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Photodegradation of folic acid in aqueous solution

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Abstract

A study of the photodegradation of folic acid by ultraviolet radiation in aqueous solution has been made. Folic acid is photolysed by an apparent first-order kinetics and the log k-pH profile shows a gradual decrease in rate in the pH range 2.0–10.0. The profile indicates the appearance of three steps which reflect the participation of different ionic species of folic acid (pK_{a_1} 2.3, pK_{a_2} 8.3) in the photolysis reaction. The rate of photodegradation varies from 0.1550×10^{-3} min^{-1} (pH 10.0) to 5.04×10^{-3} min^{-1} (pH 2.5) in the pH range studied. The photolysis of folic acid shows that it is degraded to pterine-6-carboxylic acid and *p*-amino-benzoyl-L-glutamic acid. A maximum yield of these products is obtained at 3–8 h, depending upon the pH. An HPLC method has been used for the assay of folic acid and its degradation products.

Keywords: Photolysis; Ultraviolet light; First-order kinetics; pH; Folic acid; *p*-Aminobenzoyl-L-glutamic acid; Pterine-6-carboxylic acid

1. Introduction

Ultraviolet light converts folic acid into fluorescent material [1]. When folic acid is irradiated with ultraviolet light it is first converted to 2-amino-4-hydroxy-6-formyl pteridine (pterine-6-carboxaldehyde) and a diazotizable amine (*p*-aminobenzoyl-L-glutamic acid). On further irradiation the aldehyde is converted to the corresponding 6-carboxylic acid (pterine-6-carboxylic acid) which is fluorescent and finally to the decarboxylated 2-amino-4-hydroxy pteridine [2]. Day light, pH and heat have the most destructive effects on the solution of folic acid for injections. At pH 7.6 folic acid solutions exhibit optimum stability [3–5]. The major degradation products of folic acid in aqueous solution have been identified as *p*-aminobenzoyl-L-glutamic acid and pterine-6-carboxylic acid with traces of *p*-aminobenzoic acid [6,7]. So far no quantitative information is available on the extent of photodegradation of folic acid at different pH levels. In the present investigation the rate of photodegradation of folic acid at various pH values and quantification of its photodegradation products have been studied using a specific HPLC method [8]. The object of the study is to understand the nature of these reactions, and to determine the pH-rate profile to assess its optimum stability in pharmaceutical formulations.

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Table 1
Photolysis of folic acid at pH 5.5 concentration of folic acid and degraded products

Time (h)	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[b]	
0	5.00	–	–	–	–	5.00
1	4.86	0.097	0.046	0.353	0.213	5.12
2	4.81	0.290	0.136	0.667	0.402	5.34
3	4.56	0.371	0.174	0.875	0.528	5.26
4	4.08	0.476	0.224	1.131	0.682	4.98
5	3.63	0.619	0.290	1.500	0.905	4.82

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

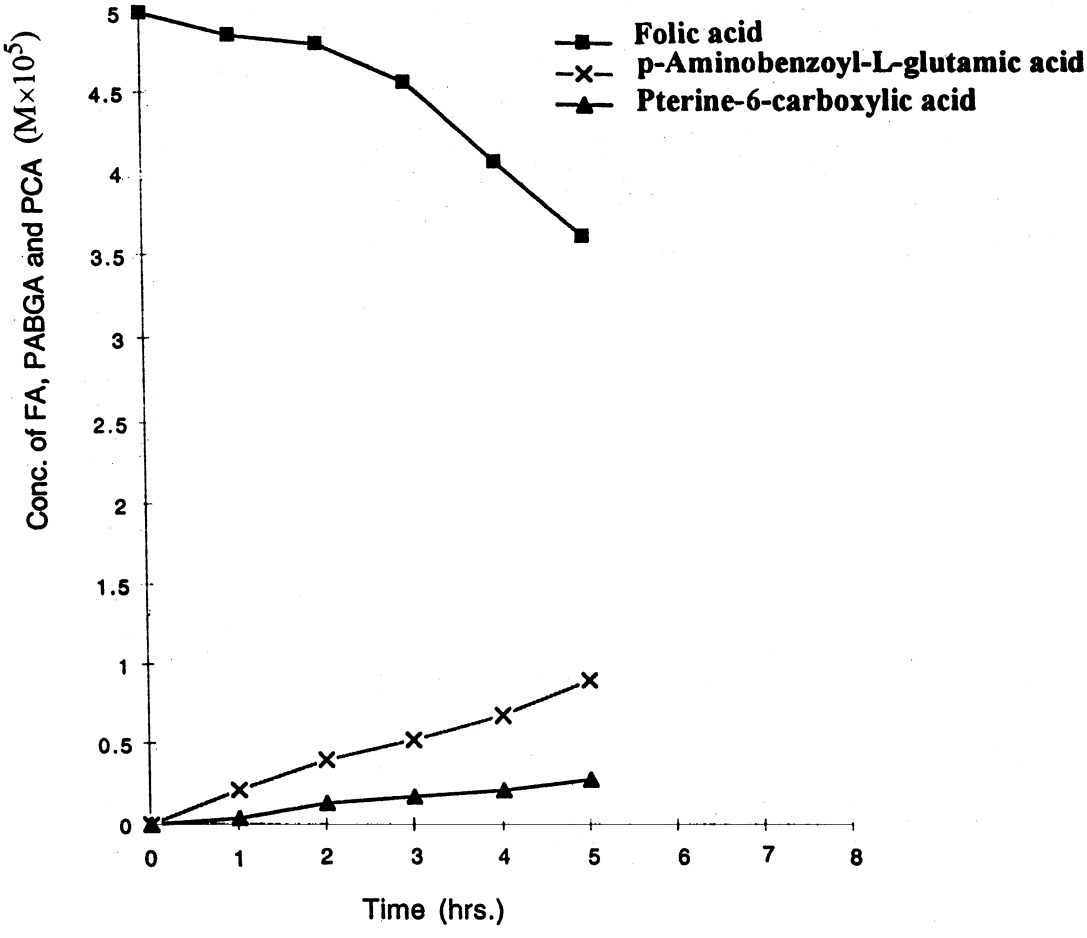

Fig. 1. Photolysis of folic acid solution at pH 5.5.

Table 2
Photolysis of 5×10^{-5} M folic acid solutions at PH 2.0–10.0

pH	2.0	2.5	3.2	3.5	4.0	4.2	4.5	5.0	5.5	5.7	6.0	6.5	6.7	7.0	7.5	8.0	8.5	9.0	10.0
Irradiation time (min)	Concentrations of folic acid ($M \times 10^5$)																		
0	0.330	0.220	0.198	0.242	0.480	0.616	5.090	5.090	4.984	4.984	4.984	5.090	4.982	5.090	5.090	5.090	5.090	4.984	4.984
15	—	—	—	—	—	—	—	—	—	—	4.939	5.055	4.950	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—	—	5.024	—	—	—	—	—	—	—	—	—
30	0.292	0.171	0.178	0.220	—	0.580	—	—	4.879	—	4.944	5.026	4.916	5.011	—	—	—	—	—
40	—	—	—	—	—	—	—	—	—	4.980	—	—	—	—	—	—	—	—	—
45	—	—	—	—	—	—	—	5.092	—	—	4.919	4.992	4.908	—	4.963	—	—	—	—
60	0.264	0.158	0.172	0.210	0.431	0.544	4.717	—	4.816	4.920	4.913	4.992	4.891	4.976	—	—	5.100	4.961	4.981
75	—	—	—	—	—	—	—	—	—	—	4.844	4.940	4.864	—	—	—	—	—	—
90	0.248	0.138	0.149	0.193	—	0.516	—	5.106	4.746	—	4.806	4.897	4.827	4.912	4.879	4.921	—	—	—
105	—	—	—	—	—	—	—	—	—	—	4.744		4.796	—	—	—	—	—	
120	0.214	0.114	0.137	0.168	0.370	0.476	0.496	—	4.678	4.801	—	—	4.752	4.863	—	—	—	4.921	4.946
135	—	—	—	—	—	—	—	4.620	—	—	—	—	—	—	4.778	—	—	—	—
150	0.176	0.102	0.113	—	—	—	—	—	4.580	—	—	—	—	4.778	—	4.798	—	—	—
160	—	—	—	—	—	—	—	—	—	4.707	—	—	—	—	—	—	—	—	—
180	0.154	—	—	0.144	0.311	—	4.282	4.394	4.490	4.652	—	—	—	—	—	—	4.870	4.879	4.880
210	—	—	—	—	—	—	—	—	4.352	—	—	—	—	—	—	4.629	—	—	—
225	—	—	—	—	—	—	—	4.308	—	—	—	—	—	—	—	—	—	—	—
240	—	—	—	—	0.250	—	3.749	—	—	—	—	—	—	—	—	—	4.765	4.819	4.853
270	—	—	—	—	—	—	—	3.912	4.022	—	—	—	—	—	—	4.537	—	—	—
300	—	—	—	—	—	—	3.459	—	—	—	—	—	—	—	—	—	4.583	4.734	4.805
315	—	—	—	—	—	—	—	3.633	—	—	—	—	—	—	—	—	—	—	—
330	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.159	—	—	—
360	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.664	4.736
420	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.581	4.698
480	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.467	4.685

2. Experimental

All the experimental work was carried out in diffused light. The solutions of folic acid 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 chemical and 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: KCI–HCI, pH 1.0–2.0; citric acid–Na_2HPO_4, pH 3.0–7.0; $Na_2B_4O_7$–HCl, 8.5–10.0; the ionic strength was 0.05 M in each case to minimise any salt effect on photolysis.

2.2. Assay method

The assay of controls and photolysed solutions was carried out using a previously reported HPLC method [8].

2.3. Photolysis

2.3.1. Radiation source

Folic acid absorbs in UV region and therefore a Philips 30W TW tube was considered suitable for photolysis. It emits 88.7% of its radiation energy at 254 nm, which corresponds to one of the absorption maxima (256 nm) of folic acid [9]. The number of quanta emitted by the radiation source has been calculated as 6.420×10^{16} quanta s^{-1} [10].

2.3.2. Method

Solutions of folic acid were prepared in various buffer systems at the required pH (2–10) and transferred to a 100 ml pyrex beaker. The concentration of folic acid solutions at pH 4.5 and above was 5×10^{-5} M, but below this pH, the concentration varied within the range of $0.2–0.6 \times 10^{-5}$ M due to low solubility of folic acid in the pH range 2.0–4.2. The beaker was placed in a radiation chamber and irradiated with the Philips 30 W TUV tube fixed horizontally at a distance of 30 cm. The temperature of the solution during irradiation was maintained at 25 ± 2°C. Samples were withdrawn at appropriate intervals for HPLC analysis. Control solutions wrapped in aluminium foil were placed in the dark and assayed for folic acid content at the end of the reaction.

3. Results and discussion

3.1. Assay of photolysed solutions

Although HPLC is a versatile technique for the determination of pharmaceutical compounds such as folic acid and its photoproducts, some variations have been observed in the assay of folic acid as evident from the deviations in the kinetic plot (Fig. 3) in spite of the most careful experimental performance regarding the photolysis technique and the assay procedure. 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

Table 3
Apparent first-order rate constants (K_{obs}) for the photolysis of folic acid solutions at pH 2.0–10.0

pH	$k \times 10^3$ min^{-1}	Correlation coefficient (r)
2.0	4.2500	0.986585
2.5	5.0366	0.991830
3.2	3.6633	0.976339
3.5	3.4600	0.976078
4.0	2.8166	0.996557
4.2	2.3241	0.992663
4.5	1.3233	0.980508
5.0	0.7666	0.971943
5.5	0.5666	0.993290
5.7	0.4600	0.999248
6.0	0.4800	0.950595
6.5	0.4333	0.978711
6.7	0.3833	0.983878
7.0	0.4083	0.990401
7.5	0.4566	1.000040
8.0	0.4466	0.994112
8.5	0.3666	0.993507
9.0	0.2366	0.97779
10.0	0.1550	0.994465

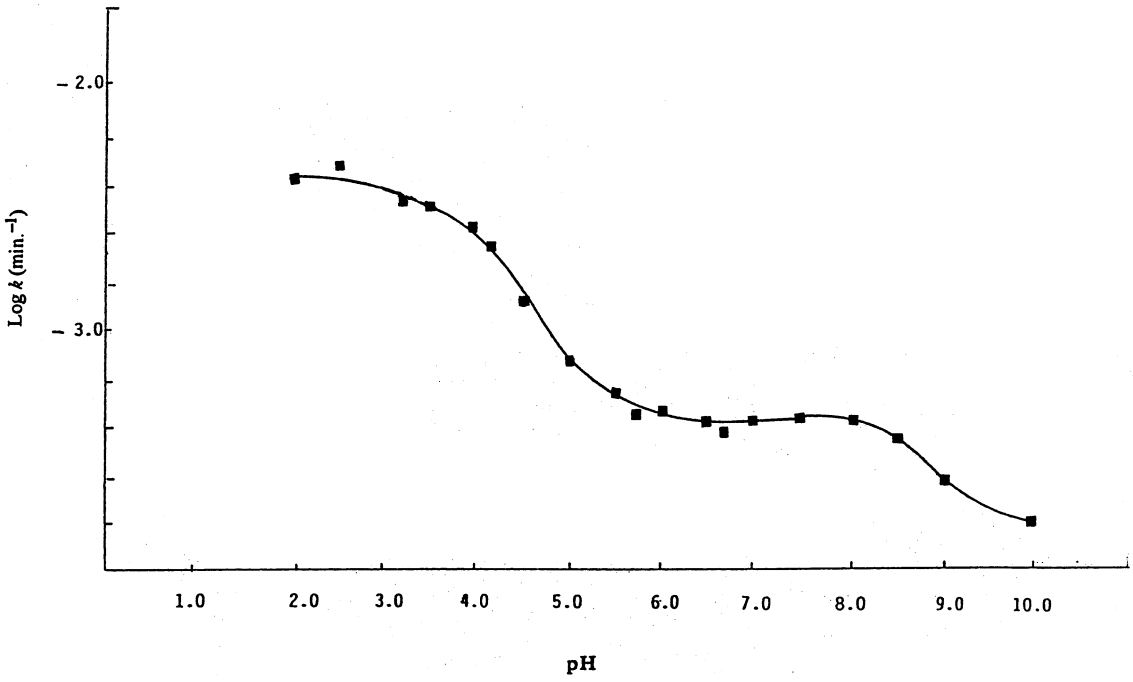

Fig. 2. Log k-pH profile for the photolysis of folic acid.

data may be considered sufficiently accurate and reliable for the purpose of kinetic treatment.

Some variation of methanol content or mobile phase pH may be required to improve resolution due to slight deterioration of the chromatographic column being used. However, chromatographic conditions described in the HPLC method [8] provide the best resolution for folic acid and its photodegradation products in a single analysis. The minimum column efficiency required to carry out this method has been found to be 6000 plates. After use, the column is washed with 30 ml each of water, water: methanol 50%(v/v) and methanol to avoid any significant deterioration in column performance. A typical chromatogram of a photolysed solution of folic acid (5×10^{-5} M) at pH 7.5 is shown in Fig. 4, which clearly demonstrate the separation of folic acid and its photodegradation products, i.e. pterine-6-carboxylic acid and *p*-aminobenzoyl-L-glutamic acid.

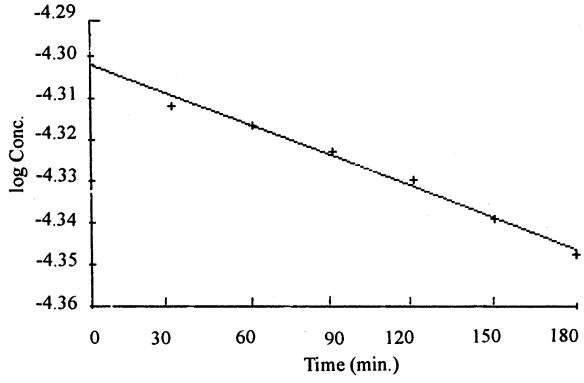

Fig. 3. Apparent first-order plot for the photolysis of folic acid at pH 5.5 (citrate–phosphate buffer).

3.2. Kinetics of photolysis

A typical set of the assay data for the photolysis of folic acid at pH 5.5 is reported in Table 1. The data were plotted as a function of time (Fig. 1) to observe the behaviour of folic acid on photolysis as well as the formation of photoproducts. It shows the folic acid is degraded, with time, and pterine-6-carboxylic acid and *p*-

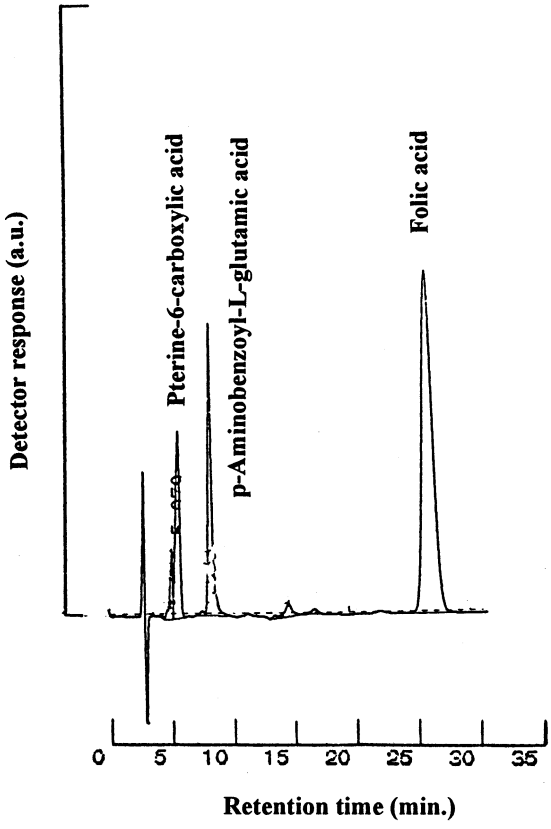

Fig. 4. Chromatogram of folic acid (5×10^{-5}M) solution photolysed at pH 7.5 (citrate–phosphate buffer).

aminobenzoyl-L-glutamic acid are formed with maximum yield obtained at 3–8 h depending upon the pH.

In order to observe the rate of reaction the data presented in Table 2 were subjected to kinetic treatment for compliance with the zero, first- and second-order reactions and were found to give the best fit when treated as first-order plots. The apparent first-order rate constants, obtained from the slopes of straight lines and their correlation coefficients are given in Table 3. The values range from 0.15×10^{-3} min^{-1} (pH 10.0) to 5.04×10^{-3} min^{-1} (pH 2.0).

3.3. pH-rate profile

In order to observe the effect of pH on the rate of photolysis of folic acid a pH-rate relationship has been established. The apparent first-order rate constants (k_{obs}) were plotted against pH and the profile is shown in Fig. 2, which demonstrates the dependence of the rate of reaction on pH. The shape of the curve represents three regions indicating the involvement of different folic acid species in the photodegradation process. The participation of different ionic states of folic acid in the photolysis at various pH values can be explained by the change in spectral behaviour of folic acid at pH 2.0–8.5 (citrate–phosphate buffer). The absorption maxima occur at 288 nm (pH 2.0), 280 nm (pH 7.0) and 285 nm (pH 10.0).

The pH-rate profile demonstrates that folic acid in acid medium is highly susceptible to photolysis because of the formation of predominantly protonated species (pK_{a_1} 2.3) [11] which undergoes photolytic degradation. The rate of reaction is high within pH 2.0–4.0 and as the pH of the medium increases towards the neutral region, the molecule becomes deprotonated and hence a gradual decrease in the rate of reaction. The curve within the pH range 5.0–7.0, where the predominantly neutral species is involved, is almost flat with little change in the rate suggesting that the neutral species is less susceptible to photolysis. The rate of reaction again appear to slow down gradually with a very low rate at pH 10.0, as the molecule proceeds towards the alkaline region where it exists in predominantly anionic form (pK_{a_2} 8.3) [12]. This may be explained on the basis of the existence of a mesomer stabilized anion [12] which is much less susceptible to the photodegradation process.

Thus the rate of photolysis of folic acid is gradually decreased on moving from the acid to the alkaline region and pH 6–7 appears to be a better choice for maintaining the pH of folic acid containing liquid vitamin preparations to achieve optimum stability on exposure to light.

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