The Desolvation and Oxidation of Crystals of Dialuric Acid Monohydrate

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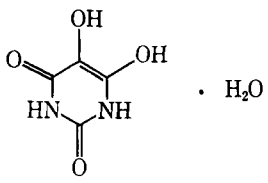
Abstract □ Examination of the influence of the solvent of crystallization on the solid-state oxidation of dialuric acid (I) monohydrate to alloxantin (II) is reported. This reaction was investigated at low and high humidities using photomicrography, X-ray crystallography, and IR and mass spectrometry. Crystals of dialuric acid desolvated somewhat anisotropically; this behavior was consistent with crystal packing. The desolvated crystals of dialuric acid monohydrate had approximately the same crystal structure as the monohydrate and were stable in air at room temperature at low humidities. At high humidity, these crystals rehydrated and rapidly oxidized to alloxantin. These studies showed for the first time that desolvation was not a necessary prerequisite to solid-state oxidation and that solid-state oxidation reactions could be accelerated by high humidity.

Keyphrases □ Dialuric acid monohydrate—desolvation and solid-state oxidation of crystals □ Crystallization—desolvation and solid-state oxidation of dialuric acid monohydrate □ Solid-state oxidation—crystals of dialuric acid monohydrate, desolvation □ Desolvation—crystals of dialuric acid monohydrate, solid-state oxidation

Recent studies (1, 2) have correlated desolvation patterns of crystalline hydrates of drugs and related compounds with crystal packing (1, 2). Several crystal solvates, including those of dihydrophenylalanine (2, 3), ergosterol (4), hydrocortisone *tert*-butylacetate (5), dialuric aicd (6), bis(salicylidene)ethylenediimine Co(II) (7), and the picket fence porphyrins, are oxygen sensitive (8). In many of these solvates, desolvation precedes or coincides with reaction with oxygen.

The present report represents an extension of previous research (1, 2) and is aimed at understanding the relationship between desolvation and oxygen reactivity; the oxidation of dialuric acid monohydrate (I) is discussed as well.



I

The results of this study, which show that moisture and factors other than oxygen permeability of the crystal influence solid-state oxidation reactions, may have important implications for the proper design and storage of drugs and dosage forms.

EXPERIMENTAL

Photomicrography of crystals was performed with a microscope[1] equipped with a hot stage[2]. The X-ray powder diffraction patterns were measured on film using CuKα radiation on a powder camera[3], and elemental analyses were obtained[4]. The extent of reaction was determined using polarographic[5] analysis (quantitative) and IR spectroscopy[6] (qualitative). The constant humidity experiments were performed in chambers with the humidity controlled by various saturated salt solutions: LiCl, 15%; NaCl + KNO_3 + $NaNO_3$, 30%; KSCN, 47%; NH_4Cl + KNO_3, 72%; KBr, 84%; and $NH_4H_2PO_4$, 93%. MS were measured on a mass spectrometer[7] in the chemical ionization mode.

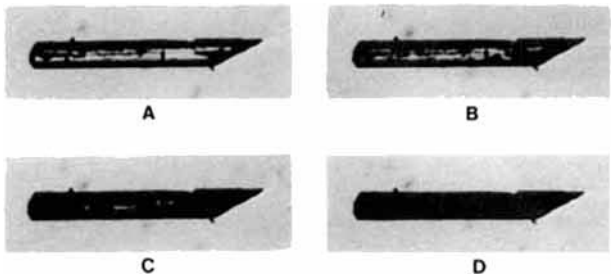


Figure 1—*Behavior of a crystal of dialuric acid monohydrate at 100°: (A) start (B) after 18 min; (C) after 28 min; (D) after 36 min.*

Dialuric acid was prepared according to a previous method (9). Two grams of recrystallized alloxan[5] was dissolved in 100 ml of freshly boiled deoxygenated water. Hydrogen sulfide[6] (5% H_2S, 95% N_2) was then passed through this solution until it became opalescent and free sulfur was liberated. Carbon disulfide (10 ml) then was added and the hydrogen sulfide passed through the solution for an additional 10 min. The carbon disulfide layer was removed and this process repeated for another 10 min. The aqueous solution then was filtered and evaporated to dryness. The product, dialuric acid monohydrate, was obtained by recrystallization from boiled, deoxygenated water. Dialuric acid gave an IR spectrum, elemental analysis, and X-ray diffraction patterns consistent with those in the literature (9).

Anal.—Calc. for $C_4H_6N_2O_5$: C, 29.64; H, 3.73; N, 17.28. Found: C, 29.56; H, 3.90; N, 17.00.

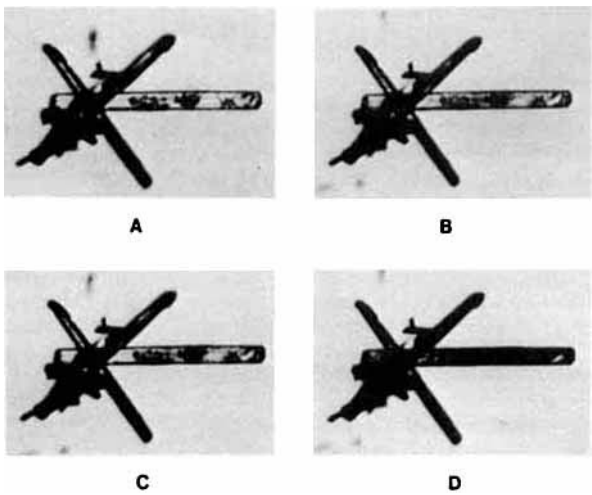


Figure 2—*Oxidation of a clump of crystals of dialuric acid monohydrate at room temperature and 100% relative humidity: (A) start; (B) after 30 min; (C) after 60 min; (D) after 120 min.*

[1] Carl Zeiss.
[2] Mettler FP5/52.
[3] Debye-Scherrer.
[4] Microanalysis laboratory, Purdue University.
[5] Eastman Organic Chemicals.
[6] Matheson.
[7] Dupont.

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Table I—Calculated *d*-Spacings for Oxidized Dialuric Acid and Anhydrous Alloxantin

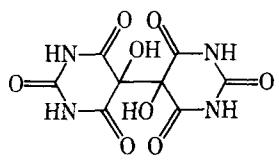
Sample 1		Sample 2		Anhydrous Alloxantin	
d-Spacing Å	Intensity[a]	*d*-Spacing Å	Intensity	*d*-Spacing Å	Intensity
6.036	w	5.996	w	6.026	m
5.556	s	5.522	vs	5.635	m
5.301	vw	5.262	vw	5.262	vw
5.082	vw	5.053	vw	5.046	vw
4.338	m	4.311	m	4.338	vw
3.754	w	3.723	w	3.708	vw
3.594	vvw	3.590	vvw	—	—
3.447	s	3.433	ms	3.417	vs
3.320	m	3.305	m	3.308	ms
3.164	m	3.145	mw	3.209	ms
3.056	vvw	3.048	vvw	3.069	vw
2.974	s	2.971	s	2.952	s
2.901	s	2.885	s	2.905	m
2.784	vw	2.767	vw	2.759	m
2.708	vw	2.692	vw	2.696	m
2.543	m	2.534	m	2.525	ms

[a] Key: (s) strong; (m) moderate; (w) weak; (v) very.

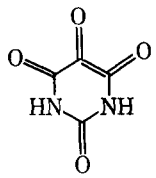
RESULTS

Dialuric acid monohydrate was prepared following literature procedures. It gave a satisfactory elemental analysis and its IR spectrum and X-ray powder pattern were identical to the published data (9). Heating at 76 or 100° led to the formation of the anhydrous form. The X-ray powder pattern of this anhydrous form, as measured on film, was approximately identical to that of the monohydrate. Crystals of dialuric acid monohydrate heated to 100° generally behaved anisotropically, with the reaction proceeding from the ends of the needle-like crystals toward the middle as shown in Fig. 1. The dehydrated solid was polycrystalline since it gave only a powder pattern, had approximately the same crystal structure as dialuric acid monohydrate, and was stable to oxidation. The dehydrated crystals had to be heated at 76° at room humidity for 56–70 days to be completely oxidized.

The structure of the oxidation product was shown to be alloxantin (II) rather than alloxan (III) as had been previously suggested (6). The X-ray powder diffraction pattern of two oxidized samples essentially was identical to that of alloxantin as shown in Table I. In addition, elemental



II



III

analysis of oxidized dialuric acid gave C, 29.77; H, 3.38; and N, 17.40%. The data calculated for alloxantin ($C_8H_6N_4O_8$) are C, 29.82; H, 3.13; and N, 17.39%; while the calculated values for alloxan are C, 30.01; H, 2.52; and N, 17.50%. Infrared and mass spectral measurements on alloxan and alloxantin and oxidized dialuric acid confirmed that dialuric acid is oxidized to alloxantin not alloxan (10).

The dehydrated crystals could be rehydrated at room temperature at humidities ranging from 30 to 84% in a constant humidity chamber. The results of these rehydration studies are shown in Table II. At 93 and 100% relative humidity, a rapid and unexpected oxidation to alloxantin occurs at room temperature. This oxidation complicates the weight gain measurements, which indicate that the equivalent of one molecule of water has been absorbed. In addition, the higher the humidity the faster the reaction, since complete oxidation occurs in <3 hr at 100% relative humidity.

Figure 2 shows the behavior of a clump of crystals of dialuric acid monohydrate at 100% relative humidity. The oxidation reaction begins at several nucleation sites and is nearly complete after 2 hr.

DISCUSSION

Dehydration of Dialuric Acid—The anisotropic behavior of dialuric acid monohydrate crystals during dehydration is probably due to the preferential exit of the water molecules along the tunnel direction (1, 2).

Table II—Structure Changes of Anhydrous Dialuric Acid at Room Temperature and Various Relative Humidities

Relative Humidity, %	Initial Weight Gain Equivalent	Structure Change[a]
2	None	None
15	None	None
30	0.018 g (1·H_2O)	Anhydrous to hydrate
47	0.018 g (1·H_2O)	
72	0.018 g (1·H_2O)	
84	0.018 g (1·H_2O)	
93	0.018 g	Anhydrous to hydrate to alloxantin hydrate (<24 hr)
100	0.018 g	Anhydrous to hydrate to alloxantin hydrate (<3 hr)

[a] As determined by IR and X-ray powder diffraction after 5-days exposure, except where noted.

An examination of the crystal packing of dialuric acid monohydrate (Fig. 3) shows that tunnels of water molecules lie approximately parallel to the needle axis of the crystal. Thus, an individual water molecule exits out these tunnels rather than penetrating the closely packed dialuric acid layers. Similar but more dramatic behavior has been observed for other crystals during desolvation (1, 2).

In addition, the crystal structure of the dehydrated crystals is approximately the same as the hydrate, since the X-ray powder patterns of dialuric acid monohydrate and anhydrous dialuric acid are nearly identical. Thus, dialuric acid is an example of crystal pseudomorphism, a term used to describe similar behavior of the solvates and anhydrous forms of the cephalosporin antibiotics (11).

Oxidation of Desolvated Dialuric Acid Monohydrate—The stability of the desolvated crystals toward oxidation in air was unexpected. Dialuric acid is unstable in solutions exposed to air and has a half-life of ~30 sec at room temperature in aqueous solution. In contrast, the desolvated crystals required 56–70 days at 76° for complete oxidation. Unlike dialuric acid, desolvated crystals of dihydrophenylalanine and bis(salicylidene)ethylenediamine Co(II) (1, 2) react with oxygen at room temperature in a few days. Also, anhydrous dialuric acid has approximately the same crystal structure as dialuric acid monohydrate and, thus, should contain voids previously occupied by water molecules. These voids do not result in facile oxidation. This shows that factors other than the ability of oxygen to penetrate the crystal can influence solid-state oxidation reactions.

Rehydration of Anhydrous Dialuric Acid—Exposure of desolvated crystals of dialuric acid to water vapor results in rehydration at humidities >30%. This is a well-known process for pharmaceuticals with the cephalosporin antibiotics exhibiting unusual ability to desolvate and resolvate (11). As with the cephalosporins, the crystal structures of dialuric acid monohydrate and anhydrous dialuric acid are nearly the same.

Rehydration and Oxidation of Anhydrous Dialuric Acid at High Humidities—If crystals of anhydrous dialuric acid are exposed to room temperature and relative humidities >93%, they rehydrate and oxidize. An increase in the rate of reaction at increased relative humidity is also apparent from the data. The rate of the decarboxylation of solid *p*-aminosalicylic acid and the hydrolysis of solid aspirin also increased with increasing relative humidities (12). The decreased stabilities of these compounds at increased relative humidities were interpreted in terms of the formation of a moisture layer a few molecules thick on the crystals and solution reaction in this moisture layer (12). A similar process may also explain the behavior of dialuric acid. However, because of the rehydration and the rapid oxidation, it is impossible to interpret the weight gain of the crystals at high humidities. For batches of crystals of

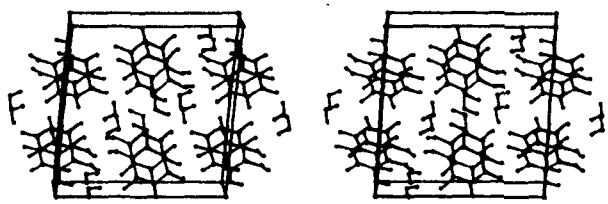


Figure 3—*Crystal packing of dialuric acid. The direction of the crystal axes are: with origin at top left a across b out of the plane of the paper, and c down. (Data from Ref. 6 were used to make this figure.)*

dialuric acid with an (assumed) area of 0.01 m^2/g, a monolayer of water molecules on a 0.100-g sample would weigh 2.14×10^{-7} g. Thus, a water layer 1000 molecules thick would barely be detectable in the absence of the complicating oxidation reaction.

These results indicate that factors other than the apparent permeability of oxygen into the crystal can govern solid-state oxidation reactions. Desolvated crystals of dialuric acid, which are expected to contain voids, are unreactive. Thus, desolvation does not always increase the reactivity of a crystal toward oxygen. Instead, solvated crystals exposed to high humidities react very rapidly, showing that high humidities can accelerate solid oxygen reactions. Also, reactions of the type: solid + gas → solid can be accelerated in high humidities presumably *via* reaction in an invisible moisture layer.

In contrast to other solids, these results indicate that stabilization of pharmaceuticals with solid-state behavior related to dialuric acid would best be accomplished, not by avoiding desolvation as indicated by some published results (1–3, 7, 8), but rather by preventing exposure to high humidities.

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A Rapid Quantitative Determination of Acetaminophen in Plasma

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Abstract □ A simple method is described for the rapid, quantitative analysis of acetaminophen in plasma. The nonconjugated acetaminophen present in the plasma following drug administration is determined after plasma protein precipitation by high-pressure liquid chromatography (HPLC) at a wavelength of 240 nm. Acetaminophen (I) is detectable at levels as low as 0.1 μg/ml. Mean recoveries of 94% with a coefficient of variation of 3% were obtained for plasma standards whose concentrations ranged from 0 to 32 μg/ml. Interassay variability of the slope of the standard curve had a coefficient of variation of 2.7%. Application and verification of this method by comparison with another procedure run simultaneously during several human bioavailability studies are described.

Keyphrases □ Acetaminophen—high-performance liquid chromatographic analysis in human plasma □ High-performance liquid chromatography—analysis, acetaminophen in human plasma and blood □ Analgesics—acetaminophen, high-performance liquid chromatographic analysis in human plasma

The widespread use of acetaminophen (I) as an analgesic and antipyretic has stimulated an interest in the development of a simple and rapid free acetaminophen plasma determination suitable for analyzing multiple samples. The pharmacology of acetaminophen is such that ~80% of a dose is conjugated predominately with glucuronic acid and to a lesser extent with sulfuric acid. These conjugated metabolites lack efficacious biological activity (1). Most of the published methods determine total (*i.e.*, free plus conjugated) acetaminophen in plasma (2–4), while those that determine free acetaminophen by a variety of techniques (5–14), including HPLC (7–14), involve time-consuming and labor intensive organic extractions, solvent evaporations (7–10), deal with toxic levels rather than the lower therapeutic levels (11, 12), or are unsuitable for routine multiple therapeutic level samples because of lack of sensitivity, insufficient sample cleanup (11–14), or long analysis time (14).

The present method involves a single plasma protein precipitation step followed by liquid chromatographic determination of acetaminophen in the clear supernatant. The plasma proteins are denatured and precipitated using 0.3 *N* $Ba(OH)_2$ and 5% $ZnSO_4$ solutions described previously (15). The method is capable of detecting <0.1 μg/ml, and the reproducibility eliminates the need for an internal standard. Furthermore, the ease and rapidity of sample workup make this method ideal for multiple sample analysis.

This method has been used routinely for over a year with good results and has been verified by a comparison with an extraction–HPLC acetaminophen method[1] and a colorimetric procedure (16).

EXPERIMENTAL

Reagents—Standard solutions were prepared in distilled water. The 0.3 *N* $Ba(OH)_2$ and 5% $ZnSO_4$ solutions were obtained commercially[2]. The combination of these two salt solutions has been known for years as an effective plasma protein precipitant.

Instrumentation and Operating Conditions—The analysis was performed using a high-performance liquid chromatograph[3] with a variable wavelength UV detector[4] set at 240 nm, an automated injection system[5] fitted with a 75-μl loop, and a 30 cm × 4-mm i.d. reversed-phase,

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