Journal of Thermal Analysis and Calorimetry, Vol. 75 (2004) 709–717

THERMAL STABILITY OF FOLIC ACID IN THE SOLID-STATE

*A. Vora, A. Riga, D. Dollimore and K. Alexander**

College of Pharmacy, Pharmacy Practice Department, The University of Toledo, Toledo, OH 43606 and Department of Clinical Chemistry, Cleveland State University, Cleveland, OH, USA

(Received July 27, 2003; in revised form October 30, 2003)

Abstract

This study attempts to identify the degradative process which folic acid undergoes in the solid-state under thermal stress. In order to facilitate the process, the various pieces of the chemical structure, namely, *p*-amino benzoic acid, pterin and glutamic acid as both its *d*- and *l*-isomers were investigated as separate entities. These structured solid-state pieces were then compared to the composite solid state folic acid degradative curves in order to identify the peaks seen and provide direction for the interpolation of the degradative mechanism. It was observed that none of the structural pieces could be superimposed as assumed earlier and hence an attempt was made to identify the decomposition products using various analytical techniques such as infrared spectroscopy, mass spectroscopy and X-ray diffraction which suggested that the glutamic acid fragment is lost first as evidenced by acid loss and amide enhancement in the IR spectra. The vitamin was ultimately degrading to carbon fragments and that further identification was not necessary.

Keywords: degradation pathway, folic acid, infrared spectroscopy, mass spectrometry, solid-state, thermal stability, X-ray crystallography

Introduction

Folic acid is a yellowish orange crystalline powder that is tasteless and odorless. It was discovered in the early 1940's. Pteroyl-glutamic acid crystallizes from cold water, in which it is only slightly soluble, as yellow spear shaped platelets [1]. Folic acid is a member of the Vitamin–B family that is necessary for the healthy function of a variety of bodily processes. Chemically the folates are a group of heterocyclic compounds based on the 4-[(pteridine-6-ylmethyl) amino] benzoic acid skeleton conjugated with one or more *L*-glutamate units. Folic acid is also known as pteroylglutamic acid. Folic acid is composed of a pteridine ring, *p*-amino benzoic acid (PABA) and glutamate moieties as seen below. Separately the three moieties have no vitamin activity [2]. Folic acid and its derivatives are widespread in nature. Folic acid is a specific growth factor for certain microorganisms, however in animals the intestinal bacteria provide

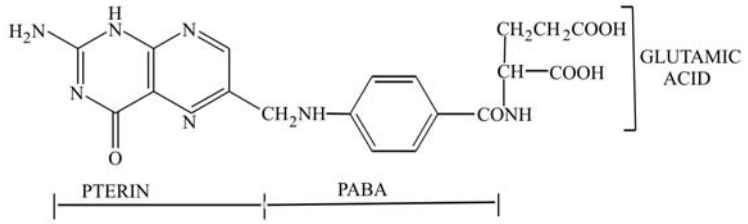
* Author for correspondence: E-mail: kalexan@utnet.utoledo.edu

1388–6150/2004/ $ 20.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

small quantities needed for growth. It acts as a co-enzyme for normal DNA synthesis and also functions as part of the co–enzyme system in amino acid and nucleoprotein synthesis.

Thermal analysis techniques have been used for the characterization of the folic acid sample (Scheme 1).



Scheme 1 Structure of folic acid

Folic acid has been extensively studied in solution and from a biochemical viewpoint and a listing of pertinent references would overshadow the meniscule number of references which report the degradation of folic acid in the solid state.

Tript and Kesselring [3] reported the loss of 1% per year decomposition rate under normal storage conditions (20°C and 65% humidity) by extrapolation of results obtained at 55, 70 and 85°C at 30, 50 and 70% relative humidity and in solid, pure and solutions of 5, 10 and 5% avicel for 18 months.

Decomposition products of folic acid were determined physicochemically using fluorometric, polarographic and spectrophotometric methods [4]. Over the years analytical methods have become more sensitive and highly specialized in that HPLC and thermal methods have come to the forefront.

Luckner [5] reviewed the formation and degradation of purines-, pteridines- and benzoteridines. Technology has advanced to the point that researchers such as Kanie *et al*. [6] have been able to produce thermotropic liquid crystals of folic acid derivatives whose hydrogen bonded complexes form layers and columns.

Experimental

The SDT 2960, simultaneous TG-DTA manufactured by TA Instruments with Universal Analysis for Windows, 95/NT Ver. 2.3 C was used to examine the thermal decomposition of folic acid. X-ray diffraction was performed on a Scintag XDS 2000 diffractometer with CuK_α; λ=1.5406 Å and solid-state Ge detector cooled by liquid nitrogen.

The GC/MS was performed using a Hewlett Packard Instrument HP5988A with direct insertion probe and a scan range of 45–450 amu. Infrared was performed on a Nicolet 60SX analytical instrument at a resolution of 4 cm^{-1}. The data was collected using KBr pellets and the final data was obtained using Omnic Software E.S.P. that was obtained from Nicolet. In order to prepare the IR samples, the Carver press was

used. The DSC samples were run on a Perkin Elmer Instrument at the Department of Clinical Chemistry, Cleveland State University, Cleveland, Ohio.

The folic acid (Lot no: 107H0337) was obtained from Sigma Chemicals Co., St. Louis, Missouri and potassium bromide (Lot no: 1854CK) was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin.

Procedure

Calcium oxalate was used to calibrate the equipment [7]. The folic acid sample was heated at a rate of 2°C min^{-1} and the flow rate for the purge gas (N_2) was 100 mL min^{-1}. The sample was heated to a temperature of 800°C, since at this temperature complete degradation of the sample was observed. In order to identify the degradation products of folic acid, experiments were performed on the anticipated individual compounds comprising folic acid. It was observed that, as assumed the degradation products did not appear as anticipated earlier. A black residue was also left in the pan, which was assumed to be carbon. Methods employed to identify the residue included X-ray diffraction, GC/MS and infrared spectroscopy [8].

Results and discussion

Thermogravimetric analysis

A typical TG/DTG plot of folic acid was obtained when using the optimum conditions that were established, which were a heating rate of 10°C min^{-1} at a flow rate of 100 mL min^{-1} of dry nitrogen gas. Figure 1 shows the TG and DTG curves for folic acid. It was observed from the plots that a mass loss occurred around 100°C due to the loss of adsorbed water [9]. As observed at the end of the entire decomposition re-

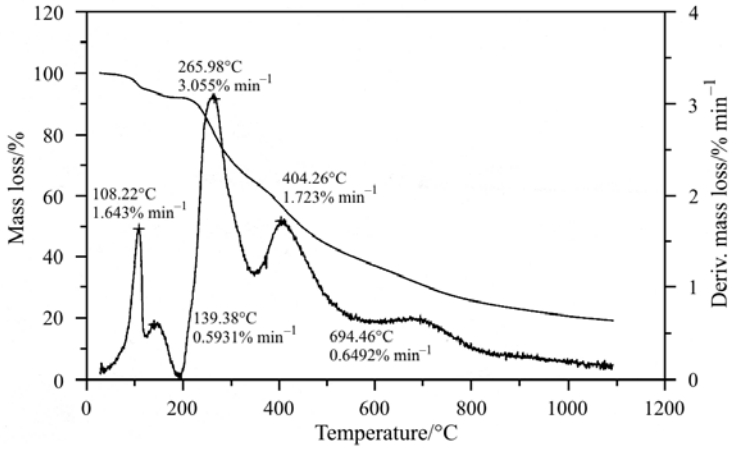


Fig. 1 TG/DTG of folic acid at a flow rate of 100 mL min^{-1} in dry nitrogen at a heating rate of 10°C min^{-1}

action a total mass loss for folic acid occurred. The TG/DTG shows four stages in the decomposition and nearly all four stages overlap as seen in Fig. 1.

X-ray diffraction analysis

The X-ray diffraction pattern for pure folic acid (Fig. 2) was compared to that of the sample treated in the tube furnace up to 349°C (Fig. 3). The data indicates an amorphous product formed suggesting that the loss of a water molecule from the surface of the solid has occurred leaving an amorphous anhydrous material or a lower carbonate. This amorphous anhydrous material then decomposes. Comparison of the X-ray diffraction patterns for pure folic acid (Fig. 2) and its residue collected at 349°C (Fig. 3) from the tube furnace showed that this residue is amorphous. There was complete loss of crystalline character for the product. Further, from the standard library of XRD patterns, it was shown to have a similar pattern to that of carbon, establishing the fact that this residue is indeed carbon. The residue at 349°C was obtained by heating the folic acid sample in a tube furnace. The sample was analyzed by carrying out thermal analysis and X-ray diffraction studies. The main purpose of this procedure was to study the decomposition products of folic acid and to identify the characteristic phases, if any, at the four distinct peak temperatures. The temperatures

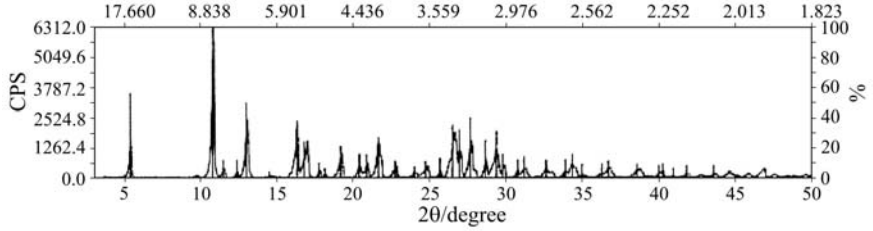


Fig. 2 X-ray diffraction pattern of folic acid

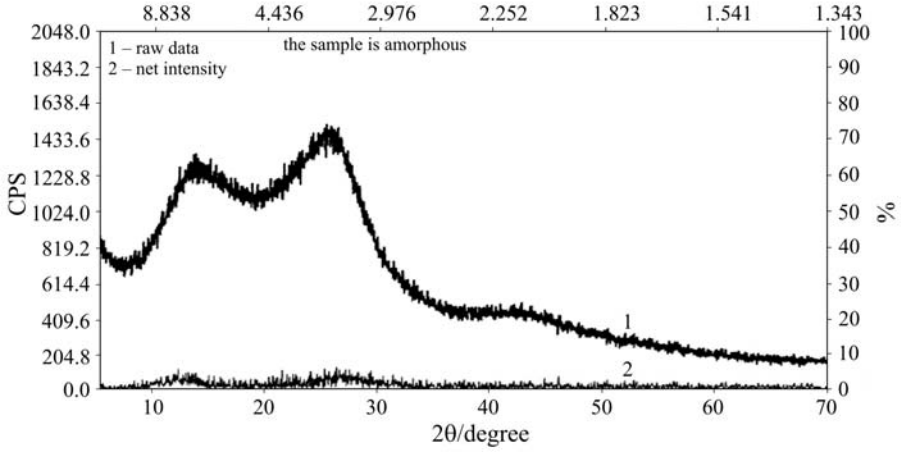


Fig. 3 X-ray diffraction pattern of folic acid treated on TG/DTA up to 349°C

were selected according to the decomposition temperatures observed in the TG plot for the folic acid sample (Fig. 1).

Infrared analysis

The infrared spectra Figs 4–7 gave a definitive pathway to the degradation of folic acid. As seen in the IR spectra of folic acid at room temperature the various functional groups could be observed. Comparing the spectra with the samples which were pretreated to temperatures of 140, 180°C in the oven and also the IR spectra of the sample which was collected from the residue of the TG experiment at 195°C the following were observed.

The IR spectra of the various samples at these key temperatures revealed significant chemical changes compared to the untreated folic acid sample Fig. 4. As seen in Figs 4–7 the important preliminary areas of examination are in the 3000–1500 cm^{-1} regions. This region is known as the functional group region and the other important region is the 900–700 cm^{-1} regions, which is characteristic of the bending regions of

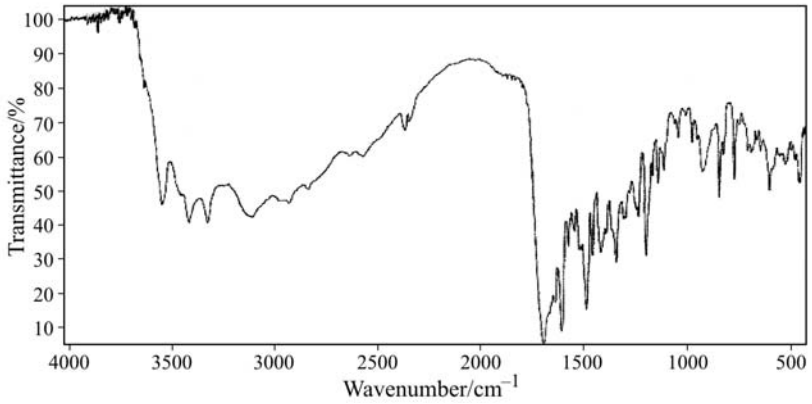


Fig. 4 IR pattern of folic acid at a temperature of 28°C treated on Carver Press

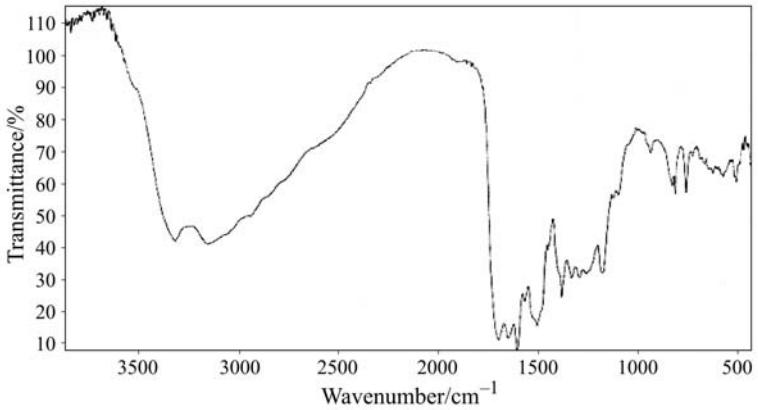


Fig. 5 IR pattern of folic acid heated to 140°C

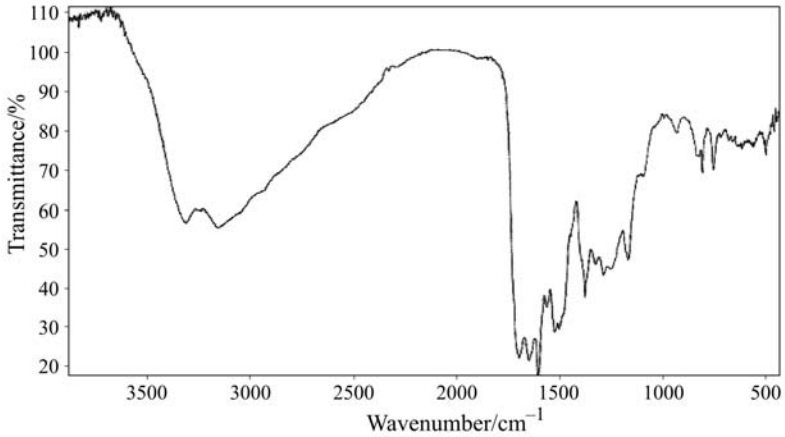


Fig. 6 IR pattern of folic acid heated to 140°C

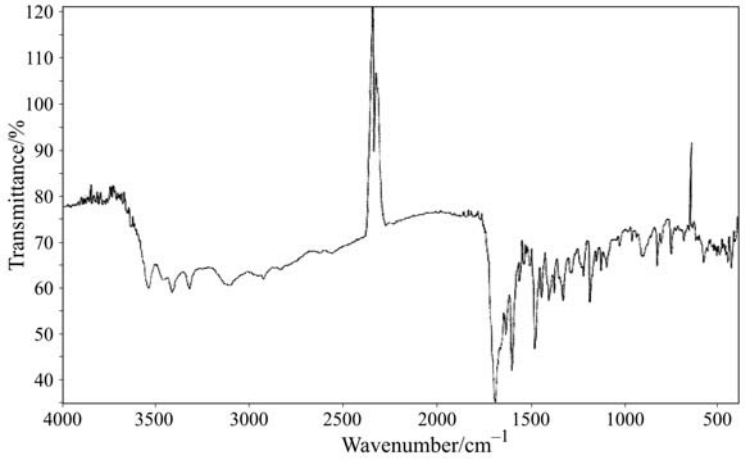


Fig. 7 IR pattern of folic acid heated to 195°C

the functional groups [4]. The absorption in the 2960–2860 cm^{-1} region gives the presence of C–H stretches both symmetric as well as asymmetric. The presence of absorption in the region of 1860–1540 cm^{-1} indicates the presence of a C=O group. At 140°C (Fig. 5) the C=O functionality at 1780 cm^{-1} representing the acid function disappears thus indicating the loss of the acid moiety. At 180°C (Fig. 6) the C=O function in the region of 1680–1650 cm^{-1} representing the amide function disappears indicating the formation of a strong amide bond. At 195°C (Fig. 7) no discernible groups were present in the compound thus indicating that folic acid was completely burnt at this temperature. The abnormal peak in the 1000 cm^{-1} regions is due to carbon dioxide and is a result of the background and was omitted from the results.

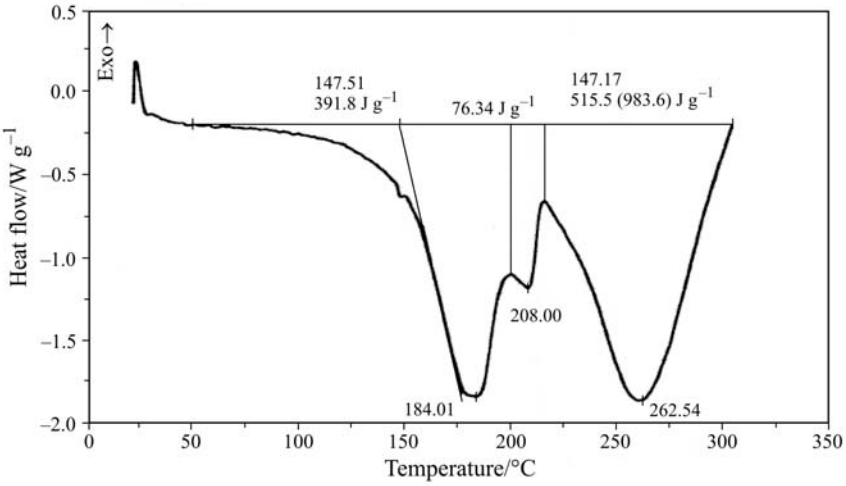


Fig. 8 Differential scanning calorimetry curve

Differential scanning calorimetry

It was observed from the DSC curve (Fig. 8) that folic acid does not have an observed melting temperature. It can be seen to apparently rapidly melt and decompose with three overlapping endothermic reactions. The total heat of degradation was 960±24 J g^{-1} from 148–262°C. The first reaction was 40% of the total reaction representing the loss of glutamic acid moiety with two molecules of water. The second reaction was 8% of the total reaction. The final temperature reaction was 52% of the total reaction which accounted the loss of pterin and *p*-amino benzoic acid based on the molecular masses of the individual compounds with respect to the molecular mass of mass of folic acid being 441.4 dalton.

It was also known at this point that the second DTG and the highly endothermic peak in the DSC were attributed to the loss of the glutamic acid moiety. This moiety begins to degrade at around 180°C, as seen in the IR and the DSC spectra. The DSC results showed an endothermic peak at 250°C, which was due to the initial melting of the sample followed by the degradation.

Gas chromatography/mass spectrometry analysis

From the plot of the electron mass spectra for folic acid Fig. 9, it was observed that it fragmented to its respective molecular mass along with one molecule of sodium, thus having a molecular mass of 464.2 dalton. The presence of sodium was due to the leaching of sodium from the glass container in which the sample was stored. The mass spectra for the temperature profiled folic acid yielded the *m/e* of 313.2 dalton representing the loss of the glutamic acid moiety supporting the IR loss of the acid. At 295.2 dalton, the peak represented the loss of two molecules of water and thereby resulted in the formation of a more stable amide bond.

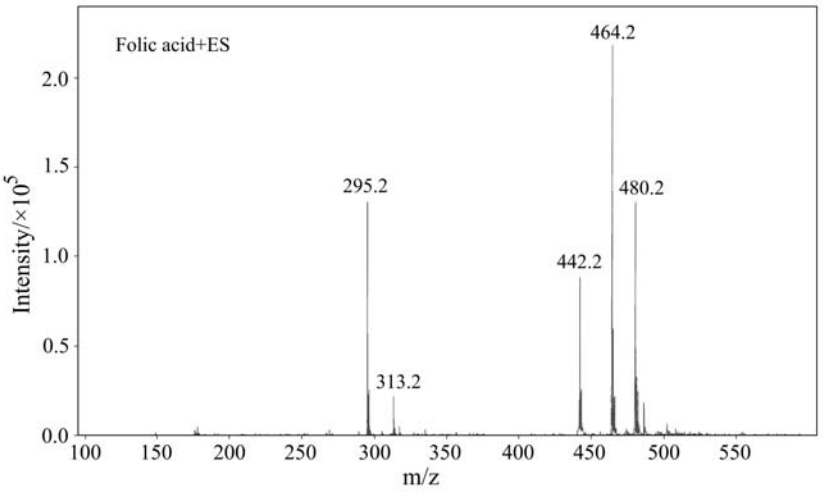


Fig. 9 An electron mass spectrum of folic acid (positive ions). Molecular mass of 480.2 is fragmented

The decomposition can be summarized as occurring in three stages. In Stage I the loss of adsorbed water takes place leaving behind the anhydrous sample. In Stage II the glutamic acid moiety is lost and thereby the other constituents of folic acid are degraded before they reach a temperature of 195°C. The third stage is the black residue left in the pan due to the burning of the compound and having only a trace of carbon.

Conclusions

Solid folic acid is crystalline at room temperature based on its powder XRD profile, as seen in Figs 2 and 3. However, it does not have an observed melting temperature, since it apparently rapidly melts and decomposes with three overlapping endothermic reactions. The total heat of degradation was 960±24 J g^{-1} from 148 to 262°C as seen from Fig. 8. The first reaction was 40% of the total heat, the second 8% and the final high temperature reaction was 52%. The FTIR analysis as a function of temperature revealed the first reaction to be the loss of glutamic acid, followed by the loss of the amide, pterin. There is a complete loss of acid and amide functionality by 195°C. The TG and DTG for folic acid at the same heating rate of 10°C min^{-1} indicates a minor mass loss at 108°C and a major mass loss at 266°C. The former DTG transition is not observed in the DSC study. The third DSC endotherm at 262°C can be associated with the 266°C DTG mass loss. The first two DSC endothermic reactions occur without a mass loss.

The room temperature crystalline folic acid becomes an amorphous mass at 349°C, if one compares Figs 2 and 3. Folic acid undergoes significant degradation by 200°C resulting in an amorphous product above this temperature. Folic acid characterization can be summarized as follows. The initial mechanism for folic acid de-

composition has been established. First, the glutamic acid component breaks away from the folic acid structure leaving the amide as a major constituent. Then the pterin and *p*-amino benzoic acid decomposes in an overlapping mechanism.

References

1 A. R. Gennaro, Remington's Pharmaceutical Sciences, 17th Ed., Easton, Pennsylvania, 1985, p. 1014, 1023, 1024.
2 United States Pharmacopoeia, 19th Ed., 1975, p. 211.
3 F. Y. Tripet and U. W. Kesselring, Pharm. Acta Helv., 50 (1975) 318.
4 H. Marciszewski, Chemia Analityczna (in Polish), 9 (1964) 1011.
5 M. Luckner, Pharmazie, 21 (1966) 142.
6 K. Kanie, M. Nishii, T. Yasuda, T. Taki, S. Ujiie and T. Kato, J. Mat. Chem., 11 (2001) 28575.
7 M. E. Brown, Introduction to Thermal Analysis, Techniques and Applications, Chapman and Hall, London 1988, p. 58.
8 A. Vora, MS Thesis, Thermal Stability of Folic Acid and Associated Excipients, The University of Toledo, December 2001
9 C. J. Keattch and D. Dollimore, An Introduction to Thermogravimetry, 2nd Ed., Heyden London, 1975, p. 28.