

## Water-catalyzed crystallization of amorphous acadesine

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### Abstract

Lyophilized, amorphous acadesine crystallizes when exposed to water vapor. Isothermal calorimetry of samples held at constant humidity showed crystallization always occurred within 1.5 h at relative humidities above about 50%, but was never observed at humidities below about 40%. The crystalline phase is anhydrous. Crystallization must proceed through an intermediate, metastable hydrate that immediately decomposes to the anhydrous crystal, a mechanism not commonly considered. The various possible mechanisms of water-catalyzed crystallization of amorphous materials can be distinguished by the dependence of the initiation time on the partial pressure of water vapor. © 1997 Elsevier Science B.V.

**Keywords:** Acadesine; Calorimetry; Crystallization; Water vapor

### 1. Introduction

Many drugs are produced as lyophilized, amorphous powders. Crystallization of such a product alters the properties of the drug. Maintenance of the drug in the amorphous state during storage requires knowledge of the conditions that prevent

or allow crystallization. Contact of amorphous materials with water vapor may lead to crystallization through several different mechanisms. This paper describes crystallization of an amorphous drug (acadesine) that crystallizes by an unusual mechanism, examines the mechanisms of water-catalyzed crystallization, and further shows the utility of calorimetry for studying the reactions of water with drugs.

Isothermal, heat conduction calorimetry has previously been used in several studies of water-drug interactions (Hansen et al., 1996a,b;

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Angberg et al., 1991a,b, 1992a,b). Angberg et al., described a method in which an open vial of water or saturated salt solution is placed in the sealed sample ampule to maintain a constant humidity over the sample. Hansen et al., 1996a described a method in which the partial pressure of water vapor over the sample is continuously scanned. The methods are complementary, The method of Angberg et al. is more useful for determination of the kinetics of water driven reactions, while that of Hansen et al. is more useful for determination of the thermodynamics of water sorption.

## 2. Materials and methods

Lyophilized acadesine (5-aminoimidazole-4-carboxamide-1-ribofuranoside) was supplied by Gen-sia Laboratories, Irvine, CA. All other materials were reagent grade. Acadesine was stored in a dessicator at room temperature in the serum vials as received. Transfer to calorimeter ampules was done in the lab atmosphere with about 20% relative humidity. As supplied, acadesine is a feathery, white material showing no evidence of crystallinity by x-ray diffraction. After exposure to water vapor, acadesine gave an x-ray diffraction pattern indicating a high degree of crystallinity.

The method and instrument (Hart Scientific model 7707 DSC) described in Hansen et al., 1996a was used in experiments in which the partial pressure of water vapor was scanned from 1 kPa to 3.5 kPa. The same calorimeter was used in isothermal mode with the method of Angberg 1991a for kinetic studies of water-catalyzed crystallization. The calorimeter has four calorimetric vessels, three are used for samples and one is used as a reference blank. The blank ampule was empty in these studies. Two to three mg of amorphous acadesine was placed in each of the sealed, 1 ml, hastelloy ampules together with a 50- $\mu$ l vial containing 40  $\mu$ l of water or of a saturated salt solution. A crystal of salt was also included to ensure saturation at all temperatures used in the study. Saturated solutions of NaCl, NaBr,  $\text{MgCl}_2$ ,  $\text{K}_2\text{CO}_3$ , and  $\text{Mg}(\text{NO}_3)_2$  were used to establish fixed relative humidities of respectively 75.3, 57.5,

32.8, 43, and 52.8% in the calorimeter ampules (Kitic et al., 1988; Nyqvist, 1983; Rockland, 1960).

A Seiko thermogravimetric balance was used for TG measurements of the amount of sorbed water in crystallized acadesine.

## 3. Results

Experiments in which the partial pressure of water vapor was scanned produced plots of heat rate versus water vapor pressure which showed only one exotherm. There was no evidence of significant sorption of water prior to the exotherm. The partial pressure at which the exotherm was observed was dependent on the rate at which the partial pressure was scanned, appearing at higher partial pressures at higher scan rates. These observations indicate that interaction of water with the sample is controlled by the kinetics of the process.

Fig. 1 shows examples of the data obtained under constant water partial pressure when crystallization was observed. Crystallization was always observed in less than 1.5 h at relative humidities above about 50%. The initiation time for crystallization was not correlated with the partial pressure of water vapor. Crystallization was never observed if the humidity was below about 40%. Table 1 summarizes the observations made under constant humidities at 15, 25, and 35°C. Crystallization usually gave one peak of about 20 min duration in the thermogram. Integration of the peaks for 12 of the determinations gave the enthalpy change for crystallization as  $41.9 \pm 6.3 \text{ J g}^{-1}$ . The data in Table 1 indicate a decrease in the relative humidity required to rapidly initiate crystallization with increasing temperature, but it is not clear whether the effect is real or is an artifact of the inability to obtain intermediate values of relative humidity with saturated salt solutions.

Thermogravimetry of the crystallized product showed a water content of less than 1.3%, corresponding to  $<0.17$  mole of  $\text{H}_2\text{O}$  per mole of acadesine. No hydrates of acadesine are known or described in the literature.

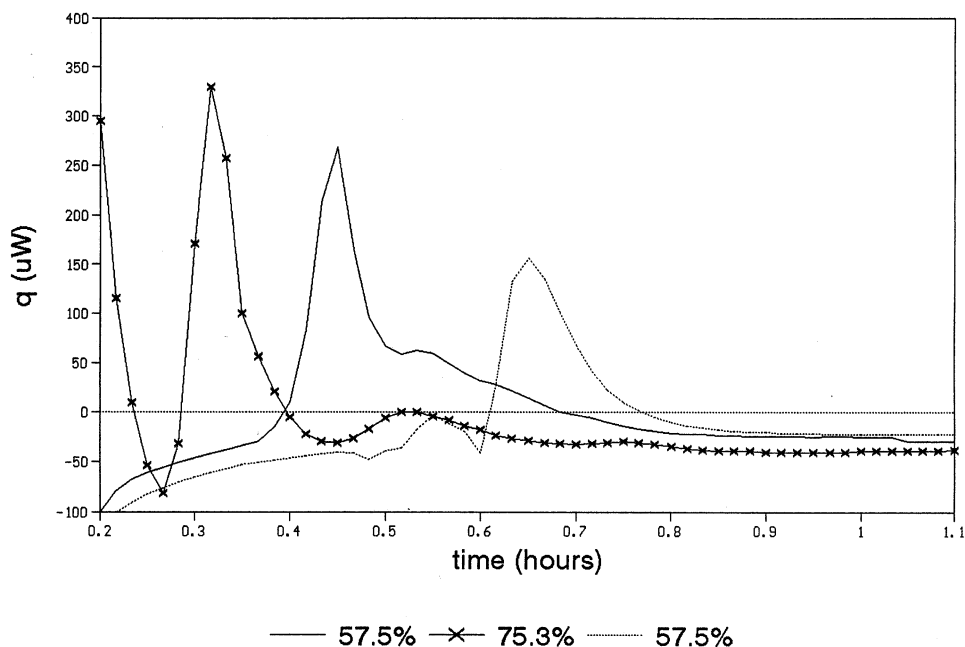


Fig. 1. Thermograms for water-catalyzed crystallization of lyophilized, amorphous acadesine at 25°C, two at 57.5% humidity (—, ...) and one at 75.3% (—X—). Thermal events at  $t < 0.25$  h are from equilibration of the samples to the measurement temperature.

#### 4. Discussion

The lyophilized, amorphous acadesine is readily soluble in large amounts of water, and is administered as an intravenous solution. However, when exposed to water vapor, the amorphous material

converts to a water-repellent, insoluble, anhydrous crystalline form very difficult to remove from the calorimeter ampules. These observations raise the question, by what mechanism can water vapor catalyze the formation of anhydrous crystals from an anhydrous amorphous material?

There are at least three different mechanisms by which water can catalyze crystallization of amorphous materials:

1. Sorbed water can dissolve the surface of the amorphous material, and when the liquid layer becomes sufficiently thick to allow nucleation, crystallization can occur. This mechanism produces thermograms similar to those in Fig. 1. However, because the rate limiting step is the rate of transport of water to the solid surface, the time to crystallization must be directly proportional to the partial pressure of water in excess of the vapor pressure of a saturated solution. This last condition is contrary to the result for acadesine where no proportionality exists between the time to crystallization and water vapor pressure in excess of about 50% humidity.

Table 1  
Summary of results of initiation times for water-catalyzed crystallization of amorphous acadesine

T/°C	Result	No. of determinations
15	$t < 1.5$ h if $p_{\text{H}_2\text{O}} > 8$ torr (58% RH)	2
	$t > 30$ h if $p_{\text{H}_2\text{O}} < 6$ torr (43% RH)	4
25	$t < 1$ h if $p_{\text{H}_2\text{O}} > 13$ torr (53% RH)	13
	$t > 30$ h if $p_{\text{H}_2\text{O}} < 11$ torr (43% RH)	5
35	$t < 1$ h if $p_{\text{H}_2\text{O}} > 19$ torr (43% RH)	4
	$t > 10$ h if $p_{\text{H}_2\text{O}} < 14$ torr (33% RH)	2

2. If the partial pressure of water exceeds the equilibrium vapor pressure for a hydrate, crystallization of the hydrate can proceed. Again, because the hydrate can form no faster than water is transported to the amorphous material, this mechanism requires that the time to crystallization be directly proportional to the partial pressure of water in excess of the equilibrium vapor pressure of the crystalline hydrate.
3. If the partial pressure of water exceeds the equilibrium vapor pressure for a metastable hydrate, crystallization of the metastable hydrate will proceed, but will often be coincident with decay of the metastable hydrate to a more thermodynamically stable form which may be anhydrous. This mechanism is in accord with Ostwald's rule, that the first phase to form from a metastable system is often the least stable that is thermodynamically accessible (Fox et al., 1995). This mechanism is not limited by the rate of water transport because, once sufficient water is present to allow nucleation of crystals of the metastable hydrate, no further water is necessary for the reaction to go to completion. Indeed, water is liberated during crystallization of an anhydrous phase. This last mechanism is consistent with the observations on acadesine.

Acadesine was not observed to crystallize if the water partial pressure was below a certain value, consistent with requirement for formation of a hydrated phase. Above a critical water vapor pressure, acadesine crystallization rate was independent of the water partial pressure, as expected for conversion of a metastable hydrate to anhydrous crystals. Although as far as we could determine, there are no previous reports in the literature of the above mechanism passing through a metastable hydrate, it may be quite common. Ostwald's rule explains why it should be commonly encountered with amorphous materials that crystallize to anhydrous phases when exposed to water vapor. The most kinetically accessible state is also usually the nearest thermodynamic state. The presence of a nearby thermodynamically metastable state facilitates the kinetics of transformation of the amorphous

state into a crystalline state. Formation of the anhydrous crystal from the hydrated crystal is autocatalytic because of the release of water during this step of the process.

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