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Enthalpies of Solution of Nucleic Acid Bases. 1. Adenine in Water

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An adiabatic solution calorimeter was used to measure the enthalpy of solution in water of various adenine samples for which a large amount of analytical information is reported.

The experimental imprecision of the measurements was 1.1 percent. However, it was necessary to assign an overall uncertainty of 3 percent because of impurity uncertainties. Thus, the best value for the enthalpy of solution is

$$\Delta H^\circ (\infty, 298.15\ \mathrm{K}) = (33.47 \pm 1.00)\ \mathrm{kJ \cdot mol^{-1}}.$$

$\Delta C_p = (78.7 \pm 10.4)\ \mathrm{J \cdot mol^{-1} \cdot K^{-1}}$ in the range 298 to 328 K at 5 $\mathrm{mmol \cdot kg^{-1}}$, and the enthalpy of dilution is $-(316 \pm 208)\ \mathrm{kJ \cdot mol^{-1}\ (mol \cdot kg^{-1})^{-1}}$ in the range 1 to 7 $\mathrm{mmol \cdot kg^{-1}}$.

The entropy of solution for adenine was calculated to be ΔS° (298.15 K) = $(72.1 \pm 3.9)\ \mathrm{J \cdot mol^{-1} \cdot K^{-1}}$, and the partial molar heat capacity at infinite dilution, $C^\circ_{p2} = (226 \pm 11)\ J \cdot \mathrm{mol^{-1} \cdot K^{-1}}$.

The density of adenine was measured by displacement as 1.47 $\mathrm{g \cdot mL^{-1}}$ with an estimated uncertainty of 1 percent.

Keywords: Adenine: density, enthalpy of solution, entropy of formation; 6-amino purine; calorimetry; nucleic acid bases; thermochemistry.

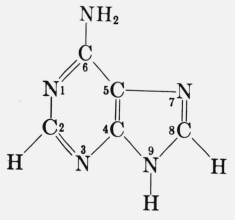
Contents

1. Introduction

This is the first in a series of papers describing our work in determining precise values for the enthalpies of solution of biologically important compounds. Thermodynamic data are needed in biology, biochemistry, and related fields, but the literature contains very few measurements of high precision and accuracy for enthalpies of solution, even for the most basic and simple compounds. It is especially important where possible to have enthalpies of solution in water for these materials as a step in linking the existing compilations of thermodynamic data for materials in well-defined classical thermodynamic standard states to measurements in other fluids such as buffers and plasmas, which are commonly used in the biological sciences.

In order to determine a precise value for the enthalpy of solution it is essential that the purity of the sample be defined. Freezing temperature measurements cannot be used to determine the purity of the nucleic acid bases because they decompose before melting. Therefore, we have attempted within the limits of our resources to characterize the adenine samples as completely as possible. However, to obtain thermochemical data of highest accuracy for these biologically important compounds, a coordinated effort is needed by specialists in the preparation and purification, analysis, and thermochemical measurements. Until this can be done, the data acquired will be subject to larger uncertainties than are desirable.

Our program has begun with measurements on the bases of the nucleic acids and the first enthalpy of solution measurements are with adenine in water. Adenine, $C_5H_5N_5$, a purine base, has the structural formula [1]:[1]



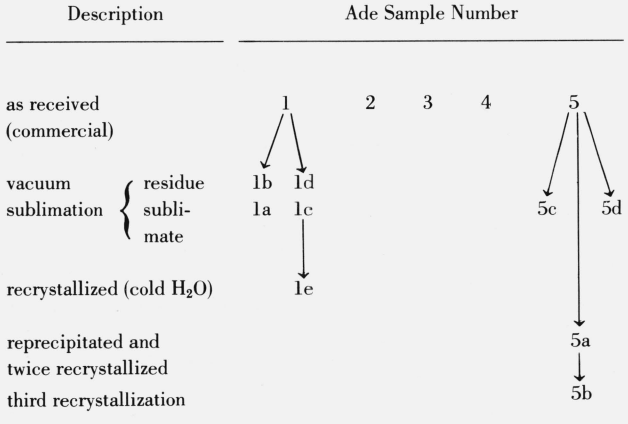
It is only sparingly soluble in water ($\sim$1 g·L^{-1} at 298 K)[2]; it sublimes and decomposes before melting. No measurements of the enthalpy of solution in water have been reported previously.

Because this is the first of a series of publications, the results of our efforts to characterize the samples used are reported here in detail in order to show the extent of our attempts to determine the impurities. Samples as received from several manufacturers are compared with one which was reprecipitated and recrystallized, and with products of sublimation. The effects of temperature, concentration, and particle size on the enthalpy of solution are reported. It is also shown that the sublimation product may contain more of an abnormal tautomer or is of lower degree of cyrstallinity than the product of recrystallization. An approximate value for the density of adenine was also measured.

2. The Adenine Samples

Our efforts to obtain specific information about the methods of preparation, purification, and analyses of the commercial samples were unsuccessful primarily because the procedures are not fixed even for a single supplier. Various starting materials and intermediate materials from many sources are often used. These products are mixed into large lots which lose their identity. Each lot is tested and sometimes reprocessed or blended with another lot until it passes specifications. Thus, the prior treatment of these samples is totally unknown. However, some methods for preparation of adenine have been described by Levene and Bass and others [3a, b, c].

Measurements of the enthalpy of solution were made on adenine (Ade) samples as received from commercial sources,[3] and samples which were products of reprecipitation and recrystallization, of sublimation, and of vacuum heating of the commercial samples in this laboratory. Results are compared for samples of three different lots from the same manufacturer, one from another manufacturer, and another from a distributor who could not identify the manufacturer. The following tabulation summarizes the derivation and treatment of the adenine samples to be described in detail:



Samples marked with ′ and ″ were the corresponding samples which were subjected to additional vacuum drying.

[1] Figures in brackets indicate literature references at the end of this paper.

[2] The National Bureau of Standards (U.S.) has recently recommended the use of "L" as the symbol for liter, the metric unit commonly used to measure volume [2].

[3] The information presented in this paper is in no way intended as an endorsement nor a condemnation of any of the materials or services used. Commercial sources are named only for specific identification.

In this work and in subsequent papers in this series we shall present all information available to us about the materials on which calorimetric measurements were made.

2.1 Sample Description

The following information for the commercial samples was obtained from labels, catalogs, and literature supplied by the manufacturer or distributor:

Ade 1. Boehringer-Mannheim Cat. No. 15512, Lot No. 6249423, 25 g, received October 1973. Catalog description: Adenine, crystallized. Typical Analysis: adenine (optical density) 98%. Chromatographically pure. Stability: Store dry at room temperature; no detectable decomposition within 12 months. At pH = 7.0, extinction coefficient = 13.3 ϵ_{260} *nm*, and extinction quotients: $\epsilon_{250}/\epsilon_{260} = 0.76 \pm 0.02$, $\epsilon_{280}/\epsilon_{260} = 0.14 \pm 0.02$, and $\epsilon_{290}/\epsilon_{260} = 0.01$. Preparation: The source of adenine . . . is ribonucleic acid. For further methods of adenine synthesis see (1) M. Ochiai, R. Marumoto, S. Kobayashi, H. Shimazu, and K. Morita, Tetrahedron 24, 5731 (1968), and (2) K. Morita, M. Ochiai and R. Marumoto, Chem. Ind. London 33, 1117 (1968).

Ade 2. Same as Ade 1 except Lot No. 7072124/Mar. 74, also received October 1973.

Ade 3. Calbiochem Cat. No. 1152, A grade, 5g received about 1970—exact date unknown. Catalog description: Synthetic, therefore free of any contamination with related natural products. Label Information: Lot 71821 Analysis: Nitrogen, 51.70%; Spectra at pH_2, 250/260 = 0.78, 280/260 = 0.35, λmax 264 mμ, εmax 13100, λmin 229 mμ, εmin 2400; Chromat. Homogeneous.

Ade 4. EM Laboratories (E. Merck) Cat. No. 838, Lot No. 1958515, 5g, received February 1974. Catalog description: Adenine for biochemistry, Type Analysis: Assay (ex N; on dried substance), 99%, chloride (Cl), 0.01%, Sulfate (SO_4), 0.01%; Heavy metals (as Pb), 0.002%; Iron (Fe), 0.002%; Loss on drying (105 °C), 1.%; Sulfated ash, 0.1%. Label: The data indicated are not guaranteed figures. (Note: The particle size of this sample was obviously larger than the others which appeared to be very fine white powders.)

Ade 5. Same as Ade 1 except Lot 7263428/July 75, 75 g received in June 1974. (See fig. 1).

Portions of the above samples were used for the following preparations in this laboratory:

Ade 5a. 49 g of Ade 5 was dissolved in 400 mL of hot 11.5 mass percent H_2SO_4 solution. Approximately 200 mL of concentrated NH_4OH was added slowly. At first large plate-like crystals formed, then a heavy, finely divided precipitate developed. This mixture was vacuum-filtered through a polyethylene Buchner-type funnel with #50 hardened filter paper, a minimal amount (~3L) of distilled H_2O was added to the precipitate to effect solution near the boiling temperature. Activated coconut charcoal was added to the solution and the heating continued with stirring for about 5 min. This mixture was again filtered while the receiving flask was kept warm to prevent premature crystallization. The solution was transferred to a large Pyrex crystallizing dish with a cover to cool slowly overnight at room temperature. A grey cast to the solution after filtration indicated that fine charcoal particles were present. Subsequent filtrations employed finer filters which effectively removed the discoloration; these filters were composed of a No. 1 paper covered by a No. 50 paper and then paper pulp from a solution of ashless tablets. The adenine crystals were redissolved in minimal hot water and filtered 3 times while the filtrate was kept warm. The filtrate was clear and colorless. It was transferred to a covered crystallizing dish and allowed to cool overnight. The adenine crystals were filtered on No. 1 under No. 50 paper. The product was white with a very faint yellow tint. There was some finely divided material but most of it was in the form of flat plates or flakes. About one-fifth of this product of 2 crystallizations was to be designated Ade 5a. It was dried in a vacuum desiccator containing $Mg(ClO_4)_2$ for about 24 hours and weighed. Heating in a vacuum oven for 24 hours at 340 K resulted in a weight loss of only 4 percent, much less than the 35 percent that would result from the loss of $3H_2O$ which is the hydration reportedly obtained in the crystallization [3a]. The dried material (3 g) was stored in a desiccator containing $Mg(ClO_4)_2$.

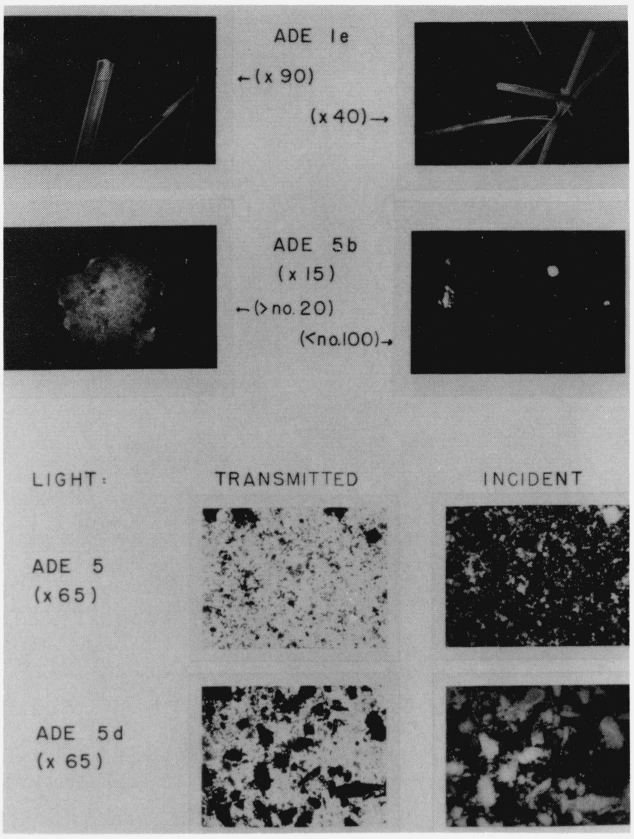


FIGURE 1. *Photomicrographs of Ade 1e (recrystallized from cold H_2O), Ade 5b (recrystallized from hot H_2O), Ade 5 (commercial sample as received), and Ade 5d (sublimate).*

Approximate magnifications are given in parentheses. The 4 photomicrographs at the top were made by Stephen Sykes, Pathologic Studies Section, Bureau of Radiological Health, Food and Drug Administration; those at the bottom, by Edgar S. Etz, Spectrochemical Analysis Section, Analytical Chemistry Division.

Ade 5b. The remainder of the product of the second crystallization above was again dissolved in minimal hot water (~1.5 L) and allowed to cool slowly in a covered crystallizing dish. The mixture was filtered and the adenine crystals were placed in a vacuum desiccator containing $Mg(ClO_4)_2$ for 24 h. When this material was further dried for 24 h in a vacuum oven at 340 K, a 26 percent mass loss was observed. Photomicrographs of this product are shown in figure 1.

Ade 5c. Ade 5 (6.8 g) was placed in a vacuum sublimation vessel (SGA Scientific Co., Cat. No. JM-8855) having a water-cooled condenser. An

electric heating mantle was used to heat the bottom of the vessel and an iron-constantan thermocouple with an ice reference junction indicated the temperature between the bottom of the vessel and the mantle. The pressure in the system was 1×10^{-3} Pa (1×10^{-5} mm Hg) or less and the maximum temperature was 438 K. The sublimation proceeded slowly and after about 40 h there was no residue visible at the bottom of the vessel and no discoloration of the product which had formed a hard crust around the condenser. This material (~4.5 g) was scraped off with a stainless steel spatula, crushed, and sieved; these operations were performed in the room atmosphere. This sample was stored in tightly capped bottles and was exposed to the atmosphere only during transfer manipulations.

Ade 5d. This sample was obtained by sublimation of Ade 5 essentially as described for Ade 5c except that the sublimation proceeded more slowly (~100 h) and the temperature reached 440 K for only a brief period, thereafter, it was maintained between 420 and 430 K. When the sublimation was complete an atmosphere of dry, CO_2-free argon was admitted to the sublimation vessel, and then the vessel was transferred to a glove box which was guarded by absorbers of CO_2 and H_2O from the air. Subsequent manipulations of this sample were performed in this environment including the crushing, sieving, and filling of the platinum calorimetric sample holder. It was observed that this product was harder to crush than the parent product, Ade 5. (See photomicrographs in fig. 1).

Ade 1a. This was the sublimate from the vacuum sublimation of Ade 1 where the maximum temperature was only 400 K. Unfortunately, even after 4 weeks the yield was only about 100 mg which was too small for the calorimetric studies, but it was used in some of the analytical work.

Ade 1b. This was the unsublimed residue from the sublimation in which Ade 1a was the sublimate.

Ade 1c. This product of vacuum sublimation of Ade 1 for a period of about 4 days at 430 K was, while still under vacuum, transferred to a glove box where it was crushed in a glass mortar and separated into two portions—that which passed and that which was retained on a No. 100 standard sieve. All manipulations of the sample were performed in the glove box containing CO_2 and H_2O absorbers.

Ade 1d. This was the unsublimed residue from the sublimation in which the sublimate was Ade 1c.

Ade 1e. This was the product of recrystallization of Ade 1c from cold H_2O (see fig. 1). The needle-like crystals were quite flexible and resisted breaking. The sublimation product, Ade 1c, remaining on the sublimation cone, the funnel, and the sieve after the bulk of the material was removed, was washed off with about 200 mL of distilled H_2O. This solution containing the crystals of Ade 1c stood overnight at room temperature (~295 K). Half of the solution was decanted to a crystallizing dish which was then placed in a refrigerator at 273–275 K. The half of the solution which remained at room temperature was stirred occasionally to hasten the saturation of the solution with adenine. At intervals of about 24 h, the refrigerated solution was decanted, leaving the small amount of crystals which had formed. Then the solution which was saturated at room temperature was added to the crystals and again cooled in the refrigerator. The other portion of the solution was then returned to the dish containing Ade 1c crystals where the saturation process was repeated at room temperature. After 10 days when a significant amount of crystals had formed, the liquid was decanted and the crystals were dried at least a week in a desiccator with concentrated H_2SO_4. The first yield was only about 100 mg, but the process was repeated until all of the Ade 1c crystals had dissolved with a total yield of about 500 mg.

The following samples were products of vacuum-heating in an oven as described in section 2.2.1:

Ade 2′ Obtained by heating Ade 2 at 373 K for 48 h.

Ade 2″ Obtained by heating Ade 2 at 430 K for 16 h. This material had previously been heated at 340 K for 12 h and subsequently exposed to the atmosphere.

Ade 5b′ Obtained by heating Ade 5b (which passed No. 200 standard sieve) at 343 K for 48 h.

These samples were stored in a desiccator, and the calorimetric samples were transferred to the sample holder in a dry box.

2.2 Characterization of Samples

Our efforts to determine the purity or the impurities in the various adenine samples were guided primarily by a publication of the National Academy of Sciences [4] and one of the International Union of Pure and Applied Chemistry [5]. The analytical methods applicable to adenine are limited by the fact that it sublimes and decomposes before melting and is only slightly soluble in water and other solvents at about 300 K.

In this laboratory the densities were measured by displacement, the H_2O content by Karl Fischer titration, the volatile matter by vacuum drying, and impurities by thin-layer and paper chromatography. Information obtained from other laboratories includes elemental analyses, and analyses of emission spectra, X-ray powder patterns, gas-liquid chromatograms, Raman spectra, and proton nuclear magnetic resonsance spectra; also some measurements were made of the enthalpy of combustion and of the heat capacity of the crystalline materials. The photomicrographs in figure 1 provide further identification of some of the samples.

2.2.1 Density, H_2O, and Volatile Matter

Some of the analytical results of our measurements on the various adenine samples are given in table 1. The densities were measured by the displacement method using spectroscopic ACS benzene and 25-mL, Gay-Lussac-type pycnometers. The pycnometer volume was determined from the mass of H_2O it contained under laboratory conditions [(295.7 ± 0.5) K, atm. pressure = $(1.00 \pm 0.01) \times 10^5$ Pa or (750 ± 10) mm Hg, the rel. humidity = (35 ± 10)%]; the standard deviation of the mean (sdm) of 6 measurements was less than 0.01 percent of the volume. The density of the benzene was determined as 0.8765 $g \cdot mL^{-1}$ under the laboratory conditions. This was the average of 4 measurements with sdm = 0.1 percent. The value is in good agreement with the literature value at 295.7 K, 0.8764 $g \cdot mL^{-1}$ [6]. After addition of enough benzene to cover an adenine sample weighed in the pycnometer, air was removed under vacuum in a bell jar with mild shaking to dislodge bubbles. Then the pycnometer was filled with benzene and weighed. The mass of the adenine samples was in the range 0.7 to 1.6 g. The mean of the 8 density measurements is (1.473 ± 0.008) $g \cdot mL^{-1}$; the uncertainty is 2 *sdm*. We estimate a maximum error of about 1 percent in these measurements. There

Table 1. Data on density, H_2O, and volatile matter for adenine samples.

Ade Sample No.	Density	[a] H_2O by Karl Fischer titration	Mass Loss on Vacuum Drying: Initial Mass	Oven Temperature	Time	Loss	Additional Losses and Notes
	g/mL	Mass %	g	K	h	Mass %	
1	1.481	0.12	1.0	341	4	0.115	0.162 } Total loss after ad-
		.08	2.1	341	4	.115	.156 } ditional 8 hours
		.14	3.0	341	4	.114	.153 } (≈0.1 $mg \cdot h^{-1}$)
		.22					
		*.35					
2	1.478	0.24(0.28)	1.2	341	4	0.106	0.166 } Total loss after ad-
		*.19	3.0	341	4	.094	.130 } ditional 8 hours
		.13	3.5	341	4	.098	.132 } (≈0.1 $mg \cdot h^{-1}$)
		*.18	*2.0	373	48	.148	
		----	*1.0	428	16	23.	14. $mg \cdot h^{-1}$ } sublimation
				398	170	13.	0.75 $mg \cdot h^{-1}$ } rates
3	1.476	0.49(0.23)	1.0	341	4	0.125	0.127 } Total loss after ad-
		*.44	1.2	341	4	.104	.123 } ditional 8 hours
			0.6	341	4	.102	.110 }
4	1.467	0.37(.20)	1.0	341	4	0.036	0.062 } Total loss after ad-
		*.40	1.0	341	4	.038	.050 } ditional 10 hours
		----	1.0	341	4	.039	.058 }
5	1.481	----	3.9	373	48	0.157	
[b] 5b	1.453	----					Retained on No. 20 std. sieve
	1.465	----					} Passed No. 20 std. sieve, but
	1.485	----					} retained on No. 100 std. sieve
			1.2	373	48	0.379	Retained on No. 200 std. sieve
			2.3	373	48	.445	Passed No. 200 std. sieve

*Samples previously vacuum dried at 341 K and subsequently exposed to the atmosphere.

[a] 500 mg samples used in the first 3 measurements; 50 mg samples, in all others. Values in parentheses were made later with an improved system. See text.

[b] After reprecipitation and recrystallization from Ade 5, this material was dried 24 h in a vacuum disiccator containing $Mg(ClO_4)_2$ and then in a vacuum oven at 341 K for 24 h; it was exposed to the atmosphere in subsequent manipulations.

appears to be no significant difference in density between the adenine samples except possibly the coarse portion of 5b.

The H_2O determinations by Karl Fischer titrations given in table 1 are probably not as accurate for this material as those determined from vacuum drying because of the low solubility of adenine in methanol and because of the low moisture content of the samples. A titrator having an automatic buret and Karl Fischer reagent calibrated with oxalic acid were used for the titrations. Only a small fraction of each adenine sample dissolved in the solvent (50 mL of methanol), and the H_2O measured was primarily adsorbed rather than occluded. Although somewhat smaller values for H_2O were obtained in the first 3 measurements using 0.5-g samples than in the others with the smaller 0.05-g samples, the higher values are not believed to be conclusive evidence of occluded moisture. The measurements marked with an asterisk in table 1 were made on samples previously vacuum-dried at 341 K and later exposed to the atmosphere. The results are essentially the same as those on samples which were not previously dried (possibly excepting the results for Ade 1) indicating that an equilibrium is reached when the samples are exposed to atmospheric moisture. The values in parentheses were made later using an improved system with a recorder, dry N_2 flushing above the solution, and calibrations with distilled H_2O delivered from a 1-mL syringe; they indicate that the earlier measurements with Ade 3 and Ade 4 gave slightly high values.

The volatile matter was determined for these adenine samples from the loss in mass on heating in a vacuum oven where the samples were protected by a liquid N_2 trap from contamination by the vacuum pump vapors (the pressure was 1 kPa or less). The samples were contained in flat aluminum moisture dishes (55-mm i.d. and 15-mm depth) with tight-fitting covers which were opened during heating and closed during cooling and weighing. Referring to table 1 the samples heated at 341 K were weighed after 4 h, and again at 2-h intervals for an additional 8 to 10 h. Ade 3 and Ade 4 were at essentially constant mass after the first heating, but Ade 1 and Ade 2 continued to lose mass (possibly by sublimation) at a nearly constant rate of about 0.1 $mg \cdot h^{-1}$ which was proportional to the surface area exposed rather than the mass of sample. None of our analytical information has explained the cause of this difference in the samples. The volatile matter in Ade 4 was significantly less than that in Ade 1, 2, 3, and 5. Another portion of Ade 2 was vacuum-heated 16 h

at 428 K with a loss of 14 $mg \cdot h^{-1}$. The same sample was heated at 398 K for an additional 170 h with weighings at intervals of 18 to 25 h; again a linear loss in mass was observed at the rate of 0.75 $mg \cdot h^{-1}$ which is apparently the sublimation rate for these conditions. Ade 5 was heated at 373 K for 48 h and the mass lost was only slightly larger than that for Ade 1, 2, and 3 at 341 K. Two samples of Ade 5b (recrystallized) were also heated at 373 K for 48 h. It is not surprising that the mass losses were nearly 3 times larger (because of occluded H_2O) than that for Ade 5; the more finely divided sample lost slightly more in mass.

The amounts of volatile matter were in fair agreement with the H_2O determined in Karl Fischer titrations although the earlier measurements on Ade 3 and 4 appear to be a little high. We conclude that a correction to the enthalpy data should be made for the presence of volatile matter (presumably H_2O) amounting to (0.15 ± 0.10) mass percent for the 5 samples as received (Ade 1, 2, 3, 4, and 5) and (0.40 ± 0.05) mass percent for Ade 5b.

2.2.2 Chromatographic Analysis

The work done to obtain a better understanding of the significance of the suppliers' expression "chromatographically pure" is described in this section.

Our general guidelines for the chromatographic work on adenine were obtained from pages 152–154 of the National Academy of Sciences (NAS) publication [4]. In that work Whatman No. 40 chromatography paper was used with the descending technique in a sealed cabinet with a presaturated atmosphere and the chromatograms were air-dried. We used this procedure and compared it with the use of Whatman No. 1 paper and with the ascending technique using glass thin-layer chromatography (TLC) plates coated with a 250-μm thickness of MN 300 Cellulose (C) and fluorescent MN 300F Cellulose (CF). The solution front traveled a distance of 35 cm on the papers and 16 cm on the TLC plates. On the plates the time required was 1 to 5 h (depending on the tank solution used); on No. 1 paper, 3 to 18 h; and on No. 40 paper, 4 to 23 h. In general we found no advantage (except lower cost of the material) in the use of paper rather than TLC plates where the time required is reduced by a factor of 3 or more. Attenuation of spots was much less on TLC plates than on the papers, and the resolution of spots was somewhat better on No. 40 than on No. 1 paper. Capillaries of 5 μL capacity (microcaps) were used for the spotting and each contained (7.0 ± 0.5) μg of the adenine samples in aqueous HCl, NH_4OH, or NaOH at the concentration of 1 $mol \cdot L^{-1}$ except as noted. Relatively concentrated solutions were used in the spottings so that impurities might be more easily detected.

The ultra-violet absorbing components in the chromatographs were observed with a 254 nm short wave lamp; a 366 nm lamp was also used but no long-wave absorbing components were detected in this work. Generally the MN 300F Cellulose plates containing zinc silicate phosphor indicator produced more well-defined and easily interpreted spots than the plates without the fluorescent indicator although there were some difficulties in the use of the latter plates especially where the samples were in aqueous HCl solutions. Some of the problems encountered will be discussed later.

Table 2 shows R_f values (distance traveled by the spot component divided by distance traveled by the solution front) for the major components for 7 adenine samples in aqueous HCl and NH_4OH with four tank solutions for which the compositions are given. The R_f values for adenine in the NAS tabulation [4] are given for comparison; the agreement is best for Soln. C and worst for Soln. D, however, the difference of ~0.1 R_f unit is acceptable. It is interesting to note the similarity in the R_f values in the two aqueous solvents, HCl and NH_4OH, except with the CF plates in tank Soln. B.

Since many of the preliminary chromatograms contained streaks, bands, or plumes near the solution front which confused or obscured spots at the higher R_f values, blanks were run to determine which of these problems could be attributed to the solvents and tank solutions. Spottings (2 μL) of distilled H_2O, and of the two solvents on C and CF plates were exposed to each of the four tank solutions with the following results:

Soln. A: Plate C showed no marks corresponding to spottings, but had three light bands (on the dark blue chromatogram) from the tank solution and cellulose effects; the lowest was at $R_f = 0.72$. The CF plate was quite clear and bright except for the very dark spot (~0.5-cm dia.) at the origin with a tail extending slightly less than 1 cm above the spot where the HCl solution was placed. At the solution front dark stalactite-type shapes extended down to about $R_f = 0.88$, but there was no correlation with the line of travel of the spottings.

Soln. B: On plate C, the field was clear blue except for a light area at the solution front with icicle-like tails extending down to $R_f = 0.72$. Plate CF was bright and clear except for a dark spot (0.5 cm dia.) at the origin of the HCl solution, and dark areas at the solution front which extended down to sharp points at $\sim R_f = 0.56$ corresponding to the line of travel of the spottings.

Soln. C: Duplicates were run about 24 h apart using the same tank solution. The CF plates were essentially identical with the characteristic dark spot at the origin of the HCl solution, a broad dark area across the solution front down to $R_f = 0.95$, and a dark tail extending down to $R_f = 0.68$ in the line of travel of the HCl spot. On the C plates there was a light band between $R_f = 0.95$ to 0.88 on the first run, and

R_f = 0.95 to 0.82 on the second; the difference in the width of the band may have been caused by the age of the tank solution. On the lower side of the bands were plume effects which correspond to the line of spot travel.

Soln. D: On plate C the light area at the solution front extended down to about R_f = 0.95. Plate CF was bright and clear except for the dark spot at the origin of the HCl solution.

Some tests were also run to determine the relative limits of detection under these conditions for five bases of the nucleic acids, adenine (Ade), guanine (Gua), uracil (Ura), cytosine (Cyt), and thymine (Thy). With Soln. A and CF plates, the pyrimidine bases (Ura, Cyt, and Thy) in NH_4OH solutions gave well-defined spots for 1-μg quantities however, Cyt and Thy are both at R_f = 0.84 and Ura at 0.78 (NAS values: 0.76, 0.78, and 0.63, respectively). A mixture containing 2 μg of each of the 5 bases in HCl showed a well defined spot for Ade at R_f = 0.92, a broad spot at R_f = 0.84 for Cyt and Thy, and a long, tailing spot for Ura and Gua R_f = 0.81. A spot containing only 1 μg of Ade in HCl was not detected but 1 μg of Gua was easily visible. Ade was not detected in a spot containing ~10 mass percent Ade (1μg) in Gua (8 μg) and a mixture of ~10 mass percent Gua (0.6 μg) in Ade (6.1 μg) resulted in the Ade spot with a long tail. Similar tests using C plates produced broad, poorly defined spots and tailing which resulted in vague interpretations. However, the C plates in Soln. B did resolve the mixtures of Ade in Gua and of Gua in Ade. We estimate that somewhat less than 5 percent of one purine base could be detected in the other by the latter procedure. With Soln. B the spots above R_f = ~0.5 were distorted, half moon or heart-shaped (apparently from interference by the tailing under the solution fronts described for the blank experiments); resolution was poor except for Gua in the mixture of the 5 bases in aqueous HCl. In Soln. B the CF plates gave no better sensitivity than the C plates but there was better resolution of the mixture. Soln. C with C plates gave the best sensitivity and resolution for the five bases and their mixtures but the similar R_f values for Ade (0.64–.68) and Cyt (0.66–.68) did not permit them to be distinguished in the mixtures. It was possible to detect 0.2 μg of Ade in 1.5 μg of Gua, but not 0.1 μg of Gua in 1.2 μg of Ade. The CF plates with Soln. C were easier to read but there was no advantage over the C plates in resolution or sensitivity. When HCl solutions were used the tailing produced ambiguous and confusing results. With Soln. D, resolution of the mixtures was precluded by the tailing from the bases in HCl solutions, and although fairly well defined spots were obtained for the pyrimidine bases in aqueous NH_4OH, their R_f values were so close together that their resolution in a mixture was impossible. Comparisons were also run of the separability of the purine bases in various solutions using both C and CF plates in Solns. B and C, with the following results: In Soln. C all chromatograms were essentially useless because of the poor definition and excessive tailing below R_f = ~0.50 and above R_f = ~0.75. However, when the same runs were made using Soln. B, well defined spots were obtained. Spots of solutions containing 4 μg of Ade 1 in HCl in unheated NH_4OH and in hot NH_4OH and 4 μg of Gua in hot HCl in unheated HCl and in NaOH gave R_f values of 0.65, 0.67, 0.67, 0.39, 0.39 and 0.35, respectively, on the C plates, and 0.56, 0.49, 0.48, 0.06, 0.09, and 0.18 on the CF plates. The solution of Gua in NaOH produced a light tail extending from R_f = 0.21 to the origin on the C plates. On the CF plates all of the HCl solutions showed the characteristic dark spot (~5 mm dia.) at the origin; with Gua the tail extended up from this spot, but with the Ade there were two additional spots at R_f = ~0.34 and 0.20 which may be hydrochlorides—these were not present in the NH_4OH solutions. There were no dark spots at the origins on the CF plates where the samples were in NH_4OH or NaOH solutions.

The results given in table 2 show essentially no difference between the 7 adenine samples, and no impurities were detected in any of the chromatograms. We estimate that 1 to 5 mole percent of the other bases (Gua, Cyt, Thy, or Ura) would have been detected as well as about 80 other compounds listed in the NAS publications [4]. We can conclude only that the 7 adenine samples are of equal purity (probably 95 mol% or more) within the limits of chromatographic detection.

2.2.3 Emission Spectra and Elemental Analyses

Samples of Ade 1, 2, 3, 4, 5, 5a, 5b, and 5c were analysed by emission spectroscopy[4] with the following limits of detection (in mass percent);

Ag, B, Ba, Be, Bi, Cu, Fe, Mg, Mn, Ni, Si, Sn, Ti, and V, <0.01; Cd, Li, and Mo, <0.02; Co, Cr, K, Na, and Pb, <0.05; and, Al, As, and Ca, <0.1.

No impurities in the samples beyond these limits of detection were identified although the recrystallized sample, 5b, showed slightly higher Si (0.02%) than the others; this may have been inhomogeneity in the sample. It is assumed that the possible presence of some of these relatively small amounts of impurities contributes no significant error to the enthalpies of solution in H_2O.

The results of duplicate analyses of some of the adenine samples for C, H, O, N, and ash are given in table 3. All of the samples were very close to the theoretical composition of anhydrous adenine. The general tendency for the analysis to

[4] Analysis by J. A. Norris, Gas and Particulate Science Division, Center for Analytical Chemistry, National Measurement Laboratory.

Table 2. R_f values for adenine samples on papers and TLC plates with four carrier solutions (A, B, C, and D).

Ade Sample No.	Solvent	A[a]				B[a]				C[a]				D[a]			
		TLC[b]		P-1	P-40	TLC[b]		P-1	P-40	TLC[b]		P-1	P-40	TLC[b]		P-1	P-40
		C	CF			C	CF			C	CF			C	CF		
1	HCl (aq,	0.85	0.94	-	0.96	0.88	0.37	0.59	0.64	0.59	0.50	0.65	-	0.65	0.37	0.48	0.49
2	1 mol·L^{-1})	.82	.92	-	.96	.86	.32	.57	.65	.59	.50	.64	.64	.67	.39	.47	.49
3		.82	.93	-	.93	.86	.32	.58	.64	.59	.50	.68	.68	.68	.37	.47	.48
4		.82	.93	-	.93	.89	.33	.58	.64	.60	.51	.67	.67	.68	.40	.45	.48
5		.82	.92	-	.93	.92	.33	.58	.64	.60	.51	.66	.66	.68	.41	.49	.49
5b		.83	.90	-	.93	.86	.34	.56	.65	.60	.51	.68	.68	.68	.41	.47	.49
5c		.83	.91	-	.92	.86	.33	.56	.64	.60	.50	.68	.68	.68	.36	.47	.47
NAS [4]		---	---	-	.88	---	---	---	.70	---	---	---	.67	---	---	---	.39
1	NH_4OH (aq,	---	.96	---	.96	---	.51	.66	.67	---	.57	.63	.69	---	.37	.40	.45
2	1 mol·L^{-1})	---	.97	---	.97	---	.51	.58	.63	---	.55	.70	.70	---	.33	.44	.43
3		---	.97	---	.97	---	.50	.61	.64	---	.55	.68	.68	---	.32	.44	.42
4		---	.97	---	.96	---	.49	.60	.65	---	.57	.68	.68	---	.34	.43	.42
5		---	.97	---	.96	---	.51	.62	.68	---	.56	.69	.69	---	.33	.42	.43
5b		---	.96	---	.96	---	.51	.62	.66	---	.56	.69	.69	---	.34	.40	.46
5c		---	.96	---	.96	---	.51	.62	.66	---	.54	.70	.70	----	.33	.42	.45

[a]The compositions of the carrier (or tank) solutions was as follows:
Soln. A: 5 parts of iso-butyric acid + 3 parts of NH_4OH (aq, 0.5 mol·L^{-1}).
Soln. B: 7 parts of iso-propyl alcohol + 1 part of conc. NH_4OH + 2 parts of H_2O.
Soln. C: 7 parts of 95% ethyl alcohol + 3 parts of sodium acetate (aq, 1 mol·L^{-1}).
Soln. D: H_2O adjusted to pH 10 with NH_4OH (~1 drop of conc. NH_4OH in 300 mL H_2O).

[b]Glass TLC plates coated with a thickness of 250 μm of
C: MN 300 Cellulose
CF: MN 300 F Cellulose (with fluorescent indicator).

Table 3. Elemental analyses of adenine samples

Substance	Empirical Formula	Molar Mass	C	H	O	N (Kjeldahl)	Sulfated Ash	Σ
		g/mol	mass percent					
Theoretical Composition:								
Ade	$C_5H_5N_5$	135.128	44.44	3.73	----	51.83	----	100.00
Ade·H_2O	$C_5H_7N_5O$	153.1432	39.21	4.61	10.45	45.73	----	100.00
Ade·$2H_2O$	$C_5H_9N_5O_2$	171.1584	35.09	5.30	18.70	40.92	----	100.01
Ade·$3H_2O$	$C_5H_{11}N_5O_3$	189.1736	31.75	5.86	25.37	37.02	----	100.00
Analyses[a]:								
Ade 1	---------	---------	44.82	3.87	0.79	51.97	0.00	101.45
	---------	---------	44.57	3.82	0.82	52.16	0.04	101.41
Ade 1e	---------	---------	44.44	3.78	0.38	52.18	0.01	100.79
	---------	---------	44.58	3.74	0.61	51.98	0.01	100.92
Ade 2	---------	---------	44.72	3.70	0.99	52.09	0.04	101.54
	---------	---------	44.80	3.62	0.89	52.35	0.00	101.66
Ade 4	---------	---------	44.59	3.69	0.67	52.19	0.05	101.19
	---------	---------	44.73	3.78	0.71	51.89	0.00	101.11
Ade 5	---------	---------	44.49	3.88	0.00	51.94	0.25	100.56
	---------	---------	44.62	3.87	0.15	52.01	0.19	100.84
Ade 5d	---------	---------	44.30	3.89	0.08	52.06	0.05	100.38
	---------	---------	44.26	3.87	0.19	51.91	0.09	100.32
	---------	---------	44.69	3.68	0.43	52.05	0.10	100.95
	---------	---------	44.69	3.76	0.42	52.12	0.14	101.13

[a]The analyses by Mirco-Analysis, Inc., Wilmington, DE, have an estimated accuracy of ± 0.2% on each determination.

be higher than the theoretical composition is reflected in the summations which are all above 100 percent. The samples of Ade 1, 1e, 2, and 4 were analysed at one time and the high oxygen values reported for these are probably the result of a calibration error. The last two sets of values given for Ade 5d were analysed 2 months before the first 4 samples but 4 months later than the other samples of Ade 5 and 5d. Since there is no reason to suspect changes in composition we assume that the differences are caused by changes in the analytical instruments and that a more conservative estimated accuracy is ±0.5 percent for the oxygen values. However, the data do eliminate the possibility of the presence of hydrates in significant amounts.

2.2.4 Other Analytical Investigations

Analysis[5] of X-ray powder diffraction patterns from Ade 1, 2, 3, 5, 5b, and 5c produced little information except that the samples are primarily crystalline rather than amorphous. The diffraction peaks were relatively broad, of low intensity, and terminated at a fairly low 2θ; thus, it was impossible to calculate cell parameters for the materials. This is not uncommon for organic materials which have been recrystallized from solutions so that strains form within the structure. The pattern for Ade 5b showed somewhat greater resolution than that of its parent material, Ade 5, which also contained an extra diffraction peak indicating a small impurity (perhaps several percent). Ade 5c showed significantly broader peaks than Ade 5 or Ade 5b, indicating smaller crystallites in the sublimed sample.

Mass spectral analyses[6] of Ade 5 and the sublimate, Ade 5d, showed no significant differences except the presence of a trace of a plasticizer (from the plastic cap) in Ade 5.

Gas chromatographic examination[7] of Ade 1, 2, 3, 4, 5, 5a, 5b, and 5c involved tri-methyl silylation of the samples based on the procedure described by Gehrke and Ruyle [7]. The chromatograph was a Hewlett-Packard Model 5750-B with an all-glass column, and detection was by flame ionization. The solvent-reagent mixture produced peaks early in the chromatograms which greatly obscured detection of impurities, especially volatile materials such as occluded solvents. A few minor peaks which might be attributed to impurities were visible in all of the samples but none of the impurities were identified because this would have been a major analytical problem. However, the conclusion that the sublimed sample, Ade 5c, appeared to have the least impurities was important because it led to closer scrutiny of the results of our enthalpy of solution measurements on the sublimed sample.

The infra-red spectra[8] of Ade 1, 1a, and 1b in Nujol (paraffin oil) can be compared in figure 2. They show no structural differences between the sample as received, the sublimate, and the residue from sublimation. Also the nuclear magnetic resonsance (NMR) proton spectra[8] (fig. 3) showed no structural differences between Ade 1a and 1b in mixtures of dimethyl sulfoxide and trimethyl silyl reagent.

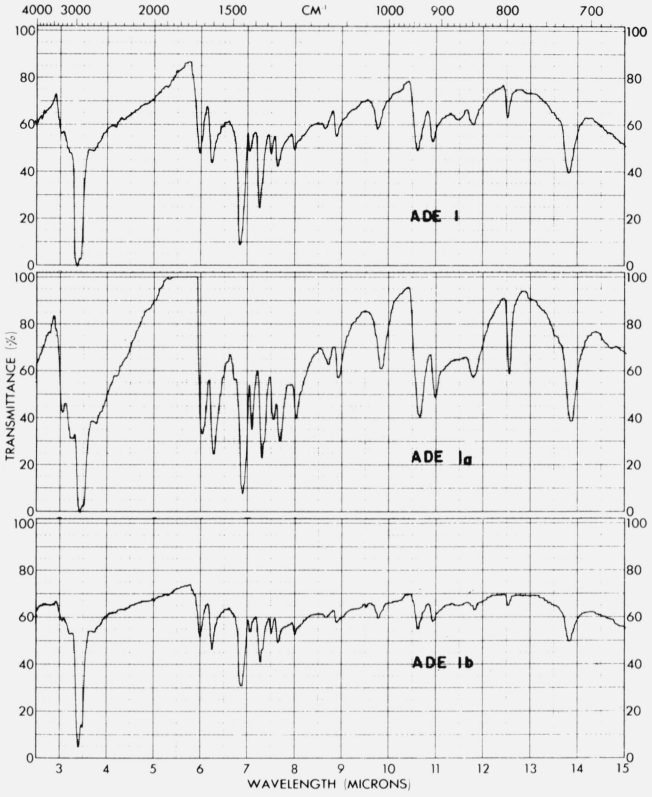


FIGURE 2. *Infra-red spectra of paraffin oil solutions of three adenine samples: 1, the commercial sample as received; 1a, the sublimate; and 1b, the unsublimed residue from sublimation.*

2.2.5 Calorimetric Characterization

Enthalpies of combustion of three of the adenine samples were measured by W. H. Johnson in an adiabatic bomb calorimeter [8] for comparison with the results of Stiehler and Huffman [9] who measured the enthalpies of combustion of adenine samples recrystallized and dried under various conditions. These results are summarized in table 4. Johnson's individual measurements have not been corrected for any impurities, but the mean of the duplicates was corrected for volatile matter or H_2O amounting to 1.1 mole percent for Ade 2 and 5, and 2.9 mole percent for Ade 5b as reported in sec. 2.2.1. The uncertainty in the correction for H_2O results in an uncertainty of about 10 $kJ \cdot mol^{-1}$ in the enthalpy of

[5] Analyses by Camden R. Hubbard, Crystallography Section, Ceramic, Glass, and Solid State Science Division, National Measurement Laboratory.

[6] Analysis by H. S. Hertz, Organic Analytical Research Division, Center for Analytical Chemistry, National Measurement Laboratory.

[7] Analysis by D. Enagonio, Organic Analytical Research Division, Center for Analytical Chemistry, National Measurement Laboratory.

[8] Analysis by A. J. Fatiadi, Organic Analytical Research Division, Center for Analytical Chemistry, National Measurement Laboratory.

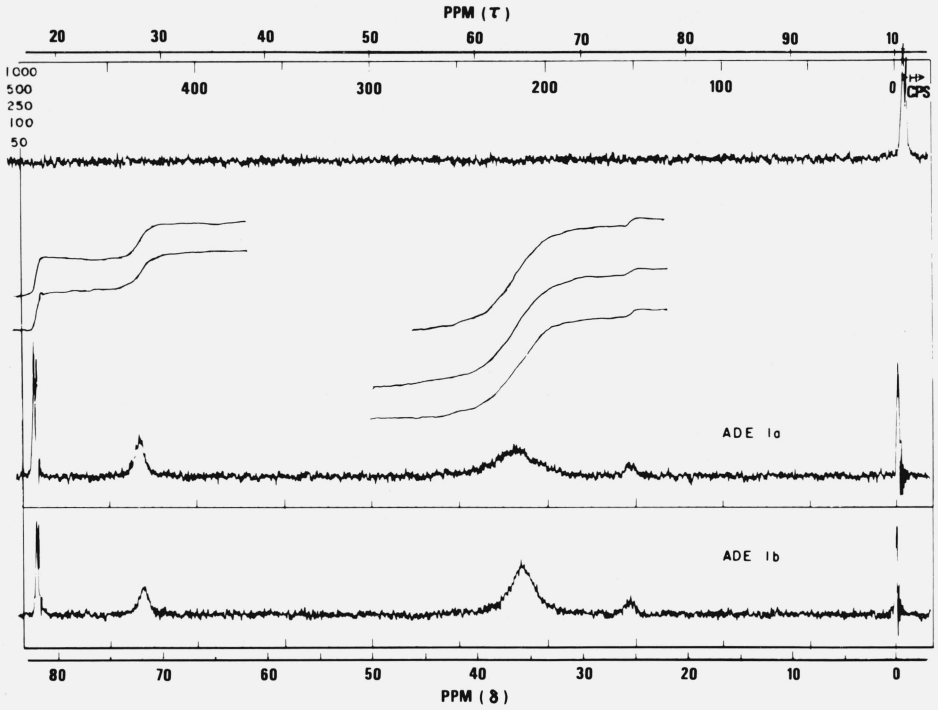


FIGURE 3. *Nuclear magnetic resonance spectra of a sublimed sample (1a) and the unsublimed residue of sublimation (1b) of adenine (at bottom).*

The background spectrum is shown (at top) with the integration lines (at center).

Table 4. Enthalpies of combustion of adenine samples.

Expt. No.	Adenine Sample No.	CO_2 formed / Theor. CO_2	ΔH_c° (298.15 K) uncorrected	ΔH_c° (298.15 K) mean corr. for H_2O
			kJ/mol	
569	2	0.9963	[a]2776.69	
570	2	.9993	2756.73	[c]2797
579	5	.9980	2773.52	
580	5	.9982	2775.15	2805
571	5b	.9927	2752.13	
572	5b	.9989	2756.02	2834
	Stiehler and Huffman		[9] [b]2776.07	
	Assigned uncertainty		= ± 0.88	

[a] Possibility exists that this value may be high because of insufficient H_2O in bomb.

[b] Corrected to current molecular mass.

[c] 2787 kJ/mol is the corrected value for the single Expt. No. 570.

combustion. Unfortunately the time available for the combustion measurements did not permit a thorough and conclusive investigation. However, these data do indicate that the ratio of C in the samples to the theoretical are about 99.5 percent or higher, and the values obtained for the enthalpy of combustion are higher than that of Stiehler and Huffman by as much as 2 percent or about 60 $kJ \cdot mol^{-1}$. Certainly the estimated uncertainties are smaller than this and we must assume unknown sample impurities.

The heat capacities of the crystalline samples at 298 K were measured by Ernesto Friere using a drop microcalorimeter at the University of Virginia. The results of these measurements are as follows: Ade 2, (1.075 ± 0.008) $J \cdot g^{-1} \cdot K^{-1}$; Ade 5b, (1.088 ± 0.008) $J \cdot g^{-1} \cdot K^{-1}$ [10]. The latter value agreed with their measurements on a sample which they had purified except that the uncertainty was 0.004 $J \cdot g^{-1} \cdot K^{-1}$. Thus, the best value at 298 K is C_p° = (147.0 ± 1.0) $J \cdot mol^{-1} \cdot K^{-1}$.

3. Enthalpies of Solution

A platinum-lined, adiabatic solution calorimeter previously described in detail [11] was used to measure the enthalpies of solution of the adenine samples. This calorimeter has proven to be capable of high precision measurements of rapid reactions (±0.1 J when complete in 20 minutes or less [11, 12]) and slow reactions (±0.5 J when complete in 2 h [13]).

The general procedures were as follows: The adenine, usually 0.2 g (or 1.5 mmol), was transferred in air or in a glove box to the platinum sample holder (see [11]) of 0.5-mL capacity. After assembly of the calorimeter containing about 300 mL of the weighed calorimetric solution and the weighed

adenine sample, the system was evacuated, the temperature controls were started, and the calorimeter was preheated. Adiabatic conditions were achieved after about one hour when the pressure within the jacket was less than 0.1 Pa (1×10^{-3} mm of Hg). Throughout the experiment the difference in temperature between the reaction vessel and the adiabatic shield was recorded on a strip chart. The calorimeter temperature, as indicated by a quartz-oscillator thermometer with direct frequency counting and digital print-out at 100-s intervals, was recorded on punched paper tape and a teletype. Rating periods consisted of 15 to 20 time-temperature readings (25 to 30 min). In each experiment an electrical calibration of the initial system was followed by the chemical reaction and then by an electrical calibration of the final system. For details of the methods and calculations for electrical calibrations and the corrected temperature rise, see [11].

3.1 Physical Constants and Calibrations

The 1975 Table of Atomic Weights [14] was used in calculating the relative mole masses for this work: adenine, 135.128 and H_2O, 18.0152. The density of adenine, 1.47 $g \cdot mL^{-1}$ (see sec. 2.2.1.) was used in calculating the buoyancy factor, 1.00066, for the mass of sample.

The calorimetric experiments reported here were made between January 1974 and October 1976. The quartz-oscillator thermometer was calibrated by comparison with a calibrated platinum resistance thermometer in October 1973, July 1974, and August 1975. Observations of this quartz thermometer system indicate that the frequency change of the quartz oscillator with temperature is linear between 295 K and 330 K. During a period of 19 months the slope of the frequency-temperature line changed by less than 0.01 percent and the intercept, by ±0.01 K; these changes are within the measurement uncertainty. (In the calorimetric measurements, the absolute temperature is needed only to the nearest 0.01 K for the temperature of reaction. The temperature change in an experiment is relatively small and the slope must remain constant only during the course of the experiment since each system is calibrated electrically.)

The voltage of the saturated standard cell, last calibrated in March 1973, is based on the value of the volt established in 1969. The 0.1-Ω, 10-Ω, and 10-kΩ standard resistors were last calibrated in January 1973. The record of periodic calibrations of these standards for more than 10 years show trends which increase confidence in the values used.

Energy conversions are based on 1 thermochemical calorie = 4.184 joules.

3.2 Estimate of Calorimetric Uncertainty for an Experiment

The uncertainty in the measurement of an enthalpy of solution in this adiabatic calorimeter varies from one experiment to another depending on the parameters of the experiment. The reaction period may vary from a few minutes to several hours; the stirring speed may be 150 to 900 revolutions per minute (rpm); the solvent may be H_2O, concentrated acids or bases, or non-aqueous solvents; and the temperature increment may be from 0.001 K to 5 K. It is useful to evaluate for each experiment the contribution to the total experimental uncertainty that is due only to the measurement process, or the calorimetric uncertainty. If the individual calorimetric uncertainties are non-uniform for a series of similar experiments, one may suspect sample inhomogeniety in either particle size or purity. Abnormally large uncertainties can also lead to the detection of other sources of error. Our method of calculating the calorimetric uncertainty for an individual experiment will be described since it has not been reported previously.

If we disregard the uncertainties in the chemical reaction and the sample purity and homogeniety, the remaining uncertainties in the enthalpy of solution are those in the measurements of the energy equivalent of the system and of the temperature change (ΔT) during the reaction. It was shown [12] that the standard error of the mean of a series of measurements of the electrical energy equivalent for this calorimeter was 0.02 percent or less, which represents the smallest calorimetric uncertainty to be expected. However, we shall refer to the uncertainty in $\Delta T_{\text{reaction}}$ as the calorimetric uncertainty for an individual experiment; this is in the range 0.01 to 0.06 percent for the series mentioned above.

Because this calorimeter is adiabatic there is no correction for transfer of heat between the calorimeter and the environment. There is also no correction for the energy of opening the sample holder which was found to be 0.00 ± 0.02 J [11]. Normally no correction is necessary for vaporization of the calorimetric solution into the space not occupied by the liquid or crystalline material in the sample holder upon opening, although appropriate calculations are made to insure that the correction is negligible. The remaining corrections to $\Delta T_{\text{reaction}}$ to be considered are those for the energies of stirring and for vaporization at the surface of the calorimetric solution (since this is a constant pressure vessel which is open to the atmosphere.) The effects of both of these energies are included in the rating period slopes which will be discussed below.

The method of calculating $\Delta T_{\text{reaction}}$ has been described [11] and essentially involves an extrapolation of the curves from the midpoints of the rating periods preceding and following the reaction to the time of initiating the reaction (see fig. 4 where $\Delta T = R_f - R_i$). The extrapolation of the initial curve is always small and its contribution to the uncertainty is negligible as is the uncertainty in the absolute values of R_{Mf} and R_i. Each rating period slope is calculated by a least squares method. The standard deviation of the mean (*sdm*) is also calculated for the difference between the

observed points and those calculated from the curve at the observed time. Table 5 shows these data for the rating periods in our experiments with Ade 2 where there are variations in the temperature of reaction, in the stirring rate, and in the length of the reaction period. An electrical calibration of the initial system followed the first rating period (RP1), the chemical reaction followed RP2, and an electrical calibration of the final system followed RP3. The

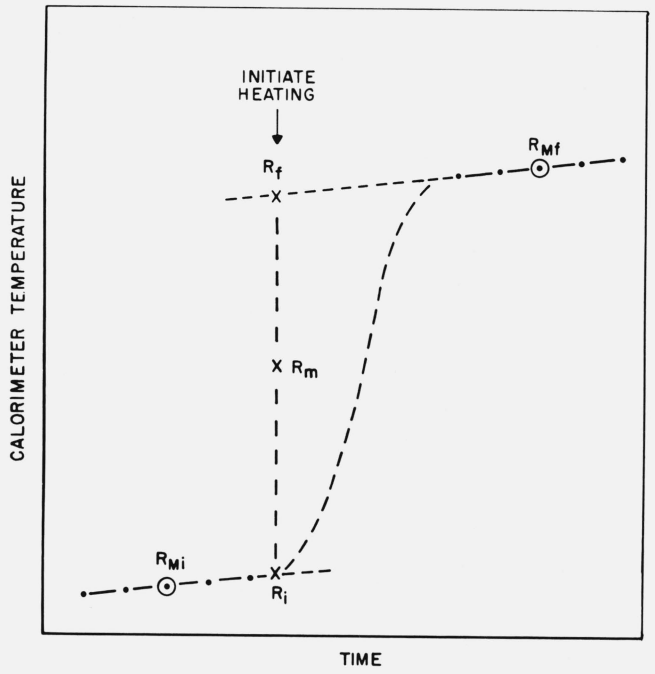


FIGURE 4. *Plot showing rating period extrapolations for obtaining* ΔT.
R_{MI} and R_{Mf} are the mean temperatures (at the mid-points) of the initial and final rating periods. R_i and R_f are the temperatures extrapolated to the time of initiating heating or reaction ($\Delta T = R_f - R_i$), and R_m is the mean temperature of the reaction or heating period.

Table 5. Rating period data under various conditions in experiments with Ade 2 and the individual calorimetric uncertainties.

Expt. No.	$T_{reaction}$	Stirring Rate	L. S. Slope and sdm for rating periods				Reaction Period Extrapolation	[e]Calorimetric Uncertainty
			RP 1	RP 2	RP 3	RP 4		
	K	rpm	μK/s				s	%
924	298.13	700	10.291 ± 0.040	10.268 ± 0.056	10.297 ± 0.034	10.278 ± 0.071	4401	0.21
925	298.18	700	10.233 ± 0.055	10.223 ± 0.051	10.286 ± 0.045	10.279 ± 0.048	4702	.29
[a]926	298.14	700	10.186 ± 0.042	10.179 ± 0.039	10.158 ± 0.056	10.109 ± 0.056	6203	.49
927	308.95	700	9.894 ± 0.072	9.999 ± 0.059	10.043 ± 0.068	10.157 ± 0.051	3501	.29
936	308.96	700	10.093 ± 0.081	10.302 ± 0.078	10.336 ± 0.047	10.401 ± 0.138	3502	.22
940	308.99	700	12.562 ± 0.091	12.616 ± 0.036	12.688 ± 0.126	12.927 ± 0.080	2603	.41
[a]941	319.05	550	6.078 ± 0.190	6.105 ± 0.189	5.477 ± 0.121	5.363 ± 0.064	2001	---
[b]942	329.14	450	3.099 ± 0.083	3.176 ± 0.059	3.196 ± 0.058	3.103 ± 0.045	2300	.16
[a]943	319.16	550	5.468 ± 0.056	5.498 ± 0.046	[d]5.381 ± 0.072	5.288 ± 0.036	2652	.24
944	329.16	550	4.772 ± 0.058	4.684 ± 0.048	4.705 ± 0.053	4.722 ± 0.044	1999	.13
[c]945	319.05	550	5.285 ± 0.052	5.352 ± 0.044	5.502 ± 0.046	5.502 ± 0.036	6501	.38

[a]Incomplete reaction.

[b]Replaced PTFE bearings for stirrer before this experiment.

[c]Sample holder failed to open completely.

[d]19 points in this rating period; 20 points in all others.

[e]See text for calculation.

sdm usually is between 0.04 and 0.06 $\mu K \cdot s^{-1}$; however, it is evident that these values had increased in Expt. Nos. 936, 940, and 941. The polytetrafluoroethylene (PTFE) bearings for the stirrer were replaced before Expt. No. 942 and the sdm values again returned to normal. Generally the slopes increase with increasing stirring rate and decreasing temperature for a given solution.

Incomplete reactions were suspected in Expt. Nos. 926, 941, and 943 of table 5 because the RP3 and RP4 slopes following the reaction were smaller than those, RP1 and RP2, preceding the reaction; this was confirmed by the presence of undissolved sample in the final calorimetric solution. Where the reactions were complete, the last two slopes were equal to or larger than the first two. In Expt. No. 945 the sample holder failed to open properly; hence, the long reaction period even at 319 K for complete reaction.

The calorimetric uncertainty (or the percentage of uncertainty in the measurement of ΔT) is primarily dependent upon (1) the uncertainty in the extrapolation of RP3 to the time of initiating the reaction, or the standard deviation of the slope; (2) the duration of the reaction period, Δt; and (3) the magnitude of ΔT. If n is the number of points in RP3, x is the point number, and Δ is the difference between the observed temperature and that calculated from the slope, then the estimated variance of the slope [15] or the standard deviation of the slope (*sds*), can be calculated from the residual standard deviation (*rsd*) and the standard deviation of the mean (*sdm*) as follows:

$$sdm = \sqrt{\frac{\Sigma(\Delta)^2}{n(n-1)}}$$

$$rsd = \sqrt{\frac{\Sigma(\Delta)^2}{n-2}} = sdm\sqrt{\frac{n(n-1)}{n-2}}$$

$$sds = \frac{rsd}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}} = f(sdm)$$

(f = 0.2402, 0.2244, 0.2108, 0.1986, 0.1879, and 0.1782 for 15, 16, 17, 18, 19, and 20 RP points, respectively.)

Then using 2(*sds*) as the uncertainty in the slope, we obtain for the percentage of uncertainty in the measurement of $\Delta T_{\text{reaction}}$ or

$$\text{Calorimetric Uncertainty} = \frac{2sds\,(\Delta t)}{\Delta T_{\text{reaction}}} \times 100$$

where *sds* is in $K \cdot s^{-1}$, Δt, in seconds, and ΔT, in kelvins. The values calculated for the experiments with Ade 2 given in table 5 used the sdm for RP3, the number of data points (given in the footnote), the reaction period extrapolation time from table 5, and the $\Delta T_{\text{reaction}}$ from table 6b in section 3.3.

3.3 Enthalpy of Solution Measurements

The enthalpies of solution of adenine in water were measured in 68 experiments for which data are given in tables 6 a, b, c, and d.

In the tables, Expt. No. is the serial number of the experiments with this calorimeter. The reaction period is the time between starting the adenine reaction and starting the final rating period. $\bar{T}_{\text{reaction}}$ is the mean temperature of the reaction period, or half the sum of the initial and final temperatures. The calorimetric uncertainty for the individual experiments was described in detail in the preceding section. The electrical energy equivalents of the initial and final systems and $\Delta T_{\text{reaction}}$ were measured and calculated as previously described [10]. q_{vap} is the heat absorbed upon vaporization of H_2O into the air space in the sample container when it was opened.

$$q_{vap} = \Delta H_{\text{vap}}\left(V - \frac{d}{m}\right)(1 - RH)$$

where ΔH_{vap} is the enthalpy of vaporization of water per unit volume [16], V is the volume of the sample container, d is the density of the sample, m is the mass of sample, and RH is the relative humidity of the air in sample container. RH is 0.35 in this work except where it is zero when the sample container was filled in the glove box.

All of the reactions described here were endothermic. The heat absorbed during the adenine reactions, Q_{reaction}, was small and so it was not necessary to add electrical energy to maintain the calorimeter temperature. The absorption of heat proceeded slowly and the stirring energy was sufficient to prevent a decrease in temperature in excess of 0.02 K.

The specific enthalpy of reaction at the mean temperature of reaction, $\Delta H(T)$, is Q_{reaction}/sample mass. The correction to 298.15 K was obtained from $\Delta Cp = 0.582\ J \cdot g^{-1} \cdot K^{-1}$, to be discussed in the following section.

In the plots of calorimetric data, the radius of a circle surrounding a point is equal to the calorimetric uncertainty of the individual experiment as discussed in section 3.2. When the least squares method was used for fitting data points, they were always fitted for linear, quadratic and cubic equations; the results of the higher order equations are not reported since their use was not justified in these cases.

In 13 of the experiments (Nos. 931–941, 989, and 994), the pH was measured for the water used as the initial calorimetric solution, and for the final calorimetric solution. The pH meter was calibrated before each measurement with a standard buffer at pH 7.0. The pH of the water was always in the range, 5.9–6.0, and of the final solutions, 6.7–7.0. The precision of the measurements was not great because of

the drifts due to the effects of atmospheric CO_2 in this neutral range, however, they are sufficient to establish the fact that the calorimetric measurements were made in the range of pH 6 to 7.

Table 6a. Pertinent data for the enthalpy of solution measurements of various adenine samples in H_2O

Expt. No.	Adenine Sample: Sieve Range[a]	Adenine Sample: Mass	H_2O Mass -300 g	Final Molality x10^6	Reaction Period	$\bar{T}$ reaction	Calorimetric Uncertainty
		g	g	mol/kg H_2O	min	K	%
Ade 1: B-M Lot 6249423							
920	T	0.21596	2.429	5284	113	298.1822	0.41
921	T	.18921	2.424	4630	113	298.1527	.42
922	T	.18405	2.499	4502	123	298.1801	.59
923	T	.18942	2.469	4634	53	309.0010	.23
935	T	.19322	2.439	4728	103	298.2098	.59
Ade 1b: Ade 1 heated at 400 K under vacuum (sublimation residue)							
1186	T	0.19665	2.409	4812	93	298.2072	0.61
Ade 1c: Sublimed Ade 1							
1187	<100	0.10086	2.414	2468	13	298.2054	0.49
1188	<100	.15038	2.359	3681	23	298.3157	.24
1189	<100	.16812	2.414	4114	13	298.3281	.49
Ade 1d: Ade 1 heated at 430 K under vacuum (sublimation residue)							
1184	T	0.14544	2.354	3560	118	298.2075	0.52
1185	T	.26084	2.404	6383	133	298.1768	.50
Ade 1e: Ade 1c recrystallized from cold H_2O							
1195	T	0.04319	2.429	1057	13	298.2203	0.79
1196	T	.10413	2.364	2548	18	298.2289	.23
1197	T	.05031	2.399	1231	18	308.8821	.37
Ade 2: B-M Lot 7072124							
924	T	0.18570	2.429	4544	58	298.1296	0.21
925	T	.19752	2.454	4832	63	298.1783	.29
926	T	.18683	2.414	4572	88	298.1358	.49
927	T	.21172	2.449	5180	43	308.9485	.29
936	T	.19337	2.459	4731	44	308.9647	.22
940	T	.20793	2.434	5088	28	308.9865	.41
942	T	.19949	2.424	4881	[b]23	329.1369	.16
943	T	.21118	2.384	5168	[b]30	319.1560	.24
944	T	.20504	2.449	5017	[b]18	329.1629	.13
945	T	.19730	2.434	4827	[b]93	319.0505	.38
Ade 2': Ade 2 vacuum dried at 373 K							
1194	T	0.16431	2.444	4094	68	298.2305	0.83
Ade 2": Ade 2 vacuum dried at 430 K							
1193	T	0.11055	2.424	2705	53	298.2374	0.78
Ade 3: Calbiochem Lot 71821							
916	T	0.23786	2.145	5825	113	298.1904	0.42
917	T	.19116	2.389	4678	86	298.1827	.37
918	T	.19034	2.419	4657	78	298.1974	.33
932	T	.19346	2.394	4734	88	298.1131	.49
933	T	.19070	2.419	4666	88	298.2300	.38
934	T	.18805	2.449	4601	78	298.4649	.37
Ade 4: E-M Lot 1958515							
937	T	0.19490	2.449	4768	53	298.1311	0.46
938	T	.18943	2.449	4634	43	298.3709	.28
964	T	.18668	2.459	4568	78	298.2005	.35

[a] T indicates that the total sample was used without sieve separation; < 100 indicates that the sample passed a No. 100 standard sieve.

[b] All stirring rates were 700 rpm except Expt. No. 942 (450 rpm) and Expt. Nos. 943-945 (550 rpm).

Table 6b. Calorimetric data for the enthalpy of solution of various adenine samples in H_2O

Expt. No.	Electrical Energy Equivalent - 1730 J/K: Initial	Final	$-\Delta T$ reaction $\times 10^5$	q_{vap}[a] $\times 10^2$	$-Q$ reaction[b]	$\Delta H(T)$		Corr. to T = 298.15 K $\times 10^3$	$\Delta H(298.15\ K)$
	J/K		K	J	J	J/g	kJ/mol	kJ/mol	kJ/mol
Ade 1: B-M Lot 6249423									
920	7.87	7.50	2846	2	49.43	228.90	30.932	- 3	30.93
921	7.77	7.52	2524	2	43.84	231.70	31.309	0	31.31
922	7.96	7.45	2427	2	42.16	229.07	30.954	- 2	30.95
923	7.64	6.47	2614	4	45.36	239.47	32.359	-854	31.50
935	7.52	6.71	2468	2	42.86	221.82	29.974	- 5	29.97
Ade 1b: Ade 1 heated at 400 K under vacuum (sublimation residue)									
1186	3.55	2.85	2595	3	44.94	228.53	30.880	- 4	30.88
Ade 1c: Sublimed Ade 1									
1187	4.43	4.16	1236	4	21.39	212.08	28.657	- 4	28.65
1188	3.55	4.79	1872	4	32.43	215.65	29.141	- 14	29.13
1189	4.93	2.18	2028	4	34.92	207.71	28.067	- 15	28.05
Ade 1d: Ade 1 heated at 430 K under vacuum (sublimation residue)									
1184	3.28	2.83	1983	4	34.33	236.04	31.896	- 5	31.89
1185	3.13	2.63	3386	3	58.65	224.85	30.384	- 2	30.38
Ade 1e: Ade 1c recrystallized from cold H_2O									
1195	2.56	3.13	626	3	10.81	250.28	33.821	- 4	33.82
1196	-5.88	1.04	1346	[c]10	23.15	222.32	30.041	- 4	30.04
1197	5.32	4.22	685	[c]19	11.70	232.56	31.425	-845	30.58
Ade 2: B-M Lot 7072124									
924	8.43	7.55	2492	2	43.28	233.06	31.493	1	31.49
925	7.07	7.19	2642	2	45.90	232.38	31.401	- 2	31.40
926	7.68	6.83	2518	2	43.74	234.12	31.636	1	31.64
927	7.17	6.55	2907	4	50.45	238.29	32.199	-850	31.35
936	7.12	6.92	2693	4	46.74	241.71	32.662	-851	31.81
940	6.69	7.24	2879	4	49.96	240.27	32.468	-853	31.61
942	11.25	10.99	2892	10	50.24	251.84	34.031	-2439	31.59
943	8.20	7.90	2970	6	51.55	244.10	32.985	-1653	31.33
944	10.28	10.67	2962	10	51.46	250.98	33.914	-2441	31.47
945	7.40	7.10	2805	6	48.66	246.63	33.326	-1645	31.68
Ade 2': Ade 2 vacuum dried at 373 K									
1194	3.27	2.19	2330	4	40.34	245.51	33.175	- 7	33.17
Ade 2": Ade 2 vacuum dried at 430 K									
1193	3.12	2.46	1486	4	25.74	232.84	31.463	- 7	31.46
Ade 3: Calbiochem Lot 71821									
916	8.40	7.77	3103	2	53.92	226.69	30.632	- 3	30.63
917	8.01	7.09	2531	2	43.96	229.96	31.075	- 3	31.07
918	8.02	7.56	2459	2	42.71	224.39	30.321	- 4	30.32
932	7.46	6.54	2598	2	45.10	233.12	31.501	3	31.50
933	6.95	6.63	2554	2	44.35	232.56	31.426	- 7	31.42
934	7.23	6.48	2407	2	41.79	222.23	30.029	- 25	30.00
Ade 4: E-M Lot 1958515									
937	7.64	7.29	2475	2	42.98	220.52	29.799	1	29.80
938	7.73	7.31	2438	2	42.34	223.51	30.203	- 18	30.18
964	6.30	5.92	2453	2	42.57	228.04	30.814	- 4	30.81

[a] Relative humidity (RH) in the sample container was 0.35 for Ade 1, 1e, 2, 3, and 4; RH = 0 for all others.

[b] Includes corrections for departures of the adiabatic shield temperature from the vessel temperature. These corrections are less than 0.01 J except as follows: No. 916, -0.01J; No. 925, -0.03J; and No. 926, -0.02J.

[c] Volume of sample holder was 3.0 cm^3 in Expt. Nos. 1196 and 1197. In all other experiments the volume was 0.7 cm^3.

Table 6c. Pertinent data for the enthalpy of solution measurements in H_2O of Ade 5 and other samples derived from it

Expt. No.	Adenine Sample: Sieve Range[a]	Adenine Sample: Mass	H_2O Mass -300 g	Final Molality x 10^6	Reaction Period	$\bar{T}$ reaction	Calorimetric Uncertainty
		g	g	mol/kg H_2O	min	K	%
Ade 5: B-M Lot 726 3428							
989	T	0.21948	2.489	5369	63	298.2292	0.34
990	T	.21748	2.494	5320	68	298.1316	.36
992	T	.19034	2.474	4656	63	298.1387	.37
993	T	.19502	2.459	4776	53	298.2927	.39
Ade 5b: Reprecipitated and recrystallized from Ade 5							
995	< 100	0.20155	2.434	4931	48	298.1504	0.24
996	20-100	.19751	2.459	4832	113	298.1459	.71
997	60-100	.20597	2.489	5039	68	298.1414	.43
998	100-150	.19228	2.494	4704	63	298.1567	.30
999	150-200	.20138	2.499	4926	38	298.1451	.22
1000	< 200	.19720	2.479	4824	28	298.1435	.22
1001	150-200	.27581	2.479	6748	83	298.1416	.25
1002	150-200	.10508	2.489	2570	38	298.1736	.39
1003	150-200	.05513	2.469	1348	18	298.1549	.39
1004	150-200	.15048	2.484	3681	28	298.1486	.19
Ade 5b': Ade 5b vacuum dried at 373 K							
1190	< 200	0.17719	2.419	4336	43	298.2236	0.76
1191	< 200	.19744	2.374	4832	38	298.2385	0.93
Ade 5c: Sublimed from Ade 5							
1067	<100	.19780	2.434	4840	15	298.1324	.20
1068	<100	.20034	2.454	4902	15	298.1277	.23
1069	<100	.20094	2.444	4917	15	298.1224	.19
1070	<100	.19179	2.419	4693	22	298.1631	.19
1077	<100	.19289	2.434	4720	17	298.4888	.25
1078	<100	.20667	2.444	5057	28	298.2030	.31
1079	<100	.20081	2.489	4913	23	298.1896	.35
1080	<100	.20540	2.484	5025	28	298.2047	.37
1081	<100	.19529	2.469	4778	28	298.1872	.32
1082	<100	.20128	2.464	4925	28	298.2050	1.62
Ade 5d: Sublimed from Ade 5 and protected from atm. H_2O							
1088	<100	0.15106	2.379	3697	18	298.5204	0.21
1089	<100	.21533	2.459	5268	28	298.1406	.18
1090	<100	.14038	2.439	3435	18	298.1937	.21
1091	<100	.20833	2.429	5098	23	298.1444	.22
1092	<100	.17631	2.429	4314	23	298.1655	.19
1125	<100	.16735	2.469	4094	28	298.1801	.16
1126	<100	.10597	2.484	2592	18	298.1856	.30

[a] T indicates that the total sample was used without sieve separation; <100 includes all of the sample which passed a No.100 standard sieve; <200, that which passed a No. 200 sieve; and the ranges indicate that the sample passed the first sieve no. and was retained on the second sieve no.

Table 6d. Calorimetric data for the enthalpy of solution of Ade 5 and other samples derived from it

Expt. No.	Electrical Energy Equivalent - 1370 J/K		-ΔT reaction x 10^5	q_{vap} [a] x 10^2	-Q reaction	ΔH(T)		Corr. to T = 298.15 K x 10^3	ΔH(298.15 K)
	Initial	Final							
	J/K		K	J	J	J/g	kJ/mol	kJ/mol	kJ/mol
Ade 5: B-M Lot 7263428									
989	----	----	2895	2	[b] 50.21	228.77	30.913	- 6	30.91
990	6.25	5.51	2769	2	48.05	220.94	29.855	2	29.86
992	6.75	5.70	2483	2	43.09	226.38	30.591	1	30.59
993	6.30	5.60	2516	2	43.66	223.87	30.252	- 11	30.24
Ade 5b: Reprecipitated and recrystallized from Ade 5									
995	5.97	5.47	2677	2	46.45	230.46	31.142	0	31.14
996	5.94	5.08	2517	2	43.66	221.05	29.870	0	29.87
997	6.37	5.59	2615	2	45.38	220.32	29.772	1	29.77
998	5.91	5.68	2525	2	43.81	227.84	30.788	0	30.79
999	6.42	5.68	2664	2	46.23	229.56	31.021	1	31.02
1000	6.29	5.52	2633	2	45.68	231.64	31.301	1	31.30
1001	6.22	5.58	3602	2	62.50	226.60	30.621	0	30.62
1002	6.18	5.27	1450	3	25.15	239.34	32.342	- 2	32.34
1003	6.03	5.27	755	3	13.08	237.26	32.060	- 1	32.06
1004	6.36	5.36	2014	3	34.93	232.12	31.366	0	31.37
Ade 5b': Ade 5b vacuum dried at 373 K									
1190	4.51	2.75	2436	3	42.20	238.16	32.182	- 6	32.18
1191	0.45	-1.29	2697	3	46.61	236.07	31.990	- 6	31.89
Ade 5c: Sublimed from Ade 5									
1067	6.20	5.20	2136	2	37.04	187.26	25.304	1	25.30
1068	5.67	5.17	2243	2	38.91	194.22	26.244	2	26.25
1069	5.62	5.11	2268	2	39.34	195.78	26.455	2	26.46
1070	5.35	4.93	2250	2	39.03	203.50	27.499	- 1	27.50
1077	3.83	4.21	2349	2	40.72	211.10	28.526	- 27	28.50
1078	0.95	3.10	2557	2	44.27	214.21	28.945	- 5	28.94
1079	3.40	4.34	2527	2	43.79	218.07	29.467	- 3	29.46
1080	6.47	4.27	2524	2	43.77	213.10	28.795	- 5	28.79
1081	5.91	3.86	2448	2	42.45	217.37	29.373	- 3	29.37
1082	4.03	-0.13	2474	2	42.83	212.79	28.754	- 5	28.75
Ade 5d: Sublimed from Ade 5 and protected from atm. H_2O									
1088	3.88	2.88	1723	4	29.83	197.47	26.684	- 29	26.65
1089	5.30	4.88	2459	3	42.64	198.07	26.764	0	26.76
1090	4.27	3.63	1573	4	27.23	193.97	26.211	- 4	26.21
1091	4.34	3.68	2253	3	39.04	187.39	25.322	1	25.32
1092	3.88	3.46	1982	3	34.33	194.71	26.311	- 1	26.31
1125	4.01	3.27	1897	4	32.85	196.30	26.525	- 2	26.52
1126	3.73	3.02	1196	4	20.70	195.34	26.396	- 3	26.39

[a] The relative humidity (R H) in the sample container was 0.35 for Ade 5, 5b and 5c; RH = 0 for all others.

[b] The electrical energy equivalents measured in this experiment were in error because a power failure had caused erroneous voltage readings with the standard cell. Therefore, the heat of reaction is based on an estimate of 1735 J/K for the mean electrical energy equivalent.

3.3.1 ΔC_p of Reaction

The change in the enthalpy of reaction with temperature was obtained from the ten experiments with Ade 2 given in tables 6a and b. A least squares fit of the data for ($\bar{T}_{reaction}$ − 298.15 K) and $\Delta H(T)$/kJ·mol^{-1} to a linear equation provided the following constants: intercept = 31.538 ± 0.081 and slope = 0.0787 ± 0.0046. The uncertainties are the standard errors, and the standard error of the estimate is 0.16 kJ·mol^{-1}. ΔCp for the reaction is 0.582 ± 0.077 J·g^{-1}·K^{-1} in the range, 298 to 328 K at 5 mmol Ade (kg H_2O)$^{-1}$; the uncertainty is at the 95 percent confidence level.

Figure 5 is a plot of these data showing that the calorimetric uncertainties for 4 of the points did not intersect the line obtained from the least squares fit. This led to the suspicion of sample inhomogeneity to be discussed later.

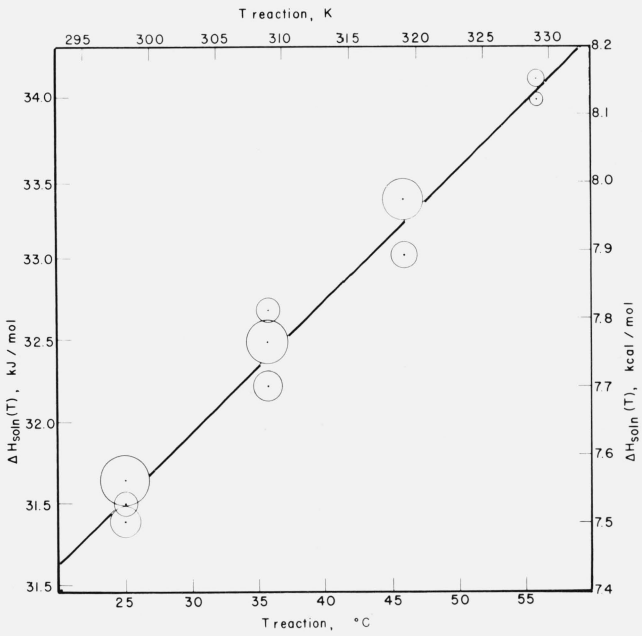


FIGURE 5. *Plot showing the relationship of the enthalpy of reaction of Ade 2 in H_2O to the temperature of the reaction.*
Radius of circles = calorimetric uncertainty (see text).

3.3.2 Particle Size and Concentration

A few, small, undissolved particles of adenine (identified by x-ray diffraction) remained in all of the final calorimetric solutions of the commercial products as received (Ade 1, 2, 3, 4, and 5) even though the reaction periods at 298 K were 1 hour or longer, and the rating period slopes gave no evidence of incomplete reaction. Nonuniform particle size was believed to be the cause of the problem; the remains of larger particles were dissolving at such a slow rate that the reaction was not detectable in the slopes.

The effects of particle size on the rate of reaction and on the magnitude of the enthalpy of solution were observed in six experiments where 0.2-g samples were used (see tables 6c and d, Expt. Nos. 995–1000). The sample, Ade 5b, had been reprecipitated and recrystallized from Ade 5 (see sect. 2.1) and dried 24 h in a vacuum desiccator containing $Mg(ClO_4)_2$, then heated 27 h in a vacuum oven at 340 K. This material was separated into 3 portions as follows: (1) retained on a No. 20 standard sieve, ~840 μm; (2) passed No. 20, but retained on No. 100, ~150 μm; and (3) passed No. 100. In Expt. No. 995 where portion (3) was used, the reaction was complete in 48 min with no visible undissolved residue in the final solution; however, in Expt. No. 996 where portion (2) was used, the reaction period was 113 min and there were still a few undissolved particles in the final solution. The sample was then separated into 4 additional portions: (4) passed No. 60 sieve, ~250 μm, but was retained on No. 100, (5) passed No. 100, but was retained on No. 150, ~100 μm, (6) passed No. 150, but was retained on No. 200, ~75 μm, and (7) passed No. 200 sieve. For portion (4), used in Exp. 997, the reaction period was 68 min and a few undissolved particles remained in the final solution. In Expt. Nos. 998, 999, and 1000 where portions (5), (6), and (7), respectively, were used, the observable reactions were completed in a shorter time and there were no undissolved particles visible in the final solution, however, a very small amount of the finely divided material was floating on the surface of the solution where it had apparently been carried by the bubble of air in the sample holder. It is estimated that the quantity of undissolved material remaining at the end of all of these experiments introduces an error much smaller than the calorimetric uncertainty; however, with the unsieved samples, there may have been a significant error when larger particles were present. Figure 6 is a plot of the enthalpy of solution data for these experiments showing that higher enthalpy values and shorter reactions periods are obtained with more finely divided samples.

The effects of adenine concentration on the enthalpy of solution are shown in figure 7 which is a plot of the data for Expt. Nos. 999, 1001–1004 (see tables 6c and d). The sample was the same as that used in the particle size study in the sieve range 150 to 200. The straight line shown in figure 7 was obtained from a least squares fit of the 5 data points. The intercept is (32.70 ± 0.89) kJ·mol^{-1} and the slope is −(316 ± 209) kJ·mol^{-1}(molal unit)$^{-1}$ (molal unit = mol·kg^{-1}); the uncertainties are at the 95 percent confidence level.

3.3.3 Comparison of Samples

Enthalpy of solution measurements were made on 5 commercial samples of adenine as received (Ade 1, 2, 3, 4, and 5), and on a portion of the latter which was reprecipitated

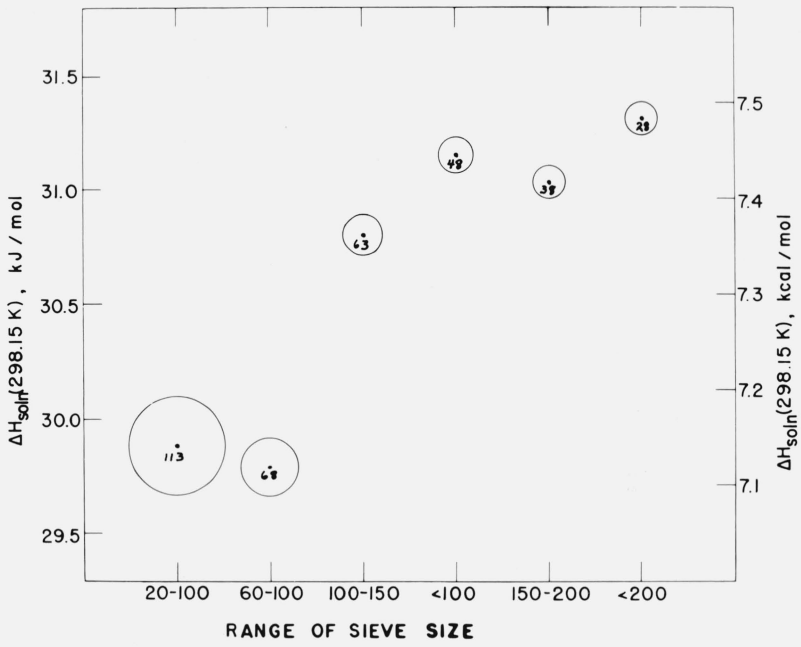


FIGURE 6. *Plot showing the effect of particle size on the enthalpy of reaction of Ade 5b in H_2O.*
The number within the circle is the reaction period in minutes, and the radius of the circle is the calorimetric uncertainty (see text). Standard Sieve Size 20, 60, 100, 150, and 200 are approximately 840, 250, 150, 100, and 75 μm, respectively.

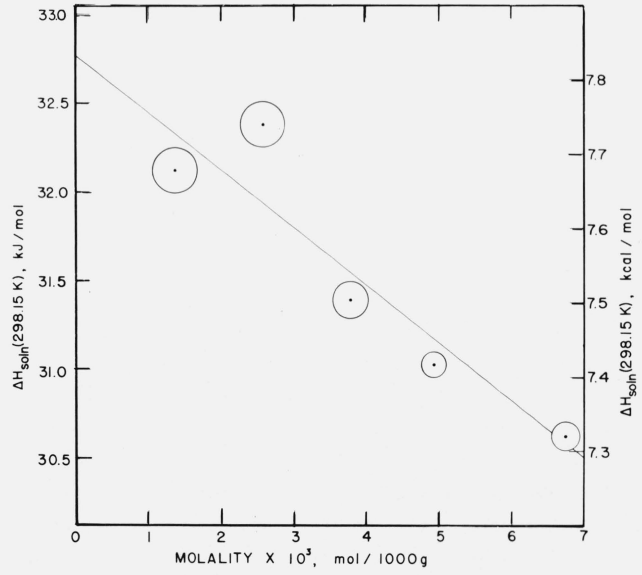


FIGURE 7. *Plot showing the effects of adenine concentration on the enthalpy of solution of Ade 5b in H_2O.*
The radius of the circle is the calorimetric uncertainty (see text).

and recrystallized twice from aqueous solution, Ade 5b. The data for these experiments are given in tables 6 a–d and are summarized in table 7. Since the number of experiments averaged was not the same for all of the samples, the Student t factor at the 95 percent confidence level was applied to the standard deviation of the mean to obtain the experimental uncertainty. The correction to infinite dilution was -316 kJ·mol^{-1} (molal unit)$^{-1}$ given above. The spread of the values for ΔH (∞, 298.15 K) is $\sim$1.6 kJ·mol^{-1} or 5 percent, which is somewhat greater than the largest experimental uncertainty. For the samples as received, Ade 2 has the highest value for the enthalpy of solution and the smallest *sdm* and experimental uncertainty (0.36%). This we attribute to the fact that it was the most finely divided of the commercial samples and apparently of more uniform particle size. Ade 1, 2, and 5 were different lots supplied by the same manufacturer, and show nonuniformity in the ΔH_{soln} of adenine samples even from a single source.

Figure 8 is a plot of the individual values for the enthalpies of solution before corrections for H_2O and dilution which are summarized in table 7. It is evident that the spread of the data is greater than expected on the basis of the magnitude of the calorimetric uncertainties (except with Ade 2). This is probably due to sample inhomogeneity with respect to impurities or particle size; the magnitude of the spread of the data is approximately the same as that in figure 6 showing the effects of particle size.

The ΔH_{soln} of Ade 5 was 1.17 kJ·mol^{-1} less than that of Ade 5b, the product of reprecipitation and recrystallization. This leads to the conclusion that the samples of higher purity have larger enthalpies of solution (although this conclusion

Table 7. Summary of enthalpy of solution measurements of adenine in H_2O for 5 commercial samples as received and 1 reprecipitated and recrystallized sample. (See section 2.1 for description of the samples.)

Ade Sample No.	1	2	3	4	5	5b
No. of expts. averaged	5	10	6	3	4	4
$\overline{\Delta H}$(298.15 K), kJ/mol	30.93	31.54	30.82	30.26	30.40	31.06
Sdm, kJ/mol	0.26	0.05	0.25	0.29	0.23	0.11
Exptl. uncertainty (95%), kJ/mol	0.73	0.11	0.64	1.26	0.72	0.34
H_2O impurity, mass%	0.15	0.15	0.15	0.04	0.15	0.40
mol%	1.11	1.11	1.11	0.30	1.11	2.92
Correction, kJ/mol	0.34	0.35	0.34	0.09	0.34	0.91
Average concentration, mol/kg	0.00476	0.00488	0.00486	0.00466	0.00503	0.00485
Correction to ∞ dil., kJ/mol	1.50	1.54	1.54	1.47	1.59	1.53
$\Delta H(\infty, 298.15\ K)$ kJ/mol	32.77	33.43	32.70	31.82	32.33	33.50
kcal/mol	7.83	7.99	7.82	7.60	7.73	8.01

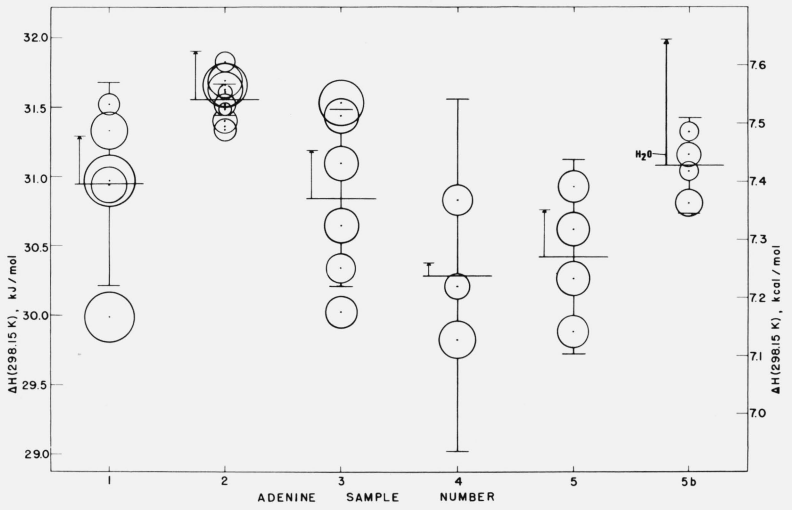


FIGURE 8. *Plot showing comparison of ΔH_{soln} measurements for 5 adenine samples as received and one purified sample.*

See section 2.1 for description of the samples. Radius of the circle is the calorimetric uncertainty. The mean for each group is shown by horizontal bars, and the vertical bars indicate the limits of the experimental uncertainties at the 95% confidence interval. The arrows extending up from the means indicate the magnitude of correction for volatile matter presumed to be H_2O.

should be used with reservations since there are conceivably impurities which could be more endothermic than adenine). Since Ade 5b has the largest ΔH of those given in table 7, we shall assume that it is the purest in the absence of definitive analytical data.

The two experiments (Nos. 1190 and 1191) using Ade 5b′ confirm the validity of the presumption that the volatile matter found in Ade 5b was H_2O or some matter equally inert to the solution reaction. If we assume that all H_2O was removed from Ade 5b during the vacuum heating at 373 K to obtain Ade 5b′, and correct the ΔH (298.15 K) values given in table 6d to infinite dilution we obtain 33.55 and 33.42 $kJ \cdot mol^{-1}$ for Ade 5b′ which are within the experimental uncertainty of the value, 33.50 $kJ \cdot mol^{-1}$, obtained for Ade 5b. However, when the result of Expt. No. 1194 with Ade 2′ (vacuum-heated at 373 K) was treated similarly, we obtain ΔH (∞, 298.15 K) = 34.46 $kJ \cdot mol^{-1}$ as compared to 33.43 $kJ \cdot mol^{-1}$ for Ade 2; this suggests the volatile matter was *not* water but perhaps some organic solvent. Furthermore, the lower value, ΔH (∞, 298.15 K) = 32.31 $kJ \cdot mol^{-1}$ for Ade 2″ (vacuum-heated at 430 K) suggests that some decomposition occurred at the higher temperature.

3.3.4 Change on Sublimation

The attempts at purification of adenine by vacuum sublimation produced surprising results. The first product, Ade 5c, was sublimed from Ade 5. (See sec. 2.1 for description of procedures). The values in four measurements of the enthalpy of solution were 4 to 5 $kJ \cdot mol^{-1}$ less than that for Ade 5; decomposition was suspected. No further measurements were made until three months later when closer examination revealed a small trend in these data. Six additional measurements showed a continuation of the trend with leveling-off near the values measured for Ade 5. The results of these 10 experiments are plotted in figure 9 as the open circles. (The large uncertainty in the last experiment was the result of a stirring problem.) The broken line indicates that approximately three months elapsed between the two experiments; there was a period of two days or less between the other experiments connected by the solid lines. It is apparent that the trend was not a function of time, but instead, of the exposure of the sample (in a capped bottle) to the atmosphere before each experiment.

The second sublimation product, Ade 5d, was prepared in essentially the same way as Ade 5c except that after preparation all manipulations of the sample were carried out in a glove box containing H_2O and CO_2 absorbers. The enthalpy of solution measurements with this sample are also plotted in figure 9: the half-filled circles represent samples protected from both H_2O and CO_2; the broken line indicates that 4 months time elapsed between experiments; and the filled circles represent samples protected only from H_2O (i.e., the CO_2 absorber had been removed from the glove box for more than 4 months.) Thus, it appears that H_2O was responsible for the slow transformation in the sublimed sample and the increasing enthalpy of solution.

There were numerous possibilities for the kind of transformation that occurred in the sublimed sample. The formation of a hydrate was ruled out because the elemental analysis of Ade 5 and 5d indicated that both were anhydrous (see table 3). Also, the mass of a 1-g sample of Ade 5d upon exposure to the atmosphere increased only 0.45 mass percent during the first day and 0.54 mass percent after nearly 12 days, which is equivalent to less than 0.1 mol of H_2O/mol Ade.

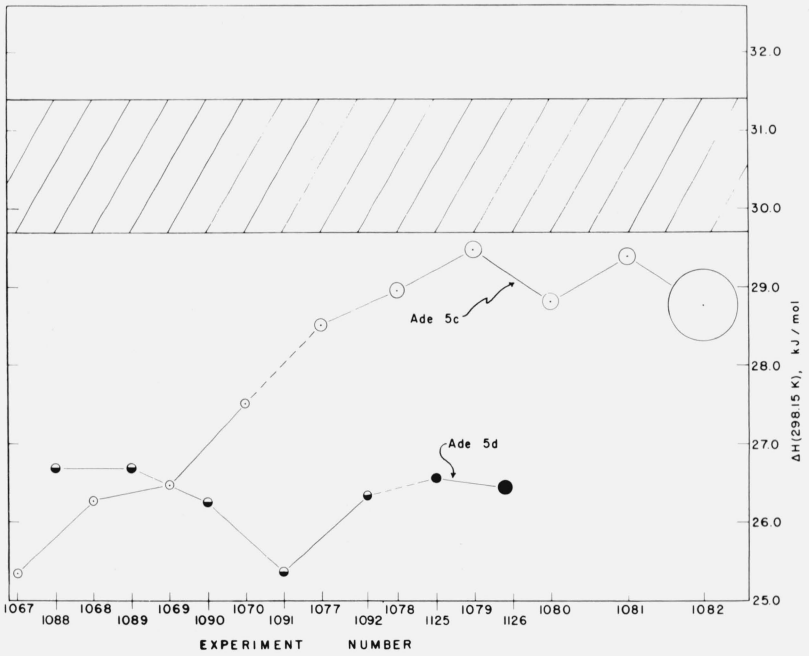


FIGURE 9. *Plot of the enthalpies of solution in H_2O measured for the adenine sublimates, Ade 5c and 5d.*

○ samples not guarded; ○ samples guarded from atmospheric CO_2 and H_2O; ● samples guarded from H_2O only. The broken lines indicate elapsed time of several months. The shaded area shows the range of measurements on the unsublimed samples for comparison.

Microscopic examination[9] of these two samples revealed a mixture of very small particles (<2 μm) and larger particles (up to 200 μm) in both samples. The sublimed material probably contained more of the larger particles than the unsublimed, and the particles appeared to be composed of agglomerates of smaller particles rather than solid crystals as in Ade 5. See figure 1.

[9] By Edgar S. Etz, Gas and Particulate Science Division, Center for Analytical Chemistry, National Measurement Laboratory.

No difference between the sublimed and unsublimed samples was detected from their mass spectra, from the infrared absorbance of their solutions in a mixture of dimethyl sulfoxide and a trimethyl silyl reagent, or from the proton NMR patterns (see sec. 2.2.4). Thus, the transformation was apparently not a change in the crystal form or bonds. Different tautomeric forms are a possible explanation. Bodor, et al [18] have calculated that about 21.5 kcal·mol^{-1} is the heat of isomerization to the next most likely tautomeric form; we are concerned with only about 1 kcal·mol^{-1} or 5 percent of the tautomer.

A difference between Ade 1 and 1a was observed in their Raman Spectra[10] (see fig. 10). The peaks were sharper and showed greater detail for Ade 1 than for Ade 1a, although

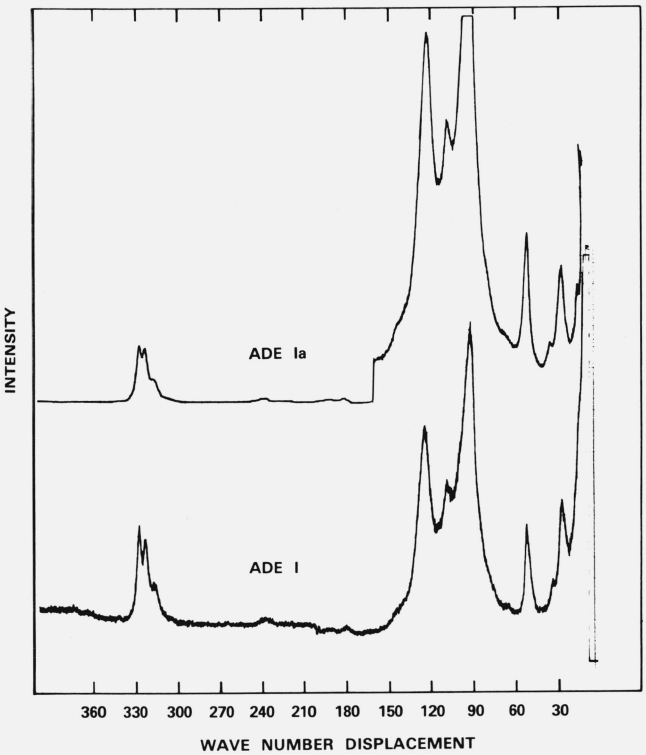


FIGURE 10. *Raman spectra for the sublimate, Ade 1a, and the commercial sample as received, Ade 1, showing the sharper, more defined peaks, hence a higher degree of crystallinity in the unsublimed material.*

the location of peaks was the same for both samples. This is interpreted as a higher order of crystallinity in the unsublimed sample or the presence of a tautomer in the sublimed sample, which is in agreement with the calorimetric results. Also, x-ray powder diffraction patterns showed broader peaks for Ade 5c indicating the presence of a tautomer or smaller crystallites than in the unsublimed sample, Ade 5.

It, therefore, appears that the commercial samples and the recrystallized sample are relatively free of other tautomers or have a high degree of crystallinity. Sublimation increases the amount of tautomer or reduces the order in the adenine crystals, and atmospheric H_2O apparently catalyses the restoration of the stable tautomer or of order in the crystals.

3.3.5 Comparison of Ade 1 and Its Derivatives

Ade 1e was obtained from a portion of the sublimate, Ade 1c, which was dissolved in H_2O and recrystallized from cold solution. The product was in the form of needlelike crystals (see photomicrograph in fig. 1) which were quite resilient and not easily broken. The appearance was very different from the product obtained in the recrystallization from hot H_2O, and must have been the same as that described by Stiehler and Huffman [9] which was assumed to be the trihydrate. However, our elemental analyses of Ade 1e (table 3) indicated that it was anhydrous.

The data for the experiments with Ade 1 and its derivatives are given in tables 6a and 6b. The mean of the values for ΔH (∞, 298.15 K)/kJ·mol^{-1} (with no correction for H_2O in the samples) are as follows: Ade 1, 32.43; Ade 1b, 32.40; Ade 1c, 29.69; Ade 1d, 32.70; and Ade 1e, 31.99. Again, the value for the sublimate, Ade 1c, is significantly smaller than the others. The values for residues from sublimation, Ade 1b and Ade 1d, show little change from that of the parent material. Ade 1e, the needlelike product of recrystallization from cold water, was in fair agreement with the other values although the large spread of the data probably indicates inhomogeneity because of occluded H_2O. The value for Ade 1 corrected for H_2O in the sample is 32.77 kJ·mol^{-1}. It is assumed that there is no H_2O in the sublimation products, Ade 1b, 1c, and 1d. Unfortunately the amount of H_2O in Ade 1e was not determined because of the small amount of the product and the unknown effects of heating the sample. However, we assume there was more H_2O in the recrystallized Ade 1e than in Ade 1 (as was true for Ade 5 and Ade 5b) and that the enthalpy value for Ade 1e corrected for H_2O would be equal to or larger than that of Ade 1. Thus it appears that the product of recrystallization from cold water is different in appearance but similar in its enthalpy of solution to the parent material and to the product of recrystallization from hot water.

4. Discussion and Summary

An important aspect of this work is the presentation of evidence that extensive analytical data are required for characterizing and defining important biological compounds for which precise thermochemical data are needed. The data in table 7 show that there was as much as 4 percent

[10] By S. A. Abramowitz, Chemical Thermodynamics Division, Center for Thermo.dynamics and Molecular Science, National Measurement Laboratory.

difference in the enthalpies of solution of the 5 commercial samples as received; the difference was nearly 5 percent after corrections for the H_2O impurity and dilution. However, none of our analytical methods (except volatile matter and H_2O titrations) identified impurities in the sample either qualitatively or quantitatively. The H_2O content in these adenine samples was small and the titration analyses were less dependable than the determination of volatile matter by vacuum heating. However, the combination of results from both methods provided greater confidence in the values.

The chromatographic analyses gave assurance that many compounds were *not* present in large amounts, but revealed no impurities in detectable quantities. X-ray powder patterns confirmed the crystallinity of the materials, and elemental analyses confirmed the anhydrous composition. Emission spectra showed no impurities within the limits of detection. No impurities were identified with gas-liquid chromatography. The enthalpies of solution showed the greatest differences among the samples, although impurities were not identifiable.

In the absence of analytical data to serve as a basis for choosing the purest sample, we assume that Ade 5b which was reprecipitated, twice recrystallized from hot water, and vacuum-dried, and had the highest values for the enthalpy of solution was the purest of the samples measured here.

If the undissociated solution is taken as the standard state, a small correction must be applied to ΔH_∞ given in table 7 to obtain ΔH_∞°. Using the values, $\log K_1 = -9.87$ kcal·mol^{-1} for the ionization at N9 (see sec. 1) and $\log K_2 = 4.20$ kcal·mol^{-1} for the protonation at N1 [1] and assuming unit activity coefficient, we calculate 0.15 percent ionization and 0.14 percent protonation which is essentially constant in these experiments. The enthalpies of ionization, 9.65 kcal/mol, and of protonation, −4.81 kcal/mol [1], agree within experimental uncertainties with those obtained by Zimmer and Biltonen [19]. From these the correction to the standard state, 0.033 kJ·mol^{-1} was obtained. This is subtracted from the value of ΔH (∞, 298.15 K) for Ade 5b given in table 7 to obtain the best value presently available for the solution of adenine in water with the estimated overall uncertainties:

$$\Delta H^\circ(\infty, 298.15\ \text{K}) = (33.47 \pm 1.00)\ \text{kJ·mol}^{-1}$$

and

$$\Delta C_p = (78.7 \pm 10.4)\ \text{J·mol}^{-1}\text{·K}^{-1}$$

as reported in section 3.3.1. The experimental imprecision of the enthalpy of solution is 0.34 kJ·mol^{-1} (1.1%), the uncertainty in the correction for H_2O impurity is 0.11 kJ·mol^{-1} (0.4%), and for dilution, 0.32 kJ·mol^{-1} (1.0%). An additional 0.5 percent for the possible impurities not detected in the emission spectra or chromatographic analysis is added for the overall uncertainty of 3 percent or 1.00 kJ·mol^{-1}. No other measurements of the reaction have been previously reported.

The entropy of solution can be calculated using $(8.0 \pm 0.4) \times 10^{-3}$ as the molal solubility [17] which gives

$$\Delta G^\circ\ (298.15\ \text{K}) = (11.97 \pm 0.60)\ \text{kJ·mol}^{-1}.$$

Combining this with our value for ΔH° (∞, 298.15 K), we obtain

$$\Delta S^\circ\ (298.15\ \text{K}) = (72.1 \pm 3.9)\ \text{J·mol}^{-1}\text{·K}^{-1}.$$

The value for C_p° reported in section 2.2.5 can be used to compute a value for the apparent molal heat capacity at infinite dilution:

$$C_{p2}^\circ = \Delta C_p + C_p^\circ = (226 \pm 11)\ \text{J·mol}^{-1}\text{·K}^{-1}$$

The average density from measurements on 6 adenine samples was 1.47 g·mL^{-1} with an estimated uncertainty of 1 percent.

Many colleagues other than those specifically cited in the text have contributed to this work through advice and consultations. Although they are too numerous to name the individuals, the author is appreciative for their interest. She is also grateful to W. A. Tvrdik of E-M Labs, and J. S. Wigand of the Boehringer-Mannheim Corporation for supplying some of the adenine samples used in this work, and to R. L. Biltonen of the University of Virginia for supplying values for the heat capacities of the crystalline samples.

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