

## Solution microcalorimetry

Dan Hallén and Ingemar Wadsö

Division of Thermochemistry, Chemical Center, University of Lund,  
P.O. Box 124, S-223 61 Lund, Sweden.

### ABSTRACT

Calorimetric techniques are important in studies of interactions between solutes and solvents. Our laboratory has for many years been involved in the measurement of enthalpies of solution in water, at different temperatures, of simple organic compounds. Results of such work are of a particular interest in discussions of thermodynamic properties of biochemical systems. During recent years the work has concentrated on studies of the hydrophobic hydration and microcalorimeters for dissolution of slightly soluble gases, liquids and solids have been developed. The different solution microcalorimeters have now been incorporated into a modular system where the different vessels all are modifications of a microtitration vessel which was reported earlier. Much of this development work has been based on joint projects with S.J. Gill, University of Colorado, Boulder, Colorado.

### INTRODUCTION

Different types of calorimetric techniques are important in studies of interactions between solutes and solvents. The enthalpy values for the dissolution of pure solutes to form infinitely dilute solutions,  $\Delta_{\text{sol}}H^{\infty}$ , are particularly useful as they refer to states where there are no interactions between the solute molecules in the solution. When microcalorimetric techniques are used, one can often assume that the directly obtained  $\Delta_{\text{sol}}H$  value is identical to  $\Delta_{\text{sol}}H^{\infty}$ . In other cases  $\Delta_{\text{sol}}H^{\infty}$  values are derived from extrapolation of  $\Delta_{\text{sol}}H$  values for different solute concentrations or by a combination with enthalpy of dilution measurements.

For solid and liquid solutes  $\Delta_{\text{sol}}H$  values contain contributions from the intermolecular forces between the molecules in the pure material. For the characterization of solute-solvent interactions it is therefore important to obtain the enthalpy values for the transfer of the solute molecules from the gaseous state to infinitely dilute solution, often called the enthalpy of solvation  $\Delta_{\text{solv}}H^{\infty}$ . These values are derived from

$$\Delta_{\text{solv}}H^{\infty} = \Delta_{\text{sol}}H^{\infty} - \Delta_{\text{vap}}H^{\ominus} \quad (1)$$

where  $\Delta_{\text{vap}}H^{\ominus}$  is the enthalpy of vaporization to form the ideal gas. Enthalpies of vaporization are, preferably, obtained from calorimetric measurements or they can be calculated from accurate values for the temperature variation of the vapor pressure.

Enthalpy determinations at constant pressure but at different temperatures will lead to values for corresponding changes in heat capacities.

$$(d\Delta H)/dT = \Delta C_p \quad (2)$$

$\Delta C_p$  values have proved to be particularly interesting in studies of aqueous solutions, but as for the corresponding changes of the enthalpy values,  $\Delta_{\text{sol}}C_p^{\infty}$  contains contributions from the properties of the solutes in their liquid or solid form. Values for  $\Delta_{\text{vap}}C_p^{\ominus}$  are not very available and  $\Delta_{\text{solv}}C_p^{\infty}$  values have therefore not been used very much in discussions of solute - solvent interactions. Another heat capacity property which has been proved to be very

useful in these connections is the partial molar heat capacity for the solute at infinitely dilute solution,  $C_{p,2}^{\infty}$ ,

$$C_{p,2}^{\infty} = \Delta_{\text{sol}} C_p^{\infty} + C_p^* \quad (3)$$

where  $C_p^*$  is the heat capacity for the pure solute (in the same state as it was used in the solution measurements).  $C_{p,2}^{\infty}$  values also can be obtained from direct calorimetric heat capacity measurements on dilute solutions.

The enthalpy and heat capacity changes for the transfer of a solute molecule between two different solvents is also very useful. Such values are easily derived as the difference between  $\Delta_{\text{sol}} H^{\infty}$  values, or  $\Delta_{\text{sol}} C_p^{\infty}$  values, for the two solvents.

In order to obtain useful entropy values for solution processes one normally depends on the corresponding Gibb's energy changes.

$$\Delta_{\text{sol}} S^{\ominus} = (\Delta_{\text{sol}} G^{\ominus} - \Delta_{\text{sol}} H^{\infty})/T \quad (4)$$

For slightly soluble compounds  $\Delta_{\text{sol}} G$ -values usually are obtained from solubility measurements. Often corresponding  $\Delta_{\text{sol}} H$  and  $\Delta_{\text{sol}} C_p$  are also derived from the solubility measurements performed at different temperatures. However, such 'van't Hoff  $\Delta H$ -values' and even more the  $\Delta C_p$  values will be uncertain unless the solubility measurements are carried out with utmost care and over a wide temperature range, cf for instance (ref. 1). In a calorimetric solution measurement it is normally the pure solute which is transferred to the solution but in solubility measurements the solute will be in equilibrium with the solution. In cases where the solute material absorbs significant amounts of solvent molecules the two techniques will thus refer to processes which are not strictly comparable. In some cases corresponding thermodynamic values can be very different (cf below microcalorimetric techniques for solid solutes).

For easily soluble compounds  $\Delta_{\text{sol}} G^{\ominus}$  values are much more difficult to determine. Such experiments will require, for instance, very careful determinations of vapor pressures or freezing points. For such compounds good solution entropy values therefore are rare.

## SOLUTION MICROCALORIMETRY

Solutions of simple organic compounds in water may to some extent be used as models in basic studies of solute-solvent interactions in biochemical systems. Our laboratory has for many years been involved in calorimetric work in this field. In particular we have been interested in studies of hydrophobic hydration i.e. the interaction between hydrophobic compounds or groups and water. This is believed to play a major role in the formation of structures of proteins and lipids (membranes) as well as in many processes where these compounds are involved. During the earlier phases of our work in this field the solution calorimetric measurements was conducted by use of precise 'macro' calorimeters. However, in studies of hydrophobic hydration it became desirable to study compounds which are very slightly soluble. This led us to develop flow micro solution vessels for measurements of liquids and gaseous compounds (ref. 2,3). These projects were carried out in collaboration with S.J. Gill, University of Colorado, Boulder, during his sabbatical leaves in Lund. Gill and coworkers also have described a flow solution vessel for slightly soluble solids (ref. 4) and a modified version of the Lund gas solution instrument (ref. 5). In our laboratory the vessel for liquid solutes was further developed as well as the methods for its operation (ref. 6) and a vessel for solid solutes was designed (ref. 7). For all these vessels, thermopile heat conduction calorimeters were used.

We have now incorporated our solution microcalorimeters into a modular system, which thus includes vessels for liquid, gaseous and solid solutes. The system will be briefly described below. The vessels are all modifications of a 'titration and perfusion vessel' (ref. 8), designed for use with our 4-channel microcalorimeter system (ref. 9) or its commercial version (Thermal Activity Monitor, Thermometric (LKB), Järfälla, Sweden). In this instrument up to 4 twin heat conduction calorimeters are immersed in a thermostated water bath

with a very high stability, about  $\pm 1 \cdot 10^{-4}$  K per week, which will make it possible to carry out the solution experiments over extended periods of time. The overall design of the modular vessel is indicated in fig. 1. The solution processes take place in the 3 ml steel container g (the 'solution chamber' shown in some detail in fig. 2, 5 and 7 for the three solution vessels).

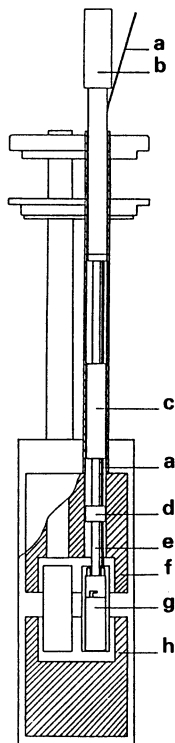


Fig. 1. Schematic picture of the modular vessel inserted into the twin calorimeter. Reference ampoule is not shown. a, titration tube (not used for solution of solids), b, stirring motor (not used for solution of liquids); c, d, brass bolts serving as heat exchangers; e, stainless steel tube; f, upper heat sink; g, 3 ml steel vessel; h, lower heat sink.

### LIQUID SOLUTES

The measurement principle for liquid solutes is the same as in the earlier designs. A flow of gas free solvent (water) is sucked through a spiralized tube positioned in the solution chamber. An accurate known volume of solute, for slightly soluble liquids typically  $3 \mu\text{l}$ , is injected into the spiralized tube where it is retained and subsequently dissolved by the solvent flow.

In the first design (ref. 2) the tube material was Teflon. The spiral was composed by an inner tube (i. d. 0.8 mm, o. d. 1.0 mm) surrounded by an outer tube (i. d. 1.3 mm, o. d. 1.9 mm). The solvent flow entered the inner tube and passed then through the concentrical space between the two tubes. The solute was injected into the inner tube. A hydrophobic solute will form a thin film on the inside of the inner Teflon tube. This solute film exposes a large surface area towards the solvent which is essential for an efficient solution process. However, it was shown that slightly soluble esters, although 'hydrophobic' adhered poorly to the Teflon. Furthermore, halogenated hydrocarbons and organic sulfides appeared to partially remain absorbed in the Teflon material over very long periods of continued solvent flow. In the second design (ref. 6) the outer Teflon tube was replaced by a stainless steel tube (several other design changes were also made). This vessel was efficient for hydrocarbon solutes as well as for compounds like slightly soluble esters and alcohols. However, the problem with absorption of some compounds in the Teflon material remained to some extent and the vessel was quite difficult to build.

With the same calorimeter vessel it was found possible to measure the solution of small amounts of water in hydrocarbons and other hydrophobic compounds (ref. 10). For the determination of  $\Delta_{\text{sol}} H^{\circ}$  of water in a hydrocarbon a small droplet of the solvent, with known water content, was injected into the calorimeter vessel containing an equilibrium mixture of the hydrocarbon and water. The enthalpy change that was measured referred to the transfer of water into the hydrocarbon phase until this became saturated with water. In these experiments there was no flow of the hydrocarbon/water mixture.

The new vessel (ref. 11) makes use of spirals which easily can be replaced and one can thus choose different tube materials for different solutes. So far we have tested spirals made from Teflon, pure gold and siliconized 18 carat gold. The latter have successfully been used for halogenated hydrocarbons and organic sulfur compounds (ref. 11,12).

A simplified picture of the solution chamber is shown in fig. 2. A triple coil, j, made from thin-walled Teflon tube (i.d. 1.0 mm, o. d. 1.3 mm) wound around a stainless steel rod, k, fits into the 'dissolution chamber', g. The gold spirals are constructed slightly different. The solvent flow enters the coil from the steel tube, e, via the lower end of the titration tube, a. The solute is injected through the hypodermic needle, i. d. 0.15 mm, i, inserted into the titration tube, a. The hypodermic needle is directly attached to a gas tight Hamilton syringe. The syringe drive is computer operated making the instrument fully automatic. In order to reduce leakage of the solute through the Teflon tip of the plunger in the Hamilton syringe, the syringe was modified to accommodate a mercury seal (ref. 6). The solution formed leaves the lower end of the spiral, passes between the outside of the spiral and the wall of the solution chamber and leaves the calorimetric zone through the steel tube l. (This tube corresponds to the stirrer shafts in the designs described below.) Tubes l and e thus form a counter current heat exchanger and the heat exchange is further improved by the brass bolts c and d (Fig. 1). The solvent flow rate is typically 30 ml/h.

The measurement procedure and methods for correction of small leakage and diffusion effects are the same as those described in reference 6. The instrument is normally calibrated by solution of propanol.

Solution of easily soluble compounds can be performed stepwise as shown for benzene in fig. 3 or as steady state measurements where a slow flow of solute (a few nl/s) is added over a rather long period of time, e.g. 1 h. The steady state procedure has the advantage that the final concentration of solute will be well defined. However, with either method it is usually possible to make measurements with an estimated maximum concentration of solute which is low enough to consider the solution as infinitely dilute.

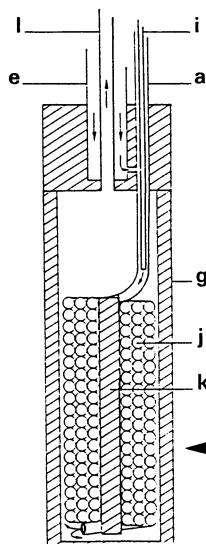


Fig. 2. Simplified picture of solution chamber for liquid solutes. a, titration tube; e, stainless steel tube; g, steel vessel; i, hypodermic needle; j, triple coil (Teflon); k, steel rod, l, stainless steel tube.

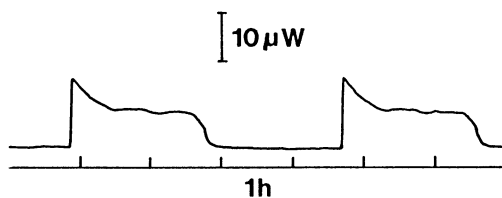


Fig. 3. Typical record from a series of solution experiments with benzene in water at 25 °C (18 carat gold coil). Water flow rate was 30 ml/h and the injected volume of benzene was 3 μl.

## GASEOUS SOLUTES

In our first report of a solution microcalorimeter for slightly soluble gases we described two different vessels (ref. 3). In both cases gas free solvent (water) was sucked through a dissolution chamber into which a controlled amount of gas could be injected. One of the vessels was given a form which allowed steady state measurements i.e. a slow flow of gas was dissolved at a constant rate by the flowing solvent. This method has been further developed by Gill and coworkers in Boulder (ref. 5) and was used in several studies on hydrocarbons and rare gases reported by this group (ref. 13-19). In the second method, the vessel was designed for complete solution of an injected gas bubble (the 'batch' method). In Lund we preferred this method which was used in our work on hydrocarbons and the rare gases (ref. 20). The  $\Delta_{\text{solv}} H^{\circ}$  values for oxygen determined with this instrument, which was calibrated

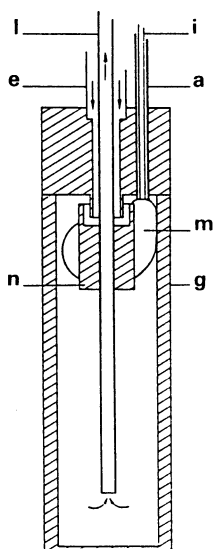
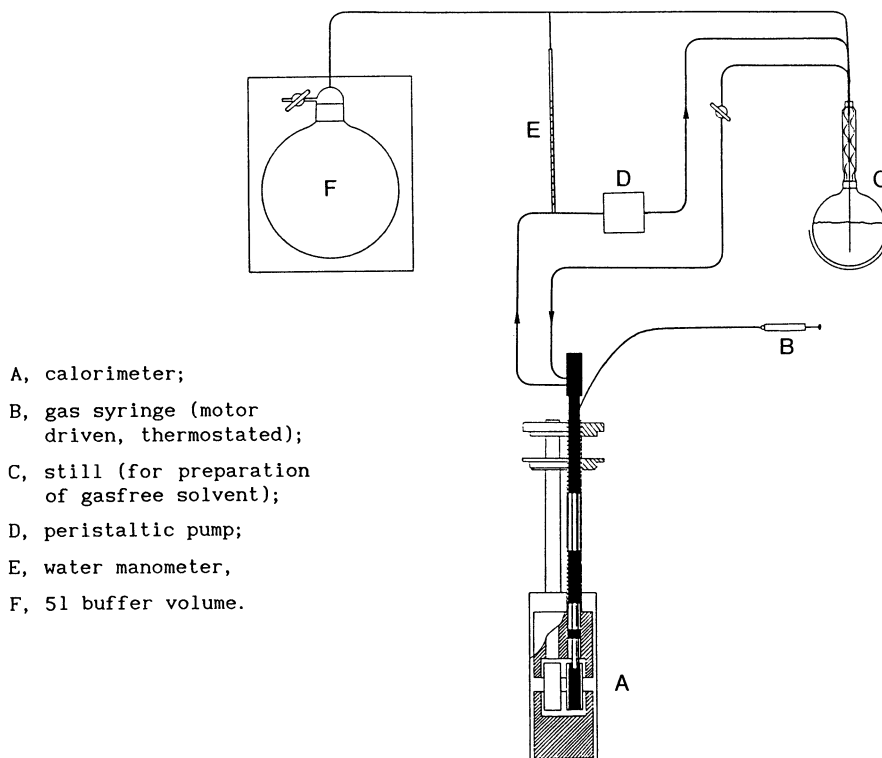


Fig. 4. Simplified picture of solution chamber for gaseous solutes. a, titration tube; e, stainless steel tube; g, steel vessel; i, stainless steel tube connected with gas syringe; l, stainless tube acting as stirrer shaft; m, gas bubble; n, water seal.

electrically, are in excellent agreement with the values obtained from solubility measurements by Benson et al (ref 21) The batch method has been further developed and the new instrument (ref. 22) forms part of the modular system described here. The main difference relative to the earlier method is that the gas - solution system is stirred which facilitates the solution process. Figure 4 shows a simplified picture of the solution chamber for this instrument. The gasfree solvent (water) is sucked from tube e through the water seal n, over and around the gas bubble m. The solution formed flows downwards until it leaves the system through the steel tube shaft l. The overall calorimetric setup is shown schematically in fig. 5.

The experimental arrangement around the calorimeter is the same as that used earlier for the batch experiments, fig. 5. Calculation of the result follows the procedure described earlier (ref. 3). With the new method the correction term due to the pressure head over the gas bubble is insignificant. The instrument is calibrated chemically by use of dissolution of oxygen in water.



- A, calorimeter;
- B, gas syringe (motor driven, thermostated);
- C, still (for preparation of gasfree solvent);
- D, peristaltic pump;
- E, water manometer,
- F, 5l buffer volume.

Fig. 5. Experimental arrangement in solution experiments with gases.

Figure 6 shows the calorimetric record from a series of calibration experiments where oxygen was dissolved in water at 25 °C. The precision obtained is typically 0.2 % corresponding to  $\pm 0.02 \text{ kJ}\cdot\text{mol}^{-1}$ . With this new instrument we have recently measured  $\text{CF}_4$  and  $\text{SF}_6$  over the temperature range 15 - 35 °C (ref. 22).

Krestov *et al.* (ref. 23), Ivanovo, USSR have recently developed an interesting isoperibol solution calorimeter by which solubilities of the gaseous solutes also can be determined. The instrument can be used for gaseous, liquid and solid solutes, but the amount of material required is quite large and it may not be called a microcalorimeter.

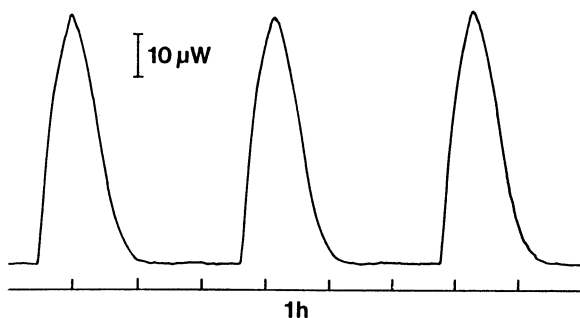


Fig. 6. Typical record from a series of solution experiments with oxygen in water at 35 °C (calibration experiments). Water flow rate was 25 ml/h and the injected volume of water vapour saturated oxygen was 0.25 ml.

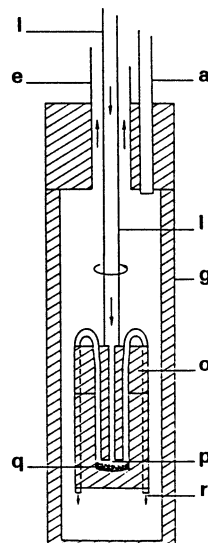


Fig. 7. Simplified picture of solution chamber for slightly soluble solids. a, titration tube (used for initial filling the ampoule with solvent); e, stainless steel tube; g, steel vessel; l, stirrer shaft acting as inlet tube for solvent; o, steel cylinder; p, cavity filled with saturated solution; q, sample; t, outlet from steel cylinder.

### SOLID SOLUTES

The instrument for use with slightly soluble solids was the first component of the present modular system of flow solution microcalorimeters (ref. 7). In this instrument the sample is contained in a small cavity where it is covered with saturated solution. After equilibration of the calorimeter a flow of pure solvent is brought in contact with the sample.

Figure 7 shows a schematic picture of the solution chamber. A small inner vessel, o, is positioned in the solution chamber, g. This is connected to the stirring shaft, l, which consists of a 4 mm (o. d.) stainless steel tube. A narrow channel (d. = 1 mm) in the inner vessel, o, leads to the small cavity, p, at the bottom of the vessel o. From the cavity two channels will lead to the upper part of vessel, o, where they are connected to Teflon tubes which exit at the lower end, g, of the vessel. The sample, q, is pressed with a steel pestle against the bottom of the cavity until it forms a thin attached layer. At the start of an experiment the cavity and the channels of the vessel are first filled with saturated solution. The stirrer shaft and the solution chamber, g, are then filled with solvent and the vessel is introduced into the calorimeter and is left for equilibration. The stirrer and the peristaltic pump which sucks the solvent through the system are then started and the solution process will begin.

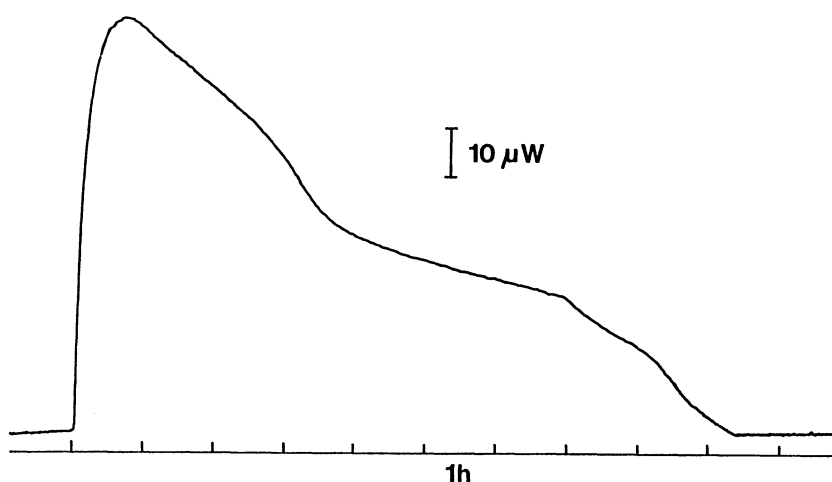


Fig. 8. Typical record from solution of 4.16 mg adenine in water at 25 °C. Water flow rate was 20 ml/h.

Figure 8 shows a typical result from a solution experiment with adenine at 25 °C. Measurements were performed with 1 - 10 mg of substance. The derived  $\Delta_{\text{sol}}H^{\circ}$  values was within uncertainty limits identical to that obtained by Kilday at National Bureau of Standards, Washington DC (ref. 24), who used about 200 mg samples in her experiments with a 'classical' solution calorimeter (300 ml vessel). Uncertainty limits were about the same in the two studies. The present method, where the solid material is in equilibrium with a saturated solution of the solvent, should be used with some care. Naturally it is not suitable for easily soluble compounds. For slightly soluble compounds two corrections should be considered. If the measurement temperature is different from the ambient temperature (at which the instrument was charged) a small amount of substance can be expected to dissolve or crystallize during the equilibration period. Further - for some compounds there might be a significant effect from dilution of the saturated solution at the start of the measurement. The sum of these two effects are expected to show up as an intercept if the non-corrected  $\Delta_{\text{sol}}H$ -values are plotted versus the amounts of substance dissolved (ref. 7). For some compounds, e.g. cyclodextrins (ref. 25), the process measured is significantly different from that where a dry substance is brought into solution e.g. by breaking an ampoule or opening a valve. Murphy and Gill (ref. 26) has very recently described a novel microcalorimetric solution technique where the solid substance also is in equilibrium with the saturated aqueous solution. In their method very small quantities of water (10  $\mu\text{l}$ ) are injected stepwise into a suspension of finally divided crystals using Gill's micro titration calorimeter. By knowing the solubility of the substance the value for  $\Delta_{\text{sol}}H$  can be calculated. (It is in principle possible to calculate both the solubility and the molar enthalpy change from measurements of solution enthalpies for a given amount of solvent at different temperatures using the van't Hoff relationship). The method described by Murphy and Gill should work with any titration microcalorimeter.

At present, we have at our laboratory another microcalorimetric method under development, where  $\Delta_{\text{sol}}H$  for both easily and for slightly soluble solids can be measured (ref. 27).

### SOME APPLICATIONS

Microsolution calorimetric work in Lund and in Boulder has so far concentrated on studies of aqueous solutions of slightly soluble compounds, in particular on hydrocarbon liquids and gases and the rare gases. These compounds have been of a particular interest to study in connection with the thermodynamic characterization of the hydrophobic effect.

In most cases the measurements were performed at 25 °C or in the temperature range of 15 °C to 35 or 40 °C. For methane (ref. 16), ethane and propane (ref. 17), butanes (ref. 18) and fluoro containing gases (ref. 19) Gill and coworkers performed measurements in a wider temperature range (0 or 5 °C to 45 or 50 °C) and values for the weak temperature dependence of  $\Delta_{\text{sol}}C_{\text{p}}^{\infty}$  could therefore be derived for these solutes. In most, but not in all cases, a satisfactory agreement was found for  $\Delta_{\text{sol}}H^{\infty}$  between the two calorimetric determinations and the van't Hoff values (ref. 28,29). For  $\Delta_{\text{sol}}C_{\text{p}}^{\infty}$  the uncertainties, as evaluated by Gill et al. (ref. 15), were in many cases large for the values derived from equilibrium measurements.

The first vessel for slightly soluble compounds was used for determinations of  $\Delta_{\text{sol}}H^{\infty}$  and  $\Delta_{\text{sol}}C_{\text{p}}^{\infty}$  of several hydrocarbons at 25 °C, table 1. The  $\Delta_{\text{sol}}H^{\infty}$  value for benzene has since been redetermined several times with the newer instruments and in all cases a slightly higher value has been found,  $\Delta_{\text{sol}}H^{\infty} = 2.20 \pm 0.03 \text{ kJ}\cdot\text{mol}^{-1}$ , as compared to the original value,  $\Delta_{\text{sol}}H^{\infty} = 2.08 \pm 0.04 \text{ kJ}\cdot\text{mol}^{-1}$ . The causes for possible systematic errors was discussed in ref. 6. The uncertainties for many of the  $\Delta C_{\text{p}}$  values in table 1 are quite large, in particular for pentane and hexane. It is judged that using our present technique, these uncertainty values could be reduced significantly. The values given in table 1, and derived quantities, are often employed in current discussions about the hydrophobic effect (ref. 30-32). It would be very useful if the determinations were extended to significantly higher temperatures,  $T \geq 100 \text{ }^{\circ}\text{C}$ .

Table 1. Enthalpies solution and partial molar heat capacities at infinitely dilute aqueous solution of some hydrocarbons at 298.15 K. The values have been taken from reference 33 except where indicated.

Compound	$\Delta_{\text{sol}}H^{\infty}$	$C_{\text{p},2}^{\infty}$
	$\text{kJ}\cdot\text{mol}^{-1}$	$\text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$
Benzene	$2.20 \pm 0.03^{\text{a}}$	$361 \pm 5$
D <sub>6</sub> -Benzene	$1.99 \pm 0.08$	$356 \pm 9$
Toluene	$1.73 \pm 0.04$	$430 \pm 13$
Ethylbenzene	$2.02 \pm 0.04$	$504 \pm 13$
Propylbenzene	$2.3 \pm 0.1$	$606 \pm 25$
Cyclohexane	$-0.1 \pm 0.1$	$516 \pm 30$
Pentane	$-2.0 \pm 0.2$	$572 \pm 70$
Hexane	$0.0 \pm 0.2$	$635 \pm 45$

<sup>a</sup>Reference 6 and 11.

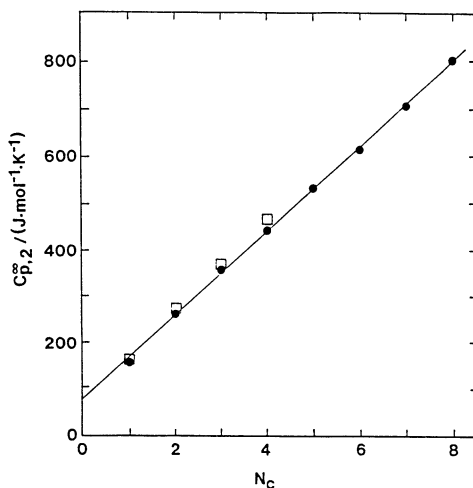


Fig. 9. Partial molar heat capacities  $C_{\text{p},2}^{\infty}$ , for n-alkanols at infinite dilution in  $\text{H}_2\text{O}$  (●) and in  $\text{D}_2\text{O}$  (□) at 25 °C plotted against number of carbon atoms,  $N_c$ .

Solution of alkan-1-ols in  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$  were studied extensively by use of the second instrument for studies of liquids (ref. 34). Figure 9 shows  $C_{\text{p},2}^{\infty}$ (water) values for methanol to octanol plotted versus the number of carbon atoms in the molecules.

A relationship is obtained which shows the notable additivity for group values of  $C_{\text{p},2}^{\infty}$  for nonionic compounds in water. The  $\text{CH}_2$ -increment is  $90 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$  which is identical to the mean value derived from several groups of compounds reported earlier (ref. 35). Such group values have been used to form an empirical additivity schemes for the calculation of  $C_{\text{p},2}^{\infty}$  (ref.36, 37).



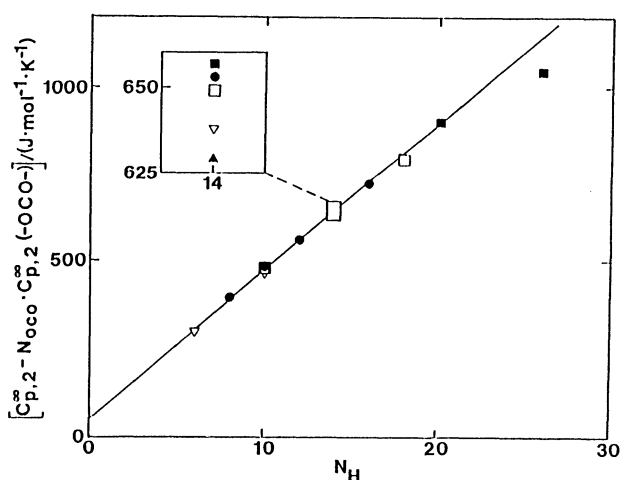


Fig. 10. Partial molar heat capacities,  $C_{p,2}^{\infty}$ , for some esters at infinite dilution in  $H_2O$  at  $25\text{ }^{\circ}C$ .  $C_{p,2}^{\infty}$ -values from which contributions from the ester group(s) have been subtracted are plotted against number of hydrogen atoms, cf the text. Methylesters ( $\nabla$ ), ethylesters ( $\bullet$ ), branched ethylesters ( $\blacktriangle$ ), diesters ( $\square$ ) and triesters ( $\blacksquare$ ).

Comparison between calculated and experimental  $C_{p,2}^{\infty}$ -values can lead to interesting conclusions. In fig. 10 a comparison is made between  $C_{p,2}^{\infty}$ -values for some mono-, di- and triesters (ref. 38). Values for the hydrocarbon parts of the molecules is obtained by subtracting the group value for the O=C-O-group(s) ( $-7\text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ ) from the experimental values for the esters. It is seen that the value for tributyrin deviates markedly from the straight line obtained. The result was interpreted as a sign of hydrophobic interaction between the alkyl chains of this triester.

At present, our measurement program involving slightly soluble liquids concentrates on halogenated (Cl, Br, I) hydrocarbons and alkyl sulfides and disulfides (ref. 11, 12).

## CONCLUSIONS

The recent developments in solution microcalorimetry have made it possible to obtain precise data of the enthalpy and heat capacity changes of slightly soluble liquids and solids in water. Intensive discussions about the interpretations of the thermodynamic results are carried out in connection with work on the hydrophobic effect and the structure of proteins (ref. 30-32). Interesting features regarding solute - water interaction can be revealed by comparison between experimental and calculated  $C_{p,2}^{\infty}$  data.

## REFERENCES

1. S.F. Dec and S.J. Gill, *J. Chem Educ.*, **62**, 879, (1985).
2. S.J. Gill, N.F. Nichols and I. Wadsö, *J. Chem. Thermodynamics*, **77**, 175, (1975)
3. S.J. Gill and I. Wadsö, *J. Chem. Thermodynamics*, **14**, 905, (1982).
4. S.J. Gill and M.L. Seibold, *Rev. Sci. Instrum.*, **47**, 1399, (1976).
5. S.F. Dec and S.J. Gill, *Rev. Sci. Instrum.*, **55**, 765, (1984).
6. S.-O. Nilsson and I. Wadsö, *J. Chem. Thermodynamics*, **16**, 317, (1984).
7. S.-O. Nilsson and I. Wadsö, *J. Chem. Thermodynamics*, **18**, 1125, (1986).

8. M. Görman-Nordmark, J. Laynez, A. Schön, J. Suurkuusk and I. Wadsö, J. Biochem. Biophys. Methods, **10**, 187, (1984).
9. J. Suurkuusk and I. Wadsö, Chemica Scripta, **20**, 155, (1982).
10. S.-Ö. Nilsson, J. Chem. Thermodynamics, **18**, 877, (1986).
11. D. Hallén and I. Wadsö, To be reported.
12. D. Hallén, T. Kimura and I. Wadsö, To be reported.
13. S.F. Dec and S.J. Gill, J. Solution Chem., **14**, 417, (1985).
14. S.F. Dec and S.J. Gill, J. Solution Chem., **13**, 27, (1984).
15. S.F. Dec and S.J. Gill, J. Solution Chem., **14**, 827, (1985).
16. H. Naghibi, S.F. Dec and S.J. Gill, J. Phys. Chem., **90**, 4621, (1986).
17. H. Naghibi, S.F. Dec and S.J. Gill, J. Phys. Chem., **91**, 245, (1987).
18. H. Naghibi, D.W. Ownby and S.J. Gill, J. Chem. Eng. Data, **32**, 422, (1987).
19. H. Naghibi, D.W. Ownby and S.J. Gill, J. Solution Chem., **16**, 171, (1987).
20. G. Olofsson, A.A. Oshodj, E. Quarnström and I. Wadsö, J. Chem. Thermodynamics, **16**, 1041, (1984).
21. B.B. Benson and D.J. Krause, J. Chem. Phys., **64**, 689, (1976).
22. D. Hallén and I. Wadsö, To be reported.
23. V.N. Prorokov, M.E. Ebanoidze and G.A. Krestov, Zhur. Fiz. Khim., **60**, 2634, (1986).
24. M.V. Kilday, J. Res. Nat. Bur. Stand., **83**, 347, (1978).
25. L.-E. Briggner and I. Wadsö, To be reported.
26. K.P. Murphy and S.J. Gill, Thermochim. Acta, In press.
27. D. Hallén, E. Quarnström and I. Wadsö, To be reported.
28. T.R. Rettich, Y.P. Handa, R. Battino and E. Wilhelm, J. Phys. Chem., **85**, 3230, (1981).
29. E. Wilhelm, R. Battino and R.J. Wilcock, J. Chem. Rev., **77**, 219, (1977).
30. S.J. Gill, S.F. Dec, G. Olofsson and I. Wadsö, J. Phys. Chem., **89**, 3758, (1985).
31. P.L. Privalov and S.J. Gill, Adv. Protein Chem., In press.
32. R.L. Baldwin, Proc. Natl. Acad. Sci. USA, **83**, 8069, (1986).
33. S.J. Gill, N.F. Nichols and I. Wadsö, J. Chem. Thermodynamics, **8**, 445, (1976).
34. D. Hallén, S.-Ö. Nilsson, W. Rothschild and I. Wadsö, J. Chem. Thermodynamics, **18**, 429, (1986).
35. N. Nichols, R. Sköld, C. Spink, J. Suurkuusk and I. Wadsö, J. Chem. Thermodynamics, **8**, 1081, (1976).
36. S. Cabani, P. Gianni, V. Mollica and L. Lepori, J. Solution Chem., **10**, 563, (1981).
37. J.P. Guthrie, Can. J. Chem., **55**, 3700, (1977).
38. S.-Ö. Nilsson and I. Wadsö, J. Chem. Thermodynamics, **18**, 673, (1986).