Use of Tris(hydroxymethyl)aminomethane as a Test Substance in Reaction Calorimetry

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The heat of reaction of tris(hydroxymethyl)aminomethane (TRIS or THAM), with 0.1 M hydrochloric acid has been measured in two different laboratories with concordant results.

The reaction is exothermic ($-\varDelta H = 7104$ cal/mole at 25.00°C, 5 g THAM per l of calorimetric liquid), rapid, has a very low differential heat of dilution and seems well suited as a "test reaction" in reaction calorimetry of fast exothermic reactions.

A ll modern calorimetric methods are characterized by being comparative, *i.e.* the heat change occurring during the reaction under investigation is compared with that for a standard reaction.

In combustion calorimetry, energy equivalents are determined by the combustion of benzoic acid. The heat of combustion of benzoic acid itself has been determined in calorimeters calibrated electrically, and the absolute values thus obtained in various laboratories have shown excellent agreement.[1,2] The benzoic acid calibration procedure in combustion calorimetry thus closely resembles the actual measurement — a highly desirable feature, as in this way a variety of possible systematic errors could be expected to cancel out. A further check on the reliability of experimental results can be made by the combustion of secondary standards,[2] that is compounds containing the same elements as the compound under investigation, preferably in approximately the same proportions and of course with a well-authenticated heat of combustion.

In reaction calorimetry direct electrical calibration is usually most convenient as well as being the most accurate method. A fact frequently overlooked, however, is that electrical calibration as it is usually performed, represents a procedure which is not as direct a comparison as is the benzoic acid calibration of combustion calorimetry.

As reaction calorimetry embraces such a wide range of reactions and very diverse calorimetric equipment, often specially designed to study a particular reaction, it is obvious that a thorough methodological investigation of the absolute reliability of the calibration method is often not undertaken. Consequently, systematic errors can, and obviously frequently do, creep into even very careful work. Evaporation or condensation effects, heat leakage from the electrical heater, slow thermal equilibrium for certain parts of the calorimetric system, or methods used in correcting for heat exchange can all contribute to systematic errors.

The obvious method of discovering these types of error is to check the calorimeter by test reactions — preferably more than one — which closely resemble the actual reaction in the nature of the calorimetric liquid (or solid), the sign and magnitude of the heat effect, kinetics and the method of initiating the reaction.

It is clear that there is need for a large number of test reactions of well-authenticated heat changes to encompass the variety of reactions and equipment necessary in the broad field of reaction calorimetry. So far there has only been minor interest in these questions and the only reaction which has been the object of a great number of investigations is the solution of potassium chloride in water. This was suggested as a test reaction by Mischenko and Kaganovich [3] and has been frequently used in this connection. Its suitability, however, has for some time been under discussion [4-7] and Gunn [6] has recently shown that different pretreatment of the potassium chloride sample causes a variation in the heat of solution value amounting to as much as 0.2 %. Other factors decreasing the suitability of this test reaction are (*i*) it is endothermal whereas most measured reactions are exothermal, (*ii*) the magnitude of the heat effect is for many purposes too low and (*iii*) the temperature coefficient is high as is the differential heat of dilution. As a substitute for the KCl reaction Gunn suggests neutralization of constant boiling sulfuric acid (or dilution thereof) in an excess of sodium hydroxide solution. Among the desirable features of this reaction are: high and exothermic heat effect, small temperature coefficient, and low sensitivity to changes in the relative quantities of acid and base. Further, as both reactants are liquids there are no uncertainty factors due to variable lattice or surface energies. Gunn concludes that the main disadvantage with this reaction is that the sulfuric acid solution is hygroscopic. The use of concentrated sulfuric acid could present difficulties in cases where the ampoule technique involves use of parts made of organic material or metal, but perhaps its biggest disadvantage compared with KCl is the difficulty of preparing and handling constant boiling sulfuric acid.

Our feelings are that the reaction proposed by Gunn is superior to the heat of solution of KCl as a test reaction for precision calorimetry, but is less convenient and is not a substitute for it in all cases. We feel that it is important to stress the desirability of having available test reactions which make use of chemicals that are readily available and can be conveniently handled as this more than anything will guarantee extensive use.

It should be noted that a reaction can be useful as a test reaction even though its heat change is not known with great precision. Thus the heats of reactions of outstanding chemical or biochemical interest which may not be

absolutely stoichiometric or which may involve substances difficult to purify can be useful even when the precision is not even 1 %. In such cases suitability of a test reaction would be judged by factors other than the precision of the heat of reaction value.

In this paper we propose the use of the reaction of tris(hydroxymethyl)-aminomethane (THAM or TRIS) with an excess of 0.1 M hydrochloric acid as a convenient test reaction for rapid, moderately exothermic reactions.

THAM is a crystalline compound, m.p. 171.1°C,[8] and is a weak base, pK_b 5.92.[9] It has a low hygroscopicity, does not adsorb carbon dioxide, is readily soluble in water and has therefore found use [8] as a primary acidimetric standard. The compound is commercially available in pure form at a reasonable price.

In the present investigation heat of solution of THAM in 0.100 M HCl has been determined at 20, 25, and 30°C and at different ratios HCl : THAM. With the same calorimetric equipment the heat of solution of KCl in water was also determined.

EXPERIMENTAL

Materials. Measurements were made on three batches (A, B, C) of THAM. Sigma "pH 7 − 9" (titrations indicated a purity of 99.8 %) was used as a starting material in preparing Batch A. The substance was further purified by the following method (*cf.* Ref.[8]).

A hot concentrated aqueous solution was prepared by dissolving 400 g of the substance in about 300 ml of near-boiling water. To the hot solution was added 1200 ml pure methanol and the solution was allowed to cool to room temperature with constant stirring. This took about 2 h. It was then cooled to about 3°C again with stirring and after an hour the crystals were filtered off. Without drying the product was again recrystallized in the same way, using the same ratio of methanol to water. After filtration the crystals were stirred thoroughly with enough cold methanol to make a thin slurry. This washing was repeated.

The pure product was dried in air for 24 h and then screened. The sample passing the 100 mesh screen was set aside for incorporation in another batch for purification. The sample remaining on the 50 mesh screen was gently crushed and rescreened and the combined sample passing the 50 mesh and retained on the 100 mesh screen was mixed thoroughly, dried at 80°C for 24 h and finally in vacuum (1 mm) for 24 h. (The substance can be kept in bottles with plastic caps, but for precise work it is recommended that before use the sample is dried overnight in vacuum.)

Batch B (Eastman, 99.94 % purity) was not further purified before screening, drying and vacuum treatment. Batch C (BDH, unspecified purity) was crystallized twice (C_1) and six times (C_2) from aqueous methanol. Both fractions were dried at 70 − 80° for 12 h followed by vacuum treatment for 24 h of the fraction between 40 and 100 mesh screen.

Potassium chloride (Merck, Darmstadt, *p.a.*) was screened, and the fraction between 50 and 100 mesh dried for 20 h at 115°C.

Calorimetric procedure. Experimental work was carried out both in Lund and in London. The calorimetric equipment was almost identical in both cases and the "isothermal jacket" type calorimeter used has already been described in detail.[4,10] The following description applies to the Lund equipment and experiments but with minor changes of detail is applicable to the London work.

The thermistor resistance was calibrated between 20 and 30°C by comparison with Beckman and Anschütz thermometers; estimated accuracy being ± 0.01°C near 25°C and ± 0.02°C in the other range. The calorimeter was charged with 105 ml of 0.1004 M hydrochloric acid and the amine was contained in a 1 ml glass ampoule. The ampoule hole was sealed by a small silicone rubber stopper and finally covered by a thin layer of "microwax". The heat equivalent, ε, of the system was determined by passing a measured current for a given length of time (180 to 300 sec) through the heating coil. Calibrations were performed on the system after the reaction had taken place. For each series of experiment

five or more electrical calibrations were performed. In calculating the ΔH-values mean ε-values, corrected to account for small variations in heat capacity and thermistor resistance range, were used.

Units of measurement. The results of the calorimetric experiments are expressed in terms of the "defined" calorie 4.1840 abs.joules. Molecular weights were computed from the 1961 Table of Atomic Weights and sample weights are corrected to true mass.

RESULTS AND DISCUSSION

Heat of solution of KCl. From 6 determinations of the heat of solution of KCl in water we arrived at $\Delta H = 4190 \pm 2$ cal/mole for the isothermal process at 25°C and for the dilution KCl·200 H_2O. This value is 9 cal/mole higher than

Table 1. Heat of solution of THAM in 105 ml of 0.1004 M HCl. Lund results.

Temp. °C	Batch	mg of THAM	$10^4 \times$ log R_i/R_f	ε'	$-\Delta H$, cal/mole
20.00	A	481.09	31.992	9070	7299
		479.38	31.852	9070	7298
		548.32	36.433	9073	7306
		563.00	37.452	9074	7312
		466.01	31.026	9069	7314
					7306 $\pm$ 5
25.00	A	515.65	32.441	9330	7110
		671.49	42.201	9334	7099
		683.35	42.969	9333	7103
		577.08	36.271	9330	7099
		544.66	34.238	9330	7102
		598.81	37.661	9333	7107
		523.03	32.900	9333	7113
		392.54	24.687	9327	7107
					7105 $\pm$ 4
25.00	A	165.92	10.441	9314	7101
		235.89	14.811	9319	7089
		174.79	10.994	9313	7095
		256.08	16.078	9323	7091
		190.34	11.994	9320	7114
					7099 $\pm$ 6
25.00	B	588.83	37.067	9327	7113
		597.07	37.602	9326	7115
		599.16	37.675	9326	7104
		566.81	35.618	9326	7099
		583.67	36.708	9327	7105
					7106 $\pm$ 5
30.00	A	531.56	31.552	9599	6904
		532.58	31.649	9599	6911
		544.37	32.302	9600	6901
		554.84	32.940	9600	6904
		536.47	31.839	9599	6901
					6904 $\pm$ 3

Table 2. Heat of solution of THAM in 99.9 ml of 0.1002 M HCl. London results.

Temp. °C	Batch	mg of THAM	$10^4 \times \log R_i/R_f$	ε'	$-\Delta H$, cal/mole
25.00	A	627.68	48.889	7531	7101
		617.90	48.175	7530	7107
		643.37	50.067	7532	7095
		560.84	43.662	7527	7096
		479.74	37.441	7521	7105
		591.35	46.041	7529	7096
					7100 ± 4
25.00	C_1	498.42	38.912	7520	7105
		488.77	38.140	7519	7102
		516.19	40.359	7521	7118
		466.56	36.433	7518	7108
		542.00	42.277	7525	7105
		515.73	40.221	7523	7102
		520.01	40.514	7524	7097
					7105 ± 4
25.00	C_2	524.77	40.936	7524	7105
		519.92	40.564	7524	7106
		511.42	39.959	7523	7115
		538.07	41.943	7525	7101
		534.04	41.675	7525	7108
					7106 ± 4

that previously reported [4] from this laboratory. The present value, however, has within uncertainty limits been obtained earlier (I.W., unpublished results) for the same sample of KCl but using the calorimeter of Ref.[4] (Type D). The value is in fair agreement with the recommended "best" values of 4194 cal/mole [3] and 4195 cal/mole,[11] as well as with the recent value arrived at by Somsen, Coops and Tolk,[7] 4185 ± 1 cal/mole. Gunn's recent [6] value 4201 ± 1 cal/mole for a KCl-sample treated similarly to that reported here is outside twice the uncertainty limit. As the histories of the KCl samples have such a profound influence on the ΔH values obtained, it seems that a deviation of up to 0.3 % from the above "best" values is not necessarily an expression of a systematic error connected with the calorimetric procedure.

Heat of solution of THAM in 0.1 M HCl. Table 1 summarizes the experimental results of the heat of solution of THAM in 0.100 M HCl obtained at the Thermochemistry Laboratory, Lund, and Table 2 those obtained at the Battersea College of Technology, London. The following symbols have been used.

$\log R_i/R_f$ is the expression proportional to the corrected temperature change.

ε' is the individual heat capacity expressed in calories per unit of $\log R_i/R_f$.

ΔH is the enthalpy change corrected to the isothermal process at 20.00, 25.00 or 30.00°C, respectively.

The uncertainty interval given for ΔH is the standard deviation of the mean including estimated systematic errors.

From the results at 20, 25, and 30°C the temperature coefficient for the reaction is calculated to be 40 cal/deg.mole. It is evident that there is no correlation between amount of THAM and ΔH-value for experiments with an ampoule content in the range 400 to 700 mg. In the experiments (Batch A) where the ampoule content is *ca.* 200 mg the mean ΔH-value is slightly lower but is doubtful if the difference is significant. It can therefore be concluded that within the concentration range investigated the differential heat of dilution is very low.

There is excellent agreement between measurements on samples of different origin as well as between the Lund and London measurements on identical samples. If the series of measurements on low-weight samples (Batch A, *ca.* 200 mg) are excluded, no preference can be made for any one series and the mean value is 7104 cal/mole.

In a comparison between KCl and THAM as test substances the latter seems to be superior on the following grounds:

THAM has been found to meet the desirable criteria of an analytical standard. As indicated by careful acidimetric titration [8,9] THAM can easily be purified to $\geq$ 99.95 % by a few crystallisations. The experimental results strongly suggest that THAM, purified to meet the specifications of an acidimetric standard, has well defined thermochemical properties in contrast to what seems to be the case for KCl. Further, the THAM-reaction is exothermic as are most reactions investigated by reaction calorimetry and has a very low differential heat of dilution.

The temperature coefficient for the two reactions are of the same magnitude (0.6 % per degree for THAM and 0.9 % per degre for KCl). The heat effect per mole is nearly twice as high for THAM, whereas the heat effect per volume of test substance is much the same.

In a comparison between the THAM-reaction and the sulfuric acid neutralisation we note that the latter reaction has a very high enthalpy change and a more favourable temperature coefficient (0.05 % per degree), but feel that in most cases these properties are more than outweighed by the greater convenience of THAM.

It is hoped that more systematic work on these reactions will be undertaken at different laboratories and in this connection we must emphasize that reactions discussed in this paper will only cover the small part of reaction calorimetry represented by fast reactions in aqueous or aqueous-like calorimetric liquids.

An obvious need for further work is in the study of slow processes where no test reaction seems ever to have been proposed.

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