indicate that the complexes formed with divalent tartrate, succinate and malonate anions are weak and, therefore, have a lower catalytic activity than that of HPO_4^{2-} or SO_4^{2-} anions. This is probably due to the bulky configuration of these anions creating steric hindrance on interaction with RF.

4.5. Photodegradation pathways

Several reviews have been published on the photodegradation pathways of RF including intramolecular photoaddition, photoreduction, and photodealkylation, and intermolecular photoreduction and photooxidation (Song, 1971; Heelis, 1982, 1991; Ahmad and Vaid, 2006). The present study is based on the photodegradation of RF involving simultaneous intramolecular photoaddition and intramolecular photoreduction reactions. The contribution of each pathway to the overall degradation of RF depends on factors such as pH, divalent ion kind and concentration and light intensity/wavelengths (Schuman Jorns et al., 1975; Ahmad and Vaid, 2006; Ahmad et al., 2004a, 2005, 2006a). The two final products, CDRF and LC, are formed by intramolecular photoaddition and intramolecular photoreduction, respectively. The ratio of the two products, CDRF/LC (or k_1/k_2') at pH 7.0, in the presence of various divalent anions (1.0 M) ranges from 0.21 (malonate) to 0.78 (phosphate) (Table 2), suggesting that the interaction of divalent anions with RF may vary in order to create sterically favourable conditions for the photoaddition reaction. This is supported by the fact that the extent of fluorescence quenching of RF depends on the nature and interaction of the divalent anions (Table 4).

It has been suggested (Schuman Jorns et al., 1975) that the photoaddition reaction occurs via the excited singlet state ($^1RF^*$) while the photoreduction is mediated by the excited triplet state ($^3RF^*$). The increase in CDRF/LC ratio (or k_1/k_2') with an increase in divalent ion concentration is indicative of the catalytic effect of these anions (e.g., HPO_4^{2-}) on the rate of RF degradation in favour of photoaddition to give CDRF. It has also been suggested (Ahmad et al., 2004a, 2005) that the monovalent phosphate species is involved in the catalysis of photoreduction reaction leading to LC, as indicated by the increase in k_2' values with an increase in phosphate concentration (Table 2).

The results of the present study have shown that the change in the product distribution and photodegradation pathways of RF is based on the extent of interaction of RF and divalent anions to facilitate photoaddition. The photodegradation of RF around pH 7 in the presence of divalent anions gives maximum yields of CDRF indicating a mechanism that favours the photoaddition pathway at neutral pH. This is in accordance with the description of the reaction (Schuman Jorns et al., 1975) as anion-catalysed neutral photochemistry.

5. Conclusion

The photodegradation of RF in the presence of divalent anions leads to the formation of CDRF and LC as final products by parallel first-order reactions through photoaddition and photoreduction pathways, respectively. The ratios of the two products, CDRF/LC, at pH 7.0 range from 0.21 (malonate) to 0.78 (phosphate) indicating that the divalent anion-catalysed photoaddition reaction occurs in the order: phosphate > sulphate > tartrate > succinate > malonate. The photoreduction (normal photolysis) pathway is deviated in favour of the photoaddition pathway by divalent anions and the extent of deviation depends on the interaction and chemical reactivity of the particular anion. The divalent anions cause quenching of RF fluorescence (8–15%) as a result of the formation of RF–divalent anion complex involved in the formation of CDRF. The rate of photodegradation of RF in the presence of phosphate anions (k_{obs}) is increased more than two fold compared to that observed in the absence of these anions (k_0). The rate–pH profiles for the reactions indicate the maximum activity of the divalent anions around pH 7. The monovalent phosphate anion has been found to catalyse the photoreduction pathway of RF giving rise to LC.

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References

Ahmad, I., Rapson, H.D.C., 1990. Multicomponent spectrophotometric assay of riboflavin and photoproducts. J. Pharm. Biomed. Anal. 8, 217–223.
Ahmad, I., Vaid, F.H.M., 2006. Photochemistry of flavins in aqueous and organic solvents. In: Silva, E., Edwards, A.M. (Eds.), Flavins Photochemistry and Photobiology. Royal Society of Chemistry, Cambridge, pp. 13–40.
Ahmad, I., Fasihullah, Q., Vaid, F.H.M., 2004a. A study of simultaneous photolysis and photoaddition reactions of riboflavin in aqueous solution. J. Photochem. Photobiol. B: Biol. 75, 13–20.
Ahmad, I., Fasihullah, Q., Vaid, F.H.M., 2005. Effect of phosphate buffer on photodegradation reactions of riboflavin in aqueous solution. J. Photochem. Photobiol. B: Biol. 78, 229–234.
Ahmad, I., Fasihullah, Q., Vaid, F.H.M., 2006a. Effect of light intensity and wavelengths on photodegradation reactions of riboflavin in aqueous solution. J. Photochem. Photobiol. B: Biol. 82, 21–27.
Ahmad, I., Fasihullah, Q., Vaid, F.H.M., 2006b. Photolysis of formylmethylflavin in aqueous and organic solvents. Photochem. Photobiol. Sci. 5, 680–685.
Ahmad, I., Ahmed, S., Sheraz, M.A., Vaid, F.H.M., 2008. Effect of borate buffer on the photolysis of riboflavin in aqueous solution. J. Photochem. Photobiol. B: Biol. 93, 82–87.
Ahmad, I., Rapson, H.D.C., Heelis, P.F., Phillips, G.O., 1980. Alkaline hydrolysis of 7,8-dimethyl-10 (formylmethyl) isoalloxazine. A kinetic study. J. Org. Chem. 45, 731–733.
Ahmad, I., Ahmed, S., Sheraz, M.A., Aminuddin, M., Vaid, F.H.M., 2009. Effect of caffeine complexation on the photolysis of riboflavin in aqueous solution: a kinetic study. Chem. Pharm. Bull. 57, 1363–1370.
Ahmad, I., Fasihullah, Q., Noor, A., Ansari, I.A., Ali, Q.N.M., 2004b. Photolysis of riboflavin in aqueous solution: a kinetic study. Int. J. Pharm. 280, 199–208.
Barbieri, Y., Massad, W.A., Díaz, D.J., Sanz, J., Amat-Guerri, F., García, N.A., 2008. Photodegradation of bisphenol A and related compounds under natural-like conditions in the presence of riboflavin kinetics, mechanism photoproducts. Chemosphere 73, 564–571.

Carney, C.F., 1987. Solution stability of ciclosidomine. J. Pharm. Sci. 76, 393–397.
Carstensen, J.T., 2000. In: Carstensen, J.T., Rhodes, C.T. (Eds.), Drug Stability Principles and Practices, 3rd edn. Marcel Dekker, New York, pp. 134–139.
Connors, K.A., Amidon, G.L., Stella, V.J., 1986. Chemical Stability of Pharmaceuticals, 2nd edn. John Wiley & Sons, New York, pp. 43–54, 163–790.
Das Gupta, V., 1983. Stability of triamcinolone acetonide solutions as determined by high-performance liquid chromatography. J. Pharm. Sci. 72, 1453–1456.
DeLuca, P., Boylan, J.C., 1992. In: Avis, K.E., Lieberman, K.A., Lachman, L. (Eds.), Pharmaceutical Dosage Forms: Parenteral Solutions, vol. 2. Marcel Dekker, New York, p. 199.
Escalada, J.P., Gianotti, J., Pajares, A., Massad, W.A., Amat-Guerri, F., García, N.A., 2008. Photodegradation of the acaricide abamectin: a kinetic study. J. Agric. Food Chem. 56, 7355–7359.
Fall, H.H., Petering, H.G., 1956. Metabolic inhibitors. 1. 6,7-Dimethyl-9-formylmethylisoalloxazine, 6,7-dimethyl-9-(2-hydroxyethyl)-isoalloxazine and derivatives. J. Am. Chem. Soc. 78, 377–381.
Florence, A.T., Attwood, D., 2006. Physicochemical Principles of Pharmacy, 4th edn. Pharmaceutical Press, London, pp. 405–413.
Fukumachi, C., Sakurai, Y., 1954. Vitamin B_2 photolysis. V. The photolytic formation of 6,7-dimethylflavin-9-acetic acid ester from riboflavin. Vitamins (Kyoto) 7, 939–943.
Görner, H., 2007. Oxygen uptake after electron transfer from amines, amino acids and ascorbic acid to triplet flavins in air-saturated aqueous solution. J. Photochem. Photobiol. B 87, 73–80.
Ha, D.O., Jeong, M.K., Park, C.U., Park, M.H., Chang, P.S., Lee, J.H., 2009. Effects of riboflavin photosensitization on the degradation of bisphenol A (BPA) in model and real-food systems. J. Food Sci. 74, C380–384.
Hansen, J., Kreilgard, B., Nielsen, O., Veje, J., 1983. Kinetics of degradation of methotrexate in aqueous solution. Int. J. Pharm. 16, 141–152.
Hatchard, C.G., Parker, C.A., 1956. A new sensitive chemical actinometer. II. Potassium ferrioxalate as a standard chemical actinometer. Proc. R. Soc. (Lond.) A 235, 518–536.
Heelis, P.F., 1982. The photophysical and photochemical properties of flavins (isoalloxazines). Chem. Soc. Rev. 11, 15–39.
Heelis, P.F., 1991. The photochemistry of flavins. In: Muller, F. (Ed.), Chemistry and Biochemistry of Flavoenzymes, vol. 1. CRC Press, Boca Raton, FL, pp. 171–193.
Heelis, P.F., Phillips, G.O., Ahmad, I., Rapson, H.D.C., 1980. The photodegradation of formylmethylflavin-a steady state and laser flash photolysis study. Photobiochem. Photobiophys. 1, 125–130.
Hoitink, M.A., Beijnen, J.H., Bult, A., van der Houwen, O.A.G.J., Nijholt, J., Underberg, W.J.M., 1996. Degradation kinetics of gonadorelin in aqueous solution. J. Pharm. Sci. 85, 1053–1059.
Juzeniene, A., Thu Tam, T.T., Iani, V., Moan, J., 2009. 5-Methyltetrahydrofolate can be photodegraded by endogenous photosensitizers. Free Radic. Biol. Med. 47, 1199–1204.
Kino, K., Kobayashi, T., Arima, E., Komori, R., Kobayashi, T., Miyazawa, H., 2009. Photoirradiation products of flavin derivatives, and the effects of photooxidation on guanine. Bioorg. Med. Chem. Lett. 19, 2070–2074.
Massad, W.A., Barbieri, Y., Romero, M., García, N.A., 2008. Vitamin B-sensitized photo-oxidation of dopamine. Photochem. Photobiol. 84, 1201–1208.
Montaña, M.P., Blasich, N., Haggi, E., García, N.A., 2009. Oxygen uptake in the vitamin B-sensitized photo-oxidation of tyrosine and tryptophan in the presence of uracil: kinetics and mechanism. Photochem. Photobiol. 85, 1097–1102.
Poochikian, G.K., Cradock, J.C., Davignon, J.P., 1983. Heroin: stability and formulation approaches. Int. J. Pharm. 13, 219–226.
Powell, M.F., 1986. Enhanced stability of codeine sulfate: effect of pH, buffer, and temperature on the degradation of codeine in aqueous solution. J. Pharm. Sci. 75, 901–903.
Powers, H.J., 2003. Riboflavin and health. Am. J. Clin. Nutr. 77, 1352–1360.
Pramar, Y., Das Gupta, V., 1991. Preformulation studies of spironolactone: effect of pH, two buffer species, ionic strength, and temperature on stability. J. Pharm. Sci. 80, 551–553.
Rivlin, R.S., 1973. Riboflavin and cancer: a review. Cancer Res. 33, 1977–1986.
Rivlin, R.S., 2007. In: Zempleni, J., Rucker, R.B., McCormick, D.B., Suttie, J.W. (Eds.), Handbook of Vitamins, 4th edn. CRC Press, Boca Raton, FL, pp. 233–251.
Schuman Jorns, M., Schollnhammer, G., Hemmerich, P., 1975. Intramolecular addition of the riboflavin side chain. Anion-catalysed neutral photochemistry. Eur. J. Biochem. 57, 35–48.
Sinko, P.J., 2006. Martin's Physical Pharmacy and Pharmaceutical Sciences, 5th edn. Lippincott Williams & Wilkins, Baltimore, pp. 270–277.
Song, P.S., 1971. In: Kamin, H. (Ed.), Flavins and Flavoproteins. University Park Press, Baltimore, pp. 37–61.
Song, P.S., Metzler, D.E., 1967. Photochemical degradation of flavins. IV. Studies of the anaerobic photolysis of riboflavin. Photochem. Photobiol. 6, 691–709.
Suelter, C.H., Metzler, D.E., 1960. The oxidation of a reduced pyridine nucleotide analog by flavins. Biochim. Biophys. Acta 44, 23–33.
Tsuji, A., Nakashima, E., Deguchi, Y., Nishide, K., Shimizu, T., Horiuchi, S., Ishikawa, K., Yamana, T., 1981. Degradation kinetics and mechanism of aminocephalosporins in aqueous solution: cefadroxil. J. Pharm. Sci. 70, 1120–1128.
Underberg, W.J.M., Lingeman, H., 1983. Determination of pK_a values of some prototropic functions in mitomycin and porfiromycin. J. Pharm. Sci. 72, 553–556.
Varia, S.A., Schuller, S., Stella, V.J., 1984. Phenytoin prodrugs IV: hydrolysis of various 3-(hydroxymethyl)phenytoin esters. J. Pharm. Sci. 73, 1074–1080.
Webster, R.P., Gawde, M.D., Bhattacharya, R.K., 1996. Modulation of carcinogen-induced DNA damage and repair enzyme activity by dietary riboflavin. Cancer Lett. 98, 129–135.
Yoshioka, S., Stella, V.J., 2000. Stability of Drugs and Dosage Forms. Kluwer Academic/Plenum Publishers, New York, pp. 126–133.
Zhang, Y., Görner, H., 2009. Flavin-sensitized photo-oxidation of lysozyme and serum albumin. Photochem. Photobiol. 85, 943–948.
Zhao, X., Zhang, Y., Hu, X., Hwang, H.M., 2007. Enhanced bio-mineralization by riboflavin photosensitization and its significance to detoxification of benzo[a]pyrene. Bull. Environ. Contam. Toxicol. 79, 319–322.