JOURNAL OF RESEARCH of the National Bureau of Standards
Vol. 83, No. 6, November–December 1978

Enthalpies of Solution of the Nucleic Acid Bases. 3. Cytosine in Water

Marthada V. Kilday

Center for Thermodynamics and Molecular Science, National Bureau of Standards, Washington, D.C. 20234

June 19, 1978

An adiabatic solution calorimeter was used for measuring enthalpies of solution in water of seven samples of cytosine for which analytical data are given.

Our best values are:

$$\Delta H^\circ(\infty, 298.15\ \mathrm{K}) = (27.2 \pm 4.0)\ \mathrm{kJ \cdot mol^{-1}},$$

and

$$\Delta C_p = (76 \pm 21)\ \mathrm{J \cdot mol^{-1} \cdot K^{-1}},\ 298\ \mathrm{K} < \mathrm{T} < 324\ \mathrm{K}\ \text{at}\ (24 \pm 2)\ \mathrm{mmol \cdot kg^{-1}}.$$

Evidence is given for an unidentified side reaction at low concentrations which is responsible for the large uncertainty assigned to the enthalpy of solution at infinite dilution. An approximate value of $(1.44 \pm 0.08)\ \mathrm{g \cdot mL^{-1}}$ for the density of cytosine was also measured. Values are calculated for ΔG° and ΔS° for the solution reaction.

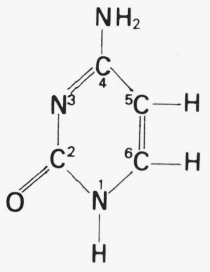
Key Words: 4-amino-2 (1H)-pyrimidinone; calorimetry; cytosine: density, enthalpy of solution, entropy of solution; nucleic acid bases; thermochemistry.

1. Introduction

This is the third in a series of papers [1, 2][1] describing the measurements of enthalpies of solution of the bases of the nucleic acids with emphasis on the characterization of the samples as an aid in establishing uncertainty limits for the measured values. The first paper in this series described in detail the general procedures which were also followed in this work.

Enthalpy of solution measurements are reported here for four samples of cytosine from commercial sources as received and for three samples which were further purified by sublimation and by recrystallization from water and from ethyl alcohol solutions. Measurements were made in the temperature range 298 to 329 K and in the concentration range 2 to 35 $\mathrm{mmol \cdot kg^{-1}}$.

Cytosine, a pyrimidine base, is slightly soluble in water at 298 K ($8.5\ \mathrm{g \cdot L^{-1}}$ [3])[2], and sublimes and decomposes before melting. It has the following structural formula [5]:



Only one measured value for the enthalpy of solution of cytosine in water has been reported previously [6]. This was a small part of a study of heat transfer coefficients and was not intended as a measurement of high accuracy.

[1] Figures in brackets indicate the 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 [4].

2. The Cytosine (Cyt) Samples

The following information about the cytosine samples from commercial sources[3] was obtained primarily from labels, catalogs, or brochures supplied by the manufacturer or distributor:

Cyt 1. Calbiochem, Cat. No. 2510, Lot 840095, 25 g, Grade A received about 1970—exact date unknown. (Listed as "hemihydrate" in the catalog; elemental analysis indicated it was anhydrous.) Analysis: Nitrogen, 37.60%; Spectra at pH 1: 250/260 0.46, 280/260 1.52, 290/260 0.77, λmax 275 mμ, ϵmax 10,200, λmin 238 mμ, ϵmin 1,000; Chromat. Homogeneous

Cyt 2. E-M Laboratories, Cat. No. 2326, Lot 1958515 (sic), 5 g received June 1974. Listed as "Cytosine for Biochemistry." Type analysis: Optical properties, measured values at pH 1 (sic): λmax 275, E_{250}/E_{260} 0.48 ± 0.02, E_{280}/E_{260} 1.50 ± 0.03, E_{290}/E_{260} 0.77 ± 0.02. Thin Layer Chromatography: Layer TLC plates PEI-Cellulose F, pre-coated, Solvent Saturated ammonium sulfate solution/1 M sodium acetate solution/2-propanol (80/18/2). R_f value: ~0.65

Cyt 3. Same as Cyt 2 except 55 g of Lot 4955544 received August 1974.

Cyt 4. Same as Cyt 2 except 5 g of Lot 4958785 received January 1975.

These samples were all finely divided white powders and there was no visible difference in their appearance. The enthalpy of solution measurements were made on the samples as received or after vacuum drying.

The following samples were purified from Cyt 3 in this laboratory:

Cyt 3a and b were twice recrystallized as described for Thy 3a and b [2]. For Cyt 3a, 20 g was dissolved in 0.25 L of boiling distilled H_2O with activated charcoal as a decolorizer; it was then filtered and slowly cooled. The yield from a second crystallization was 6.9 g. For Cyt 3b, 10 g was dissolved in 0.5 L of boiling 90 percent ethyl alcohol, activated charcoal was added, the solution was filtered and slowly cooled. The yield from a second crystallization was 7.9 g.

Cyt 3d. This was the product of vacuum-sublimation as described [1] for Ade 1c and 5c except that the maximum temperature here was about 475 K. The sublimation was stopped after 4 days although only about one-half of the 5 g sample had sublimed. The product was removed from the sublimation vessel in a glove box containing an H_2O absorber, and all subsequent manipulations of this sublimate were performed in the glove box.

Cyt 3e. This was the unsublimed residue from sublimation of Cyt 3d. The residue had a brown discoloration of the portion in contact with the glass vessel which was evidence of apparent decomposition.

2.1 Characterization of the Samples

The purity of cytosine could not be determined from melting-temperature measurements because it decomposes before melting. Therefore, it was necessary to search for impurities and to characterize as completely as possible the samples on which enthalpies of solution were measured as an aid in the assignment of uncertainty limits for the measured values.

In this laboratory densities were determined by displacement, volatile matter by vacuum drying, H_2O by Karl Fischer titrations, and other impurities by paper and thin layer chromatography (TLC). Other laboratories contributed analyses of the elemental compositions, emission spectra, x-ray diffraction patterns, and the measurement of the heat capacity of the crystalline cytosine.

2.1.1. Density, Volatile Matter, and H_2O

The densities of two of the cytosine samples were measured by a benzene displacement method; details of the method have been described [1]. Duplicate density measurements were: for Cyt 1, 1.475 and 1.491 $g \cdot mL^{-1}$, and for Cyt 3, 1.418 and 1.388 $g \cdot mL^{-1}$. The mean value of (1.44 ± 0.08) $g \cdot mL^{-1}$ was used in calculating the buoyancy factor, 1.0007, for the cytosine mass corrections in this work.

The volatility and hygroscopicity of the cytosine samples were determined from changes in mass after vacuum-drying (see [1] for details) and exposure to atmospheric moisture (~35% relative humidity). The samples, contained in aluminum moisture dishes, were vacuum-dried 4 hours and weighed, then dried again for 2-h intervals and weighed, until the total drying time was 10 to 14 hours. At 340 K, samples of Cyt 1 (1, 2, and 3 g) had reached constant mass after 4 h with a loss of 0.5 $mg \cdot g^{-1}$. The 3-g sample was then exposed to the atmosphere before drying at 375 K along with 3-g samples of Cyt 2, 3, and 4; the losses were 1.6, 2.9, 2.8, and 4.4 $mg \cdot g^{-1}$, respectively, and they were at constant mass after about 6 hours of drying. This sample of Cyt 4 was exposed to the atmosphere for 64 hours and gained 1.2 $mg \cdot g^{-1}$, but there was no further gain in mass after an additional 30 hours exposure to the atmosphere. This agrees with the findings of Falk [7] that cytosine does not form hydrates even at 93 percent relative humidity. When this sample was vacuum dried for 2 hours at 375 K, it again returned to the same constant mass as before. Again exposure to the atmosphere resulted in the following gains in mass in $mg \cdot g^{-1}$: 0.1 after 1 min, 0.6 after 10 min, 0.9 after 25 min, 1.0 after 40 min, and 1.2 after 18 hours; thus, one-half of the mass increase occurred during the first 10 min and the mass at equilibrium with the atmosphere was reproduced. This sample was then included in observations of mass changes after relatively long periods of vacuum drying at 375 K; samples of Cyt 3a and b and Thy 2 and 4 [2] were heated simultaneously. Cyt 3a and b had previously been dried at 340 K at atmospheric pressure and reached constant mass. After 24 hours of vacuum drying at 375 K, they each lost about 0.7 $mg \cdot g^{-1}$; and the masses remained essentially constant for an additional 110 hours of drying. As previously

[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.

reported [2], Thy 2 and 4 were losing mass at the rate of 600 and 100 $\mu g \cdot g^{-1} \cdot h^{-1}$, respectively. The sample of Cyt 4 which had proven stable during the vacuum heating lost only about 0.1 $mg \cdot g^{-1}$ during 30 hours of continuous vacuum heating, but then surprisingly began to gain mass: 1.6 $mg \cdot g^{-1}$ after 22 hours, and additional 1.3 $mg \cdot g^{-1}$ after the next 22 hours, and finally a gain of about 4.5 $mg \cdot g^{-1}$ after a total of 130 hours. When the two thymine samples were removed from the oven, the gain in mass of Cyt 4 ceased. It is assumed that the thymine samples were subliming onto this cytosine sample. However, it is not understood why it was apparently selective; Cyt 3a and b were in cylindrical glass weighing bottles while the Cyt 4, Thy 2, and Thy 4 were in aluminum moisture dishes, and only the Cyt 4 sample showed the gain in mass.

From these observations it is concluded that cytosine is essentially non-hygroscopic and that the volatile matter, presumed to be H_2O, is (in mass %): Cyt 1, 0.16; Cyt 2, 0.29; Cyt 3, 0.28; and Cyt 4, 0.44.

Karl Fischer titrations for H_2O in Cyt 1, 2, 3, and 4, indicated 0.26, 0.31, 0.33, and 0.43 mass percent, respectively. (See [2] for details of the procedure.) The cytosine samples dissolved completely in the methanol solvent, and the values represent the total H_2O in the samples. These results are in good agreement with those obtained for volatile matter. Corrections to the calorimetric data for H_2O in the sample use the mean of the titration results and the volatile matter measurements, namely, 0.21 mass percent for Cyt 1; 0.30, for Cyt 2; 0.30, for Cyt 3; and 0.44, for Cyt 4. The other samples had been vacuum dried and required no corrections for H_2O.

2.1.2. Other Analyses

The emission spectra for Cyt 1, 2, 3, and 4[4] showed no impurities beyond the background limits listed for adenine [1].

The results of duplicate elemental analyses of Cyt 1, 3, and 3a are given in table 1. Within the estimated accuracy of 0.2 percent for each determination, the analyses for all three samples correspond to the composition of anhydrous cytosine, except the C and O in Cyt 1 and the O for Cyt 3 which are a little high, and the N for Cyt 3 which is a little low. However, none of the analytical values are close to the theoretical composition of the hemihydrate (the catalog for Cyt 1 had listed the material as the hemihydrate). Since the samples are anhydrous, no significant error in the enthalpy of solution can be attributed to the presence of hydrates of cytosine.

Stirred solutions of Cyt 1 and of Cyt 3 (15 $mmol \cdot kg^{-1}$) in equilibrium with air (as in the calorimetric solutions) were at pH 6.4 ± 0.1. The pH meter was calibrated with a standard buffer at pH 7.00 and checked at the end of the measurements.

Table 1. Elemental analysis of cytosine samples.

Substance	Empirical Formula	Molecular Mass	C	H	O	N (Kjeldahl)	Sulfated Ash	Σ
		g/mol	mass percent					
Theoretical Composition:								
Cyt	$C_4H_5N_3O$	111.103	43.24	4.54	14.40	37.82	----	100.00
Cyt·1/2 H_2O	$C_4H_6N_3O_{1.5}$	120.1106	40.00	5.03	19.98	34.98	----	99.99
Cyt·H_2O	$C_4H_7N_3O_2$	129.1182	37.21	5.46	24.78	32.54	----	99.99
Analyses[a]:								
Cyt 1	--------	--------	43.78	4.77	15.00	37.73	0.06	101.34
	--------	--------	43.50	4.75	14.89	37.67	0.02	100.83
Cyt 3	--------	--------	43.46	4.54	14.99	37.29	0.05	100.33
	--------	--------	43.33	4.63	15.02	37.59	0.04	100.61
Cyt 3a	--------	--------	43.23	4.38	14.56	37.68	0.20	100.05
	--------	--------	43.41	4.65	14.82	37.60	0.06	100.54

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

The procedures followed in our paper and thin-layer (TLC) chromatography work and the observations regarding backgrounds and sensitivity have been previously discussed in detail [1]. R_f values obtained for the four commercial cytosine samples as received are compared in table 2 with those reported in the National Academy of Sciences (NAS) publication [8]. Our values have in general tended to be somewhat higher than those of NAS; this is probably due to differences in laboratory temperature or other experimental conditions.

The estimated uncertainty in reading the chromatograms is ±0.02 R_f unit. Within this uncertainty, the cytosine samples listed in table 2 showed no significant differences in any of the solutions and no spots other than that of the major component were visible. Therefore, it is concluded that the four cytosine samples are of equal purity (probably 95% or more) within the limits of chromatographic detection.

2.1.3. Calorimetric Characterization

The heat capacities at 298 K of two of our crystalline samples were measured by Ernesto Friere using a drop microcalorimeter at the University of Virginia. His values were reported as (1.197 ± 0.004) $J \cdot g^{-1} \cdot K^{-1}$ for Cyt 1 and (1.188 ± 0.004) $J \cdot g^{-1} \cdot K^{-1}$ for Cyt 3 [9]. The mean value is $C_p^\circ = (132.5 \pm 0.8)\ J \cdot mol^{-1} \cdot K^{-1}$.

3. Enthalpies of Solution

The enthalpies of solution in H_2O of four commercial samples of cytosine as received and products of recrystallization and of sublimation were measured in the platinum-lined adiabatic solution calorimeter which has been previously described in detail [10, 11]. The solution volume was approximately 300 mL and the capacity of the platinum sample holder [10] was 0.7 or 3.0 mL depending on which of two interchangeable cylinders was used. Measurements were

[4] Analyses by J. A. Norris, Center for Analytical Chemistry, National Measurement Laboratory, National Bureau of Standards.

Table 2. R_f values for the cytosine samples in NH_4OH (aq, 1 $mol \cdot L^{-1}$) solutions on fluorescent TLC plates and two papers with four carrier solutions, A, B, C, and D.

Cytosine Sample No.	A[a]			B[a]			C[a]			D[a]		
	TLC	P-1	P-40	TLC	P-1	P-40	TLC	P-1	P-40	TLC	P-1	P-40
1	0.79	0.78	0.84	0.56	0.58	0.60	0.60	0.71	----	0.83	0.76	0.73
2	.79	.78	.83	.58	.56	.60	.60	.70	----	.82	.74	.69
3	.79	.78	.82	.56	.56	.60	.60	.70	----	.82	.73	.71
4	.80	.78	.82	.54	.56	.60	.60	.70	----	.84	.74	.72
NAS [8]			0.76			0.50			0.70			0.71

[a]The composition 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 $mol \cdot L^{-1}$).

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 $mol \cdot L^{-1}$).

Soln D: H_2O adjusted to pH 10 with NH_4OH (~1 drop of conc. NH_4OH in 300 mL H_2O).

Table 3. Data for the measurements of the enthalpies of solution of various cytosine samples in water.

Expt. No.	[a]S.H. cyl.	Sample Mass x10^5	H_2O Mass -300 g	Conc. x10^6	Reaction Period	$T_{reaction}$	Cal. unc. x10^2	Electrical Energy Equivalent -1730 J/K Initial	Final	$-\Delta T_{react}$ x10^5	EIt x10^2	[b]q_{vap} x10^2	$-Q_{react}$ x10^2	$-q_{H_2O}$ x10^2	$\Delta H_m(T)$ J/g	J/mol	corr. to T = 298.15 K	ΔH_m (298.15 K)
		g	g	mol/kg	min	K	%	J/K		K	J	J	J	J			J/mol	J/mol
Cyt 1: Calbiochem Lot 840095																		
897	S	30817	2.459	9171	157	298.170	85	8.06	7.11	3464	-----	2	6016	13	195.63	21735	- 2	(21733)
898	S	20727	2.454	6168	57	298.210	25	8.35	7.37	2378	-----	2	4130	9	199.68	22184	- 5	22179
899	S	19840	2.459	5904	27	298.205	13	8.75	7.80	2305	-----	2	4005	8	202.29	22475	- 4	22411
900	S	20219	2.444	6017	17	298.186	25	8.16	7.24	2340	-----	2	4063	8	201.37	22373	- 3	22370
901	S	21219	2.424	6315	18	298.180	8	8.11	7.61	2457	-----	2	4268	9	201.56	22394	- 2	22392
902	S	19977	2.459	5945	27	308.913	11	8.38	7.07	2372	-----	4	4118	9	206.57	22950	- 822	22128
Cyt 2: E-M Lot 1958515																		
1223	L	77313	2.384	23013	17	298.210	6	5.16	4.29	8699	88140	9	15081	45	195.65	21737	- 5	21732
1224	L	56970	2.379	16958	22	298.227	6	4.06	3.78	6435	88246	9	11149	33	196.29	21808	- 6	21802
Cyt 3: E-M Lot 4955544																		
1202	L	85496	2.414	25446	22	298.405	5	4.07	4.56	9666	85995	9	16755	50	196.56	21839	- 19	21820
1204	L	79298	2.389	23603	22	318.777	4	7.39	7.49	9441	87730	25	16378	49	207.16	23016	-1576	21440
1205	S	19336	2.424	5755	37	308.691	26	3.05	3.81	2508	88136	4	4343	13	225.28	25029	- 805	24224
1215	S	20866	2.384	6211	62	308.451	30	6.28	4.01	3003	-----	4	5207	16	250.29	27808	- 787	27021
Cyt 3a: Twice recrystallized from H_2O																		
1206	L	82483	2.389	24552	32	323.838	5	9.34	8.96	10345	87506	31	17960	--	217.74	24192	-1962	22229
1207	L	82159	2.409	24453	37	313.707	7	7.06	7.09	9791	87440	19	16989	--	206.78	22974	-1188	21786
1208	S	20958	2.449	6237	96	303.626	46	4.35	2.98	2539	87510	3	4399	--	209.90	23320	- 418	22902
1210	L	118576	2.389	35294	92	298.377	9	5.26	5.17	13618	86763	8	[c]23617	--	199.17	22128	- 17	22111
1211	L	99953	2.409	29749	117	298.189	14	4.08	3.67	11571	86682	8	20054	--	200.80	22310	- 3	22307
1212	L	50608	2.429	15062	82	298.225	15	6.34	4.32	6015	86758	10	10428	--	206.05	22893	- 6	22887
1213	S	10071	2.364	2998	32	297.987	37	4.18	3.99	1269	-----	2	2199	--	218.35	24259	+ 12	24271
1214	S	5199	2.364	1548	17	298.096	53	3.81	3.24	730	-----	3	1263	--	242.93	26990	+ 4	26994
1217	L	76644	2.404	22812	50	321.222	13	9.61	9.14	9369	86677	26	16271	--	212.29	23586	-1763	21823
1218	L	51554	2.439	15342	27	303.612	9	4.92	4.91	6139	88135	13	10637	--	206.33	22924	- 417	22507
1275	S	19158	2.299	5704	42	298.100	42	4.06	1.85	2793	-----	2	4838	--	252.53	28057	+ 4	28061
1276	S	27799	2.294	8277	132	298.093	51	1.78	0.74	3294	-----	2	5701	--	205.08	22785	+ 4	22789
1277	S	23027	2.264	6857	127	298.104	70	2.00	0.99	2855	-----	2	4941	--	214.57	23840	+ 4	23844
1278	S	9275	2.274	2896	37	298.109	43	1.57	1.03	1299	-----	2	2247	--	231.05	25671	+ 3	25674
1289	L	79552	2.344	23682	42	303.190	7	5.24	4.47	9395	88741	12	16287	--	204.73	22746	- 385	22361
1290	L	80293	2.264	23909	42	308.496	6	4.97	4.87	9600	89383	15	16640	--	207.24	23025	- 790	22225
Cyt 3b: Twice recrystallized from 90% EtOH																		
1219	L	79805	2.419	23752	22	298.413	6	5.00	4.14	9063	88265	9	15712	--	196.88	21874	- 20	21854
1220	L	79342	2.364	23618	17	298.423	5	3.93	3.80	9005	88145	9	15604	--	196.67	21850	- 21	21829
Cyt 3d: Sublimed																		
1221	L	46488	2.399	13837	32	298.486	8	3.92	3.90	5294	88119	15	9164	--	197.13	21901	- 26	21875
1222	L	33553	2.414	9986	22	298.448	14	3.86	3.64	3883	88153	15	6717	--	200.19	22242	- 23	22219
Cyt 4: E-M Lot 4958785																		
1225	L	63996	2.394	19048	27	298.220	8	4.03	3.92	7315	87538	9	12674	56	198.92	22101	- 5	22097

[a]Two sizes of sample holder cylinders were used; the volumes are S = 0.7 mL and L = 3.0 mL.

[b]The sublimed samples (Cyt 3d) were transferred to the sample holder in a glove box where the relative humidity (RH) was zero; all other samples were transferred in the laboratory atmosphere where RH = 0.35 ± 0.10.

[c]This value includes a correction of -0.05 J for the energy lost to the surroundings during a brief period after starting the reaction when the vessel temperature was less than that of the adiabatic shield.

made over the temperature range, 298 K to 324 K, and the concentration range, 1.5 to 35 mmol·kg^{-1}. The endothermic reactions of cytosine in H_2O were moderately rapid (~30 min) at 298 K if the samples were loosely packed in the sample holder, however, some of the reactions required more than two hours. In most experiments a moderate stirring rate, 550 revolutions per min, was used.

Detailed descriptions of the samples and their analyses are given in section 2. All calorimetric samples were transferred to the sample holder in the laboratory atmosphere except the product of sublimation, Cyt 3d.

The general calorimetric procedures and methods of calculation have been described [10, 11]. The electrical energy equivalents of the initial and final systems were measured in each experiment. In experiments where the heat absorbed during the endothermic reaction was expected to exceed 100 J, precisely measured electrical energy was added during the reaction to prevent a decrease in the vessel temperature and a loss of adiabatic conditions; if less than 100 J of heat was absorbed, the stirring energy was sufficient to maintain the calorimeter temperature.

The calorimeter temperature was measured with a quartz-oscillator thermometer system [1, 10]. The calibrations of this system and those of the standard cell and standard resistors used in electrical energy measurements were given previously [1]. The calorimetric experiments reported in this work were completed between December 1973 and April 1977. Uncertainties are given at the 95 percent confidence level except as noted.

The 1975 Table of Atomic Weights [12] was used to obtain molecular masses for this work as follows: cytosine, 111.103, and H_2O, 18.0152. Energy conversions were made according to the following relationship: 1 thermochemical calorie = 4.184 joules.

Data for 33 experiments in which enthalpies of solution of cytosine in water were measured are given in table 3. The Experiment Number is a serial number for experiments with this calorimeter and indicates the chronological order of the experiments. S. H. Cyl. indicates the size of the sample holder cylinder used in each experiment. The Reaction Period is the elapsed time between initiating the reaction and the beginning of the rating period which follows the reaction. T_{reaction} is the mean of the initial and final temperatures of reaction. The estimated calorimetric uncertainty for an experiment, Cal. Unc., is based on the duration of the reaction, the magnitude of the temperature change from the reaction, and the standard deviation of the slope of the rating period following the reaction (see [1] for details). The heat of the solution reaction, Q_{reaction}, is given by the following equation:

$$Q_{\text{react}} = \Delta T_{\text{react}}\left(\frac{\epsilon_i + \epsilon_f}{2}\right) - q_{\text{vap}}$$

where the electrical energy equivalents (in J·K^{-1}) of the initial and final systems are ϵ_i and ϵ_f, the temperature change due to the solution reaction is ΔT_{react}, and the heat of vaporization of water into the air space in the sample holder upon opening is q_{vap}. $\Delta T_{\text{react}} = \Delta T - \text{EIt}\left(\frac{\epsilon_i + \epsilon_f}{2}\right)^{-1}$ where ΔT (not given in table 3) is the net temperature change resulting from the endothermic solution reaction and the electrical energy added, EIt. $q_{\text{vap}} = \Delta H_{\text{vap}}\left(V - \frac{s}{d}\right)(1 - RH)$ where ΔH_{vap} is the enthalpy of vaporization of water per unit volume at the mean temperature of reaction, V is the internal volume of the sample holder, s is the mass of sample, d is the density of the sample, and RH is the relative humidity of the atmosphere in which the sample was transferred to the sample holder (for the transfer in the dry box, $RH = 0$; in the laboratory atmosphere, $RH = 0.35 \pm 0.10$). A correction, $q_{H_2O} = Q_{\text{react}}$ (mass % H_2O), is added to Q_{react} for the water in the samples of Cyt 1, 2, 3, and 4 given at the end of section 2.1.1. The enthalpy of solution per gram at the temperature and concentration of the experiment, $\Delta H_m(T) = -(Q_{\text{react}} + q_{H_2O})(\text{sample mass})^{-1}$.

The change in the enthalpy of solution with temperature, ΔC_p, was obtained from the 10 experiments in table 3 in the concentration range, 22 to 26 mmol·kg^{-1}. The data for T_{reaction} and $\Delta H_m(T)$ from these experiments are plotted in figure 1. (The point marked "A" on the plot was for Expt. No. 1215 which was rejected and not included in table 3.)

A least squares fit of these 10 points to a linear equation gave a slope of 76.4 J·mol^{-1}·K^{-1}; the standard error was 9.5 J·mol^{-1}·K^{-1}. Thus in the range, 298 K $< T <$ 324 K $\Delta C_p = (76 \pm 21)$ J·mol^{-1}·K^{-1}. This was used to calculate the corrections to $T = 298.15$ K given in table 3 which were added to $\Delta H_m(T)$ to obtain the enthalpy of solution at the standard temperature, $\Delta H_m(298.15\text{ K})$.

A plot of these values given in the last column of table 3 versus the concentration of the final solutions is shown in figure 2. The points representing various samples are distinguished by different symbols. The estimated calorimetric uncertainty for the experiment is equal to the radius of a circle (or a circle circumscribed by the other geometric figures). The straight lines shown were obtained from the fits of the data points by the method of least squares; the solid lines are from the fits of only the points for Cyt 3A (open circles) with 7 points below and 9 points above 9 mmol·kg^{-1}, and the broken lines are from the fit of all 14 points below and 19 points above 9 mmol·kg^{-1}. The linear equations obtained are as follows (in kJ·mol^{-1}):

Cyt 3a, >9 mmol·kg^{-1}

$$\begin{matrix}\Delta H_m\ (298.15\text{ K}) = 22.91 - 0.027\ (C) \\ \pm 0.31 \quad \pm 0.43 \quad \pm 0.018\end{matrix} \qquad (1)$$

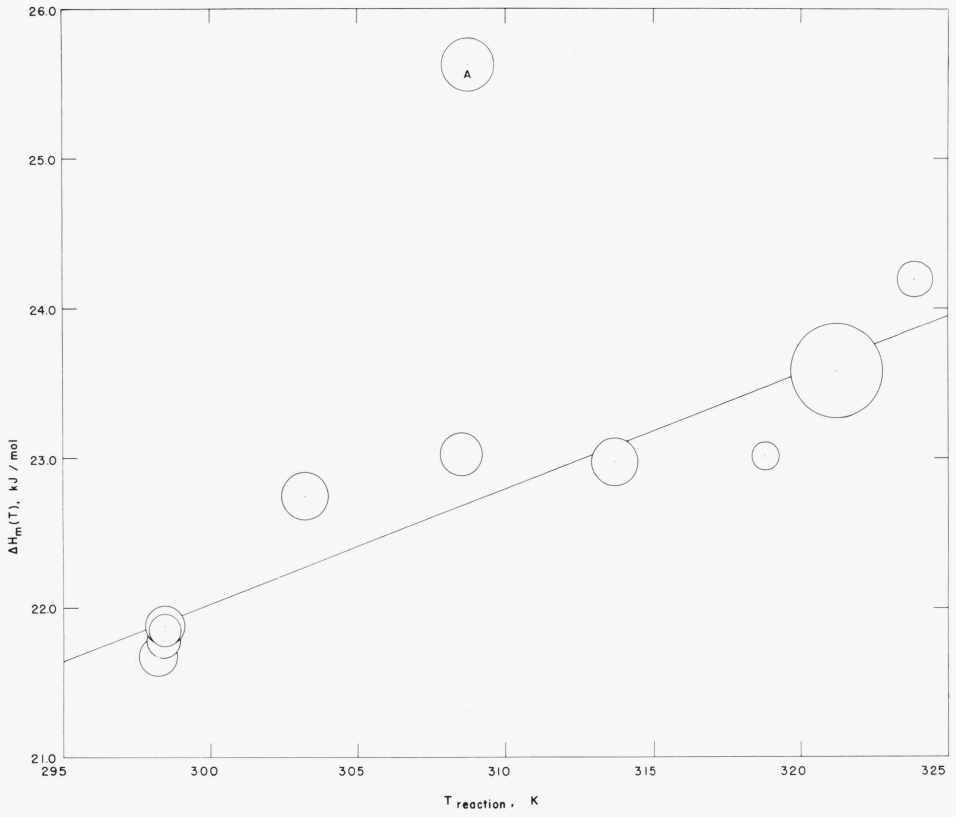


FIGURE 1. *Plot showing the relationship between the mean temperature of reaction, $T_{reaction}$, and the enthalpy of solution of cytosine in H_2O at the concentration (24 ± 2) mmol·kg^{-1}.*

The point marked "A" was omitted from the data for the least squares fit to obtain the straight line shown (slope = ΔCp = 76 ± 21 J·mol^{-1}·K^{-1}). The radius of a circle is equal to the estimated calorimetric uncertainty (see text).

All samples, >9 mmol·kg^{-1}

$$\Delta H_m\ (298.15\ \text{K}) = 22.13 - 0.005\ (C) \qquad (2)$$
$$\pm 0.35 \quad \pm 0.28 \quad \pm 0.013$$

Cyt 3a, <9 mmol·kg^{-1}

$$\Delta H_m\ (298.15\ \text{K}) = 27.2 - 0.46\ (C) \qquad (3)$$
$$\pm 1.8 \quad \pm 1.7 \quad \pm 0.31$$

All samples, <9 mmol·kg^{-1}

$$\Delta H_m\ (298.15\ \text{K}) = 27.2 - 0.56\ (C) \qquad (4)$$
$$\pm 1.9 \quad \pm 1.7 \quad \pm 0.29.$$

where C is the concentration in mmol·kg^{-1} and the uncertainties are the standard deviations.

In figure 2 it can be seen that generally above 9 mmol·kg^{-1} the values for Cyt 3a are larger than those for the other samples. This may indicate that impurities were removed in the recrystallization from H_2O. There is some evidence of inhomogeneity in the two low values at 23 and 24 mmol·kg^{-1}. There appears to be no significant difference between the values with Cyt 3 (filled circles) and those with sublimate, Cyt 3d (right half-filled circles). The spread of the data in figure 2 is larger than the estimated calorimetric uncertainties which suggests inhomogeneity in the samples.

The reason for the change in the slope of the lines near the concentration, 10 mmol·kg^{-1}, shown in figure 2, is still not understood. The first supposition was that a protonation was completed at that concentration. However, Izatt et al [5] indicate N_3 protonation of cytosine at pK = 4.6 and ΔH (298.15 K) = −5.14 kcal·mol^{-1}, an exothermic reaction which would be more complete as the concentration decreases, hence, the value for the endothermic enthalpy of solution should decrease as the concentration decreases; in figure 2, the opposite is observed. The possibility of the removal of the N_1 proton from cytosine in aqueous solution (pH = 6.4) is very unlikely since pK for the reaction is 12.15 [5]. The remaining possibility is an exothermic side reaction occurring at the lower concentrations.

Our previous experience with tris(hydroxymethyl)aminomethane gave evidence of an exothermic side reaction under some conditions in the presence of CO_2 and O_2 [11]. It was suspected, but not proven, that the amino group was involved

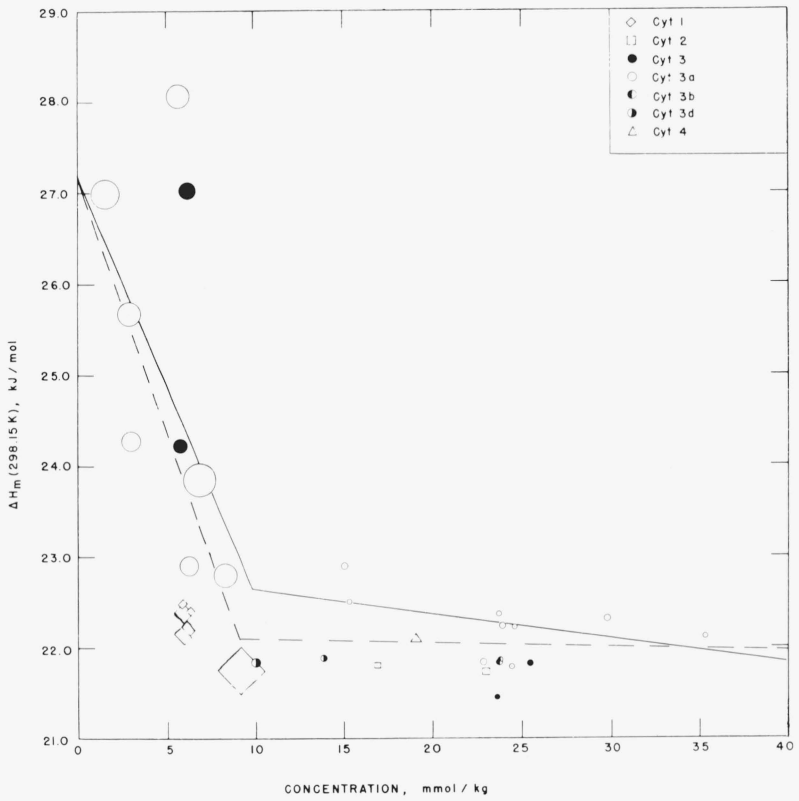


FIGURE 2. *Plot showing the relationship of concentration and the enthalpy of solution of cytosine in H_2O at 298.15 K using various samples distinguished by different symbols.*
The solid lines result from a least squares fit of the data for only Cyt 3a above and below 9 mmol·kg^{-1}, and the dashed lines, for all points above and below 9 mmol·kg^{-1}. The estimated calorimetric uncertainty (see text) is equal to the radius of a circle (or a circle circumscribed by the other symbols).

in the side reaction. Cytosine also has an amino group which could participate in a similar reaction with the CO_2 and O_2 in equilibrium with the distilled H_2O used for the calorimetric solution. The relatively small quantity of CO_2 dissolved in the H_2O could react with the cytosine until the solution was depleted of CO_2, then at higher concentrations the side reaction would continue only with CO_2 drawn through limited access from the atmosphere. This would account for the sharp increase in the enthalpy of solution as the concentration decreases. It is also interesting to note that plots comparable to figure 2 for thymine (in the preceding work) and for uracil (in the subsequent work) show no change in the enthalpy of solution with concentration. The structure of thymine and uracil are similar to that of cytosine except that they have an oxygen atom in place of the amino group (thymine also has a methyl group in place of a hydrogen). Thus, a side reaction with the amino group in cytosine is a possible but not a proven explanation for the change in slope at the lower concentrations as shown in figure 2.

The increase in the enthalpy of solution of cytosine in H_2O below 10 mmol·kg^{-1} (fig. 2) must be the result of an unidentified side reaction rather than an ionization. The exothermic heat effect of this side reaction at infinite dilution is taken as the difference between the intercepts for equations (2) and (4), or -5.1 kJ·mol^{-1} and the sum of the uncertainties is 2.0 kJ·mol^{-1}. This uncertainty was doubled for assignment to the enthalpy of solution of cytosine in water at infinite dilution which was taken as the difference of the intercept in equation (2), 22.13 kJ·mol^{-1}, and the heat of the side reaction, -5.1 kJ·mol^{-1}. Thus our best value is ΔH° (∞, 298.15 K) = (27.2 ± 4.0) kJ·mol^{-1}. The uncertainty is large enough to include reasonable uncertainties in the purity of the samples and other possible errors in the measurements.

4. Discussion and Summary

Larsen and Magid [6] reported the only direct measurement of the enthalpy of solution of cytosine in water prior to this work. No information about the purity of the sample or the concentrations at which their measurements were made was given in the paper or the supplemental material. The 20 percent discrepancy between their value, (21.55 ± 0.25) kJ·mol^{-1}, and ours is probably due primarily to the apparent

side reaction discussed at the end of the previous section. Our values above 9 mmol·kg^{-1} agree with their value within about 2 percent.

Scruggs, Achter, and Ross [3] calculated the enthalpy of solution of cytosine in the saturated solution at 335 K, 32.09 kJ·mol^{-1}, from their solubility measurements; using our value for ΔC_p, we obtain 31.17 kJ·mol^{-1} at 298 K. With our equation (2) we calculate ΔH (298.15 K) = 21.75 kJ·mol^{-1} at saturation, 76 mmol·kg^{-1}; then correcting for the side reaction, we obtain 26.85 kJ·mol^{-1} which is about 14 percent smaller than the value of Scruggs et al.

Our measurements with various samples of cytosine showed differences of several percent in the enthalpies of solution, and yet our analytical data could not explain these differences. Until measurements can be made with cytosine samples which are known to be of higher purity than ours, the value for the enthalpy of solution of cytosine in water at infinite dilution is taken as

$$\Delta H^\circ (\infty, 298.15\ \text{K}) = (27.2 \pm 4.0)\ \text{kJ}\cdot\text{mol}^{-1}.$$

The change in the heat capacity of the reaction with temperature at (24 ± 2) mmol·kg^{-1} is

$$\Delta C_p = (76 \pm 21)\ \text{J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}, \qquad 298\ \text{K} < T < 324\ \text{K}.$$

Because of the side reaction this may not apply to low concentrations.

Other useful thermodynamic data may be derived from these values. Scruggs, et al. [3] measured the solubility of cytosine at 277.8 K and at 309.8 K. Assuming linearity between these temperatures, we calculate 76 mmol·kg^{-1} as the solubility at 298.15 K; the estimated uncertainty is 5 percent. Then

$$\Delta G^\circ (298.15\ \text{K}) = -RT\ \ell n\ m = (6.4 + 0.3)\ \text{kJ}\cdot\text{mol}^{-1}$$

and

$$\Delta S^\circ (298.15\ \text{K}) = \frac{\Delta H^\circ - \Delta G^\circ}{T} = (70 \pm 14)\ \text{J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}.$$

The uncertainty for the entropy value is the sum of the uncertainties on ΔH° and ΔG° divided by 298 K.

The density of cytosine was measured at approximately 295 K as (1.44 ± 0.08) g·mL^{-1}.

The author expresses her appreciation to R. L. Biltonen at the University of Virginia for providing heat capacity measurements of the cytosine samples and for his consultations during this work.

5. References

[1] Kilday, M. V., J. Res. Nat. Bur. Stand. (U.S.), **83,** No. 4, 347–370 (July–August 1978).

[2] Kilday, M. V., J. Res. Nat. Bur. Stand. (U.S.), **83,** No. 6, 529–537 (Nov.–Dec. 1978).

[3] Scruggs, R. L., Achter, E. K., and Ross, P. D., Biopolymers **11,** 1961–1972 (1972).

[4] Nat. Bur. Stand. (U.S.) Spec. Publ. 330, 1977 edition, pg. 11, 41 pages (1977).

[5] Izatt, R. M., Christensen, J. J., and Rytting, J. H., Chem. Rev. **71,** 439–481 (1971).

[6] Larsen, J. W., and Magid, L. J., J. Phys. Chem. **78,** 834–839 (1974).

[7] Falk, M., Can. J. Chem. **43,** 314–318 (1965).

[8] Committee on Specifications and Criteria for Biochemical Compounds, "Specifications and Criteria for Biochemical Compounds," Third Edition, National Academy of Sciences, Washington, D.C. (1972).

[9] Biltonen, R. L., Department of Pharmacology, University of Virginia School of Medicine, Charlottesville VA, private communication, July 1977.

[10] Prosen, E. J., and Kilday, M. V., J. Res. Nat. Bur. Stand. (U.S.) **77A,** No. 2 (Phys. and Chem.), 179–203 (1973).

[11] Prosen, E. J., and Kilday, M. V., J. Res. Nat. Bur. Stand. (U.S.) **77A,** No. 5 (Phys. and Chem.), 581–597 (1973).

[12] Commission on Atomic Weights, Pure and Applied Chem. **47,** 75–95 (1976).