CSIRO PUBLISHING

Aust. J. Chem.
http://dx.doi.org/10.1071/CH12457

Full Paper

Effect of pH, Buffer, and Viscosity on the Photolysis of Formylmethylflavin: A Kinetic Study

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The kinetics of the photolysis of formylmethylflavin, a major intermediate product in the aerobic and anaerobic photolysis of riboflavin, was studied in the pH range 2.0–11.0. Formylmethylflavin and its photoproducts, lumichrome and lumiflavin, were determined in degraded solutions using a specific multicomponent spectrophotometric method. The photolysis of formylmethylflavin in alkaline medium takes place by first-order kinetics and the rate constants (k_{obs}) at pH 7.5–11.0 range from 0.27×10^{-4} to 3.88×10^{-4} and 0.36×10^{-4} to $5.63 \times 10^{-4}\,s^{-1}$ under aerobic and anaerobic conditions respectively. In acid medium, the photolysis involves a second-order mechanism and the rate constants at pH 2.0–7.0 range from 1.37 to 2.11 and 2.03 to $2.94\,M^{-1}\,s^{-1}$ under aerobic and anaerobic conditions respectively. The rate–pH profiles for the photolysis reactions indicate the highest rate of formylmethylflavin degradation is at ~pH 4 and above pH 10. In the alkaline region, the increase in rate with pH is due to higher reactivity of the flavin triplet state. The photolysis of formylmethylflavin is catalyzed by phosphate ions and is affected by the solvent viscosity.

Manuscript received: 7 October 2012.
Manuscript accepted: 29 January 2013.
Published online: 22 February 2013.

Introduction

Formylmethylflavin (2-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)ethanone, FMF), was isolated by Smith and Metzler[1] and identified as a major intermediate product in the photolysis of riboflavin (RF, vitamin B_2). It is degraded by hydrolysis,[2,3] thermolysis,[4] and photolysis[5–12] to lumichrome (LC), lumiflavin (LF), and carboxymethylflavin (CMF) in aqueous and organic solvents.[1–14] The kinetics of formation of FMF on the photodegradation of RF, followed by its disappearance in aqueous solution, have been studied using a specific multicomponent spectrophotometric method.[9,15–21] The mechanism of the photochemical formation of FMF from RF and its degradation to further photoproducts has been discussed by several workers.[2,22–26]

FMF is more sensitive to light than RF and its degradation is affected by pH in acid and alkaline media.[5–8] The photolysis of FMF in aqueous solution is a controlling factor in the photodegradation sequence of RF and is important to understanding the mode of RF degradation leading to the formation of the final products (LC, LF, CMF).

RF is the prosthetic group of flavoenzymes involved in biological redox reactions. It is an essential component of vitamin preparations and its fate on photodegradation involves the participation of FMF and ultimately its loss during the reaction. This may be affected by factors such as pH, oxygen content, and light intensity. The present work involves a study of the kinetics of FMF photolysis under aerobic and anaerobic conditions over a wide range of pH and in organic solvents using a low-intensity visible irradiation source. It would throw light on the extent of the involvement of FMF in the kinetics of RF degradation by different pathways and whether the reaction is catalyzed by phosphate buffer, as in the case of RF,[17] and is affected by solvent viscosity, in addition to solvent polarity.[11,27] The chemical structures of RF, FMF, and photoproducts (LC, LF, and CMF) are shown in Fig. 1.

Results and Discussion

Characterization and Assay of FMF and Photoproducts

FMF and its photoproducts (LC, LF, and CMF) were isolated from an anaerobically photolyzed solution of RF using cellulose column chromatography (Whatman CC 31) and solvent system (b) described in the Experimental section under *Thin-Layer Chromatography*. These compounds were characterized by chromatographic (solvent systems (a) and (b)) and spectroscopic techniques[28] as follows:

FMF: R_f 0.69 (a), 0.70 (b). λ_{max}/nm (log ε) (pH 7.0) 445 (4.023), 376 (3.996), 266 (4.450); fluorescence (pH 7.0) λ_{ex}/nm 460, λ_{em}/nm 528. ν_{max} (KCl)/cm^{-1} 3350 (–NH), 1705, 1660 (C=O), 1580 (C=C), 1545 (C=N). ^{1}HNMR (CF_3COOH): τ, 7.20

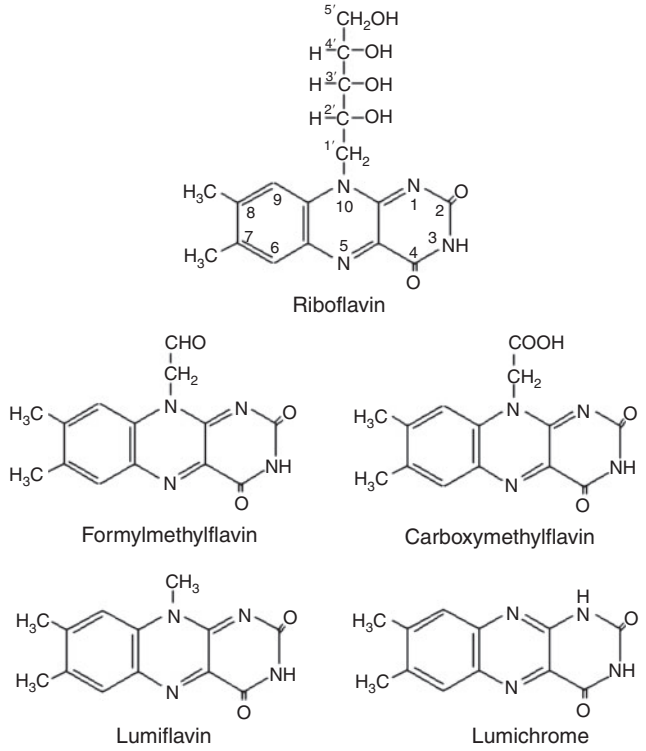

Fig. 1. Chemical structures of riboflavin, formylmethylflavin and photoproducts.

(s, CH_3), 7.09 (s,CH_3), 4.42(d, N–CH_2), 1.97 (s, Ar–H). *m/z* 284 [M^+] ($C_{14}H_{12}O_3N_4$).

LC: R_f 0.66 (a), 0.63 (b). λ_{max}/nm (log ε) (pH 7.0) 356 (3.929), 262 (4.435); fluorescence (pH 7.0) λ_{ex}/nm 368, λ_{em}/nm 478. ν_{max} (KCl)/cm^{-1} 3380 (–NH), 1720, 1695 (C=O), 1575 (C=C), 1560 (C=N). ^{1}HNMR (CF_3COOH): τ, 7.31 (s, CH_3), 7.26 (s,CH_3), 1.80 (s, Ar–H). *m/z* 242 [M^+] ($C_{12}H_{10}O_2N_4$).

LF: R_f 0.53 (a), 0.41 (b). λ_{max}/nm (log ε) (pH 7.0) 445 (3.914), 370 (3.806), 263 (4.354); fluorescence (pH 7.0) λ_{ex}/nm 460, λ_{em}/nm 528. ν_{max} (KCl)/cm^{-1} 3350 (–NH), 1705, 1660 (C=O), 1575 (C=C), 1560 (C=N). ^{1}HNMR (CF_3COOH): τ, 7.32 (s, CH_3), 7.19 (s,CH_3), 5.48 (d, NCH_3), 1.80 (s, Ar–H). *m/z* 256 [M^+] ($C_{13}H_{12}O_2N_4$).

CMF: R_f 0.38 (a), 0.20 (b). λ_{max}/nm (log ε) (pH 7.0) 445 (4.008), 376 (3.981), 266 (4.435); fluorescence (pH 7.0) λ_{ex}/nm 460, λ_{em}/nm 528.

FMF is photodegraded under aerobic and anaerobic conditions to LC in acid medium and to LC and LF in alkaline medium as detected by TLC. These compounds exhibit characteristic fluorescence emission (FMF, LF, yellow-green; LC, sky blue) and were identified by comparison of their R_f values with those of the reference standards. Minor amounts of CMF (yellow-green fluorescence), as an oxidation product of FMF under aerobic conditions, were also detected in acid and alkaline medium. LC, LF, and CMF have previously being reported as photoproducts of FMF.[6,9,11]

FMF, LC, and LF were determined in degraded solutions during photolysis by the multicomponent spectrophotometric method of Ahmad and Rapson.[9] This method has been applied to study the hydrolysis,[3] thermolysis,[4] and photolysis[8,9,11] of FMF in aqueous and organic solvents. The results of a typical assay of FMF and photoproducts (LC and LF) during the reaction at pH 9.0 are reported in Table 1. The values of the molar balance

Table 1. Photolysis of 10^{-4} M formylmethylflavin (FMF) solution at pH 9.0 and concentrations of FMF and photoproducts

Experimental conditions: wavelength, visible radiation; exposure time, 1 h; temperature, 25 ± 1°C

Time [min]	FMF [M × 10^5]	LC [M × 10^5]	LF [M × 10^5]	Total [M × 10^5]
0	10.00	–	–	10.00
10	8.55	0.23	1.20	9.98
20	7.40	0.41	2.29	10.10
30	6.34	0.61	3.12	10.07
40	5.25	0.74	3.99	9.98
60	3.78	0.93	5.26	9.97

obtained during the reaction are in good agreement with the initial concentration of FMF based on mole-to-mole conversion. The accuracy of the analytical data is an important factor in the evaluation of the kinetics of these reactions.

CMF, a minor photoproduct, could not be accounted for in the assay scheme. It was, therefore, separated by TLC of the photolyzed solutions (R_f 0.38, solvent system (a)), extracted with phosphate buffer (pH 7.0), concentrated under reduced pressure and determined spectrophotometrically at 445 nm using 10200 M^{-1} cm^{-1} as the value of molar absorptivity. At 50 % photolysis of FMF (i.e. 5×10^{-5} M), the concentration of CMF in aerobically photolyzed solutions at pH 2–7 was found to be ~$0.1–0.2 \times 10^{-6}$ M and at pH 8–10, ~$0.2–0.4 \times 10^{-6}$ M. The value was slightly higher, i.e. 0.5×10^{-6} M at pH 11.0, indicating that the reaction is facilitated in alkaline medium.

Spectral Characteristics of Photolyzed Solutions of FMF

FMF (in the protonated state) exhibits an absorption maximum at 385 nm at pH 2.0 (aqueous layer),[9] and the loss of absorption at this wavelength, with time, is an indication of the photodegradation of FMF in the reaction. The photoproducts (LC and LF) were extracted in chloroform and do not interfere with the absorption of FMF. The absorbance values of FMF at 385 nm during photolysis at various pH values were used to determine the rates of reaction.

Kinetics of Photolysis

The kinetics of photolysis of FMF in acid and alkaline media were studied. The rate constants reported were determined under constant irradiation conditions to avoid any variation in the results. The values of the rate constants are relative and may be used for comparative purposes.

The hydrolysis and photolysis of FMF in alkaline solution follows apparent first-order kinetics[3] and can be represented by Eqn 1.

$$\text{FMF} \xrightarrow{k_1} \text{LC} \qquad \text{FMF} \xrightarrow{k_2} \text{LF} \tag{1}$$

The differential equations for the reactant and the products are:

$$\frac{-\mathrm{d}\,[\text{FMF}]}{\mathrm{d}t} = k_1\,[\text{FMF}] + k_2\,[\text{FMF}] \tag{2}$$

$$\frac{\mathrm{d}\,[\text{LC}]}{\mathrm{d}t} = k_1\,[\text{FMF}] \tag{3}$$

Table 2. Apparent first-order rate constants for aerobic photolysis of formylmethylflavin (FMF) (k_{obs}), for the formation of lumichrome (LC) (k_1) and lumiflavin (LF) (k_2) in alkaline solution and second-order rate constants for the photolysis of FMF (k') in acid solution

Experimental conditions: initial concentration of FMF, 10^{-4} M; wavelength, visible radiation; exposure time, 1 h; temperature, 25 ± 1°C

pH	$k_{obs} \times 10^4$ [s^{-1}] ± s.d.[A]	$k_1 \times 10^4$ [s^{-1}] ± s.d.[A]	$k_2 \times 10^4$ [s^{-1}] ± s.d.[A]	k' [$M^{-1}\,s^{-1}$] ± s.d.[A]
2.0	–	–	–	1.37 ± 0.056
3.0	–	–	–	1.70 ± 0.067
4.0	–	–	–	2.11 ± 0.066
5.0	–	–	–	1.84 ± 0.055
6.0	–	–	–	1.73 ± 0.047
7.0	–	–	–	1.69 ± 0.071
7.5	0.27 ± 0.017	0.23 ± 0.014	0.03 ± 0.001	–
8.0	0.36 ± 0.022	0.32 ± 0.018	0.05 ± 0.002	–
9.0	1.13 ± 0.038	0.95 ± 0.033	0.17 ± 0.010	–
10.0	2.72 ± 0.091	2.01 ± 0.081	0.70 ± 0.026	–
11.0	3.88 ± 0.108	2.43 ± 0.087	1.45 ± 0.058	–

[A] $n = 3$.

Table 3. Apparent first-order rate constants for anaerobic photolysis of formylmethylflavin (FMF) (k_{obs}), for the formation of lumichrome (LC) (k_1) and lumiflavin (LF) (k_2) in alkaline solution and second-order rate constants for the photolysis of FMF (k') in acid solution

Experimental conditions are as in Table 2

pH	$k_{obs} \times 10^4$ [s^{-1}] ± s.d.[A]	$k_1 \times 10^4$ [s^{-1}] ± s.d.[A]	$k_2 \times 10^4$ [s^{-1}] ± s.d.[A]	k' [$M^{-1}\,s^{-1}$] ± s.d.[A]
2.0	–	–	–	2.03 ± 0.081
3.0	–	–	–	2.41 ± 0.075
4.0	–	–	–	2.94 ± 0.097
5.0	–	–	–	2.61 ± 0.071
6.0	–	–	–	2.44 ± 0.080
7.0	–	–	–	2.39 ± 0.079
7.5	0.36 ± 0.019	0.33 ± 0.024	0.03 ± 0.001	–
8.0	0.55 ± 0.030	0.48 ± 0.031	0.07 ± 0.002	–
9.0	1.73 ± 0.058	1.46 ± 0.064	0.26 ± 0.013	–
10.0	4.08 ± 0.109	3.02 ± 0.128	1.08 ± 0.051	–
11.0	5.63 ± 0.158	3.51 ± 0.133	2.10 ± 0.108	–

[A] $n = 3$.

$$\frac{\mathrm{d}\,[\mathrm{LF}]}{\mathrm{d}t} = k_2\,[\mathrm{FMF}] \tag{4}$$

The values of k_1 and k_2 for the parallel first-order reactions leading to the formation of LC and LF respectively were determined according to the method of Ahmad et al.[15]

In acid solution, FMF undergoes photolysis and thermolysis by second-order kinetics[4,11] as follows:

$$2\ \mathrm{FMF} \xrightarrow{h\nu,\ k'} \mathrm{LC} \tag{5}$$

The differential equations for the reactant and the product are:

$$\frac{-\mathrm{d}\,[\mathrm{FMF}]}{\mathrm{d}t} = k'\,[\mathrm{FMF}]^2 \tag{6}$$

$$\frac{\mathrm{d}\,[\mathrm{LC}]}{\mathrm{d}t} = k'\,[\mathrm{FMF}]^2 \tag{7}$$

The oxidation of flavin semiquinone radicals formed in this reaction involves a bimolecular mechanism leading to the final product.[11,25] The same mode of photolysis of FMF was observed in the present study and the rate constants for the reactions carried out in acid and alkaline media under aerobic and anaerobic conditions are reported in Tables 2 and 3 respectively. A difference in the rates of photolysis (k_{obs}) was observed under aerobic and anaerobic conditions. As reported in the photolysis of RF,[29] the rates under anaerobic conditions are higher (~1 1/2 times) than those under aerobic conditions. This may be due to the quenching of the flavin singlet state [^{1}FL*] and the flavin triplet state [^{3}FL*][7] under aerobic conditions. This would lead to a decrease in the concentration of the flavin triplet state [^{3}FL*] and hence a lower rate of photolysis of FMF compared with that under anaerobic conditions. The mechanism of these reactions has been discussed by Heelis,[25] Ahmad and Vaid,[26] and Ahmad et al.[11]

The degradation of FMF in the dark at pH 2.0–11.0 at 25 ± 1°C was also studied to determine its contribution in the photolysis process. At the exposure times taken for 50% photolysis of FMF ($t_{1/2}$), the percentage of FMF degraded during the same period in the dark increased with pH and is given in Table 4.

Table 4. Degradation of 10^{-4} M formylmethylflavin (FMF) at 25 ± 1°C in the dark during the exposure times ($t_{1/2}$) required for 50 % photolysis of FMF (aerobic) at pH 2.0–11.0
First-order reactions at pH 8.0–11.0; second-order reactions at pH 2.0–7.0

pH	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0
% FMF degraded (in dark)	0.3	0.8	3.6	6.2	9.1	14.7	19.3	25.2	31.1	36.9
$t_{1/2}$ [min] (in light)	122	98	79	91	96	99	315	102	42.5	29.7

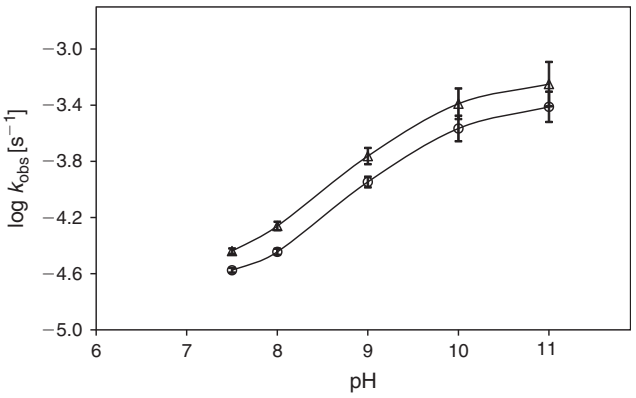

Fig. 2. Log *k*–pH profiles for the photolysis of formylmethylflavin (FMF) in alkaline solution under aerobic (○) and anaerobic (△) conditions.

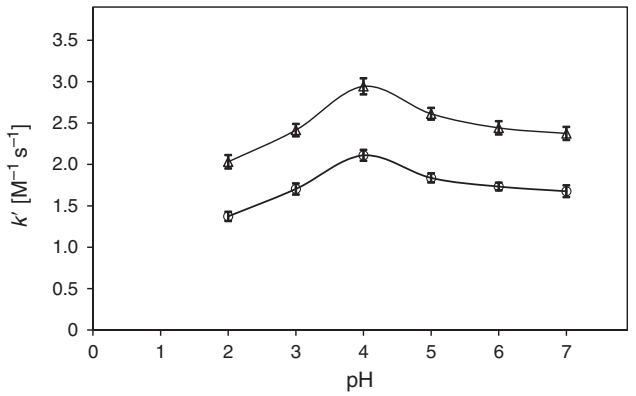

Fig. 3. *k*′–pH profiles for the photolysis of formylmethylflavin (FMF) in acidic solution under aerobic (○) and anaerobic (△) conditions.

Effect of pH

The photolysis of FMF is markedly affected by the pH of the solution, which is a controlling factor in the kinetics of the photodegradation of RF.[7,16] The *k* versus pH profiles for the photolysis of FMF under aerobic and anaerobic conditions in alkaline and acidic solutions are shown in Figs 2 and 3 respectively. As the photolysis of FMF in alkaline medium takes place by hydrolytic degradation,[2,3] an increase in the rate with pH was observed in the range 7.5–11.0. The reduced enhancement in the rate constant above pH 10.0 may be due to the ionization of the molecule to form an anion. The pK_a of RF, which possesses the same isoalloxazine nucleus as that of FMF (N–3), is 10.2,[30] and as the ionized form of RF is less susceptible to photolysis,[16] the ionized form of FMF also appears to have low susceptibility to photolysis above pH 10.0. A 15-fold increase in the rate of photolysis on increasing the pH from 7.5 to 11.0 (Tables 2 and 3) is probably due to a change in the reactivity of ^{3}FL*. The observed increase in rate constants with increasing pH could also be accounted for by the higher rate of hydrolysis of ground-state FMF at 25°C at high pH. The unprotonated form of ^{3}FL* (pK_a 6.5) is more reactive than the protonated form,[7] leading to a higher rate of FMF photolysis in the alkaline region. It has also been suggested that the higher rate of photolysis of flavins at high pH values results from both the high pK_a and increased reactivity of ^{3}FL*, which exists in a bent, diradical form in this range.[7]

The photolysis of FMF in the acid range results from a bimolecular reaction and is affected by the protonation of FMF (pK_a 3.5).[31] The protonated form of FMF (97 %, pH 2.0) is non-fluorescent owing to the quenching of ^{1}FL* and is resistant to photolysis. This is evident from the decrease in rate with a decrease in pH below 4. In the pH range 5–6, the redox potentials of RF are at their lowest,[32] hence the lower rates of reaction in this region.[16] This also applies to FMF, which possesses the same nucleus as that of RF and undergoes similar redox reactions. This is supported by the fact that the pK_a of the reduced form of FMF is 6.1,[33] which results in a decrease in the rate of photolysis in the pH range 5–6.

The ratios of k_2 to k_1 (Tables 2 and 3) increase with pH in the region 7.5–11.0, indicating that the formation of LF increases with pH. LF formation results from the alkaline hydrolysis of the side chain of a ground-state FMF molecule according to the mechanism suggested by Song et al.[2] The formation of LC also takes place in alkaline medium by cleavage of the side chain on the addition of a water molecule, leading to the formation of an isoalloxazine ring and glycolaldehyde. This is followed by the rearrangement of the isoalloxazine ring to alloxazine, the nucleus present in LC. However, the formation of LC in acid medium results from a bimolecular mechanism as discussed above in this section.

The photolytic degradation of FMF in both acid and alkaline solutions is different from that of RF, which follows first-order kinetics in the entire pH range of 2.0–12.0.[16] RF photolysis leading to LC and LF takes place through the formation of FMF as an intermediate in this reaction.[1] Therefore, the role of FMF in the photodegradation sequence of RF is an important factor in understanding the photolytic behaviour of RF in chemical, biological, and pharmaceutical systems.

Effect of Phosphate Buffer

Phosphate buffer is known to catalyze the photolysis of RF. The rate of photolysis of RF has been found to be proportional to the buffer concentration.[34] Several studies have been conducted on the effect of phosphate buffer on the photodegradation of RF and the rate constants for $H_2PO_4^-$ and HPO_4^{2-} ion-catalyzed reactions have been determined.[15,17,18] In order to observe the effect of phosphate species on the rate of photolysis of FMF, reactions were carried out at pH 7.0. At this pH value, FMF undergoes second-order kinetics[11] and the rate constant is given in Table 5. It was found that the phosphate species catalyze the photodegradation of FMF in aqueous solution. A graph

Table 5. Second-order rate constant for the aerobic photolysis of formylmethylflavin (FMF) (k') at pH 7.0 in the presence of phosphate buffer (0.2–1.0 M)

Experimental conditions are as in Table 2

Buffer concentration [M]	k' [$M^{-1}s^{-1}$] ± s.d.
0.00	2.39 ± 0.079
0.25	5.50 ± 0.175
0.50	9.70 ± 0.272
0.75	12.48 ± 0.312
1.00	15.28 ± 0.367

Table 6. Second-order rate constants (k_{obs}) for the anaerobic photolysis of formylmethylflavin (FMF) in organic solvents

Experimental conditions are as in Table 2

Solvent	Viscosity^{-1} [mPa s]$^{-1}$	k' [$M^{-1}s^{-1}$] ± s.d.
Water, pH 7.0	1.000	2.39 ± 0.079
Acetonitrile	2.898	1.50 ± 0.073
Methanol	1.838	1.25 ± 0.055
Ethanol	0.931	1.16 ± 0.051
1-Propanol	0.514	1.02 ± 0.036
1-Butanol	0.393	0.98 ± 0.039
Dichloroethane	1.193	0.95 ± 0.042

of second-order rate constants (k') versus phosphate concentration is linear ($R^2 = 0.998$), indicating that the phosphate species exert a catalytic effect on the photolysis of FMF.

The effect of phosphate buffer on the reaction may also be explained based on fluorescence studies. A decrease in the fluorescence intensity of FMF solutions in the presence of 0–1.0 M phosphate concentration (100–88 % decrease) indicated an interaction between FMF and the phosphate ions similar to that observed in the case of the RF–HPO_4^{2-} complex leading to the formation of cyclodehydroriboflavin (CDRF) and the RF–$H_2PO_4^-$ complex forming LC [17]. This complexation caused ~12 % quenching of the excited singlet state [^{1}FMF]. As the RF–HPO_4^{2-} complex can only form CDRF by photoaddition, which is not possible in the case of FMF, which lacks a ribose side chain, the FMF–phosphate complex could form LC by photoreduction as suggested previously for the photoreduction pathway of RF.[17] In view of the fact that phosphate ions exert a catalytic effect on the photolysis of FMF, there is a strong possibility that the $H_2PO_4^-$ and/or HPO_4^{2-} ions are involved in the photolysis reaction.

Effect of Solvent Viscosity

The solvent may exert a significant effect on the rate of a chemical reaction.[35–37] The dependence of the rate of photolysis of FMF on solvent dielectric constant has been reported.[11] In the present study, an attempt was made to correlate the rate of photolysis with solvent viscosity (Table 6). A graph of rate constants versus the inverse of solvent viscosity was found to be linear ($R^2 = 0.995$). It appears that the rate of reaction increases linearly with the inverse of solvent viscosity. A similar observation was made by Ahmad and Tollin[27] for the dependence of flavin triplet state quenching on solvent viscosity. The rate constants for diffusion-controlled processes have been reported as a function of solvent viscosity.[38] Thus, the rate of reaction would be affected by the solute diffusion processes and, therefore, the degree of redox reactions undergone by the molecule in a particular solvent.[11] A similar effect of viscosity on the rate of photolysis of ascorbic acid[39] and levofloxacin[40] in organic solvents has been observed.

Mode of RF and FMF Photolysis

RF is photodegraded in aqueous solution by intramolecular photoreduction[7,22,25,26] as follows:

$$RF \xrightarrow{h\nu} {}^1RF^*_{ox} \quad (8)$$

$${}^1RF^*_{ox} \xrightarrow{\text{intersystem crossing}} {}^3RF^*_{ox} \quad (9)$$

$${}^3RF^*_{ox} + RF_{ox} \longrightarrow RFH\bullet + RF_{ox}\bullet \quad (10)$$

$$2RFH\bullet \longrightarrow RF_{ox} + RFH_2 \text{ (cylic intermediate)} \quad (11)$$

$$RFH_2 \xrightarrow{O_2} FMF + \text{side chain products} \quad (12)$$

In the above reaction scheme, RF is excited to the excited singlet [$^1RF^*_{ox}$] and excited triplet [$^3RF^*_{ox}$] states. This is followed by the conversion of [$^3RF^*_{ox}$] to the semiquinone radical [$RFH^\bullet$]. The semiquinone radicals disproportionate to form an oxidized RF and a reduced RFH_2 cyclic intermediate, which is oxidized to FMF and side chain products.

The role of FMF as an intermediate product in the photolysis of RF is well established.[1,5,7,9,16] It is photolyzed to LC in acidic solutions and to LC and LF in alkaline solutions under aerobic and anaerobic conditions directly[2,3,6–8,10–13] or through the photolysis of RF.[1,5–7,9,13] FMF may lead to the formation of LC in acidic solutions by a bimolecular photoredox reaction involving an excited triplet state [^{3}FMF*] and a ground state [FMF] molecule as suggested earlier for flavins.[11,25–27]

$${}^3FMF + FMF \longrightarrow FMF\bullet^- + FMF\bullet^+ \quad (13)$$

$$FMF\bullet^- \xrightarrow{H^+} FMFH\bullet \quad (14)$$

$$FMF\bullet^+ \xrightarrow{-H^+} FMF\bullet \quad (15)$$

$$2\ FMFH\bullet \longrightarrow FMF + FMFH_2 \quad (16)$$

$$FMF\bullet + FMF \longrightarrow FMFH\bullet + LC \quad (17)$$

The reaction of a triplet [^{3}FMF*] with a ground state [FMF] molecule leads to the formation of an anionic and a cationic FMF radical. These radicals may accept or lose a proton to form a reduced or an oxidized radical. The reduced FMF radicals may lead to the formation of an oxidized and a reduced FMF molecule. The oxidized FMF radical may react with a ground state FMF molecule to give rise to a reduced FMF radical and LC.

FMF is photolyzed in alkaline solutions by hydrolytic degradation.[2,3] The increase in rate with pH is due to the higher reactivity and a change in the configuration of ^{3}FMF.[7]

$${}^3FMF \xrightarrow{OH^-} LF + HCOO^- \quad (18)$$

$${}^3FMF + H_2O \xrightarrow{H^+} LC + OHCCH_2OH \quad (19)$$

Thus FMF on photolysis in aqueous solutions forms both LC and LF by different mechanisms.

Conclusion

The photolysis of FMF follows first-order kinetics in alkaline medium and second-order kinetics in acid medium both under aerobic and anaerobic conditions. The *k*–pH profiles for these reactions indicate the maximum rate of reaction at ~pH 11 and 4. The rates are higher under anaerobic conditions owing to the existence of a greater number of flavin singlet states and are affected by the ionization of the molecule. The ionized species of FMF, like those of RF, are less susceptible to photolysis both in acid and alkaline media. The increase in rate in the pH range 7.5–11.0 is due to greater reactivity of the flavin triplet state to base hydrolysis. The lower rate in the pH range 5–6 is probably due to the molecule having its lowest redox potentials in this region. FMF on photolysis undergoes a bimolecular reaction in acid media to produce LC and a unimolecular reaction in alkaline media to form LC and LF. The photodegradation of RF in aqueous solution is dependent on the photolytic behaviour of FMF as an intermediate product in this reaction. Phosphate ions exert a catalytic effect on the photolysis of FMF in aqueous solution. The rates of FMF photolysis are inversely proportional to the solvent viscosity.

Experimental

LC and LF were obtained from Sigma Chemical Co. FMF and CMF were synthesized by the method of Fall and Petering[41] and Fukumachi and Sakurai[42] respectively. All reagents and solvents were of the purest form available from BDH and Merck. The following buffer systems were used throughout the photolysis reactions: KCl/HCl, pH 2.0; citric acid/Na_2HPO_4, pH 2.5–8.0; $Na_2B_4O_7$/HCl, pH 8.5–9.0; $Na_2B_4O_7$/NaOH, pH 9.5–11.0; the ionic strength was 0.02 M in each case.

Precautions

The experimental work was carried out in a dark chamber under subdued light. FMF solutions were protected from light at all times before irradiation and during the various procedures. The solutions were freshly prepared for each experiment to avoid degradation.

Photolysis

A 10^{-4} M aqueous solution of FMF (pH 2.0–11.0) was placed in a 100-mL volumetric flask (Pyrex), deoxygenated by bubbling with nitrogen for 1 h and irradiated with a Phillips 25-W fluorescent lamp (emission in visible region) fixed horizontally at a distance of 30 cm from the centre of the flask. Nitrogen was continuously bubbled through the solution during irradiation. The temperature of the solution was maintained at 25 ± 1°C using a constant-temperature water bath. The procedure was repeated under aerobic conditions by bubbling air through the solution during irradiation to maintain the solution in free equilibrium with oxygen. The irradiation was carried out for a period of 1–5 h depending on the pH of the solution. Samples of the irradiated solution were withdrawn at appropriate intervals for thin-layer chromatography and spectrophotometric assay.

Thin-layer Chromatography

Thin-layer chromatography (TLC) for the separation and identification of FMF and its photoproducts was carried out on 250-µm cellulose plates (Whatman CC 41). The following solvent systems were used: (a) 1-butanol/acetic acid/water (40 : 10 : 50 v/v, organic phase); and (b) 1-butanol/propanol/acetic acid/water (50 : 30 : 2 : 18 v/v).[3] FMF and its photoproducts were detected by their characteristic fluorescence emission under UV (365 nm) excitation using a Uvitech lamp (Cambridge, UK).

Spectral Determinations

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

Light Intensity Measurement

The intensity of the Phillips 25-W fluorescent lamp was determined using potassium ferrioxalate actinometry[43] and a value of $4.52 \pm 0.15 \times 10^{16}$ quanta s^{-1} was obtained.

Spectrophotometric Assay

A multicomponent spectrophotometric assay method was used for the determination of FMF and its major photoproducts, LC and LF.[9] The method involved pre-adjustment of the photolyzed solution to pH 2.0 (HCl/KCl buffer) and extraction with chloroform to remove LC and LF, followed by their determination at pH 4.5 (acetate buffer) by a two-component assay at 445 and 356 nm. The aqueous phase (pH 2.0) was used to determine FMF concentrations at 385 nm. It is stable in its protonated form (pK_a 3.5)[31] and is not extractable into chloroform.

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