ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
23 (2000) 1039–1044

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

# Effect of riboflavin on the photolysis of folic acid in aqueous solution

M. Jamil Akhtar [a,*], M. Ataullah Khan [b], Iqbal Ahmad [c]

[a] *Research and Development Laboratories, GlaxoWellcome Pakistan Ltd., D/43, S.I.T.E. Karachi, Pakistan*
[b] *Department of Chemistry, Organic Section, University of Karachi, Karachi-75270, Pakistan*
[c] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan*

Received 6 November 1999; received in revised form 28 April 2000; accepted 26 May 2000

**Abstract**

A study of the photolysis of folic acid in aqueous solution by visible radiation in the presence of riboflavin has been made. The second-order rate constants for the bimolecular interaction of folic acid and riboflavin have been determined in the pH range 4.0–9.0. The rate–pH profile shows a gradual increase in the rate up to pH 6.2 ($pH_{max.}$) followed by a decrease up to pH 9.0, depending upon the susceptibility of ionic species involved in the interaction. The rate of photolysis varies from $0.50 \times 10^3$ $M^{-1}$ $min^{-1}$ (pH 9.0) and $0.63 \times 10^3$ $M^{-1}$ $min^{-1}$ (pH 4.0) to $3.0 \times 10^3$ $M^{-1}$ $min^{-1}$ (pH 6.2) in the pH range studied. A HPLC method has been used for the assay of folic acid and its photoproducts, pterine-6-carboxylic acid and *p*-aminobenzoyl-L-glutamic acid in the presence of riboflavin. 

*Keywords:* Folic acid; Riboflavin; Photolysis; Bimolecular interaction

## 1. Introduction

Folic Acid is a sensitive compound and is inactivated by heat, sunlight, ultraviolet light, oxidation, reduction, acid and alkalis. A detailed study of the photolysis of folic acid in aqueous solution has recently been reported [1]. The oxidative cleavage of folic acid by visible light is markedly intensified by riboflavin. The products of riboflavin-induced photooxidation of folic acid are *p*-aminobenzoyl-glutamic acid and a carbonyl compound, presumably 2-amino-4-hydroxy-6-pteridine carboxaldehyde. It has been reported that folic acid is not stable in aqueous solution containing B vitamins [2–5] and therefore an interaction between these vitamins may be stipulated. So far no quantitative information is available on the extent of photolytic degradation of folic acid in the presence of riboflavin at various pH values. In the present investigation the rate of photolysis of folic acid in the presence of riboflavin at various pH values has been studied using a specific HPLC method [6]. The object of this study is to develop-

* Corresponding author. Tel.: +92-21-2578600/0665; fax: +92-21-2564373/3668.

0731-7085/00/$ - see front matter 
PII: S0731-7085(00)00383-6

an understanding of the nature of photo reactions of folic acid in the presence of riboflavin and to determine the rate pH profile for their interaction to assess the optimum stability of folic acid in pharmaceutical preparations.

## 2. Experimental

All the experimental work was carried out in diffused light, i.e. protected from direct exposure. The solutions of folic acid containing riboflavin and its degradation products were well protected from light by wrapping the containers with aluminium foil. Freshly prepared solutions were used for each experiment to avoid any chemical or photochemical effects.

### 2.1. Materials

All reagents and solvents were analytical grade, obtained from Merck. Reference standards were obtained from Sigma. The following buffers were used throughout: citric acid–$Na_2HPO_4$, pH 3.0–8.0; $Na_2B_4O_7$–HCl, 8.5–9.0. The ionic strength was 0.05 M in each case.

### 2.2. Assay method

The assay of controls and photolysed solutions of folic acid in the presence and absence of riboflavin were carried out using a previously reported HPLC method, which describes correlation coefficient for the calibration curves and accuracy within the range of 0.9926–0.9997 and 98.62–102.47%, respectively. The validity of this method for the assay of photolysed solution of folic acid in the presence and absence of riboflavin was also discussed in terms of constant molar balance with 96.80 and 95.62%, respectively [6].

### 2.3. Photolysis of folic acid

#### 2.3.1. Radiation source

In order to study the influence of riboflavin on the photolysis of folic acid, it was felt necessary to choose a radiation source with strong emission in

Table 1
Regression analysis of the kinetic plots (curves) at pH 4.0

| Irradiation time (min) | Folic acid ($M \times 10^5$) | Riboflavin ($M \times 10^5$) | Range for folic acid ($M \times 10^5$) | Slope | Intercept | Correlation coefficient |
|---|---|---|---|---|---|---|
| 3–12 | 5.090 | 1.0 | 4.059–5.090 | −0.0090 | −4.2835 | −0.99713 |
| 3–12 | 5.090 | 1.5 | 3.775–5.090 | −0.0092 | −4.3100 | −0.99783 |
| 3–09 | 5.090 | 2.0 | 3.869–5.090 | −0.0161 | −4.2660 | −0.99984 |

Table 2
Photolysis of folic acid solution in the presence of riboflavin at pH 4.0 concentrations of folic acid and degraded products

| Time (min) | Folic acid ($M \times 10^5$) | Pterine-6-carboxylic acid ($M \times 10^5$) | | *p*-Aminobenzoyl-L-glutamic acid ($M \times 10^5$) | | Total[b] |
|---|---|---|---|---|---|---|
| | | X | X[a] | Y | Y[a] | |
| 0 | 5.00 | | | | | 5.000 |
| 3 | 4.57 | 0.190 | 0.089 | 0.606 | 0.365 | 5.020 |
| 6 | 4.33 | 0.308 | 0.144 | 0.869 | 0.524 | 4.998 |
| 9 | 4.05 | 0.462 | 0.216 | 1.198 | 0.723 | 4.989 |
| 12 | 3.77 | 0.589 | 0.276 | 1.510 | 0.911 | 4.957 |

[a] Moles equivalent to folic acid.
[b] Total moles equivalent to folic acid.

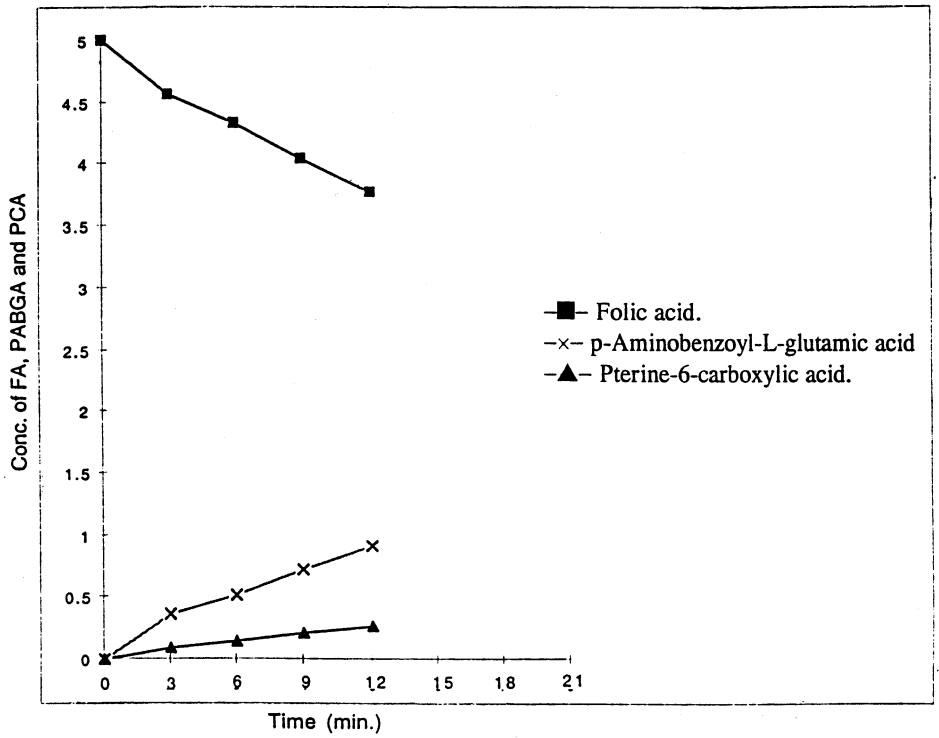


Fig. 1. Photolysis of folic acid solution in presence of riboflavin at pH 4.0 (citrate–phosphate buffer).

Table 3
Photolysis of folic acid solution at pH 4.0 in the presence of riboflavin

| Riboflavin ($M \times 10^5$) | Time (min) | Peak height (a.u.) | Concentration of folic acid ($M \times 10^5$) | Log concentration |
|---|---|---|---|---|
| 1.0 | 0 | 9056 | 5.090 | −4.292 |
| | 3 | 8646 | 4.867 | −4.312 |
| | 6 | 8237 | 4.637 | −4.334 |
| | 9 | 7630 | 4.295 | −4.367 |
| | 12 | 7211 | 4.059 | −4.391 |
| 1.5 | 0 | 9383 | 5.090 | −4.292 |
| | 3 | 8613 | 4.571 | −4.340 |
| | 6 | 8167 | 4.334 | −4.363 |
| | 9 | 7634 | 4.051 | −4.392 |
| | 12 | 7113 | 3.775 | −4.423 |
| 2.0 | 0 | 9054 | 5.090 | −4.292 |
| | 3 | 8588 | 4.835 | −4.315 |
| | 6 | 7712 | 4.341 | −4.362 |
| | 9 | 6873 | 3.869 | −4.412 |

the visible region corresponding to the absorption bands of riboflavin. A Philips ML-160 W lamp was considered suitable for the present work. It has strong emission lines at 405 and 436 nm which are absorbed by riboflavin only and hence there is a possibility of energy transfer through

mutual interaction of riboflavin and folic acid on irradiation in the solution.

### 2.3.2. Method

An aqueous solution of $5 \times 10^{-5}$ M folic acid (30 ml) containing varying molar concentrations ($0.25–2.00 \times 10^{-5}$ M) of riboflavin were prepared at pH 4.0–9.0 and irradiated in a 50 ml volumetric flask with Philips ML-160 W lamp. The solutions were placed at a distance of 30 cm from the irradiation source on a magnetic stirrer and had free access to air. The irradiation was carried out in a chamber maintained at a temperature of $25 \pm 2$°C. Sample were withdrawn at appropriate time intervals and subjected to HPLC analysis. Control solutions wrapped in aluminium foil were placed in the dark and assayed for folic acid content at the end of the reactions.

Table 4
First-order rate constants, $k \times 10^3$ ($min^{-1}$) of folic acid in presence of riboflavin at pH 4.0–9.00

| Ph | Riboflavin concentrations ($M \times 10^5$) | | | | |
|---|---|---|---|---|---|
| | 0.25 | 0.50 | 1.0 | 1.50 | 2.00 |
| 4.0 | | | 19.56 | 22.68 | 32.01 |
| 4.7 | | 14.16 | 22.30 | 28.66 | |
| 5.0 | | 12.16 | 23.20 | | 46.20 |
| 5.5 | 6.63 | 16.20 | 28.83 | 34.78 | |
| 5.9 | 7.43 | 16.41 | 28.98 | | |
| 6.2 | 7.66 | 17.35 | 28.68 | | |
| 6.5 | 10.86 | 19.30 | 28.68 | | |
| 7.0 | | 10.75 | 15.08 | 24.08 | |
| 7.5 | 3.86 | 9.76 | 16.06 | | |
| 8.0 | | 7.80 | 12.70 | 18.23 | 22.58 |
| 8.2 | | 6.11 | 8.56 | 13.95 | 16.26 |
| 8.5 | 2.75 | | 11.71 | | 16.91 |
| 9.0 | | 2.49 | 4.77 | 7.83 | |

Table 5
Second-order rate constants, ($k_2$) for the photolysis of folic acid solutions in presence of riboflavin at pH 4.0–9.0

| pH | $k_2 \times 10^{-3}$ ($M^{-1}$ $min^{-1}$) | Correlation coefficient, $r$ |
|---|---|---|
| 4.0 | 0.625 | 0.960868 |
| 4.7 | 1.428 | 0.997535 |
| 5.0 | 2.400 | 0.999948 |
| 5.5 | 2.947 | 0.976507 |
| 5.9 | 2.941 | 0.995547 |
| 6.2 | 3.000 | 0.989530 |
| 6.5 | 2.428 | 0.987331 |
| 7.0 | 1.571 | 0.980152 |
| 7.5 | 1.750 | 0.985381 |
| 8.0 | 1.148 | 0.999035 |
| 8.2 | 0.888 | 0.962370 |
| 8.5 | 0.842 | 0.972605 |
| 9.0 | 0.500 | 0.996379 |

## 3. Results and discussion

### 3.1. Assay of photolysed solutions

The assay results for photolysed solutions subjected to the kinetic treatment exhibits slight variation as evident from the regression analysis of the kinetic plot, log concentration of folic acid versus time in minutes (Table 1). This may possibly be due to factors such as minute light intensity variations during photolysis, sensitivity of the compound to HPLC detection at various stages of the reaction and fluctuations in the column performance and pH of the mobile phase. However, the data may be considered sufficiently precise and reliable for the purpose of kinetic treatment.

### 3.2. Kinetics of photolysis

A typical set of assay data for the photolysis of an equimolar solution ($5 \times 10^{-5}$ M) of folic acid and riboflavin at pH 4.0 is presented in Table 2. The data were plotted as a function of time (Fig. 1) to observe the photolysis behaviour of folic acid as well as the formation of photoproducts. The plot shows the degradation of folic acid and the formation of pterine-6-carboxylic acid and *p*-aminobenzoyl-L-glutamic acid as photoproducts. The sum of the residual folic acid and its photoproducts (M/l) is in good agreement with the initial concentration of folic acid.

In order to observe the rate of reaction, the data presented in Table 3, were subjected to kinetic treatment and were found to comply with first- order reaction. The regression analysis of these typical plots, log concentration of folic acid versus time in minutes, for the photolysis of folic acid in the presence of riboflavin (varying concen-

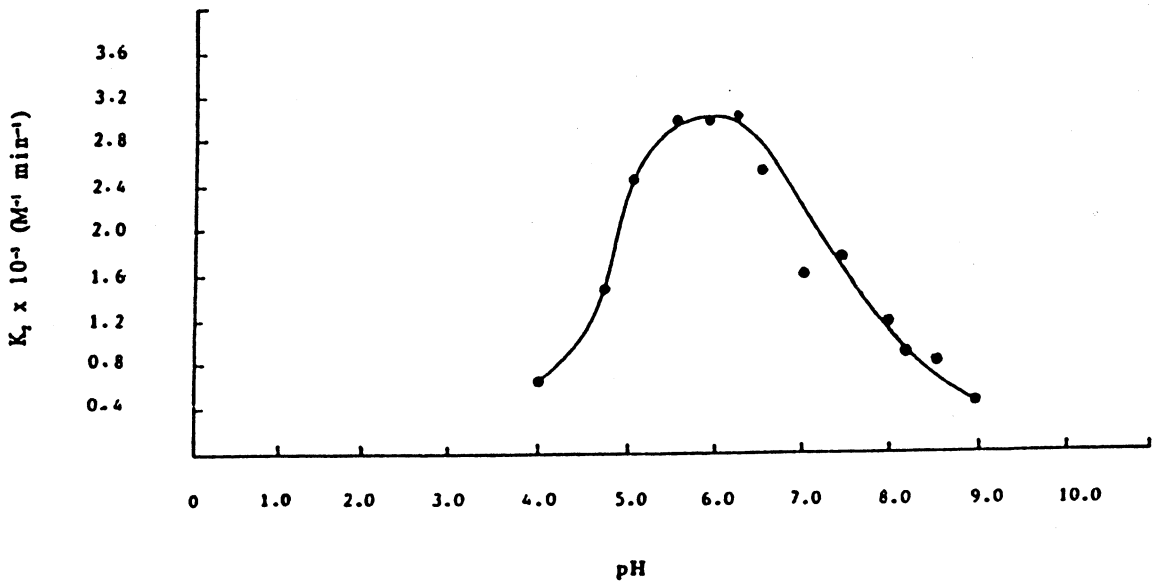


Fig. 2. $k_2$–pH profile for the photolysis of folic acid in the presence of riboflavin.

trations) at pH 4.0 are presented in Table 1. The first – order rate constant for the degradation of folic acid at pH 4.0–9.0 are presented in Table 4. The first-order rate constant ($k_{obs}$) were plotted against the molar concentration of riboflavin and the second-order rate constants ($k_2$) for the bimolecular interaction of folic acid and riboflavin, determined from the slopes of the straight lines are given in Table 5.

Earlier, it has been reported [1] that first order rate constant for the photolysis of folic acid in absence of riboflavin at pH 2.0–10.0 lies within the range of $4.2500–0.1550 \times 10^{-3}$ $min^{-1}$.

### 3.3. Rate pH profile

The $k_2$–pH profile (Fig. 2) for the photodegradation of folic acid in the presence of riboflavin indicates that with an increase in pH from acidic to neutral region, the rate of degradation increases up to pH 6.2 ($pH_{max.}$) and then appears to be decreases gradually towards the alkaline region. The rate of reaction is dependent upon the extent of interaction of folic acid and riboflavin. Since the stability of riboflavin itself is pH dependent, the rate of photodegradation of folic acid in presence of riboflavin is also pH dependent.

In the acidic medium, riboflavin is protonated and therefore the extent of its interaction appears to be reduced and hence the rate of degradation of folic acid (also protonated species) in this region is slow. As the reaction proceeds towards the neutral medium the extent of protonation of riboflavin is decreased [7] and hence its interaction with folic acid (also neutral species) appears to be increases resulting in the enhancement of the rate of reaction. Thus the neutral riboflavin species appears to be more interactive with folic acid resulting in a greater amount of destruction of the later compared to that in the acidic medium.

In the alkaline medium riboflavin molecule is itself susceptible to the nucleophilic attack of the solvent ($OH^-$) and therefore its own breakdown takes place [8]. As the pH increases the susceptibility of nucleophilic attack ($OH^-$) on riboflavin increases therefore its interaction with folic acid (also anionic species) is considerably suppressed, resulting in a decrease in the rate of degradation of folic acid. It may thus be concluded that the neutral species of riboflavin and folic acid are more susceptible to interaction in the presence of light and therefore riboflavin leads to the destruction of folic acid in the pH range studied with maximum effect at pH 6.20.

Riboflavin is known to form a complex with divalent phosphate ions at pH 7.0 in the ground state [9], therefore it was considered to be appropriate to use 0.05 M citrate-phosphate buffer to maintain the pH of the solution of folic acid containing riboflavin, subjected to irradiation.

## References

[1] M. Jamil Akhtar, M. Ataullah Khan, I. Ahmed, J. Pharm. Biomed. Anal. 25 (1) (1999) 269–275.
[2] A.R. Biamonate, G.H. Schneller, J. Am. Pharm. Assoc. 40 (1951) 313–320.
[3] B. Borey, Arch. Sci. Med. 93 (1952) 108–110.
[4] P. Reusser, Int. Z. Vitaminforsch. 40 (1) (1970) 64–72.
[5] S. Scheindlin, A. Lee, I. Griffith, J. Am. Pharm. Assoc. 51 (8) (1952) 420–427.
[6] M. Jamil Akhtar, M. Ataullah Khan, I. Ahmad, J. Pharm. Biomed. Anal. 16 (1) (1997) 95–99.
[7] A.C. Moffat, Clark's Isolation and Identification of Drugs, The Pharmaceutical Press, London, 1986, p. 633.
[8] H.J. Roth, K. Eger, R. Troschutz, Pharmaceutical Chemistry, vol. 2, Drug Analysis, Ellis Horwood, New York, 1991, p. 655.
[9] M. Schuman-Jorns, G. Schollnhammer, P. Hommerich, Eur. J. Biochem. 57 (1975) 35.