Effect of Caffeine Complexation on the Photolysis of Riboflavin in Aqueous Solution: A Kinetic Study

Iqbal AHMAD,[a] Sofia AHMED,[a] Muhammad Ali SHERAZ,*[,a] Muhammad AMINUDDIN,[a] and Faiyaz Hussain Madni VAID[b]

[a] *Institute of Pharmaceutical Sciences, Baqai Medical University; Toll Plaza, Super Highway, Gadap Road, Karachi–74600, Pakistan: and* [b] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi; Karachi–75270, Pakistan.* Received June 6, 2009; accepted July 21, 2009; published online September 14, 2009

The effect of caffeine complexation with riboflavin on the kinetics of riboflavin photolysis in the pH range 2.0—10.5 has been studied. The photolysis of riboflavin solutions (5×10^{-5} M) was carried out in the presence of caffeine ($0.5—2.5\times10^{-4}$ M) using a visible radiation source. A specific multicomponent spectrophotometric method was used for the determination of riboflavin and photoproducts in photolysed solutions. The apparent first-order rate constants (k) for the photolysis reactions range from 2.71×10^{-4} to 4.26×10^{-2} min^{-1}. The values of the rate constants decrease with increasing concentrations of caffeine indicating its inhibitory effect on the reactions. The second-order rate constants (k') for the caffeine inhibited reactions lie in the range of 0.13 to 5.10×10^{-3} M^{-1} min^{-1}. The log k–pH profiles for the photolysis reactions at various caffeine concentrations involve multiple steps indicating a gradual increase in the rate up to pH 10. The lower rates at pH 2.0 and 10.5 are due to the ionization of riboflavin as evident from fluorescence measurements. The k'–pH profile for the interaction of riboflavin with caffeine represents a bell-shaped curve in the pH range 3—6 followed by a sigmoid curve in the pH range 7—10. The inhibition of photolysis of riboflavin in the presence of caffeine is a result of the monomeric interaction and complex formation of caffeine with riboflavin. The photochemical interaction of riboflavin with caffeine suggests that a pH around 6 is most appropriate for the stabilization of the vitamin. At this pH the complex shows the highest stability constant.

Key words photolysis; kinetics; complexation; riboflavin; caffeine

Riboflavin is a highly photosensitive compound and is degraded in aqueous solution by several mechanisms[1—3] including intramolecular photoreduction[4,5] and intramolecular photoaddition.[6—9] These reactions are affected by pH,[4,5,10—12] solvent polarity and viscosity,[13,14] ionic strength,[15] buffer kind and concentration[6—9,16] and light intensity and wavelengths.[6—9,17] Various methods including the use of photostabilizers, incorporation into liposomes and cyclodextrin complexation have been used to stabilize riboflavin solutions in the presence of light.[18—21] Another approach to achieve stabilization of drugs is through complexation with caffeine and other agents.[22—26] Riboflavin is known to form molecular complexes with caffeine[27—32] which has been found to influence the rate of its chemical[33] and photochemical degradation.[17] Riboflavin–caffeine complexation may improve the bioavailability[34] and therapeutic activity[35] of the vitamin. NMR studies have been conducted to understand the nature of association between caffeine and flavin mononucleotide[36] that may help in the understanding of the mode of riboflavin stabilization. The present work is based on a detailed study of the kinetics of riboflavin photolysis in the presence of caffeine over a wide range of pH using a specific multicomponent spectrophotometric method[12] for the determination of riboflavin and photoproducts. The kinetic data have been used to determine the stability constants for riboflavin–caffeine complex. The quantitative and kinetic evaluation of such interactions may suggest the possibility of achieving the stabilization of photolabile compounds and thus enhance their biological action. The chemical structures of riboflavin, its photoproducts and caffeine are shown in Fig. 1.

Experimental

The materials and methods used are the same as previously described for a similar study.[5] The photolysis of 5×10^{-5} M riboflavin (RF) solutions was carried out in the presence of $0.5—2.5\times10^{-4}$ M caffeine using a Philips HPLN 125 W high pressure mercury vapor fluorescent lamp (emission at 405 and 435 nm)[9] as the irradiation source. The spectral measurements on RF and degraded solutions were performed on a Shimadzu UV-1601 recording spectrophotometer using silica cells of 10 mm path length. The intensity of the fluorescent lamp was determined by potassium ferrioxalate actinome-

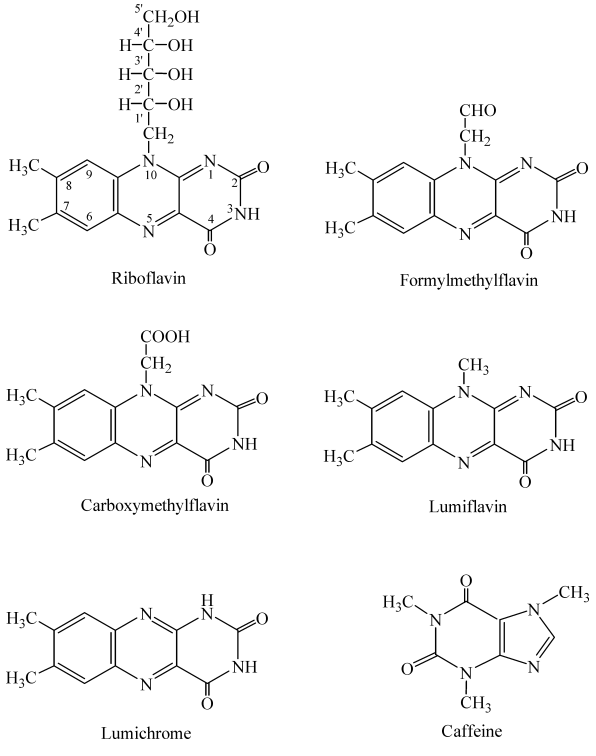

Fig. 1. Chemical Structures of Riboflavin, Its Photoproducts and Caffeine

* To whom correspondence should be addressed. e-mail: ali_sheraz80@hotmail.com

try[37] as $1.15\pm0.10\times10^{17}$ quanta s^{-1}.

The fluorescence measurements were performed on a Versa Fluor fluorimeter (Bio-Rad Laboratories, U.S.A.) equipped with a 20 W quartz halogen lamp (350—390 nm), a photodiode detector and excitation and emission filters. The measurements were carried out at 25—27 °C using 10 mm quartz cells. The 0.05 mM RF solutions without caffeine and maintained at a particular pH were used to calibrate the fluorescence intensity scale of the instrument. The excitation wavelength (374 nm) and the emission wavelength (525 nm)[10] were set by using appropriate filters and the relative fluorescence intensity of RF solutions in the presence of caffeine in the pH range 2.0—10.5 was measured.

Results and Discussion

Riboflavin Photoproducts In order to confirm the presence of known photoproducts of RF in the degraded solutions (30—50% photolysis), thin-layer chromatography (TLC) was performed on cellulose plates using the solvent systems: (A) 1-butanol–acetic acid–water (40 : 10 : 50, v/v, organic phase) and (B) 1-butanol–1-propanol–acetic acid–water (50 : 30 : 2 : 18, v/v).[38] The photoproducts were identified by their characteristic fluorescence under UV (365 nm) excitation and comparison of the *Rf* values with those of the reference standards as follows (the fluorescence is given in parenthesis):

Acid Photolysis (pH 2.0—6.0). Major: RF (undegraded) and formylmethylflavin (FMF, yellow green), lumichrome (LC, sky blue). Minor: Carboxymethylflavin (CMF, yellow green).

Neutral and Alkaline Photolysis (pH 7.0—10.5). Major: RF (undegraded), FMF, LC and lumiflavin (LF, yellow green). Minor: CMF and trace amounts of unknown compounds with yellow green fluorescence.

The products detected in this study are known and have previously been identified in the photolysis of RF and FMF (an intermediate in this reaction) by Smith and Metzler,[39] Treadwell *et al.*[40] and Ahmad *et al.*[5,12,14,38] The confirmation of the products formed on the photolysis of RF in the presence of caffeine is necessary to carry out the assay of RF and the photoproducts in degraded solutions. The intensity of the spots of the photoproducts in degraded solutions appeared to decrease with an increase in the concentration of caffeine indicating its inhibitory effect on the rate of photolysis of RF in the pH range studied.

Spectral Characteristics of Fresh and Degraded Solutions of RF The absorption spectra of aqueous solutions of RF (5×10^{-5} M) containing various concentrations of caffeine ($0.5—2.5\times10^{-4}$ M) at pH 2.0—10.5 show variations in the 500 to 300 nm region before irradiation. A loss of absorbance of RF solutions in the presence of caffeine at pH 3.0—10.0 has been observed at the absorption maxima of RF at 444 and 373 nm[41] to the extent of 0.2—1.0%. The loss of absorbance at these wavelengths at pH 2.0 and 10.5 due to the ionized forms of RF (pK_{a1} 1.9, pK_{a2} 10.2)[42] is greater and is in the range of 0.4—2.0%. A similar change in absorbance has been observed at 385 nm, the wavelength, along with 445 nm, used for the assay in this study. It has been observed that the addition of caffeine in aqueous solutions of RF causes a decrease in the intensity of the absorption at 375 and 450 nm[43] and at 445 nm[33] and 446 nm.[35] These changes were considered to occur due to the formation of an intermolecular complex between the isoalloxazine ring of RF and the aromatic ring of caffeine. This may affect the chromophoric system of RF causing a decrease in absorbance at the maxima. A typical set of the absorption spectra of RF solutions photolysed at pH 8.0 in the presence of caffeine and measured after chloroform extraction at pH 2.0 (aqueous phase) (Fig. 2a) showed gradual disappearance of the 445 nm band with the progress of photolysis indicating the formation of the major final product, LC. This is evident from the gradual increase in absorbance at the maximum of this compound (356 nm)[12] in the chloroform extract determined at pH 4.5. An increase in the concentration of caffeine results in a slower decrease in absorbance at 445 nm in the aqueous phase indicating the inhibition of RF photolysis as a result of complexation. A similar change in the formation of LC is observed due to a relative increase in absorbance at 356 nm in the chloroform extract (Fig. 2b). The overall spectral changes of the photolysed solutions are similar to those observed by Holzer *et al.*[44] for the photo-induced degradation of RF in aqueous solution.

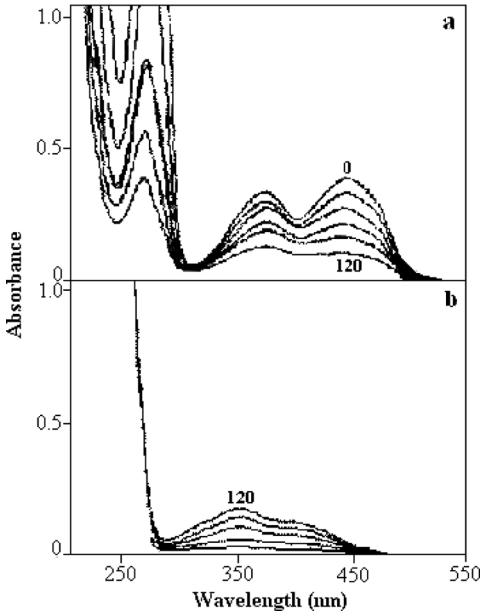

Fig. 2. UV and Visible Absorption Spectra of (a) the Aqueous Phase (pH 2.0) at 0, 15, 35, 60, 90 and 120 min and (b) the Chloroform Extract (pH 4.5) at 15, 35, 60, 90 and 120 min during the Photolysis of 5×10^{-5} M Riboflavin Solution at pH 8.0 in the Presence of 2.5×10^{-4} M Caffeine

Assay of RF and Photoproducts The assay of RF and photoproducts (FMF, LC, LF) was carried out by a specific multicomponent spectrophotometric method developed by Ahmad and Rapson[12] and previously applied to the study of RF and FMF photolysis reactions.[5,7,11,14,38] In the presence of caffeine the assay results could be affected due to the loss of absorbance at the analytical wavelengths as discussed above on the spectral characteristics of RF solutions. The assay method is based on preadjustment of the photolysed solutions to pH 2.0 (HCl–KCl buffer) and extraction with chloroform to remove LC and LF. The chloroform is evaporated and the residue dissolved at pH 4.5 (acetate buffer) to determine LC and LF by a two-component assay at 356 and 445 nm. Similarly the aqueous phase is used to determine RF and FMF by a two-component assay at 385 and 445 nm. The reproducibility of the method is of the order of ±5%.[12]

Caffeine is highly soluble in chloroform (1 g/5.5 ml) and exists from 3.8 to 6.1% in the protonated form at pH 2.0

(amine pK_a 0.6).[42,45,46] On the extraction of photolysed solutions of RF at pH 2.0, 94—96% of the added caffeine would be transferred to chloroform as indicated by an increase in absorption around 273 nm (absorption maximum of caffeine)[42] on measurement at pH 4.5 (Fig. 2b). Since the aqueous phase (pH 2.0) (Fig. 2a) contains only about 4—6% of the added caffeine, there would be negligible effect on the absorption of the mixture of RF and FMF at the analytical wavelength (385, 445 nm) as observed in this work.

To examine the effect of caffeine on the absorption of the mixture of LC and LF, a RF solution (pH 9.0) photolysed in the absence of caffeine was extracted with chloroform and on evaporation the residue dissolved in pH 4.5 acetate buffer. The absorbance of the solution was measured in the presence and absence of the highest concentration of caffeine (2.5×10^{-4} M) used in the photolysis reactions. A loss of absorbance to the extent of 3% and 1% was observed at the analytical wavelengths of 356 and 445 nm, respectively, suggesting some interaction between caffeine and LC and caffeine and LF. Since LC is the major final product in this reaction, there is a greater possibility of interaction between caffeine and LC. In view of the absorbance loss in the chloroform extract in the presence of caffeine, the analytical data have been corrected for LC and LF concentrations to account for the respective absorbance loss at the two wavelengths. A typical assay of RF and photoproducts in the solutions photolysed at pH 9.0 in the presence of caffeine is reported in Table 1, indicating a gradual decrease in the concentration of RF and an increase in the concentrations of FMF (up to 40 min and then its gradual loss to form LC and LF) and LC and LF, with time, giving an almost constant molar balance. CMF is a minor photoproduct formed on the oxidation of FMF[40] and its presence does not appear to affect the assay results as the molar balance of RF and photoproducts is almost constant during the reactions. Control solutions of RF stored in the dark at all pH values during the photolysis reactions (120—240 min at pH 2.0—8.0, 40—60 min at pH 9.0—10.5) did not show any change in absorbance at the analytical wavelengths.

Kinetics of RF Photolysis The kinetics of RF photolysis has been evaluated by using the analytical data obtained for various reactions under the same experimental conditions (*i.e.* pH, analytical wavelengths, irradiation conditions). This is necessary to avoid any variations in the use of kinetic data for comparative purposes. A kinetic plot for the photolysis of RF in the presence of caffeine at pH 10.0 is shown in Fig. 3. During photolysis, the RF values gradually decreased with a concomitant increase in FMF (up to 40 min) and LC and LF values. The highest values are obtained for LC which is formed by the degradation of FMF (an intermediate in this reaction), along with LF. The photolysis of RF in aqueous solution follows first-order kinetics[5] and the values of the apparent first-order rate constants, k_{obs}, for the photolysis reactions carried out at various pH values are reported in Table 2. It is evident from these values that the rate of photolysis is inhibited in the presence of caffeine. Further treatment of the kinetic data by plotting the values of k_{obs} *versus* caffeine concentration at all the pH values resulted in straight lines indicating that the rate of photolysis is a linear function of caffeine concentration in the range studied (Fig. 4). The slopes of these plots yielded the second-order rate constants (k') for the caffeine inhibited reaction (Table 3). The values of k_0 were determined by back-extrapolation of the second-order plots to a zero concentration on the vertical axis (Fig. 4) and are reported in Table 3. These values are about one and a half to four times greater than those obtained at the highest caffeine concentration (2.5×10^{-4} M) except at pH 6.0 (about one and a quarter times greater) and indicate the gradual inhibitory effect of caffeine on the rate of reaction.

Rate–pH Profiles The present work involves the study

Table 1. Photolysis of 5×10^{-5} M RF Solution in the Presence of 2.5×10^{-4} M Caffeine at pH 9.0 Concentrations of RF and Photoproducts

Time (min)	RF (M×10^5)	FMF (M×10^5)	LF (M×10^5)	LC (M×10^5)	Total (M×10^5)
0	5.00	0.00	0.00	0.00	5.00
10	3.75	0.29	0.09	0.89	5.02
20	2.84	0.42	0.17	1.60	5.03
30	2.17	0.49	0.22	2.12	5.00
40	1.64	0.50	0.26	2.58	4.98
50	1.31	0.48	0.34	2.84	4.97

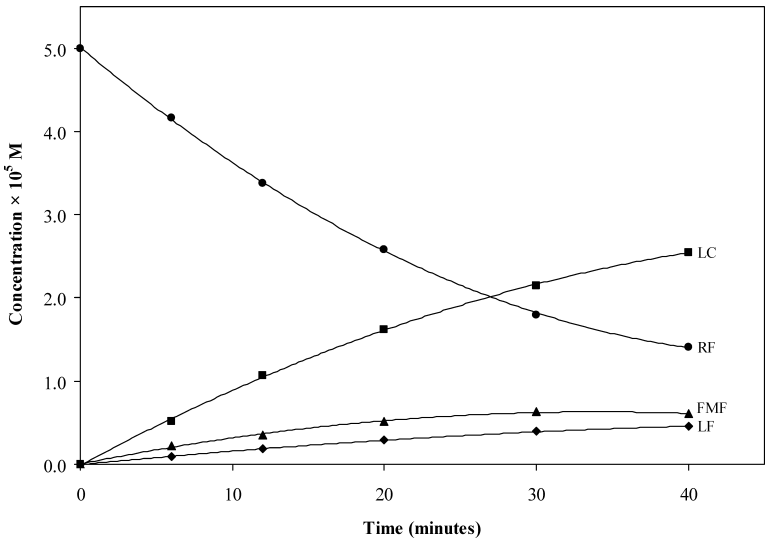

Fig. 3. Photolysis of 5×10^{-5} M Riboflavin Solution at pH 10.0 in the Presence of 2.5×10^{-4} M Caffeine

Table 2. First-Order Rate Constants (k_{obs}) for the Photolysis of Riboflavin at pH 2.0—10.5 in the Presence of Caffeine (0.5—2.5×10^{-4} M)

pH	$k_{obs}\times10^2$ min^{-1}				
	0.5[a)]	1.0[a)]	1.5[a)]	2.0[a)]	2.5[a)]
2.0	0.052	0.046	0.040	0.032	0.027
	(0.997)	(0.999)	(0.998)	(0.999)	(0.998)
3.0	0.067	0.059	0.050	0.039	0.030
	(0.998)	(0.998)	(0.999)	(0.996)	(0.999)
4.0	0.263	0.226	0.181	0.131	0.088
	(0.996)	(0.997)	(0.996)	(0.997)	(0.996)
5.0	0.357	0.295	0.243	0.192	0.141
	(0.999)	(0.997)	(0.999)	(0.997)	(0.996)
6.0	0.304	0.294	0.284	0.274	0.266
	(0.996)	(0.999)	(0.999)	(0.997)	(0.997)
7.0	0.351	0.329	0.301	0.276	0.245
	(0.998)	(0.998)	(0.996)	(0.996)	(0.996)
8.0	1.270	1.179	1.091	0.995	0.920
	(0.997)	(0.999)	(0.999)	(0.998)	(0.997)
9.0	3.650	3.402	3.160	2.894	2.671
	(0.999)	(0.998)	(0.998)	(0.999)	(0.999)
10.0	4.260	4.032	3.840	3.503	3.250
	(0.998)	(0.997)	(0.997)	(0.998)	(0.997)
10.5	3.439	3.220	2.941	2.772	2.520
	(0.998)	(0.998)	(0.997)	(0.996)	(0.996)

a) Caffeine concentration (M×10^4). The values in parenthesis are correlation coefficients.

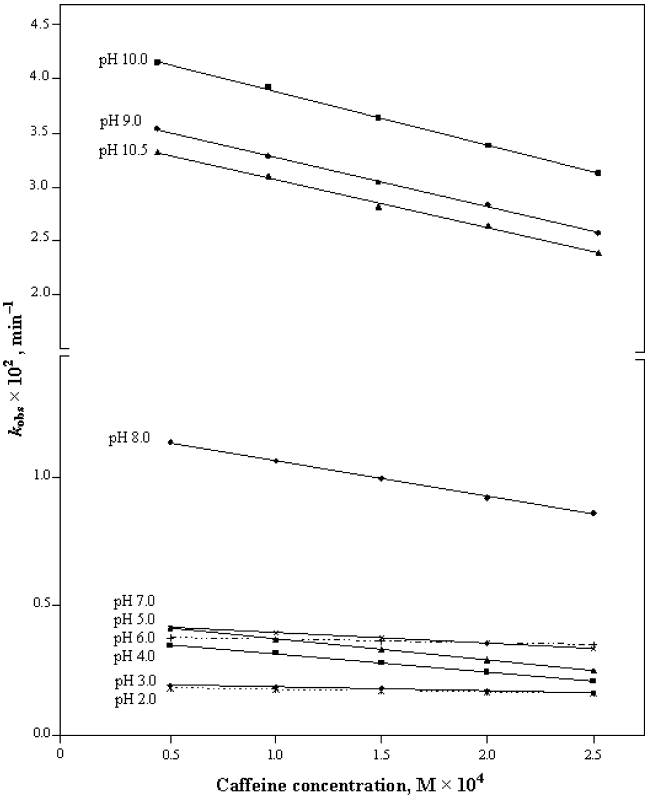

Fig. 4. Plots of k_{obs} *versus* Caffeine Concentration for the Photolysis of Riboflavin at pH 2.0—10.5

of the kinetic behavior of RF in the presence of caffeine over a wide range of pH. The rate–pH profiles (Fig. 5) depict the highest rate at a pH value around 10, with a gradual decrease on increasing the caffeine concentration at all pH values and exhibit a pattern similar to that observed for the photolysis of

Table 3. First-Order Rate Constants (k_0) for the Photolysis of Riboflavin in the Absence of Caffeine and Second-Order Rate Constants for the Photolysis of Riboflavin (k') in the Presence of Caffeine

pH	$k_0\times10^2$ (min^{-1})	$k'\times10^{-2}$ (M^{-1} min^{-1})	Correlation coefficient
2.0	0.056	0.013	0.998
3.0	0.078	0.019	0.997
4.0	0.310	0.089	0.998
5.0	0.406	0.105	0.999
6.0	0.314	0.019	0.998
7.0	0.380	0.053	0.997
8.0	1.360	0.177	0.999
9.0	3.890	0.468	0.999
10.0	4.540	0.510	0.999
10.5	3.650	0.458	0.996

RF in the absence of caffeine.[5)] The profiles indicate gradually rising steps and a pH-independent plateau extending over the pH range 5—7. The rates are lowest at pH 2.0 and decrease at pH 10.5 due to ionization of RF (pK_{a1} 1.9, pK_{a2} 10.2).[42)] The ionized forms of the molecule are less susceptible to photolysis than the non-ionized form.[5)] The increase in the rate of photolysis in the pH range of 2—5 is due to the gradual deprotonation of the molecule (pK_{a1} 1.9, N−10) and in the alkaline range as a result of the increased reactivity of the triplet state.[4)] The presence of a plateau in the pH range 4—5 may be explained on the basis of the lowest redox potentials of RF in this range (E° pH 5.0=−0.117, pH 7.0=−0.207 V, pH 8.6=−0.274 V)[47,48)] since the primary reaction involved in the photolysis of RF is photoreduction.[3)] Thus, the ionization state, redox potentials and triplet state reactivity appear to influence the rate of photolysis of RF over the pH range studied. These characteristics may also play a significant role in the photolysis of RF in the presence of caffeine and thus influence the rate of reaction. The rate–pH profile for the photochemical interaction of RF and caffeine represents a bell-shaped curve in the pH range 3—6 followed by a sigmoid curve in the pH range 7—10 (Fig. 6). The initial step of the bell-shaped curve (around pH 2—5) may result from the gradual increase in the non-ionized form of RF and a subsequent greater interaction between RF and caffeine. This is followed by a decrease in the rate (around pH 5—6) which may be due to the involvement of an intermediate species and consequently a change in the rate-determining step of the reaction as observed in the hydrolysis of hydrochlorothiazide.[49)] The involvement of an intermediate species in this range may be substantiated by the fact that RF shows lower reactivity (lowest redox potentials around pH 5—6) in this region and caffeine has greater influence (highest stability constant around pH 6) in suppressing the rate of reaction. The sigmoid curve extending over the pH range 7—10 suggests a lower interaction of caffeine with RF *vis-à-vis* greater reactivity of RF triplet state in enhancing the rate of reaction. Above pH 10 the ionization of RF (N−3) appears to be the major factor in causing the decrease in the rate of reaction. The photolysis of RF in the presence of caffeine suggests that the molecule is most stable at a pH value around 6. This is in agreement with a previous study on the photolysis of pure RF solutions.[5)]

Fluorescence Studies Aqueous solutions of RF exhibit intense yellow green fluorescence which is destroyed on the

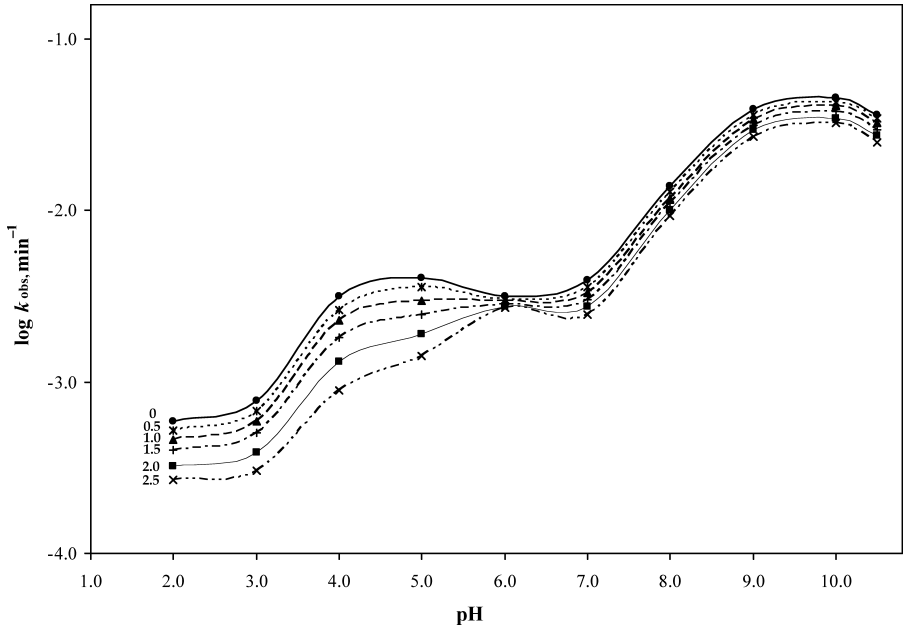

Fig. 5. log k_{obs}–pH Profiles for the Photolysis of Riboflavin in the Presence of Caffeine (M×10^4)

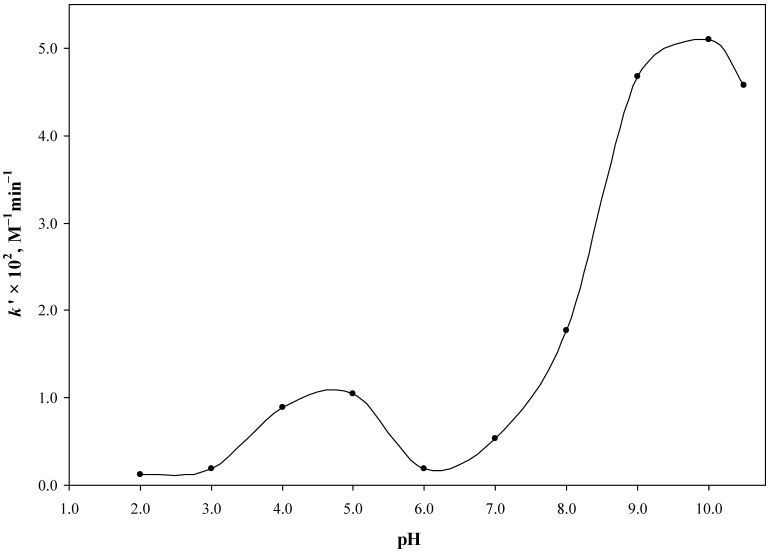

Fig. 6. k'–pH Profile for the Photolysis of Riboflavin in the Presence of Caffeine

addition of mineral acids or alkalis.[1,41,43,50] In the present work a loss of RF fluorescence has been observed in the presence of caffeine at pH 2.0 (5%) and 10.5 (18%) (Table 4) compared to that of the pure solutions. This may result from the formation of a complex between RF and caffeine as reported by previous workers.[27,30,33,43,51] The relatively greater loss of fluorescence at pH 2.0 and 10.5, compared to the negligible loss at pH 4—9, is due to the ionization of RF,[43,50] in addition to complex formation. The loss of RF fluorescence due to complex formation probably involves the interaction of the chromophoric systems of RF and caffeine and thus may affect the RF excitation process resulting in the inhibition of the rate of photolysis.

Stability Constants of RF–Caffeine Complex The complexation of RF with caffeine may be represented by the following reaction:

Table 4. Fluorescence Intensity of 5×10^{-5} M Riboflavin Solutions at pH 2.0—10.5 in the Presence of 0.5—2.5×10^{-4} M Caffeine[a)]

Caffeine concentration (M×10^4)	Relative fluorescence intensity at pH				
	2.0	4.0	7.0	9.0	10.5
0	100.0	100.0	100.0	100.0	100.0
0.5	98.5	100.0	100.0	100.0	95.2
1.0	97.2	100.0	100.0	100.0	90.6
1.5	96.4	99.7	100.0	100.0	87.5
2.0	95.6	99.4	100.0	99.8	84.8
2.5	95.0	99.0	100.0	99.5	82.2

a) Measurements were made on 1 : 10 dilute solutions using appropriate buffers at all pH values.

$$\text{RF} + \text{caffeine} \rightleftarrows \text{RF: caffeine} \quad (1)$$

The stability constants of 1 : 1 RF–caffeine complex can be determined by using the following Eq. 2 developed by Carstensen[22]:

$$k_{obs} = \frac{k + k^*K[B]}{(1 + K[B])} = k + \frac{(k^* - k)K[B]}{1 + K[B]} \quad (2)$$

which may be expressed reciprocally by Eq. 3 as:

$$\frac{1}{k_{obs} - k} = \frac{1}{k^* - k} + \frac{1}{K(k^* - k)} \cdot \frac{1}{[B]} \quad (3)$$

where k is the rate constant for the degradation of RF (k_0), k_{obs} is the overall rate constant for the degradation of RF in the presence of caffeine, k^* is the rate constant for the degradation of RF–caffeine complex ($k_{obs} - k$), [B] is the molar concentration of caffeine in solution, and K is the stability constant of RF–caffeine complex.

The value of K can be obtained from a linear plot of $1/(k_{obs} - k)$ *versus* 1/[caffeine] according to Eq. 3 as the slope-to-intercept ratio as shown for the photolysis reaction at pH 6.0 (Fig. 7).

The values of K for 1 : 1 RF–caffeine complex, determined at pH 2.0—10.5 (Table 5), range from 0.121—2.490$\times 10^{-2}$ M^{-1} and vary with pH (Fig. 8). The highest value is close to the value (2.72×10^{-2} M^{-1}, 25 °C) determined by Guttman[33] in alkaline solution (0.05 M NaOH). A comparison of the graphs of second-order rate constants (k') for the interaction of RF and caffeine *versus* pH (Fig. 6) and the graph of the stability constants (K) *versus* pH (Fig. 8) indicates a nearly inverse relationship between the two values. The K values increase at pH 2.0 and 10.5 (cationic and anionic forms of RF, respectively) probably due to higher interaction (*e.g.* dipole–dipole interaction, van der Waal's force, hydrogen bonding)[52] between the two molecules. These values are lower in the pH range 3—5 probably due to lower interaction and reach the maximum value at pH 6 *vis-à-vis* the lowest k' value at that pH due to the lowest redox potentials of RF in this region. The redox potentials of RF increase with pH[53] and appear to influence the rate of reaction and the interaction with caffeine. In the pH range 7—10 the K values are decreasing while the k' values are increasing with pH. In this region the predominant factor appears to be the RF triplet state reactivity[4] leading to a greater increase in k' values although the K values are decreasing in this region. Thus several factors such as RF ionization, redox potentials and triplet state reactivity contribute to the values of the stability constants in the pH range studied.

Structure of 1 : 1 RF–Caffeine Complex Caffeine is a hydrotropic agent and it forms a hetero-association complex

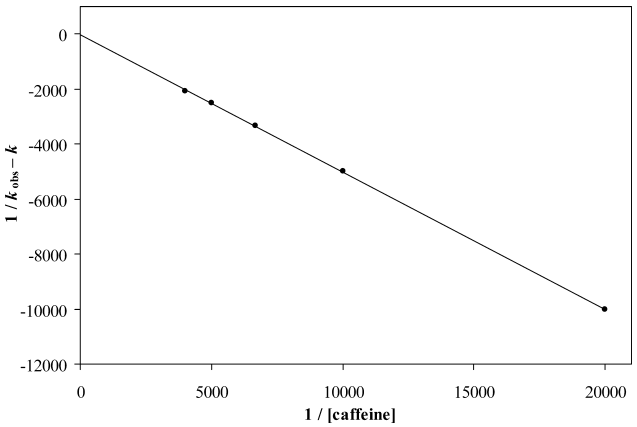

Fig. 7. A Plot of $1/(k_{obs} - k)$ *versus* 1/[caffeine] for the Photolysis of Riboflavin at pH 6.0

Table 5. Stability Constants of Riboflavin–Caffeine Complex (K) at pH 2.0—10.5

pH	$K\times10^2$ (M^{-1})	pH	$K\times10^2$ (M^{-1})
2.0	1.533	7.0	0.140
3.0	0.121	8.0	0.554
4.0	0.140	9.0	0.415
5.0	0.614	10.0	0.175
6.0	2.488	10.5	0.812

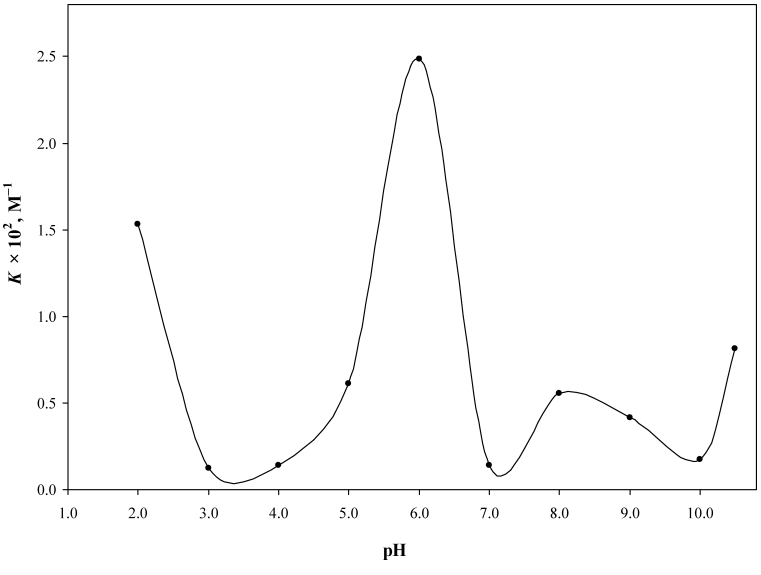

Fig. 8. A Plot of Stability Constants for Riboflavin–Caffeine Complex *versus* pH

with flavin mononucleotide (FMN) in aqueous solution as a result of vertical stacking interaction between the aromatic chromophores of the two molecules. The possible structure of the 1 : 1 FMN–caffeine complex has been studied by molecular dynamics simulations and an analysis of the induced proton chemical shifts for these molecules.[36] Similar heteroassociation complexes of caffeine with nucleic acids,[54] actinomycin D,[55] theaflavin,[56] novatrone (mitoxantrone)[57,58] and aromatic drugs[59—60] have been studied by NMR spectroscopy. It has been suggested that the imidazole moiety of caffeine is involved in the interaction with aromatic species.[25] The structure of RF–caffeine complex may be stabilized by hydrogen bonds between caffeine oxygens (in positions 2 and 6) and NH group (in position 3) of riboflavin as observed in the case of caffeine–novatrone complex.[57] Intermolecular hydrogen bonding has also been suggested in the interaction of caffeine with biological compounds.[61] The crystal structure of caffeine complexes with inorganic bromides and iodides has indicated the presence of a network of hydrogen bonds between the two molecules[62] which may stabilize these complexes.

It is evident from the above discussion that caffeine has been used to form complexes with a number of compounds including RF. However, the efficacy of these complexes to stabilize a drug depends on their relative strength and chemical reactivity. The complexation of RF with caffeine may improve the bioavailability[34] and therapeutic activity[35] of the vitamin. Most of the investigations conducted on caffeine complexes by earlier workers are based on structural considerations. The present study provides a detailed account of the kinetic behavior of the RF–caffeine complex over a wide range of pH and may be useful in similar studies.

Conclusion

The rate of photolysis of RF in the pH range 2.0—10.5 is inhibited by caffeine as a result of 1 : 1 complex formation between the two molecules as indicated by the kinetic data. The $\log k_{obs}$–pH profiles for the photolysis of RF in the presence of varying concentrations of caffeine are similar to that obtained in the absence of caffeine and involve multiple steps with a gradual increase in the rate reaching a maximum value around pH 10. The increase in caffeine concentration results in a gradual decrease in the rate of reaction at all pH values. The lower rate of photolysis at pH 2 and above pH 10 appears to be due to the cationic and anionic forms of RF and a lower susceptibility of the ionized form to undergo photolysis in the presence of caffeine.

The k'–pH profile for the photochemical interaction of RF and caffeine represents a bell-shaped curve in the acid region and a sigmoid curve in the alkaline region. The photochemical interaction of the two compounds may involve an intermediate species in the rate-determining step resulting in the suppression of the rate of reaction in the pH range 5—6. The increase in the rate in the pH range 7—10 is due to the greater reactivity of the RF triplet state in this region. The kinetic behavior of RF in the pH range 3—9 appears to be due to the interaction of non-ionized form of the molecule with caffeine. The photolysis of RF in the presence of caffeine is influenced by complex formation, ionization state, redox potentials, triplet state reactivity and fluorescence characteristics in the pH range studied. RF is most stable to photolysis in the presence of caffeine at a pH value around 6.

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