

8 Thermochemistry

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1 Introduction

The previous Annual Report on Thermochemistry was made in 1987,¹ and a large number of publications have been considered in producing the present report. Since 1987 the number of research groups actively engaged in calorimetric measurements of enthalpies of reaction has diminished, but the decrease in the number of reported experimental results is barely perceptible. Computer control of apparatus has made the task of measurement simpler and more rapid but, more importantly, commercial suppliers of chemicals have in recent years greatly improved the purity of samples on offer, thus making the final stages of purification, especially important for combustion calorimetry, much less onerous. There has been an increase in the number of results from non-calorimetric techniques; especially from the application of mass-spectrometry, involving, for example, ion cyclotron resonance, tandem guided beam methods, and photo-ionization, which has provided a large contribution to our knowledge of the bond dissociation energies in molecules, radicals, cations, and anions. The main body of results in this area has been in the field of organometallic species and provide a great deal of information on the strengths of bonding in comparatively simple species and, when subjected to theoretical interpretation, improves our understanding of chemical binding. Organometallic species are of great importance in the field of catalysis, and thermochemical information can aid explanation of catalytic processes and will, perhaps, assist in catalyst design.

Although marginally outside the ambit of this report, progress has been made in recent times in the development of *ab initio* calculations: such calculations have improved greatly so that when carefully applied, particularly to minimize the effect of changes in electron correlation energies, they can predict enthalpies of simple reactions to probably within $\pm 4 \text{ kJ mol}^{-1}$. Although at present the number of such

¹ M. N. Jones and G. Pilcher, *Annu. Rep. Prog. Chem., Sect. C*, 1987, **84**, 65.

reliable results is small, it is certain that this number will grow and in future will become a significant source of thermochemical information.

The reporting of results in SI units is still not universal, but the proportion of those doing so has grown since the last report and they now seem to be in a majority. Experimental results quoted without a statement of the experimental uncertainty are without significance; it is surprising that some editors of journals permit this to happen. In some cases, from consideration of other work done using the same techniques, a reasonable value for the uncertainty can be assumed, but where this has not been possible the results have been excluded from this report.

2 Apparatus and Methods

The International Temperature Scale has been revised and a clear description of ITS90 has been given by McGlashan.² Several developments have been reported in microcalorimetry, especially for flow microcalorimeters operating over ranges of temperature and pressure. Randzio *et al.*³ describe a pressure scanning calorimeter operating between 303 to 503 K and 0.1 to 400 MPa which was tested by measuring the isobaric expansivity of hexane. Ernst *et al.*⁴ report a flow microcalorimeter for measuring the isobaric heat capacities of gases and gaseous mixtures between 300 and 450 K at pressures up to 100 MPa. Hallen and Wadsö⁵ have described a new microcalorimeter vessel for measuring the enthalpies of solution of slightly soluble gases, and report the enthalpies of solution of CF₄(g) and SF₆(g) in water; Hallen *et al.*⁶ describe a vessel for measuring enthalpies of solution of slightly soluble liquids. Rogers and Duffy⁷ report a calibration method for flow heat capacity calorimeters that is more precise by an order of magnitude than previous methods, and recommend C_p of NaCl(aq) (1–6 mol kg⁻¹) to 598 K and 20 MPa as a suitable reference standard. Of notable new microcalorimeters, that of Koval'chuk and Tsymarnaya⁸ has a sensitivity of 10⁻⁶ W and can measure thermal powers in the range 10⁻¹ to 10⁻⁴ W to a precision of $\pm 1\%$; Pivovarov and Starodubtsev⁹ have designed a Calvet-type microcalorimeter of reproducibility $\pm 0.5\%$ suitable for measuring the enthalpies of slow reactions, and tested by measuring the enthalpy of solution of GeO₂(cr) in HF(aq). A high temperature microcalorimeter for measurements up to 1400 K, with integration of the heat flux over the major fraction of the surface, has been described by Kleppa and Topor.¹⁰ Olofsson *et al.*¹¹ have designed a heat conduction calorimeter for the study of electrochemical processes and tested the apparatus by carrying out the electrolysis of H₂O and D₂O, thus demonstrating the accuracy to be better than $\pm 0.2\%$.

Knauth and Sabbah¹² have developed the Seteram CRMT calorimeter for

² M. L. McGlashan, *J. Chem. Thermodyn.*, 1990, **22**, 653.

³ S. L. Randzio, D. J. Eatough, E. A. Lewis, and L. D. Hansen, *J. Chem. Thermodyn.*, 1988, **20**, 937.

⁴ G. Ernst, G. Maurer, and E. Wiederuh, *J. Chem. Thermodyn.*, 1989, **21**, 53.

⁵ D. Hallen and I. Wadsö, *J. Chem. Thermodyn.*, 1989, **21**, 519.

⁶ D. Hallen, S. O. Nilsson, and I. Wadsö, *J. Chem. Thermodyn.*, 1989, **21**, 529.

⁷ P. S. Z. Rogers and C. J. Duffy, *J. Chem. Thermodyn.*, 1989, **21**, 595.

⁸ B. A. Koval'chuk and O. V. Tsymarnaya, *Russ. J. Phys. Chem.*, 1989, **63**, 1570.

⁹ M. M. Pivovarov and A. M. Starodubtsev, *Russ. J. Phys. Chem.*, 1987, **61**, 1789.

¹⁰ O. J. Kleppa and L. Topor, *Thermochim. Acta*, 1989, **139**, 291.

¹¹ G. Olofsson, I. Wadsö, and L. Eberson, *J. Chem. Thermodyn.*, 1991, **23**, 95.

¹² P. Knauth and R. Sabbah, *J. Chem. Thermodyn.*, 1989, **21**, 203, 779.

measuring the enthalpies of combustion of liquid samples of 5–10 mg within polythene containers and have measured a series of α, ω -diols; the results appear to be accurate to $\pm 0.3\%$. Lavut *et al.*¹³ claim to improve the precision of conventional bomb calorimetric measurements by using low inertia thermometry and have also devised a new method for controlling the electrical energy input for the ignition of samples.¹⁴ Li Shaofeng *et al.*¹⁵ have redetermined the specific energy of combustion of benzoic acid in an electrically calibrated bomb calorimeter, obtaining $-\Delta_c u/Jg^{-1} = 26433.4 \pm 4.0$ in exact agreement with the generally accepted value.

Enthalpies of vaporization and sublimation are of importance in determining enthalpies of formation in the gaseous state. Švab *et al.*¹⁶ have described a new calorimeter for measuring enthalpies of vaporization in the range 298–600 K at pressures up to 3 MPa, and the results with benzene were in good agreement with accepted values. Torres-Gomez *et al.*¹⁷ have used the isothermal operation of a DSC apparatus to obtain good results for the enthalpies of sublimation of test substances such as naphthalene and benzoic acid. Ribeiro da Silva and Monte¹⁸ have described a new Knudsen apparatus, with the simultaneous operation of three cells, giving good results for the enthalpies of sublimation of benzoic acid and of ferrocene.

Thermochimica Acta occasionally devote single issues to papers concerning design and developments in calorimetry and of particular interest are the volumes **151** (1989) and **154** (1989); the latter is devoted to papers in the memory of J. Christensen who contributed so much to thermochemical measurements, especially in titration calorimetry.

3 Enthalpies of Formation $\Delta_f H^\circ$

Organic Compounds. – The measurement of enthalpies of combustion in oxygen is the basic method for determining $\Delta_f H^\circ$ values of organic compounds. Enthalpies of other reactions are used to derive differences between enthalpies of formation, leading to $\Delta_f H^\circ$ values for other compounds, and can also be combined with combustion data to improve the quality of the final results. For compounds containing C, H, O, N only, the conventional static-bomb calorimeter is satisfactory, but for compounds containing other elements in addition, such as S and the halogens, the rotating-bomb calorimeter is used.

Since the previous report some long term projects have been completed. Colomina *et al.*¹⁹ report $\Delta_f H^\circ$ (cr) and $\Delta_f H^\circ$ (g) values for 1,2,4,5-tetramethylbenzene, penta-methylbenzene, and hexamethylbenzene, thus values for all the methyl substituted benzenes in the gaseous state are now available. Colomina *et al.*²⁰ also, report $\Delta_f H^\circ$ values for the crystalline and gaseous states for the three tetramethylbenzoic

- ¹³ E. G. Lavut, B. I. Timofeev, N. V. Chelovskaya, and V. M. Yuldashev, *Russ. J. Phys. Chem.*, 1989, **63**, 486.
- ¹⁴ E. G. Lavut and N. V. Chelovskaya, *Russ. J. Phys. Chem.*, 1990, **64**, 760.
- ¹⁵ Li Shaofeng, G. Peizhen, Yu Xiuhui, and He Xiheng, *J. Chem. Thermodyn.*, 1990, **22**, 319.
- ¹⁶ L. Švab, L. Pětroš, V. Hynek, and V. Svoboda, *J. Chem. Thermodyn.*, 1988, **20**, 545.
- ¹⁷ L. A. Torres-Gomez, G. Barriero-Rodriguez, and A. Galarza-Mondragon, *Thermochim. Acta*, 1988, **124**, 229.
- ¹⁸ M. A. V. Ribeiro da Silva and M. J. S. Monte, *Thermochim. Acta*, 1990, **171**, 169.
- ¹⁹ M. Colomina, P. Jiménez, M. V. Roux, and C. Turrión, *J. Chem. Thermodyn.*, 1989, **21**, 275.
- ²⁰ M. Colomina, P. Jiménez, R. Pérez Ossorio, M. V. Roux, and C. Turrión, *J. Chem. Thermodyn.*, 1988, **20**, 575.

acids, and also pentamethylbenzoic acid, thus completing the series of methylbenzoic acids. The thermochemistry of carboxylic acids and related derivatives has been reviewed.²¹ Chirico *et al.*²² report the thermodynamic properties of 4-methylphenanthrene including $\Delta_f H^\circ$ (cr). Ribeiro da Silva *et al.*²³ report $\Delta_f H^\circ$ (g) for the two hydroxynaphthalenes and of four dihydroxynaphthalenes; the $\Delta_f H^\circ$ (g) values fit the Cox scheme²⁴ in which each group when substituted into the aromatic ring produces a characteristic increment in $\Delta_f H^\circ$ (g) with additional corrections for adjacent substituents. Ribeiro da Silva *et al.*²⁵ report $\Delta_f H^\circ$ (cr) and $\Delta_f H^\circ$ (g) for 1,4-naphthoquinone, 9,10-anthraquinone, 9,10-phenanthraquinone, 1,4,9,10-antradiquinone, naphthazarin and quinizarin: the diquinone on contact with water reduces to quinizarin, but it was shown that consequent oxidation of water to hydrogen peroxide was not thermodynamically possible.

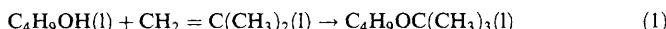
Combustion studies have been continued by Acree *et al.* on compounds containing the (N—O) dative covalent bond with the purpose of deriving values for the dissociation enthalpy of this bond: this $D(N—O)$ is sensitive to its immediate environment. $D(N—O)/k\text{J mol}^{-1}$ in benzylidene *t*-butylamine *N*-oxides was found to be 285.6 ± 6.0 ;²⁶ in 2,4,6-trimethylbenzonitrile *N*-oxide 222.2 ± 4.6 ; in 2,4,6-trimethoxybenzonitrile *N*-oxide 232.8 ± 3.8 ;²⁷ in phenazine *N*-oxide 280.7 ± 5.6 ; and in benzofuran 250.9 ± 3.0 ;²⁸ and the mean value in 1,4-dicyanobenzene di-*N*-oxide, 223.1 ± 1.7 .²⁹ Li Shaofeng and Pilcher,³⁰ from the enthalpy of formation of pyridine *N*-oxide, found from its enthalpy of reduction using Ti^{III} chloride solution, derived $D(N—O) = 301.7 \pm 2.8 \text{ kJ mol}^{-1}$ in this compound. Aioldi and Gonçalves³¹ using the same method determined $\Delta_f H^\circ$ (cr) for the picoline *N*-oxides but the absence of enthalpies of sublimation precluded derivation of $D(N—O)$.

Rogers *et al.* have continued microcalorimetric studies of the enthalpies of hydrogenation of alkenes, the hydrogenations were carried out in hexane solution with about 2–6 mg of alkene per injection; the enthalpies in such dilute solutions can be compared with gas state values. Values were reported for heptenes and methylhexenes,³² the dimethylpentenes,³³ and some octenes.³⁴ The results agree reasonably

- ²¹ G. Pilcher, 'The Chemistry of Acid Derivatives, Vol. 2', ed. S. Patai, John Wiley and Sons, New York, 1992, p51.
- ²² R. D. Chirico, I. A. Hossonlopp, A. Nguyen, W. V. Steele, and B. E. Gammon, *J. Chem. Thermodyn.*, 1989, **21**, 179.
- ²³ M. A. V. Ribeiro da Silva, M. D. M. C. Ribeiro da Silva, and G. Pilcher, *J. Chem. Thermodyn.*, 1988, **20**, 969.
- ²⁴ J. D. Cox, 'A method of estimating enthalpies of formation of benzene derivatives in the gas state', NPL Report CHEM 83, June, 1978.
- ²⁵ M. A. V. Ribeiro da Silva, M. D. M. C. Ribeiro da Silva, J. A. S. Teixeira, J. M. Bruce, P. M. Guyan, and G. Pilcher, *J. Chem. Thermodyn.*, 1989, **21**, 265.
- ²⁶ W. E. Acree, J. J. Kirchner, S. A. Tucker, M. D. M. C. Ribeiro da Silva, and G. Pilcher, *J. Chem. Thermodyn.*, 1989, **21**, 443.
- ²⁷ W. E. Acree, S. A. Tucker, A. I. Zvaigzne, Yang Meng-Yan, G. Pilcher, and M. D. M. C. Ribeiro da Silva, *J. Chem. Thermodyn.*, 1991, **23**, 31.
- ²⁸ M. L. P. Leitão, G. Pilcher, W. E. Acree, A. I. Zvaigzne, S. A. Tucker, and M. D. M. C. Ribeiro da Silva, *J. Chem. Thermodyn.*, 1990, **22**, 923.
- ²⁹ W. E. Acree, S. A. Tucker, and G. Pilcher, *J. Chem. Thermodyn.*, 1992, **24**, 213.
- ³⁰ Li Shaofeng and G. Pilcher, *J. Chem. Thermodyn.*, 1988, **20**, 463.
- ³¹ C. Aioldi and L. J. Gonçalves, *Thermochim. Acta*, 1992, **194**, 259.
- ³² D. W. Rogers and K. Dejroongraung, *J. Chem. Thermodyn.*, 1988, **20**, 675.
- ³³ D. W. Rogers and K. Dejroongraung, *J. Chem. Thermodyn.*, 1989, **21**, 1115.
- ³⁴ D. W. Rogers, K. Dejroongraung, S. D. Samuel, Wei Fang, and Y. Zhao, *J. Chem. Thermodyn.*, 1992, **24**, 561.

closely with previous values derived from the difference in the enthalpies of combustion of the corresponding alkene and alkane, but appear to be more precise.

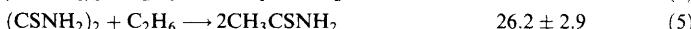
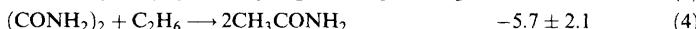
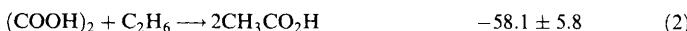
Sharanov *et al.*³⁵ by studying equilibria in the liquid phase derived enthalpies of reaction of formation of some ethers, *e.g.*:



and subsequently measured the enthalpies of combustion of these ethers, t-butoxybutane, 2-methyl-1-t-butoxypropane, and 1-methyl-1-t-butoxypropane,³⁶ obtaining excellent agreement with the equilibrium results. The same group using similar methods report $\Delta_f H^\circ$ for 2-ethyl-2-methoxypropane in the liquid and gaseous states.³⁷ Bastos *et al.*³⁸ report $\Delta_f H^\circ$ for glycerol in the liquid and gaseous states, the enthalpy of solution in water and the heat capacities of its aqueous solutions. Knauth and Sabbah³⁹ have measured $\Delta_f H^\circ$ of four 1,2-alkanediols using the CMRT calorimeter. Ribeiro da Silva *et al.*⁴⁰ report $\Delta_f H^\circ$ of 3-methylpentane-2,4-dione in the liquid and gaseous states. $\Delta_f H^\circ$ of the Diels–Alder product of anthracene and maleic anhydride has been reported.⁴¹ Wiberg *et al.*⁴² have determined $\Delta_f H^\circ$ for some aldehydes, *i.e.* ethanal to butanal, and also some ketones, *i.e.* propanone, cyclopentanone, and cyclohexanone, from their enthalpies of reduction using lithium triethylborohydride in triglyme. The derived enthalpies of formation agree with literature values but are more precise and hence to be preferred. Abboud *et al.*,⁴³ from combustion measurements, report $\Delta_f H^\circ$ in the crystalline and gaseous states for 1-adamantylmethyl ketone and 1,1-diadamantyl ketone; surprisingly the strain energy in the latter compound appears to be no larger than in di-t-butylketone. Alekseev *et al.*,⁴⁴ from a combination of combustion and solution–reaction calorimetry, determined $\Delta_f H^\circ$ for phthalic acid, pyromellitic acid, phthalic anhydride and the dianhydride of pyromellitic acid. Verevkin *et al.*⁴⁵ measured the enthalpies of combustion and of vaporization of eleven esters of the general formula $(\text{R}_1\text{R}_2\text{R}_3)\text{CCO}_2\text{R}_4$ with $\text{R}_2, \text{R}_3 = \text{H}$ or CH_3 ; $\text{R}_4 = \text{CH}_3$ or C_2H_5 ; and $\text{R}_1 = \text{CO}_2\text{CH}_3$, $\text{N}(\text{CH}_3)_2$, or C_2H_5 : the results fit very well into a group additivity scheme. Nuñez *et al.*⁴⁶ report the enthalpies of formation of oxamic acid, oxamide, and dithioxamide, and the $\Delta_f H^\circ(\text{g})$ values would not fit any additivity scheme.

- ³⁵ K. G. Sharanov, Y. B. Mishentseva, A. M. Rozhnov, E. A. Miroshnichenko, and L. I. Korchatova, *J. Chem. Thermodyn.*, 1991, **23**, 141.
- ³⁶ K. G. Sharanov, Y. B. Mishentseva, A. M. Rozhnov, E. A. Miroshnichenko, and L. I. Korchatova, *J. Chem. Thermodyn.*, 1991, **23**, 637.
- ³⁷ A. M. Rozhnov, V. V. Safronov, S. P. Verekin, K. G. Sharanov, and V. I. Alenin, *J. Chem. Thermodyn.*, 1991, **23**, 629.
- ³⁸ M. Bastos, S-O Nilsson, M. D. M. C. Ribeiro da Silva, M. A. V. Ribeiro da Silva, and I. Wadsö, *J. Chem. Thermodyn.*, 1988, **20**, 1353.
- ³⁹ P. Knauth and R. Sabbah, *Thermochim. Acta*, 1990, **164**, 145.
- ⁴⁰ M. A. V. Ribeiro da Silva, M. L. C. C. H. Ferrão, M. J. S. Monte, R. M. G. Esteves da Silva, and J. C. Ribeiro, *J. Chem. Thermodyn.*, 1992, **24**, 585.
- ⁴¹ M. Kar, T. G. Lenz, and J. D. Vaughan, *J. Chem. Thermodyn.*, 1992, **24**, 151.
- ⁴² K. B. Wiberg, L. S. Crocker, and K. M. Morgan, *J. Am. Chem. Soc.*, 1991, **113**, 3447.
- ⁴³ J-L. M. Abboud, P. Jiménez, M. V. Roux, C. Turrión, C. Lopez-Mardomingo, and G. Sanz, *J. Chem. Thermodyn.*, 1992, **24**, 217.
- ⁴⁴ V. G. Alekseev, V. D. Kiznev, N. V. Fedyainov, and V. I. Bushinshu, *Russ. J. Phys. Chem.*, 1989, **63**, 1280.
- ⁴⁵ S. P. Verevkin, H-D. Beckhaus, and C. Rüchart, *Thermochim. Acta*, 1992, **197**, 27.
- ⁴⁶ L. Nuñez, L. Barral, and G. Pilcher, *J. Chem. Thermodyn.*, 1988, **20**, 1211.

The enthalpies of the gaseous reactions, $\Delta_f H^\circ/\text{kJ mol}^{-1}$:



demonstrate as the series is descended that the polar repulsion is reduced and intramolecular hydrogen bonding produces stabilization.

Kirklin *et al.*⁴⁷ from $\Delta_f H^\circ$ of 1,4-dimethylcubane dicarboxylate deduce a strain energy of 590.7 kJ mol⁻¹ and this result suggests that the value of $\Delta_f H^\circ$ for cubane reported by Kybett *et al.*⁴⁸ is in error. Both $\Delta_f H^\circ(\text{cr})$ and $\Delta_f H^\circ(\text{g})$ for adamantane-1-methylcarboxylate and adamantane-1-carbonitrile have been reported.⁴⁹

Vasil'ev *et al.* have measured $\Delta_f H^\circ$ for several aminocarboxylic acids, *i.e.* imino-diacetic acid and ethylenediamine tetraacetic acid,⁵⁰ L-histidine and L-proline,⁵¹ iminodisuccinic acid and β -hydroxyethylamino diacetic acid,⁵² and of nitrilotriacetic acid.⁵³ The contribution of repeating glycine and *dl*-alanine groups to $\Delta_f H^\circ$ of peptides was derived by Diaz *et al.*⁵⁴ from $\Delta_f H^\circ$ of glycylglycine and of *dl*-alanine-*dl*-alanine, and also from $\Delta_f H^\circ$ of hexaglycine.⁵⁵ Abboud *et al.*⁵⁶ report $\Delta_f H^\circ$ for some amides, *i.e.* 2-methylpropanamide, 2,2-dimethylpropanamide, and 1-adamantyl-carboxamide; Kulagina and Kiparisova give $\Delta_f H^\circ$ for benzamide, phenylcarbamide and dimethylcarbamide.⁵⁷

Surprisingly, urea derivatives have received much attention from several groups of workers. Imamura *et al.* give $\Delta_f H^\circ(\text{cr})$ and $\Delta_f H^\circ(\text{g})$ for acetylurea and trimethyl-isocyanurate;⁵⁸ trimethylcyanurate, malonamide, and 1,3-dimethyluracil.⁵⁹ Kozyro *et al.*⁶⁰ give $\Delta_f H^\circ$ for tetramethylurea, and Davies *et al.*⁶¹ give $\Delta_f H^\circ$ for 1,3-dimethylurea and 1,3-dimethylurea nitrate, and proposed a correlation of the thermochemical data for urea derivatives with the degree of N substitution.⁶² The enthalpies of sublimation of eleven alkylureas are reported by Piacente *et al.*⁶³

It is well known from previous studies on monocyclic compounds, such as cycloalkanes, cycloalkenes, cyclic ethers, *etc.*, that the conventional strain-energy in the

⁴⁷ D. R. Kirklin, K. L. Churney, and E. S. Domalski, *J. Chem. Thermodyn.*, 1989, **21**, 1105.

⁴⁸ B. D. Kybett, S. Carroll, P. Natalis, D. W. Bonnell, J. L. Margrave, and J. L. Franklin, *J. Am. Chem. Soc.*, 1966, **88**, 626.

⁴⁹ J.-L. Abboud, P. Jiménez, M. V. Roux, C. Turrión, and C. López-Mardomingo, *J. Chem. Thermodyn.*, 1992, **24**, 1299.

⁵⁰ V. P. Vasil'ev, V. A. Borodin, and S. B. Kopnyshev, *Russ. J. Phys. Chem.*, 1988, **62**, 1156.

⁵¹ V. P. Vasil'ev, V. A. Borodin, and S. B. Kopnyshev, *Russ. J. Phys. Chem.*, 1989, **63**, 891.

⁵² V. P. Vasil'ev, V. A. Borodin, and S. B. Kopnyshev, *Russ. J. Phys. Chem.*, 1989, **63**, 1566.

⁵³ V. P. Vasil'ev, V. A. Borodin, and S. B. Kopnyshev, *Russ. J. Phys. Chem.*, 1989, **63**, 742.

⁵⁴ E. L. Diaz, E. S. Domalski, and J. C. Colbert, *J. Chem. Thermodyn.*, 1992, **24**, 1311.

⁵⁵ J. C. Colbert, E. S. Domalski, B. Coxon, and D. L. Vanderhart, *Thermochim. Acta*, 1989, **153**, 123.

⁵⁶ J.-L. Abboud, P. Jiménez, M. V. Roux, C. Turrión, and C. Lopez-Mardomingo, *J. Chem. Thermodyn.*, 1989, **21**, 859.

⁵⁷ T. G. Kulagina and E. G. Kiparisova, *Russ. J. Phys. Chem.*, 1987, **61**, 261.

⁵⁸ A. Imamura, S. Murata, and M. Sakiyama, *J. Chem. Thermodyn.*, 1988, **20**, 389.

⁵⁹ A. Imamura, K. Takahashi, S. Murata, and M. Sakiyama, *J. Chem. Thermodyn.*, 1989, **21**, 237.

⁶⁰ A. A. Kozyro, A. P. Krasulin, V. V. Simirskii, and V. S. Markovnik, *Russ. J. Phys. Chem.*, 1988, **62**, 895.

⁶¹ R. H. Davies, A. Finch, and J. O. Hill, *Thermochim. Acta*, 1991, **184**, 243.

⁶² R. H. Davies, A. Finch, and J. O. Hill, *Thermochim. Acta*, 1991, **188**, 321.

⁶³ V. Piacente, D. Ferro, and G. Della Gatta, *Thermochim. Acta*, 1990, **158**, 79.

Table 1 $S(6\text{-ring}) - S(5\text{-ring})$ in monocyclic compounds (kJ mol^{-1})

cycloalkanes	-26.4 ± 1.1	lactones	7.5 ± 1.2
cycloalkenes	-18.3 ± 1.6	lactams	5.6 ± 2.3
cyclic ethers	-18.6 ± 1.3	cyclic anhydrides	16.1 ± 2.5
cyclic thioethers	-8.8 ± 1.6	cyclic imides	2.5 ± 2.3
cyclic amines	-23.2 ± 1.0		
cyclic ketones	-13.4 ± 2.8		

6-ring compound is less than that in the 5-ring compound and that the strain-energies in the 6-ring compounds are close to zero. Recent measurements have shown that this is not a universal truth. If we assume the difference in $\Delta_f H^\circ(\text{g})$ between 5- and 6-unstrained ring structures is due to the insertion of a $-\text{CH}_2-$ group between two $-\text{CH}_2-$ groups, which from the $-\text{CH}_2-$ increment in $\Delta_f H^\circ(\text{g})$ for alkanes is $-20.6 \pm 0.1 \text{ kJ mol}^{-1}$, then $S(6\text{-ring}) - S(5\text{-ring})$ is readily calculated from $\Delta_f H^\circ(\text{g})$ for the two compounds and the values are listed in Table 1. For those compounds in the left-hand column of Table 1, the $\Delta_f H^\circ(\text{g})$ values were taken from standard compilations and for these cases $S(6\text{-ring}) < S(5\text{-ring})$. For those compounds in the right-hand column, the combustion measurements by Leitão *et al.*^{64,65} were confirmed by reaction-solution calorimetric measurements of the enthalpies of hydrolysis and of reduction of lactones by Wiberg and Waldron.⁶⁶ Yang Meng-Yan and Pilcher⁶⁷ deduced the values for the cyclic anhydrides and cyclic imides from combustion measurements. For those cases where $S(6\text{-ring}) < S(5\text{-ring})$, the 5-rings are nearly planar whereas the 6-rings are non-planar. When $S(6\text{-ring}) > S(5\text{-ring})$, the introduction of a structural group for which the preferred conformation is planar causes greater distortion in the 6-ring than in the 5-ring. Wiberg and Waldron⁶⁶ examined the C_4-C_{13} monocyclic lactones, deducing strain-energies for each, and analysed the results using molecular mechanics and some *ab initio* calculations. Kozina *et al.*⁶⁸ determined $\Delta_f H^\circ(\text{g})$ for a series of cycloalkene oxides, from cyclopentene oxide to cyclooctene oxide, from combustion and vaporization measurements, also deriving strain-energies. Yu Ya Van Chin-Syan and Kachurina⁶⁹ report $\Delta_f H^\circ$ values for 14 oxirane derivatives of the general formula $\text{O}-\text{CH}_2\text{CH}_2\text{CH}_2\text{OR}$, deriving bond-energy terms for the oxirane ring.

Of miscellaneous thermochemical results for aliphatic compounds, worth noting are: Kazakov *et al.*,⁷⁰ from a study of equilibria in the nitration of glycerol, derived $\Delta_f H^\circ$ for 2-nitroglycerol and two dinitroglycerols; Kirpichev *et al.*⁷¹ from combustion and reaction calorimetric studies, derived $\Delta_f H^\circ$ for some onium salts

⁶⁴ J. M. Brown, A. D. Conn, G. Pilcher, M. L. P. Leitão, and Yang Meng-Yan, *J. Chem. Soc., Chem. Commun.*, 1989, 1817.

⁶⁵ M. L. P. Leitão, G. Pilcher, Yang Meng-Yan, J. M. Brown, and A. D. Conn, *J. Chem. Thermodyn.*, 1990, **22**, 885.

⁶⁶ K. B. Wiberg and R. F. Waldron, *J. Am. Chem. Soc.*, 1991, **113**, 7697.

⁶⁷ Yang Meng-Yan and G. Pilcher, *J. Chem. Thermodyn.*, 1990, **22**, 893.

⁶⁸ M. P. Kozina, L. P. Timofeeva, V. A. Luk'yanova, S. M. Pimenova, and L. I. Kas'yan, *Russ. J. Phys. Chem.*, 1988, **62**, 609.

⁶⁹ Y. V. Chin-Syan and N. S. Kachurina, *Russ. J. Phys. Chem.*, 1987, **61**, 622.

⁷⁰ A. I. Kazakov, G. V. Lagodzinskaya, E. P. Kirpichev, L. P. Andrienko, N. G. Yunda, A. M. Korolev, Yu. I. Rubtsov, G. V. Manelis, and L. T. Eremenko, *Dokl. Akad. Nauk. SSSR. Phys. Chem.*, 1989, **305**, 287.

⁷¹ E. P. Kirpichev, Yu. M. Rubtsov, T. V. Sorokina, G. N. Shirakova, V. V. Aleshin, and V. Ya. Rosolovskii, *Russ. J. Inorg. Chem.*, 1991, **36**, 1002.

of hyponitrous and hyponitric acids, e.g. $(\text{CN}_3\text{H}_6)_2\text{N}_2\text{O}_2$ and $(\text{CN}_3\text{H}_6)_6\text{N}_2\text{O}_3$ and of CN_4H_7^+ (aq); Kozyro *et al.*⁷² report the thermodynamic properties of cyclohexanone oxime; Kabo *et al.*⁷³ give the thermodynamic properties of ϵ -caprolactam; and Miroshnichenko *et al.*⁷⁴ made combustion measurements on methylidinitramine, $\text{CH}_3\text{N}_3\text{O}_4$ (l), mixed with dimethyl phthalate to avoid explosion.

Of aromatic derivatives, Finch *et al.*, from combustion measurements, give $\Delta_f H^\circ$ for 2,4-dinitroresorcinol, 4,6-dinitroresorcinol,⁷⁵ of 2,4,6-trinitroresorcinol,⁷⁶ and of two crystalline modifications of 2,6-dinitrotoluene.⁷⁷ Ribeiro da Silva *et al.*⁷⁸ report $\Delta_f H^\circ(\text{cr})$ and $\Delta_f H^\circ(\text{g})$ for 3-nitrophenol. Steele *et al.*⁷⁹ report the thermodynamic properties of 2-aminobiphenyl. Dias *et al.*⁸⁰ give $\Delta_f H^\circ(\text{cr})$ and $\Delta_f H^\circ(\text{g})$ for *cis*- and *trans*-azobenzene; $\Delta_f H^\circ$ for *trans*-azobenzene was from combustion measurements, and that for the *cis*-isomer was derived from the enthalpy of isomerization *cis* \rightarrow *trans* determined by reaction calorimetry, and confirmed by *trans* \rightarrow *cis* measured by photomicrocalorimetry. In the gas state the enthalpy of isomerization, *cis* \rightarrow *trans*, is $-48.4 \pm 3.4 \text{ kJ mol}^{-1}$, large when compared with the corresponding value for stilbene, $-17.5 \pm 3.8 \text{ kJ mol}^{-1}$. The enthalpy of formation of benzophenone oxide, Ph_2CO_2 , was determined by Harstock *et al.*⁸¹ using photoacoustic calorimetry. For the decomposition:



$$\Delta_f H^\circ = -318 \pm 25 \text{ kJ mol}^{-1}.$$

By rotating-bomb calorimetry, Ribeiro da Silva *et al.*⁸² measured $\Delta_f H^\circ$ of thiobenzamide, *N,N*-dimethylthiobenzamide and *N,N*-diethylthiobenzamide, and proposed a bond-energy scheme for PhCSNR_2 molecules. Sabbah and Watik⁸³ measured the energy of combustion of thianthrene using the CRMT calorimeter, obtaining agreement with previous values. Ribeiro da Silva *et al.*⁸⁴ measured the enthalpies of decomposition of some dialkylammoniumdialkyldithiocarbamates by microcalorimetry thus deriving $\Delta_f H^\circ$ for some dialkyldithiocarbamic acids.

The $\Delta_f H^\circ$ of several perfluoro compounds have been determined using

- ⁷² A. A. Kozyro, G. J. Kabo, V. S. Krouk, M. S. Sheiman, I. A. Yurska, V. V. Simirsky, A. P. Krasulin, V. M. Sevruck, and V. I. Gogolinsky, *J. Chem. Thermodyn.*, 1992, **24**, 785.
- ⁷³ G. J. Kabo, A. A. Kozyro, V. S. Krouk, V. M. Sevruck, I. A. Yurska, V. V. Simirsky, and V. I. Gogolinsky, *J. Chem. Thermodyn.*, 1992, **24**, 1.
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- ⁷⁵ A. Finch and J. Payne, *Thermochim. Acta*, 1991, **189**, 109.
- ⁷⁶ A. Finch and J. Payne, *Thermochim. Acta*, 1990, **170**, 209.
- ⁷⁷ A. Finch and J. Payne, *Thermochim. Acta*, 1990, **164**, 55.
- ⁷⁸ M. A. V. Ribeiro da Silva, A. M. M. V. Reis, M. J. S. Monte, M. M. S. S. F. Bartolo and J. A. R. G. O. Rodrigues, *J. Chem. Thermodyn.*, 1992, **24**, 653.
- ⁷⁹ W. V. Steele, R. D. Chirico, S. E. Knipmeyer, and A. Nguyen, *J. Chem. Thermodyn.*, 1991, **23**, 957.
- ⁸⁰ A. R. Dias, M. E. Minas da Piedade, J. A. Martinho Simões, J. A. Simoni, C. Teixeira, H. P. Diogo, Yang Meng-Yan, and G. Pilcher, *J. Chem. Thermodyn.*, 1992, **24**, 439.
- ⁸¹ F. W. Hartstock, J. N. Kanabus-Kaminska, and D. Griller, *Int. J. Chem. Kinet.*, 1989, **21**, 157.
- ⁸² M. D. M. C. Ribeiro da Silva, P. Souza, and G. Pilcher, *J. Chem. Thermodyn.*, 1989, **21**, 173.
- ⁸³ R. Sabbah and L. El. Watik, *Thermochim. Acta*, 1989, **138**, 241.
- ⁸⁴ M. A. V. Ribeiro da Silva, A. M. M. V. Reis, and G. Pilcher, *Thermochim. Acta*, 1988, **124**, 319.

rotating-bomb calorimetry by Zhogina *et al.*,^{85–90} i.e. perfluorodecalin,⁸⁵ perfluorobicyclo[4,4,0]-dec-1,6-ene,⁸⁶ perfluoro-2,7-dimethyloctane,⁸⁷ perfluoro-2-methylpent-2-ene and perfluoro-2-methyl-3-isopropyl-pent-2-ene,⁸⁸ *cis*-perfluorobicyclo[4,3,0] nonane,⁸⁹ and *trans*-perfluorobicyclo[4,3,0] nonane.⁹⁰

The established rotating-bomb method for Cl-compounds is to place a solution of either arsenious oxide or hydrazine hydrochloride in the bomb to reduce chlorine to chloride; this involves analysis of the final solution and a consequent thermal correction. Lyubarskii *et al.*^{91,92} have proposed a new method by which the compound is burnt with sulfur, which acts as a reducing agent for the chlorine, giving the final solution as a mixture of sulfuric and hydrochloric acids. The results for *p*-chlorobenzoic acid,⁹¹ *o*-chlorobenzene sulfonamide, 6-chloro-2-amino-benzothiazole, and 2,6-dichlorobenzothiazole⁹² appear to be convincing, but for this promising technique to become generally acceptable similar measurements must be undertaken by other groups. Measurements by conventional methods have been made for 2-chloro-6-(trichloromethyl) pyridine,⁹³ in which preoxidation of the arsenious oxide solution was examined and correction made, for 1,2-dichlorohexafluoropropane,⁹⁴ and for 1,1,2-trichloroethane.⁹⁵

Heterocyclic compounds have received much attention in the last five years. Chirico *et al.* report $\Delta_f H^\circ$ for dibenzofuran,⁹⁶ and dibenzothiophene,⁹⁷ with complete determination of the thermodynamic properties: similarly for chroman and isochroman.⁹⁸ The thermodynamic properties of quinoline and of isoquinoline have been reported.⁹⁹ Ribeiro da Silva *et al.*,^{100–102} from combustion measurements to determine $\Delta_f H^\circ$ (cr) and Knudsen vapour pressure measurements to determine enthalpies of sublimation, report $\Delta_f H^\circ$ values for 8-hydroxyquinoline, 5-nitro-8-hydroxyquinoline, and 2-methyl-8-hydroxyquinoline,¹⁰⁰ and for 2-hydroxyquinoline, 4-methyl-2-hydroxyquinoline, 4-hydroxyquinoline, and 2-methyl-4-hydroxyquinoline;¹⁰¹ these latter

- ⁸⁵ E. V. Zhogina, T. S. Papina, V. P. Kolesov, B. A. Mel'nichenko, M. A. Zapol'skaya, and I. P. Prokudin, *Russ. J. Phys. Chem.*, 1987, **61**, 1523.
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- ⁸⁷ E. V. Zhogina, T. S. Papina, V. P. Kolesov, L. N. Kosareva, and T. Ya. Ivanov, *Thermochim. Acta*, 1989, **139**, 43.
- ⁸⁸ E. V. Zhogina, T. S. Papina, V. P. Kolesov, L. L. Gervits, K. N. Makarov, and V. P. Vorob'eva, *Russ. J. Phys. Chem.*, 1990, **64**, 1514.
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- ⁹⁰ E. V. Zhogina, T. S. Papina, V. P. Kolesov, I. P. Prokudin, V. S. Asovich, and B. A. Mel'nichenko, *Russ. J. Phys. Chem.*, 1989, **63**, 747.
- ⁹¹ M. V. Lyubarskii and T. I. Gromova, *Russ. J. Phys. Chem.*, 1989, **63**, 986.
- ⁹² M. V. Lyubarskii, T. I. Gromova, R. I. Smolyanets, and S. V. Rudakova, *Russ. J. Phys. Chem.*, 1989, **63**, 1204.
- ⁹³ Zhi-Ching Tan, A. Kamaguchi, Y. Naganu, and M. Sakiyama, *J. Chem. Thermodyn.*, 1989, **21**, 615.
- ⁹⁴ T. S. Papina, V. P. Kolesov, and Yu. G. Golovanova, *Russ. J. Phys. Chem.*, 1987, **61**, 1168.
- ⁹⁵ T. S. Papina and V. P. Kolesov, *Russ. J. Phys. Chem.*, 1987, **61**, 1170.
- ⁹⁶ R. D. Chirico, B. E. Gammon, S. E. Knipmeyer, A. Nguyen, M. M. Strube, C. Tsonopoulos, and W. V. Steele, *J. Chem. Thermodyn.*, 1990, **22**, 1075.
- ⁹⁷ R. D. Chirico, S. E. Knipmeyer, A. Nguyen, and W. V. Steele, *J. Chem. Thermodyn.*, 1991, **23**, 431.
- ⁹⁸ R. D. Chirico, D. G. Archer, I. A. Hossenlopp, A. Nguyen, W. V. Steele, and B. E. Gammon, *J. Chem. Thermodyn.*, 1990, **22**, 665.
- ⁹⁹ W. V. Steele, D. G. Archer, R. D. Chirico, W. B. Collier, I. A. Hossenlopp, A. Nguyen, N. K. Smith, and B. E. Gammon, *J. Chem. Thermodyn.*, 1988, **20**, 1223.
- ¹⁰⁰ M. A. V. Ribeiro da Silva, M. J. S. Monte, and M. A. R. Matos, *J. Chem. Thermodyn.*, 1989, **21**, 159.
- ¹⁰¹ M. A. V. Ribeiro da Silva, M. A. R. Matos, and M. J. S. Monte, *J. Chem. Thermodyn.*, 1990, **22**, 609.

results cast suspicion on an earlier value for 4-hydroxypyridine, a redetermination of $\Delta_f H^\circ$ (g) for which¹⁰² showed that the increments in $\Delta_f H^\circ$ (g) for substituents into the pyridine ring of quinoline agree with the corresponding values for substitution into pyridine. Steele *et al.*¹⁰³ report the thermodynamic properties of acridine, phenanthridine, and 7,8-benzoquinoline, of 1,2,3,4-tetrahydroquinoline and 5,6,7,8-tetrahydroquinoline,¹⁰⁴ of benzothiazole and benzoxazole,¹⁰⁵ and of 1,2,3,4-tetrahydro-9-methylcarbazole.¹⁰⁶ Vilcu *et al.*¹⁰⁷ give $\Delta_f H^\circ$ (cr) for α - and β -perhydro-acridine, the α being the more stable by $10.5 \pm 3.4 \text{ kJ mol}^{-1}$. Jiménez *et al.* report $\Delta_f H^\circ$ (cr) and $\Delta_f H^\circ$ (g) for 1,2,4-triazole and benzotriazole,¹⁰⁸ 9-H-carbazole, 9-methyl-carbazole and 9-ethyl-carbazole,¹⁰⁹ and also 2-methylimidazole and 2-ethylimidazole.¹¹⁰ Kozyro *et al.*¹¹¹ give $\Delta_f H^\circ$ (cr and g) for nine tetrazole derivatives. Finch *et al.*¹¹² measured the enthalpies of combustion of 1,2,4-triazol-5-one and of 3-nitro-1,2,4-triazol-5-one and their enthalpies of neutralization with alkali. Sabbah *et al.*, using the CMRT calorimeter, report $\Delta_f H^\circ$ of phenazine,¹¹³ and of phenoaxazine and phenothiazine.¹¹⁴ The enthalpies of sublimation of six halogen substituted 8-hydroxyquinolines derived from Knudsen vapour pressure measurements by Ribeiro da Silva and Monte¹¹⁵ demonstrated a linear correlation between the enthalpy of sublimation and the temperature at which the vapour pressure was 0.5 Pa.

Nagano *et al.* from combustion measurements, give $\Delta_f H^\circ$ (cr) for tetramethylammonium iodide,¹¹⁶ and tetra-n-butylammonium iodide,¹¹⁷ and when combined with enthalpies of solution, the crystal lattice and hydration energies of the tetralkylammonium ions were derived. The $\Delta_f H^\circ$ values and molar lattice energies were determined by de Souza *et al.*¹¹⁸ for some dialkylammonium halides.

Inorganic Compounds. – The measurement of the enthalpies of formation of inorganic compounds inspires the use of a greater variety of experimental techniques. Calorimetric methods, combustion in oxygen or in fluorine, and reaction–solution calorimetry provide the major proportion of results, but many useful data are now obtained by mass spectroscopic examination of the effusate from Knudsen cells

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and by the study of ion–molecule reactions. In recent years the application of solid state electrochemical cells has yielded useful results.

O'Hare *et al.* have continued to produce excellent results using fluorine bomb calorimetry, paying great attention to the state of the sample and the effect of impurities. With this technique $\Delta_f H^\circ$ values have been reported for: WS_2 (cr) with revision of the value for MoS_2 (cr),¹¹⁹ $\text{S}_2\text{N}^+\text{AsF}_6^-$ (cr),¹²⁰ MoTe_2 (cr);¹²¹ WTe_2 (cr);¹²² WSi_2 (cr),¹²³ GeS (cr),¹²⁴ PF_3 (g) and the enthalpy difference, P (α , white) \rightarrow P (orthorhombic, black), $\Delta_f H^\circ = -21.2 \pm 2.1 \text{ kJ mol}^{-1}$,¹²⁵ As_2Se_3 (cr),¹²⁶ and SF_6 (g), measured with a much purer sample of sulfur leading to a more precise value of $\Delta_f H^\circ (\text{SO}_4^-, \text{aq}) = -909.51 \pm 0.21 \text{ kJ mol}^{-1}$.¹²⁷ The enthalpies of formation of two crystal forms of BN were determined by combustion in fluorine by Leonidov *et al.*: BN (cr, cubic), $\Delta_f H^\circ = -266.8 \pm 2.2 \text{ kJ mol}^{-1}$,¹²⁸ and BN (cr, wurtzite), $\Delta_f H^\circ = -263.2 \pm 2.3 \text{ kJ mol}^{-1}$.¹²⁹

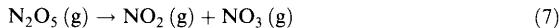
Lavut *et al.* use the technique of carrying out the chemical reaction in an electrically heated microfurnace contained within the calorimetric vessel. Chlorinations appear to be particularly successful with results given for VCl_2 (cr),¹³⁰ CrCl_3 (cr),¹³¹ CoCl_2 (cr),¹³² the result for the latter being in precise agreement with that from a reaction-calorimetric measurement by Efimov and Evdokimova.¹³³ The method has also been applied to the combustion of metals in oxygen to obtain $\Delta_f H^\circ (\text{Y}_2\text{O}_3, \text{cr})$,¹³⁴ and $\Delta_f H^\circ (\text{V}_2\text{O}_5, \text{cr}) = -1557.4 \pm 1.4 \text{ kJ mol}^{-1}$.¹³⁵ However, this value differs from both an earlier combustion measurement, $-1550.6 \pm 2.1 \text{ kJ mol}^{-1}$,¹³⁶ and a recent determination by reaction–solution calorimetry, $-1552.51 \pm 0.90 \text{ kJ mol}^{-1}$,¹³⁷ and hence there is uncertainty concerning the correct value.

Efimov and co-workers have recently carried out some excellent work in reaction–solution calorimetry deriving $\Delta_f H^\circ$ of some simple halides. The values are an improvement over those in present use and so are quoted here, all in kJ mol^{-1} : FeCl_2 (cr) -340.87 ± 0.47 , FeCl_3 (cr) -395.30 ± 0.67 ,¹³⁸ FeBr_2 (cr) -244.74 ± 0.22 , FeBr_3 (cr) -262.63 ± 0.20 ,¹³⁹ FeI_2 (cr) -118.10 ± 0.30 , NiI_2 (cr) -96.40 ± 0.40 ;¹⁴⁰

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NiCl_2 (cr) -304.46 ± 0.53 ,¹⁴¹ NiBr_2 (cr) -211.93 ± 0.35 ,¹⁴² CoCl_2 (cr) -311.07 ± 0.29 ,¹³³ CoBr_2 (cr) -215.43 ± 0.27 ,¹⁴³ CoI_2 (cr) -94.30 ± 0.30 ,¹⁴⁴ ZrCl_2 (cr) -531.0 ± 5.0 .¹⁴⁵

An interesting study by McDaniel *et al.*¹⁴⁶ of the enthalpy of hydrolysis of N_2O_5 (cr), with its enthalpy of sublimation, led to $\Delta_f H^\circ (\text{N}_2\text{O}_5, \text{g}) = 5.0 \pm 2.6 \text{ kJ mol}^{-1}$, and when coupled with the enthalpy of the decomposition, Equation 7,



derived from the equilibrium constant as $f(T)$,¹⁴⁷ led to $\Delta_f H^\circ (\text{NO}_3, \text{g}) = 64.4 \pm 3.1 \text{ kJ mol}^{-1}$, a value not previously available.

Of the vast quantity of work done in the field of reaction-solution calorimetry we select:

(a) Measurements of high accuracy yielding results which should supersede previous values. These include $\Delta_f H^\circ$ for LiOH (cr),¹⁴⁸ KOH (cr) and CsOH (cr),¹⁴⁹ La(OH)_3 (cr),¹⁵⁰ In_2O_3 (cr),¹⁵¹ Nd_2O_3 (cr),¹⁵² BaO (cr), SrO (cr), BaCl_2 (cr), SrCl_2 (cr), Ba^{2+} (aq) and Sr^{2+} (aq),¹⁵³ Ba(OH)_2 (cr) and Sr(OH)_2 (cr),¹⁵⁴ KBrO_3 (cr) and BrO_3^- (aq),¹⁵⁵ Nd(OH)_3 (cr),¹⁵⁶ YCl_3 (cr), YBr^3 (cr), YI_3 (cr) and Y^{3+} (aq),¹⁵⁷ $\text{K}_2\text{Pt}(\text{CN})_4$ (cr),¹⁵⁸ GaCl_3 (cr),¹⁵⁹ Ti^{4+} (aq),¹⁶⁰ Co^{2+} (aq) and Ni^{2+} (aq),¹⁶¹ Dy^{2+} (aq).¹⁶²

(b) Measurements on compounds of technical interest, several of which are regarded as possible products of nuclear reactor accidents, include, Cs_2TeO_3 (cr), $\text{Cs}_2\text{Te}_2\text{O}_5$ (cr), $\text{Cs}_2\text{Te}_4\text{O}_9$ (cr), and Cs_2TeO_4 (cr),¹⁶³ Cs_2RuO_4 (cr),¹⁶⁴ SrSiO_3 (cr) and Sr_2SiO_4 (cr),¹⁶⁵ BaCuO_2 (cr), Y_2BaO_4 (cr), and $\text{Y}_2\text{Cu}_2\text{O}_5$ (cr),¹⁶⁶ Li_2CrO_4 (cr),

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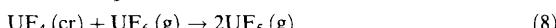
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and Li_2MoO_4 (cr),¹⁶⁷ BaSiO_3 (cr) and Ba_2SiO_4 (cr),¹⁶⁸ Li_2ZrO_3 (cr), $\text{Li}_6\text{Zr}_2\text{O}_7$ (cr), and Li_8ZrO_6 (cr),¹⁶⁹ KAlO_2 (cr),¹⁷⁰ LiAsO_3 (cr),¹⁷¹ K_2TeI_6 (cr), Rb_2TeI_6 (cr) and Cs_2TeI_6 (cr),¹⁷² NH_4VO_3 (cr),¹⁷³ $\text{Zn}_3(\text{PO}_4)_2$ (cr), and of four hydrates,¹⁷⁴ $\text{NH}_4\text{F} \cdot \text{H}_2\text{O}_2$ (cr),¹⁷⁵ a series of rare earth isothiocyanates, $\text{M}(\text{NCS})_3$ (cr) where the curve of $\Delta_f H^\circ$ plotted against the element parallels that for the corresponding chlorides,¹⁷⁶ Na_2MoO_4 (cr), $\text{Na}_2\text{Mo}_2\text{O}_7$ (cr), $\text{Na}_2\text{Mo}_3\text{O}_{10}$ (cr), and $\text{Na}_2\text{Mo}_4\text{O}_{13}$ (cr),¹⁷⁷ $\text{Ce}(\text{ClO}_4)_3$ (cr), $\text{Cs}[\text{Ce}(\text{ClO}_4)_4]$ (cr), $\text{Cs}_2[\text{Ce}(\text{ClO}_4)_5]$ (cr), and $\text{Cs}_3[\text{Ce}(\text{ClO}_4)_6]$ (cr),¹⁷⁸ silver iodide acetylide determined using three independent methods,¹⁷⁹ N_3^- (aq.) determined from the enthalpy of combustion of ammonium azide and its enthalpy of solution extrapolated to infinite dilution.¹⁸⁰

(c) Transuranium-element thermochemistry has received a first class review by Fuger in the Huffman Memorial Lecture of 1991.¹⁸¹ The most recent calorimetric studies have been concerned with mixed oxides and halides, clearly of importance in reactor technology. These include BaU_2O_7 (cr) and $\text{Ba}_2\text{U}_2\text{O}_7$ (cr),¹⁸² dehydrated schoepite $\text{VO}_3 \cdot 0.9\text{H}_2\text{O}$ determined from its enthalpy of solution in HF (aq),¹⁸³ BaCeO_3 (cr), SrCeO_3 (cr), BaTbO_3 (cr), SrTbO_3 (cr), BaAmO_3 (cr), and SrAmO_3 (cr),¹⁸⁴ Li_2NpO_4 (cr), Na_2NpO_4 (cr), K_2NpO_4 (cr), Cs_2NpO_4 (cr), and Na_4NpO_5 (cr),¹⁸⁵ the lattice energies of a series of mixed halides $\text{Cs}_2\text{NaMCl}_6$ (cr), $\text{M} = \text{U}, \text{Np}, \text{Pu}, \text{Am}, \text{Cf}$ were derived from $\Delta_f H^\circ$'s measured by solution calorimetry.¹⁸⁶ Of non-calorimetric measurements of note in this area, two independent determinations of $\Delta_f H^\circ$ (UF_5, g) are in precise agreement; Borshchevskii *et al.*¹⁸⁷ made a study of ion–molecule equilibria using Knudsen cell plus mass spectrometer, and Bondarenko *et al.*¹⁸⁸ studied the equilibrium.



using mass spectroscopic methods. The Knudsen effusion–mass spectrometer technique has been used to obtain $\Delta_f H^\circ$ (g) for NpF_4 ,¹⁸⁹ and NpO_2F_2 (cr).¹⁹⁰ From

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the vapour pressures of decomposition of small samples, $\Delta_f H^\circ$ was obtained for AmH_2 (cr) and CmH_2 (cr).¹⁹¹ By means of high temperature drop calorimetry, Jung and Kleppa¹⁹² determined $\Delta_f H^\circ$ for Ru_3U (cr), RhU_3 (cr), and PdU_3 (cr).

The thermochemistry of the Ga/Cl system has been investigated by measuring vapour pressures over Ga/GaCl₃ mixtures¹⁹³ and by Knudsen cell-mass spectrometer studies¹⁹⁴ leading to $\Delta_f H^\circ(\text{g})$ values for GaCl, Ga₂Cl₂, GaCl₂, Ga₂Cl₄ in reasonably good agreement. Vapour pressure measurements have led to $\Delta_f H^\circ$ for FeS (cr) and FeS₂ (cr), in good agreement with calorimetric values,¹⁹⁵ to $\Delta_f H^\circ$ (Al₂Cl₂, g),¹⁹⁶ to $\Delta_f H^\circ$ for ZnSe (cr), and ZnTe (cr),¹⁹⁷ P₄S₃ (cr),¹⁹⁸ and Li₄As₂O₇ (cr),¹⁹⁹ and SnBr₂ (cr).²⁰⁰ The Knudsen-mass spectrometer method has led to $\Delta_f H^\circ$ values for SnBr (g),²⁰¹ InCl₃ (g), InCl (g), In₂Cl₆ (g), In₂Cl₄ (g),²⁰² OsO₃ (g),²⁰³ LiCrO₂ (cr), and NaCrO₂ (cr),²⁰⁴ SbNbO₄ (cr),²⁰⁵ SrBO₂ (g) and SrB₂O₄ (cr),²⁰⁶ CrCl (g) and CrClO (g).²⁰⁷

High temperature mixing calorimetric measurements have led to $\Delta_f H^\circ$ (cr) values for IrZr, IrHf, IrTi, and OsTi,²⁰⁸ Ni_{2.55}P,²⁰⁹ Pd₃P,²¹⁰ GeS₂, GeSe₂, SnSe₂, SnSe, and PbSe,²¹¹ and Cu₃P.²¹²

Solid state electrochemical cells are useful for obtaining the thermodynamic quantities for chemical reactions and recent measurements have been employed to derive $\Delta_f H^\circ$ (cr) values of the following: MoTe₂,²¹³ RuO₂,²¹⁴ DyCl₂,²¹⁵ TmCl₂,²¹⁶ NdCl₂,²¹⁷ LaCl₃ and PrCl₃,²¹⁸ CeCl₃,²¹⁹ K₂MgF₄ and KMgF₃.²²⁰

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Empirical correlations of thermochemical data can be of value in the estimation of unknown values and in assessing the reliability of existing data. Hisham and Benson,²²¹ have proposed a two parameter relation,

$$[\Delta_f H(MX_n) - \Delta_f H(MY_n)] = a[\Delta_f H(MX_n) - \Delta_f H(MZ_n)] + b \quad (9)$$

which correlates $\Delta_f H$ for any three classes of compounds, MX_n , MY_n , and MZ_n , where a and b are constants for any particular group. Dias *et al.*²²² have demonstrated good linear plots of $\Delta_f H^\circ(\text{cr})$ and of $\Delta_f H^\circ(\text{g})$ of homoleptic halides MX_n against $\Delta_f H(HX, \text{g})$, a simple and useful correlation.

Organometallic Compounds. – The thermochemistry of organosilicon compounds has been reviewed by Walsh.²²³ The enthalpies of formation of alkylsilanes have been discussed in terms of bond additivity and SCF calculations by Gordon *et al.*²²⁴ The enthalpy of formation of hexamethyldisilane was determined from the enthalpy of bromination to form trimethylsilicon bromide by Pilcher *et al.*²²⁵ to give $\Delta_f H(Si_2Me_6, l) = -341.1 \pm 5.5 \text{ kJ mol}^{-1}$, about 50 kJ mol^{-1} less negative than previous values determined by combustion calorimetry. Of the various consequences of this new value, the enthalpies of formation of the silylenes were derived as $SiH_2 266 \pm 6$, $SiHMe 201 \pm 6$, and $SiMe_2 140 \pm 6 \text{ kJ mol}^{-1}$. Carson *et al.*²²⁶ from rotating-bomb calorimetry and Knudsen-torsion vapour pressure measurements report $\Delta_f H^\circ(\text{cr})$ and $\Delta_f H^\circ(\text{g})$ for $Ph_3GeCh=CH_2$, $Ph_3GeC\equiv CC_6H_5$ and $(Ph_3Ge)_2O$: the same group have measured $Ph_2Ge-(CH_2)_4$ and showed that the 5-membered ring appeared to be strain-free.²²⁷ Carson *et al.*²²⁸ applied similar techniques and analysis to both $Ph_2Sn-(CH_2)_4$ and $Ph_2Sn-(CH_2)_5$ and there was no evidence of strain-energy in either of these cyclic compounds. Kirklin and Domalski have made rotating-bomb measurements on phosphorus compounds with complete analyses of the final products and report $\Delta_f H^\circ(\text{cr})$ for triphenylphosphine and triphenylphosphine oxide,²²⁹ these authors also report $\Delta_f H^\circ$ for triphenylphosphate.²³⁰ Barnes *et al.*²³¹ report $\Delta_f H^\circ$ for triphenylarsine oxide and derived $D(Ph_3As-O)$ as $429.3 \pm 30.5 \text{ kJ mol}^{-1}$, which may be compared with $D(Ph_3P-O) = 566.6 \pm 8.4 \text{ kJ mol}^{-1}$. Mortimer and Waterhouse report the enthalpies of formation of $PhSeBr(\text{cr})$ and $PhSeBr_3(\text{cr})$ from the enthalpy of reaction of Ph_2Se_2 with bromine.²³² Dimethyltelluride and divinyltelluride were measured by Tel'noi *et al.*²³³ using static-bomb calorimetry and the mean dissociation enthalpies $\langle D(\text{Te-Me}) \rangle = 212.1 \pm 5.4 \text{ kJ mol}^{-1}$ and $\langle D(\text{Te-C}_2\text{H}_3) \rangle = 226.8 \pm 9.6 \text{ kJ mol}^{-1}$ were derived.

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Complexes. – Reaction–solution calorimetry is the most widely used method for determining $\Delta_f H^\circ$ of complexes as these generally hydrolyse rapidly and completely in an acidic solvent. Most results reported are for metal β -diketonates, for example of pentane-2,4-dione (HPD), 2,6-dimethylheptane-3,5-dione (HPIRM), 2,2,6-trimethylheptane-3,5-dione (HIBPM), 2,2,6,6-tetramethylheptane-3,5-dione (HDPM), trifluoro and hexafluoropentane-2,4-diones (HTFAC) and (HHFAC), respectively, and benzoylacetone (HBZAC). To derive $\langle D(M—O) \rangle$ in metal β -diketonates from $\Delta_f H^\circ(g)$, $D(O—H)$ in the enol form of the β -diketone is required and various estimates have been proposed ranging from 365 to 420 kJ mol⁻¹. Sharpe and Richardson²³⁴ by Fourier transform ion cyclotron resonance, obtained $D(O—H)$ in the enol form of pentane-2,4-dione as 368 ± 25 kJ mol⁻¹, the large uncertainty reflects the difficulty of measurement.

$\Delta_f H^\circ$ of all the group II pentane-2,4-dionates, Be to Hg, were reported by Ribeiro da Silva *et al.*²³⁵ A linear correlation was obtained by plotting $\langle D(M—O) \rangle$ versus $\langle D(M—O) \rangle$, oxide), the latter derived from the enthalpy of decomposition, $MO(\text{cr}) \rightarrow M(\text{g}) + O(\text{g})$. For Hg(PD)₂, the binding energy of the ligand was of the same order of magnitude as $\langle D(Hg—C) \rangle$ in Hg(CH₃)₂, hence this ligand could be bound to Hg through C and not O.

Ribeiro da Silva and Ferrão²³⁶ report $\Delta_f H^\circ$ for Fe(BZAC)₃, Fe(DPM)₃, Fe(TFAC)₃, and Fe(HFAC)₃; $\langle D(Fe—O) \rangle$ in these complexes was found to be constant within the experimental uncertainty, and equal to that found previously in Fe(PD)₃. Other $\Delta_f H^\circ$ values reported for β -diketonates by Ribeiro da Silva *et al.* include Cu(TFAC)₂ and Cu(HFAC)₂,²³⁷ Zr(PD)₄ and Zr(TFAC)₄,²³⁸ Co(BZAC)₃, Co(DPM)₃ and Co(TFAC)₃,²³⁹ Mn(TFAC)₂ · H₂O (cr), Mn(HFAC)₂ · 2H₂O (cr), Co(HFAC)₂ · 2H₂O (cr), and Ni(HFAC)₂ · 2H₂O (cr), and the enthalpies of formation of the anhydrous complexes in the gaseous state were derived from microcalorimetric measurements of the enthalpies of decomposition, e.g. Mn(TFAC)₂ · H₂O (cr) → Mn(TFAC)₂ (g) + H₂O (g),²⁴⁰ and complexes with 3-methylpentane-2,4-dione (MPD), Cu(MPD)₂,²⁴¹ Fe(MPD)₃,²⁴² Murata *et al.*²⁴³ have measured $\Delta_f H^\circ(\text{cr})$ for Be(PD)₂, showing no difference between the α and γ crystal forms, and also Be₄O(C₂H₅CO₂)₆ (cr) and Be₄O(NO₃)₆ (cr). The enthalpy of combustion of Al(PD)₃ was reported by Mosin *et al.*²⁴⁴ Ribeiro da Silva *et al.*²⁴⁵ report $\Delta_f H^\circ(\text{cr})$ and $\Delta_f H^\circ(g)$ for Cu₂(acetate)₄, Cu(dimethylglyoxime)₂,

²³⁴ P. E. Sharpe and D. E. Richardson, *J. Am. Chem. Soc.*, 1991, **113**, 8339.

²³⁵ M. A. V. Ribeiro da Silva, G. Pilcher, and R. J. Irving, *J. Chem. Thermodyn.*, 1988, **20**, 985.

²³⁶ M. A. V. Ribeiro da Silva, and M. L. C. C. H. Ferrão, *J. Chem. Thermodyn.*, 1988, **20**, 79.

²³⁷ M. A. V. Ribeiro da Silva, and M. L. C. C. H. Ferrão, *J. Chem. Thermodyn.*, 1988, **20**, 359.

²³⁸ M. A. V. Ribeiro da Silva, M. L. C. C. H. Ferrão, R. M. C. Marques, and J. M. T. Lima, *J. Chem. Thermodyn.*, 1992, **24**, 595.

²³⁹ M. A. V. Ribeiro da Silva and M. L. C. C. H. Ferrão, *Bull. Chem. Soc. Jpn.*, 1988, **61**, 1755.

²⁴⁰ M. A. V. Ribeiro da Silva and M. L. C. C. H. Ferrão, *Thermochim. Acta*, 1989, **139**, 33.

²⁴¹ M. A. V. Ribeiro da Silva, M. L. C. C. H. Ferrão, M. J. S. Monte, R. M. G. Esteves da Silva, and J. C. Ribeiro, *J. Chem. Thermodyn.*, 1992, **24**, 585.

²⁴² M. A. V. Ribeiro da Silva, M. L. C. C. H. Ferrão, and R. M. G. Esteves da Silva, *J. Chem. Thermodyn.*, 1992, **24**, 1293.

²⁴³ S. Murata, M. Sakiyama, and S. Seki, *J. Chem. Thermodyn.*, 1988, **20**, 1203.

²⁴⁴ A. M. Mosin, V. G. Genchel, N. Kh. Samorukova, R. M. Aizatullova, S. L. Gershkoken, and N. N. Korneev, *Russ. J. Phys. Chem.*, 1990, **64**, 1205.

²⁴⁵ M. A. V. Ribeiro da Silva, M. D. M. C. Ribeiro da Silva, M. C. S. S. Rangel, G. Pilcher, M. J. Akello, A. S. Carson, and E. H. Jamea, *Thermochim. Acta*, 1990, **160**, 267.

$\text{Cu(4-phenylamino-3-penten-2-onate)}_2$, and Cu(glycine)_2 . It was found that glycine was the most strongly bound ligand and that if a (Cu—Cu) bond is postulated in $\text{Cu}_2(\text{acetate})_4$, its contribution to the total binding energy will be very small.

Dialkyldithiocarbamate complexes, $M(S_2\text{CNR}_2)_n$, where $R = -C_2H_5$, $-C_3H_7$, $-CH(CH_3)_2$, $-C_4H_9$, $-CH_2CH(CH_3)_2$ have been studied. Ribeiro da Silva and Reis have determined $\Delta_f H(\text{cr})$ and $\Delta_f H(\text{g})$ for $\text{Cu}(S_2\text{CNR}_2)_2$,²⁴⁶ $\text{Ni}(S_2\text{CNR}_2)_2$,²⁴⁷ and for $\text{Fe}(S_2\text{CNR}_2)_3$, $\text{Cr}(S_2\text{CNR}_2)_3$, $\text{Co}(C_2\text{CNR}_2)_3$ and $\text{Mn}(S_2\text{CNR}_2)_3$;²⁴⁸ for each metal $\langle D(M-S) \rangle$ appears constant, except for $R = -CH(CH_3)_2$ when a lower value suggests steric hindrance in the complex. Airoldi and de Souza²⁴⁹ have determined $\Delta_f H^\circ(\text{cr})$ for the tris(di-n-butylthiocarbamate) complexes of P, As, Sb, and Bi, with estimated enthalpies of sublimation $\langle D(M-S) \rangle$ falls through the series from 214 to 156 kJ mol⁻¹ as expected; the same authors report $\Delta_f H^\circ(\text{cr})$ for the tris(diethyldithiocarbamate) complexes of Sb and Bi and $\langle D(Sb-S) \rangle$ is larger than $\langle D(Bi-S) \rangle$.²⁵⁰

Souza *et al.*²⁵¹ made microcalorimetric measurements on the Cu^{II} and Ni^{II} complexes with the Schiff base derived from 2-(2-aminophenyl) benzimidazole and salicylaldehyde, the average binding energy of the ligand to Ni was greater than that to Cu by about 31 kJ mol⁻¹.

Ribeiro da Silva *et al.* have reported $\Delta_f H^\circ$ values for amino acid complexes with Cu^{II}²⁵² and Ni^{II}²⁵³ with the amino acids, glycine, DL-alanine, DL-valine, L-(α)-leucine, L-(α)-isoleucine, L-(α)-phenylglycine, and L-(α)-phenylalanine. It is observed experimentally that for any complex ML_n , $\langle D(M-L) \rangle - D(H-L)$ is constant in the absence of steric hindrance in the complex, and a consequence is that a plot of $\Delta_f H^\circ(ML_n, \text{g})$ versus $\Delta_f H(H-L, \text{g})$ should be linear with slope n , and such a correlation should also apply in the condensed state.²⁵⁴ For the copper amino acid complexes, least-squares fitting yielded, $\Delta_f H^\circ(\text{CuL}_2, \text{cr}) = 119.60 + 2.014\Delta_f H^\circ(\text{HL}, \text{cr})$ with a correlation coefficient of 0.997, and for the nickel amino acid complexes $\Delta_f H^\circ(\text{NiL}_2, \text{cr}) = 17.09 + 1.947\Delta_f H^\circ(\text{HL}, \text{cr})$ with a correlation coefficient of 0.997, demonstrating that these enthalpies of formation fit well into these relations.

Santos and Airoldi²⁵⁵ report $\Delta_f H$ for a series of urea adducts of group II halides, ZnX_2 , CdX_2 , HgX_2 , with the order of binding energy $Zn < Cd > Hg$. A similar order of binding energies with the same halides was observed for adducts of thioacetamide and thiobenzamide.²⁵⁶ Santos *et al.*²⁵⁷ report $\Delta_f H$ values and binding energies for tetramethylurea adducts with SbI_3 and BiI_3 .

The thermochemistry of adducts $\text{AsX}_3 \cdot nL$ ($X = \text{Cl}$, Br , I , $n = 1$, 1.5, 2,

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²⁴⁸ M. A. V. Ribeiro da Silva and A. M. M. V. Reis, *J. Chem. Thermodyn.*, 1992, **24**, 401.

²⁴⁹ C. Airoldi and A. G. de Souza, *J. Chem. Thermodyn.*, 1989, **21**, 283.

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²⁵¹ P. Souza, M. I. Paz Andrade, and G. Pilcher, *Thermochim. Acta*, 1991, **190**, 203.

²⁵² M. A. V. Ribeiro da Silva, M. D. M. C. Ribeiro da Silva, M. M. C. Bernado, and L. M. N. B. F. Santos, *Thermochim. Acta*, 1992, **205**, 99.

²⁵³ M. A. V. Ribeiro da Silva, M. D. M. C. Ribeiro da Silva, M. M. C. Bernado, and L. M. N. B. F. Santos, *Thermochim. Acta*, 1992, **205**, 115.

²⁵⁴ G. Pilcher, *Pure Appl. Chem.*, 1989, **61**, 855.

²⁵⁵ M. R. M. C. Santos and C. Airoldi, *Thermochim. Acta*, 1988, **125**, 295.

²⁵⁶ C. Airoldi and E. A. Digiampietri, *J. Chem. Thermodyn.*, 1992, **24**, 33.

²⁵⁷ L. C. R. Santos, S. F. de Oliveira, and C. Airoldi, *Thermochim. Acta*, 1992, **206**, 13.

$L =$ pyridine, β -picoline, γ -picoline) was reported by Dunstan and Airoldi,²⁵⁸ also that of the corresponding pyridine N -oxide and picoline N -oxide adducts,²⁵⁹ and of the pyridine complexes of PhAsX_2 ($X = \text{Cl}, \text{Br}, \text{I}$).²⁶⁰ In all these adducts $\langle D(\text{As}-\text{N}) \rangle$ showed large variations, in the latter case varying from 112 to 217 kJ mol^{-1} , in the order I > Br > Cl.

Birkenshaw *et al.*²⁶¹ report $\Delta_f H^\circ$ ($\text{Co}(\text{cytosine})_2\text{Cl}_2$, cr) and derived $\langle D(\text{Co-cytosine}) \rangle = 216 \pm 13 \text{ kJ mol}^{-1}$, and Evans and Mortimer²⁶² studied *trans*- $\text{PdCl}_2(\text{TeEt}_2)_2$. Kuriyama and Sakiyama,²⁶³ by reaction-solution calorimetry, determined $\Delta_f H^\circ$ ($\text{Ni}(\text{NH}_3)_6\text{Cl}_2$, cr).

4 Bond Dissociation Energies, Radicals and Ions

Organic Species. — Although experimental measurements show that the largest (C—H) bond dissociation energy is in acetylene, its precise value is still uncertain. Measurement in a single experiment of the translational energy release in $\text{C}_2\text{H}_2 \rightarrow \text{C}_2\text{H} + \text{H}$, in a crossed molecular beam photodissociation, gave $552 \pm 8 \text{ kJ mol}^{-1}$.²⁶⁴ From photodissociation in the 201–216 nm region, Baldwin *et al.*²⁶⁵ obtained $D(\text{HC}_2-\text{H}) = 548 \pm 4 \text{ kJ mol}^{-1}$, but Green *et al.*,²⁶⁶ from Stark anti-crossing spectroscopy, obtained an upper limit of $529.9 \pm 0.1 \text{ kJ mol}^{-1}$, and Segall *et al.*,²⁶⁷ from 193.3 nm photolysis with sub-Doppler resolution, also claim an upper limit of $531.4 \pm 6.3 \text{ kJ mol}^{-1}$. An *ab initio* calculation by Curtiss and Pople²⁶⁸ gave $558.6 \pm 9.6 \text{ kJ mol}^{-1}$. At present the two upper limit determinations seem the most acceptable, but further experimental studies of this quantity are to be expected. In contrast, Bartmess and Griffith²⁶⁹ have found the smallest $D(\text{C}-\text{H})$ so far in 3-methylene-1,4-cyclohexadiene, $268 \pm 13 \text{ kJ mol}^{-1}$, from its enthalpy of isomerization to toluene by reaction-solution calorimetry.

A novel method of determining bond dissociation energies has been proposed by Chen *et al.*²⁷⁰ from the activation energy for dissociative thermal electron attachment, requiring knowledge of the electron affinity of the molecule. The method was tested against a large number of known values and appears to be accurate to $\pm 10 \text{ kJ mol}^{-1}$. Sawyer²⁷¹ claimed to measure bond dissociation energies from redox potentials in aqueous solution, but it was pointed out that solvent effects were not taken into account.²⁷²

Some recently determined bond dissociation energies in organic molecules are listed in Table 2, most were determined by well established methods from kinetics

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- ²⁶¹ P. M. Birkenshaw, C. T. Mortimer, and E. G. Tyler, *Thermochim. Acta*, 1988, **131**, 95.
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Table 2 Bond Dissociation Energies in Organic Molecules (kJ mol^{-1})

		Ref.		Ref.
CH_3-H	436.2 ± 1.3	273	$\text{Br}_2\text{CH}-\text{H}$	417.1 ± 4.2
$\text{C}_2\text{H}_3-\text{H}$	446.8 ± 3.3	274	$\text{ClFCH}-\text{H}$	421.7 ± 4.2
$\text{CH}_3\text{CO}-\text{H}$	373.8 ± 1.5	275	$\text{Cl}_2\text{FC}-\text{H}$	410.0 ± 4.2
HSCH_2-H	387 ± 8	276	$\text{C}_6\text{H}_5\text{O}-\text{H}$	351.5 ± 4.0
$\text{CH}_3\text{S}-\text{H}$	360 ± 3	276	$p\text{-CF}_3\text{C}_6\text{H}_4\text{O}-\text{H}$	364.8 ± 4.0
$\text{C}_6\text{H}_5\text{CH}_2-\text{H}$	378 ± 4	277	$p\text{-ClC}_6\text{H}_4\text{O}-\text{H}$	353.1 ± 4.0
$\text{C}_6\text{H}_5\text{CH}_2-\text{I}$	190 ± 4	277	$p\text{-Bu}^t\text{C}_6\text{H}_4\text{O}-\text{H}$	343.5 ± 4.0
$\text{C}_6\text{H}_5\text{CH}_2-\text{CH}_3$	329 ± 4	277	$p\text{-MeOC}_6\text{H}_4\text{O}-\text{H}$	326.8 ± 4.0
$\text{C}_6\text{H}_5\text{O}-\text{CH}_3$	255 ± 8	278	$\text{C}_2\text{F}_5-\text{Cl}$	343.1 ± 5.0
$\text{NC}-\text{CN}$	563.5 ± 1.9	279	CF_3-I	222 ± 4
ClCH_2-H	421.7 ± 4.2	280	$\text{C}_2\text{F}_5-\text{I}$	215 ± 4
BrCH_2-H	425.1 ± 4.2	280	$\text{C}_3\text{F}_7-\text{I}$	209 ± 4
$\text{Cl}_2\text{CH}-\text{H}$	411.7 ± 4.2	280	$\text{C}_4\text{F}_9-\text{I}$	206 ± 4

and photoionization, but for the parasubstituted phenols the values derive from photoacoustic calorimetry.

Time resolved photoacoustic calorimetry has led to the enthalpies of formation of some singlet carbene radicals $\text{RR}'^1\text{C}$, with $\text{R} = \text{C}_6\text{H}_5$ and $\text{R}' = \text{F}, \text{Cl}, \text{Br}^{284}$ and to $\Delta_f H$ for cyclopentane-1,3-diyl and 2-isopropylidinycyclopentane-1,3-diyl.²⁸⁵ From the kinetics of decomposition of azoalkanes, $\Delta_f H$ values for the azoradicals CH_3N_2 , $\text{C}_2\text{H}_5\text{N}_2$, and $(\text{CH}_3)_2\text{CHN}_2$ have been derived.²⁸⁶ Clauberg *et al.*,²⁸⁷ using photoionization, determined $\Delta_f H$ of the cyclopropylidene radical. The enthalpy of formation of the cyclopentadienyl radical is of importance in deriving dissociation energies in metal cyclopentadienyl derivatives. A careful kinetic study of the reaction of atomic iodine with cyclopentadiene by Puttemans *et al.*²⁸⁸ has led to a revision of the value of this $\Delta_f H$ to be $242.7 \pm 8.3 \text{ kJ mol}^{-1}$, which differs from the previously accepted value, $264.4 \pm 9.0 \text{ kJ mol}^{-1}$, obtained from ion–cyclotron resonance studies.²⁸⁹

Inorganic Species. – Recent years have been productive in the number of determinations of bond dissociation energies in inorganic species. The main experimental methods have been mass spectroscopic analysis of the effusate from Knudsen cells

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Table 3 Bond Dissociation Energies in Simple Inorganic Species (kJ mol^{-1})

		Ref.			Ref.
NH_2-H	447.7 ± 1.3	291	$\text{Al}-\text{Br}$	425 ± 6	299
$\text{N}_2\text{H}_3-\text{H}$	366 ± 12	292	$\text{Ag}-\text{F}$	356 ± 8	300
$\text{CO}-\text{Cl}$	32.2 ± 2.5	293	$\text{OTh}-\text{F}$	594 ± 8	301
$\text{C}-\text{S}$	710.4 ± 3.6	294	$\text{FTh}-\text{O}$	813 ± 8	301
$\text{SC}-\text{S}$	434.3 ± 3.8	294	$\text{V}-\text{N}$	523 ± 37	302
$\text{SC}-\text{O}$	664.0 ± 2.9	294	$\text{Cu}-\text{In}$	184 ± 8	303
$\text{F}_5\text{S}-\text{F}$	397 ± 13	295	$\text{Ag}-\text{In}$	163 ± 5	303
$\text{Na}-\text{O}$	266 ± 4	296	$\text{Au}-\text{In}$	282 ± 6	303
$\text{Na}-\text{ONa}$	228 ± 8	296	$\text{Ti}-\text{Ir}$	418 ± 13	304
$\text{Mg}-\text{O}$	247 ± 21	297	$\text{Ni}-\text{Pr}$	270.0 ± 0.3	305
$\text{Mg}-\text{OH}$	280 ± 25	297	$\text{Ni}-\text{Pd}$	≈ 141	306
$\text{Ca}-\text{I}$	280 ± 8	298	$\text{Pt}-\text{Pd}$	≈ 191	306
$\text{Ba}-\text{I}$	319 ± 8	298	Pd_2	< 136	306

held at high temperatures and the guided ion beam tandem mass spectroscopic method as described by Schultz and Armentrout,²⁹⁰ which has been applied effectively to produce values for simple species and, very importantly, values for cations which have only recently become available.

A selection of recent values for bond dissociation energies for simple species is given in Table 3. Of note, in the sequence CuIn, AgIn, and AuIn, the high value for the latter suggests the possibility of multiple bonding. The low values for Pd diatomics are a consequence of the highly stable $4d^{10}5s^0, ^1\text{S}_0$ ground state of Pd. The high value for V—N would indicate multiple bonding.

The Knudsen cell plus mass spectrometer technique has been exploited to determine successive dissociation energies in UF_6 ,³⁰⁰ PuF_6 ,³⁰⁷ RuF_4 ,³⁰⁸ ThF_4 ,³⁰¹ ThCl_4 ,³⁰⁹ ThBr_4 ,³¹⁰ ZrF_4 ,³¹¹ and OsO_4 .³¹² These determinations represent a splendid achievement and the values are listed in Table 4. It does appear that the sequence of values in corresponding series are similar and there seem to be no anomalous values.

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- ³⁰⁵ S. Taylor, E. M. Spain, and M. D. Morse, *J. Chem. Phys.*, 1990, **92**, 2698.
- ³⁰⁶ S. Taylor, E. M. Spain, and M. D. Morse, *J. Chem. Phys.*, 1990, **92**, 2710.
- ³⁰⁷ P. D. Kleinschmidt, *J. Chem. Phys.*, 1988, **89**, 6897.
- ³⁰⁸ D. L. Hildenbrand and K. H. Lau, *J. Chem. Phys.*, 1988, **89**, 5825.
- ³⁰⁹ K. H. Lau and D. L. Hildenbrand, *J. Chem. Phys.*, 1990, **92**, 6124.
- ³¹⁰ D. L. Hildenbrand and K. H. Lau, *J. Chem. Phys.*, 1990, **93**, 5983.
- ³¹¹ N. V. Barkovskii, L. N. Gorokov, A. M. Emel'yanov, Yu. S. Khodeev, and V. I. Tsirel'nikov, *Russ. J. Phys. Chem.*, 1988, **62**, 1521.
- ³¹² D. L. Hildenbrand and K. H. Lau, *J. Phys. Chem.*, 1992, **96**, 2325.

Table 4 Successive Bond Dissociation Energies (kJ mol^{-1})

$\text{F}_5\text{U}-\text{F}$	297 ± 8	$\text{F}_5\text{Pu}-\text{F}$	222 ± 8	$\text{F}_3\text{Ru}-\text{F}$	361 ± 8
$\text{F}_4\text{U}-\text{F}$	410 ± 8	$\text{F}_4\text{Pu}-\text{F}$	452 ± 50	$\text{F}_2\text{Pu}-\text{F}$	339 ± 8
$\text{F}_3\text{U}-\text{F}$	615 ± 8	$\text{F}_3\text{Pu}-\text{F}$	473 ± 21	$\text{F}\text{Ru}-\text{F}$	464 ± 8
$\text{F}_2\text{U}-\text{F}$	619 ± 8	$\text{F}_2\text{Pu}-\text{F}$	590 ± 60	$\text{Ru}-\text{F}$	402 ± 8
$\text{FU}-\text{F}$	565 ± 8				
$\text{U}-\text{F}$	648 ± 8				
$\text{F}_3\text{Th}-\text{F}$	666 ± 8	$\text{Cl}_3\text{Th}-\text{Cl}$	504 ± 8	$\text{Br}_3\text{Th}-\text{Br}$	510 ± 6
$\text{F}_2\text{Th}-\text{F}$	653 ± 8	$\text{Cl}_2\text{Th}-\text{Cl}$	507 ± 8	$\text{Br}_2\text{Th}-\text{Br}$	477 ± 6
$\text{FTh}-\text{F}$	702 ± 8	$\text{Cl}\text{Th}-\text{Cl}$	454 ± 8	$\text{Br}\text{Th}-\text{Br}$	439 ± 6
$\text{Th}-\text{F}$	652 ± 8	$\text{Th}-\text{Cl}$	489 ± 8	$\text{Th}-\text{Br}$	364 ± 6
$\text{F}_3\text{Zr}-\text{F}$	610 ± 10	$\text{O}_3\text{Os}-\text{O}$	435 ± 21		
$\text{F}_2\text{Zr}-\text{F}$	624 ± 12	$\text{O}_2\text{Os}-\text{O}$	570 ± 17		
$\text{FZr}-\text{F}$	670 ± 15	$\text{O}\text{Os}-\text{O}$	542 ± 17		
$\text{Zr}-\text{F}$	672 ± 15	$\text{Os}-\text{O}$	575 ± 20		

Table 5 Bond Dissociation Energies in Inorganic Cations (kJ mol^{-1})

		Ref.		Ref.
Mg^+-OH	314 ± 17	297	Ti^+-N	500 ± 13
Mg^+-O	222 ± 13	297	Co^+-NH_2	257 ± 9
Ca^+-O	344 ± 5	313	Ni^+-NH_2	233 ± 8
Cr^+-O	359 ± 12	313	Cu^+-NH_2	202 ± 12
Mn^+-O	385 ± 13	313	V^+-NH_2	307 ± 9
Fe^+-O	340 ± 6	313	V^+-NH	414 ± 6
Co^+-O	320 ± 6	313	V^+-N	449 ± 6
Ni^+-O	264 ± 7	313	Fe^+-NH	255 ± 21
Cu^+-O	156 ± 14	313	Fe^+-S	255 ± 25
Zn^+-O	159 ± 12	313	Fe^+-S_2	200 ± 21
Sc^+-O	686 ± 8	314	FeS^+-S	205 ± 21
Ti^+-O	664 ± 7	314	FeS_2^+-S	205 ± 21
V^+-O	560 ± 16	314	FeS_3^+-S	180 ± 21
SAc^+-NH_2	356 ± 7	315	FeS_4^+-S	159 ± 21
Sc^+-NH	498 ± 10	315	Ni^+-Pt	233 ± 14
Ti^+-NH_2	356 ± 13	315	Pt^+-Ni	345 ± 33
Ti^+-NH	466 ± 12	315		

The determination of bond dissociation energies in simple inorganic cations is a relatively new activity; they are mainly determined either by using the guided ion beam tandem mass spectroscopic method or by photoionization of molecular beams. Recent values for M^+-O , M^+-N , and M^+-S bonds are listed in Table 5.

With so many results there are some instances of duplication by different workers using different methods and in all such cases agreement within experimental uncertainty was obtained. A detailed discussion of the results in Table 5 is inappropriate here but some simple trends are immediately apparent. The bonds Ti^+-X , V^+-X and Sc^+-X have such high dissociation energies that multiple bonding must be

³¹³ E. R. Fisher, J. L. Elkend, D. E. Clemmer, R. Georgiadis, S. K. Loh, N. Aristov, L. S. Sunderlin, and P. B. Armentrout, *J. Chem. Phys.*, 1990, **93**, 2676.

³¹⁴ D. E. Clemmer, J. L. Elkend, N. Aristov, and P. B. Armentrout, *J. Chem. Phys.*, 1991, **95**, 3387.

³¹⁵ D. E. Clemmer, L. S. Sunderlin, and P. B. Armentrout, *J. Phys. Chem.*, 1990, **94**, 3008.

³¹⁶ D. E. Clemmer and P. B. Armentrout, *J. Phys. Chem.*, 1991, **95**, 3084.

³¹⁷ D. E. Clemmer, L. S. Sunderlin, and P. B. Armentrout, *J. Phys. Chem.*, 1990, **94**, 208.

³¹⁸ T. J. MacMahon, T. C. Jackson, and B. S. Freiser, *J. Am. Chem. Soc.*, 1989, **111**, 421.

involved. The low values for $\text{Cu}^+—\text{O}$, $\text{Zn}^+—\text{O}$, and the drop in $\text{FeS}_n^+—\text{S}$ as n increases, are to be expected from comparison with the behaviour of neutral species.

Organometallic Species. — The recent increase in activity in investigation of bond strengths in organometallic species is very great. A NATO Advanced Study Institute on the Energetics of Organometallic Species was held in Portugal in 1991, and the lectures have been published:³¹⁹ these included discussion of calorimetry, electrochemical methods and cyclic voltammetry, enthalpies of sublimation, kinetic studies, mass spectroscopic techniques of ion cyclotron resonance and guided beams, together with critical examination of bond strength, estimation methods and theoretical investigations. Transition metal–hydrogen and metal–carbon bond strengths as keys to catalysis were reviewed by Martinho Simões and Beauchamp.³²⁰

An area of interest is in the dissociation energies of the ions $\text{M}^+—\text{H}$, $\text{M}^+—\text{CH}_3$, and $\text{M}^+—\text{CH}_2$, some of which are listed in Table 6. Trends in these $D(\text{M}^+—\text{X})$ values can be understood by considering the energy of promotion of the metal atom from its ground state to a $4s^1 3d^{n-1}$ configuration where the $4s$ electron is spin decoupled from the $3d$ electrons. For ($\text{M}^+—\text{CH}_2$) a double bond is involved and for the promotion energy two electrons from the metal atom must be spin decoupled. A clear indication is that the values for Cr and Cu are low as these metals have stable half-filled and fully filled $3d$ electron shells. Linear plots of dissociation energy versus promotion energies

Table 6 Bond Dissociation Energies in Organometallic Cations (kJ mol^{-1})

	$\text{M}^+—\text{H}$	Ref.	$\text{M}^+—\text{CH}_3$	Ref.	$\text{M}^+—\text{CH}_2$	Ref.
Sc	239 ± 9	321	247 ± 13	321	412 ± 22	321
Ti	231 ± 9	322	241 ± 12	323	391 ± 15	323
V	198 ± 8	322	209 ± 10	324	318 ± 6	329
Cr	116 ± 8	322	127 ± 7	325	225 ± 15	330
Mn	199 ± 14	322	215 ± 10	326	295 ± 13	326
Fe	197 ± 16	322	242 ± 10	327	347 ± 17	331
Co	190 ± 10	322	205 ± 15	328	324 ± 10	332
Ni	161 ± 13	322	188 ± 10	328	315 ± 8	332
Cu	91 ± 9	322	124 ± 7	328	267 ± 7	332
Y	261 ± 6	321	249 ± 5	321	398 ± 13	321
La	249 ± 9	321	231 ± 14	321	411 ± 6	321
Lu	204 ± 15	321	190 ± 20	321		

³¹⁹ 'Energetics of Organometallic Species', NATO ASI Series C, Vol. 367: ed. J. A. Martinho Simões, Kluwer, Dordrecht, Netherlands, 1992.

³²⁰ J. A. Martinho Simões and J. L. Beauchamp, *Chem. Rev.*, 1990, **90**, 629.

³²¹ L. S. Sunderlin and P. B. Armentrout, *J. Am. Chem. Soc.*, 1989, **111**, 3845.

³²² V. L. Elkind and P. B. Armentrout, *Inorg. Chem.*, 1986, **25**, 1078.

³²³ L. S. Sunderlin and P. B. Armentrout, *J. Phys. Chem.*, 1988, **92**, 1209.

³²⁴ N. Aristov and P. B. Armentrout, *J. Am. Chem. Soc.*, 1986, **108**, 1806.

³²⁵ E. R. Fisher and P. B. Armentrout, *J. Am. Chem. Soc.*, 1992, **114**, 2039.

³²⁶ L. S. Sunderlin and P. B. Armentrout, *J. Phys. Chem.*, 1990, **94**, 3589.

³²⁷ R. H. Schultz, J. L. Elkind, and P. B. Armentrout, *J. Am. Chem. Soc.*, 1988, **110**, 411.

³²⁸ R. Georgiadis, E. R. Fisher, and P. B. Armentrout, *J. Am. Chem. Soc.*, 1989, **111**, 4251.

³²⁹ N. Aristov and P. B. Armentrout, *J. Phys. Chem.*, 1987, **91**, 6178.

³³⁰ R. Georgiadis and P. B. Armentrout, *J. Phys. Chem.*, 1988, **92**, 7067.

³³¹ P. B. Armentrout, 'C—H Activation'; ed. J. Lieberman and A. Greenberg, VCH Publishers, New York, 1990, p.467.

³³² E. R. Fisher and P. B. Armentrout, *J. Phys. Chem.*, 1990, **94**, 1674.

are observed for these species.³¹⁹ Intrinsic bond energies, which include the promotion energies for ($M^+—CH_3$) species, are reported by Armentrout *et al.*³³³

Dissociation energies of metal cyclopentadienyl ions from Fourier transform ion cyclotron resonance mass spectrometry for V, Mn, and Ni are reported by Ryan *et al.*,³³⁴ and by the same method for the pentane-2,4-dionates of Cr, Mn, Fe, and Co and their negative ions.²³⁴ The dissociation energies of tris(1,3,5-tri-t-butylcyclopentadienyl) derivatives of Y, Gd, Dy, Ho, and Er derived from reaction calorimetric studies gave a linear plot against the enthalpies of sublimation of the metals.³³⁵

The first three dissociation ion energies for Mo(CO)₆ were determined from the pressure dependence of recombination rate constants.³³⁶ The successive bond dissociation energies for Mn(CO)₆⁺ were reported by Dearden *et al.*³³⁷ and the guided beam mass spectrometric method has led to the successive dissociation energies in Fe(CO)₅⁺.³³⁸

It is to be expected that this expanding area of the determination of thermochemical results from mass spectrometric methods will continue and will provide a valuable source of information for theoretical calculations. Theoretical calculations of energies of reaction are improving continually. Pople *et al.*^{339,340} title their research 'Theoretical Thermochemistry' and calculate by *ab initio* methods the enthalpies of formation of the hydrides of the elements of the first two periods and their ionization potentials. Agreement with experimental values is excellent, especially for some cases, *e.g.* SiH₂, PH, PH₂, where experimental values have become available after the theoretical predictions were made. It is anticipated that results from such calculations could be properly included in future reports on thermochemistry.

5 Biochemical Thermochemistry

The last report on biochemical thermochemistry was in 1987.¹ This report concerns the work published in the five year period 1987–92 and is primarily concerned with thermochemical studies which have been carried out on a range of biochemical systems, or which have a direct bearing on our understanding of the wider issue of the thermodynamics of biochemical systems. Biochemical thermodynamics is now a very well established area of study and encompasses an extremely wide range of biological and biochemical phenomena, from the thermodynamic behaviour of whole cells to the detailed thermodynamics of the biochemical interactions and reactions of small biologically important molecules. We first draw attention to some recent texts covering the general area of biochemical thermodynamics. At an introductory level the text of Smith & Wood³⁴¹ highlights the importance of energy in biological systems. It is a well produced attractive text containing overviews of

³³³ P. B. Armentrout, L. S. Sunderlin, and E. R. Fisher, *Inorg. Chem.*, 1989, **28**, 4436.

³³⁴ M. F. Ryan, J. R. Eyler, and D. E. Richardson, *J. Am. Chem. Soc.*, 1992, **114**, 8611.

³³⁵ W. A. King, T. J. Marks, D. M. Anderson, D. J. Duncalf, and F. G. N. Cloke, *J. Am. Chem. Soc.*, 1992, **114**, 9221.

³³⁶ J. A. Ganske and R. N. Rosenfeld, *J. Phys. Chem.*, 1990, **94**, 4315.

³³⁷ D. V. Dearden, K. Hayashibara, J. L. Beauchamp, N. J. Kirchner, P. A. M. van Koppen, and M. T. Bowers, *J. Am. Chem. Soc.*, 1989, **111**, 2401.

³³⁸ R. H. Schultz, K. C. Crellin, and P. B. Armentrout, *J. Am. Chem. Soc.*, 1991, **113**, 8590.

³³⁹ J. A. Pople, B. T. Luke, M. J. Frisch, and J. S. Binkley, *J. Phys. Chem.*, 1985, **89**, 2198.

³⁴⁰ J. A. Pople and L. A. Curtiss, *J. Phys. Chem.*, 1987, **91**, 155; 3637.

³⁴¹ C. A. Smith and E. J. Wood, 'Energy in biological systems', Chapman & Hall, London, 1991.

thermodynamics and bioenergetics and their importance and applications. A more specialized text by Blaxter considers the details of the energetics of metabolism in animals and man.³⁴² This text will be of interest to biothermodynamists who want to know how energy is used in maintaining organisms, particularly the energetic 'balance sheet' of biochemical processes.

At the research level, a second edition of 'Biochemical Thermodynamics'³⁴³ contains reviews of a number of areas including, for example, peptides and model systems (T. H. Lilley), conformational transitions in proteins (W. Pfeil) and nucleic acids (H. H. Klump), the thermal behaviour of lipids (M. N. Jones), conformational changes associated with energy transduction (A. G. Lowe & A. R. Walmsley) and the thermochemistry of cellular systems (I. Wadsö). The general text on 'Solution Calorimetry'³⁴⁴ edited by Marsh and O'Hare also contains chapters on biochemical systems.

Instrumentation. – Biochemical thermochemistry is largely concerned with measurements made with three types of calorimeter; (i) the differential scanning calorimeter, (ii) the batch and flow microcalorimeters, and (iii) the isothermal titration calorimeter. The differential scanning calorimeter (DSC), in which heat is supplied to both the sample and reference cells and a feed-back circuit is used to compensate for temperature differences between the two, is particularly useful for the measurement of thermal transitions in macromolecule and lipid systems. The year 1989 marked the 25th anniversary of the first publication, by Privalov *et al.*, describing a high sensitivity instrument for the measurement of very small heat effects in heated liquids. Privalov and Plotnikov³⁴⁵ have presented an interesting historical review of the development of DSCs, and recognize three significant stages in their development. The first generation of differential adiabatic scanning microcalorimeters suffered from baseline instability that was linked with the problem of loading the samples into the cells. This necessitated dismounting the adiabatic system to remove the cells. After replacement the baseline position and shape changed such that reproducible runs were difficult to achieve. The second generation of instruments used undismountable calorimetric blocks, which improved baseline stability, but the washing and refilling of cells without leaving air bubbles was a problem. The problem of free volume and loading was overcome in these instruments by using definite volumes, rather than masses as in the first generation machines. The change from mass to volume was an essential development. In the third generation of instruments, cylindrical cells with small surface to volume ratios were replaced with capillary tubes which not only have a very high surface to volume ratio but can also be easily filled and washed. Privalov and Plotnikov suggest that the sensitivity of DSCs is unlikely to be increased in the future although one can foresee improvements in the selection of materials for their construction, the accessible temperature range, and computerization and data handling. There is now a broad choice of

³⁴² K. Blaxter, 'Energy metabolism in animals and man', Cambridge University Press, Cambridge, UK, 1989.

³⁴³ 'Biochemical Thermodynamics', ed. M. N. Jones, Studies in Modern Thermodynamics 8, Elsevier, Amsterdam, 1988.

³⁴⁴ 'Experimental Chemical Thermodynamics – Solution Chemistry' ed. K. N. Marsh and P. A. G. O'Hare, Blackwell, Oxford, 1991.

³⁴⁵ P. L. Privalov and V. V. Plotnikov, *Thermochim. Acta*, 1989, **139**, 257.

commercial DSC instruments with high sensitivities available, that are capable of making measurements over a wide range of temperature from around -150°C to 750°C . The manufacturers offering a range of DSC equipment together with other forms of thermal analysis (e.g. differential thermal, thermogravimetric, and thermo-mechanical analysis) include Perkin–Elmer, DuPont, Marlin Scientific, Netzsch–Gerätebau, Polymer Laboratories, TA Instruments, Mettler Instruments, Setaram and Seiko. These instruments operate with small sample sizes, usually in a range up to approximately 0.4 cm^3 . The samples are hemetically sealed in metal pans; a procedure which not infrequently fails either at the sealing stage or during a heating run. It is unfortunate that a more sophisticated system has yet to be developed.

The batch and flow heat conduction microcalorimeters operate through detection of the heat effects in sample and reference vessels with banks of thermocouples (thermopile), located between the vessels and a heat sink. Mixing of components in the sample and reference vessels is brought about by either a flow system or by rotation of sealed bicompartimented vessels. The thermal activity monitor (Thermo-Metric/LKB 227) is an example of a conduction microcalorimeter having a 4-channel system, each ‘channel’ being a twin (sample and reference) heat conduction calorimeter. Such an arrangement enables several unwanted heat effects to be eliminated at the same time. Heat conduction microcalorimeters operate isothermally but are restricted to the measurement of heats of interaction, or reaction, of components at specific concentrations. Extension of such measurements to enable successive additions, one component to another, leads to isothermal titration calorimetry (ITC).

The calibration of conduction calorimeters is usually done electrically by the inclusion of heaters of known resistance in the sample and reference vessels. In an ideal heat conduction calorimeter all the heat exchange between vessel and heat sink occurs through the thermopiles, however, batch microcalorimeters are generally not ideal and the fraction of the heat which flows through the heat sink can be as little as 50%. Under these circumstances the heat flow pattern in the actual measurements and the calibration should be the same. It is important to check that this is so by comparison of the electrical calibration with an alternative solution calibration. Briggner and Wadsö³⁴⁶ have evaluated a number of aqueous systems which can be used as alternatives to electrical calibrations, including the hydrolysis of triacetin, the binding of Ba^{2+} ions to 1,4,7,10,13,16-hexaoxacyclooctadecane, dissolution and dilution of propan-1-ol, dilution of sucrose (which was used many years ago by Gucker and Pickard), and acid–alkali neutralization. They present power–time relationships and enthalpy data which can be used for the calibration of microcalorimeters, and discuss problems associated with particular types of calorimeter vessels.

Many biochemical studies involve binding interactions between, for example, a macromolecule and a ligand, and while measurements can be made as a series of single batch microcalorimetric experiments it is very much quicker to use an isothermal titration calorimeter (ITC). The precision of ITC has been steadily increasing; in the 1970s heat effects of the order of 10^{-3} J were measurable, but during the 1980s sensitivity improved to around 10^{-6} J . This was due to several

³⁴⁶ L.-K. Briggner and I. Wadsö, *J. Biochem. Biophys. Meth.*, 1991, **22**, 101.

developments, but principally to improvements in thermopile design. The use of semiconductor bismuth telluride thermopiles having a thermocouple density of 70 per cm^2 , giving greater voltage change for a given temperature change, led to improved sensitivity. Minimization of baseline noise has also been improved by greatly improving the thermostatting of the whole calorimeter. Another major development is the use of power compensation, which is carried out by continuously regulating the heat supplied to the titration cells to drive the temperature difference between reference and sample vessels toward the baseline. These developments have been usefully summarized by Freire *et al.*,³⁴⁷ together with a discussion of the application of ITC to several biochemical systems. In contrast to using the heat conduction mode which allows the temperature difference (and hence thermopile power-time response) to go to zero by flow of heat to the heat sink, the power compensation method gives a greatly improved time response so that the power-time curve comes back to the baseline faster. This is particularly important in ITC when successive addition of, for example, a ligand to a macromolecule are required.

The problem of increasing the rate of addition of aliquots of titrant in titration microcalorimetry has been addressed by Bastos *et al.*³⁴⁸ New titration vessels have been designed for the ThermoMetre (LKB) 2277 microcalorimeter, with very small reaction volumes (0.2 cm^3), which are almost completely covered with semiconductor thermocouples. Using this vessel it was possible to titrate with a rate of addition every 6.5 minutes and if the 'dynamic correction' procedure³⁴⁹ is used, so that the thermograms do not have to come back to the baseline after each addition, the rate could be increased to additions every 1.5 minutes.

Studies on Low-Molecular Weight Compounds of Biochemical Interest. – The study of the thermochemistry of interactions of amino acids, amides, and small peptides in aqueous solutions as background information leading towards a better understanding of the problem of the stability of native protein structures continues to be pursued. The thermodynamics of the formation of hydrogen bonds by the amide group is central to any discussion of secondary protein structure. Approximately 75% of the backbone amide groups in proteins are involved in secondary structures (α -helices and β -pleated sheets) and two-thirds of these are buried in the apolar core of the protein.³⁵⁰ The stability of amide hydrogen bonds in an apolar environment relative to those formed in water is of particular interest. Sneddon *et al.*³⁵¹ have investigated this for the formation of hydrogen bonds by formamide in both an apolar (carbon tetrachloride) environment and in water by molecular dynamics. Their simulations involved the formamide in the centre of a rectangular box containing approximately 240 water molecules or 210 CCl_4 molecules. They found that the hydrogen bonded formamide dimer forms with a free energy change of $-35.3 \text{ kJ mol}^{-1}$ in CCl_4 and $-1.42 \text{ kJ mol}^{-1}$ in water. The very small free energy of amide hydrogen bonding in water suggests that hydrogen bonds are rapidly formed and broken. A study of the partial molar heat capacities of some *N*-acetyl amino

³⁴⁷ F. Freire, O. L. Mayorga, and M. Straume, *Anal. Chem.*, 1990, **62**, 950A.

³⁴⁸ M. Bastos, S. Hägg, P. Lönnbro, and I. Wadsö, *J. Biochem. Biophys. Meth.*, 1991, **23**, 255.

³⁴⁹ S. L. Randzio and J. Surkuusk, in 'Biological Microcalorimetry', ed. A. E. Beezer, Academic Press, London, 1980, pp 311.

³⁵⁰ C. L. Brooks III, M. Karplus, and B. M. Pettitt, *Adv. Chem. Phys.*, 1988, **71**, 1.

³⁵¹ S. F. Sneddon, D. J. Tobias, and C. L. Brooks III, *J. Mol. Biol.*, 1989, **209**, 817.

acid amides and *N*-acetyl peptide amides in water at 25°C has shown that it is possible to use a simple additivity scheme to predict partial molar heat capacities within 2%, at least for substances containing glycyl and alanyl residues.³⁵² Apolar residues make a large positive contribution to the net heat capacity while the primary peptide group makes little contribution. The study suggests that it may well be possible to predict the heat capacities of denatured proteins given sufficient data and making allowances for charged residues.

The enthalpies of interaction of formamide³⁵³ and a range of alkyl substituted amides^{354,355} and peptides³⁵⁶ with alkali metal halides, and the interaction of amides and substituted amides with urea^{357–359} and guanidinium chloride³⁶⁰ have been investigated as an approach to understanding the factors which contribute to the interactions of ions with proteins and the mechanism of urea/guanidinium chloride denaturation. The enthalpies of interaction (ΔH^*) of equimolal (m) solutions of salt (MX) and amide (A) may be written for a flow-calorimetric experiment as shown in Equation 10,

$$\Delta H^* = m^2 y_A y_{MX} (B_1 + B_2 m + B_3 y_A m) \quad (10)$$

where y_A and y_{MX} are the mole fractions of amide and salt respectively and the B_n s are the enthalpic coefficients representing heterotactic interactions of the solute species. For a batch calorimetric experiment in which the final molalities of amide and salt are m'_A and m'_{MX} (Equation 11):

$$\Delta H^* = B_1 m'_A m'_{MX} + B_4 m'^2_A m'_{MX} + B_5 m'_A m'^2_{MX} \quad (11)$$

The B_n terms can be written in terms of the enthalpic virial coefficients (h) as follows:

$$B_1 = 2(h_{A,M} + h_{A,X}) \quad (12)$$

$$B_2 = 3(h_{A,M,M} + h_{A,X,X} + 2h_{A,M,X}) \quad (13)$$

$$B_3 = 3(h_{A,A,M} + h_{A,A,X} - h_{A,M,M} - h_{A,X,X} - 2h_{A,M,X}) \quad (14)$$

$$B_4 = 3(h_{A,A,M} + h_{A,A,X}) \quad (15)$$

$$B_5 = 3(h_{A,M,M} + h_{A,X,X} + 2h_{A,M,X}) \quad (16)$$

It is not possible to separate the cationic and anionic contributions in equations (12)–(16) without extra-thermodynamic assumptions, hence attention has been largely directed to the B_1 term containing pair wise interactions between solvated species. Table 7 shows the virial coefficients $h_{A,M} + h_{A,X}$ for formamide, *N*-methylacetamide and *N,N*-dimethylformamide on interaction with alkali metal halides. The cationic series (LiCl to CsCl) do not follow a regular trend although for the three

³⁵² G. R. Hedwig, J. F. Reading, and T. H. Lilley, *J. Chem. Soc., Faraday Trans.*, 1991, **87**, 1751.

³⁵³ P. J. Check, M. A. Gallardo-Jimenez, and T. H. Lilley, *J. Chem. Soc., Faraday Trans.*, 1988, **84**, 3435.

³⁵⁴ K. G. Davis, M. A. Gallardo-Jimenez, and T. H. Lilley, *J. Chem. Soc., Faraday Trans.*, 1989, **85**, 2901.

³⁵⁵ M. A. Gallardo-Jimenez and T. H. Lilley, *J. Chem. Soc., Faraday Trans.*, 1989, **85**, 2909.

³⁵⁶ K. G. David, M. A. Gallardo-Jimenez, and T. H. Lilley, *Fluid Phase Equilibria*, 1990, **57**, 191.

³⁵⁷ A. H. Sijpkens, A. A. C. M. Oudhuis, G. Somsen, and T. H. Lilley, *J. Chem. Thermodyn.*, 1989, **21**, 343.

³⁵⁸ A. H. Sijpkens, G. Somsen, and T. H. Lilley, *J. Chem. Soc., Faraday Trans.*, 1990, **86**, 2943.

³⁵⁹ M. Abbate, G. Barone, G. Castronuovo, P. J. Check, C. Giancolas, T. E. Leslie, and T. H. Lilley, *Thermochim. Acta*, 1990, **173**, 261.

³⁶⁰ G. R. Hedwig, T. H. Lilley, and H. Linsdell, *J. Chem. Soc., Faraday Trans.*, 1991, **87**, 2975.

Table 7 Heterotactic virial coefficients $(h_{A,M} + h_{A,X})/J\text{ kg mol}^{-2}$ for interactions between amides and alkali-metal halides in water at 25°C (Ref. 353, 354, 357)

<i>Salt</i>	<i>Formamide</i>	<i>N</i> -Methylacetamide	<i>N,N</i> -Dimethylformamide
LiCl	-293	46	-202
NaCl	-452	266	-79
KCl	-451	189	-168
CsCl	-580	-231	-299
KF	-213	424	323
KCl	-451	189	-168
KBr	-474	175	-329
KI	-602	115	-364

amides the interactions are more exothermic for CsCl than for LiCl. The anionic series (KF to KI) all become more exothermic (or less endothermic in the case of *N*-methylacetamide) with increasing anion size. Despite the irregular behaviour of the cationic series there are some similarities between these observations and salt-induced denaturation of globular proteins. The tendency of anions to denature proteins follows the series $\text{I}^- > \text{Br}^- > \text{Cl}^- > \text{F}^-$, which is consistent with the I^- ion interacting most exothermically with the amides. For the cations, denaturation tendency increases in the series $\text{Li}^+ > \text{Cs}^+ > \text{Na}^+, \text{K}^+$ which is only consistent with the virial coefficient data for *N,N*-dimethylformamide.

The enthalpies of solution of amino acids, *N*-alkylamides and peptides in water and denaturant solutions (commonly urea or guanidinium chloride) can be used to obtain both the pairwise enthalpic interaction coefficients of the solutes in these media and the enthalpies of transfer between water and denaturant. Such measurements have a direct bearing on the enthalpic factors which contribute to protein denaturation. From a study³⁵⁷ of a range of eight peptides, *N*-acetylglycinamide, *N*-acetyl-L-alaninamide, *N*-acetyl-L-leucinamide, *N*-methyl-*N'*-methylglycinamide, *N*-acetyl-*N'*-methyl-L-alaninamide, *N*-acetyl-*N'*-methyl-L-leucinamide, *N*-acetyl-L-prolinamide and *N*-acetyl-*N'*-methyl-L-prolinamide, it was found that the enthalpies of transfer between aqueous and aqueous–urea solutions in the concentration range upto 8 mol dm⁻³ were all negative apart from the leucinamide and *N*-acetyl-*N'*-methyl-L-alaninamide peptides. Introduction of larger alkyl groups into the peptides makes the transfer to aqueous urea less favourable showing that the urea–alkyl interaction is repulsive. In contrast the glycine and proline peptides make a favourable interaction with urea. It is an interesting observation that this favourable interaction would contribute to the denaturation process in that glycine and proline residues are frequently located in the β -turn regions of proteins, often on the outer surface of the tertiary structure, so that a favourable interaction with urea would help to initiate unfolding.

Calorimetric studies on amides in 6 mol dm⁻³ guanidinium chloride³⁶⁰ enable us to make a comparison between homotactic pairwise interaction coefficients in this denaturant, urea and water (Table 8). For the three solvents the interaction coefficients become more positive with increase in ‘alkyl’ content; the increase is particularly dramatic for the acetamides. This increase is considered to reflect the hydrophobic hydration of the alkyl groups. There are two reasons for the increasing repulsive interactions. Firstly the relaxation of water from the hydrophobic co-regions to the bulk solvent when the co-regions overlap and, secondly, the

Table 8 Homotactic enthalpic pairwise interaction coefficients for amides in water, 8 mol dm⁻³ urea and 6 mol dm⁻³ guanidinium hydrochloride (GuHCl) at 25°C (Ref. 360)

Solute		$h_{AA}/\text{J dm}^3 \text{mol}^{-2}$	
	Water	8 mol dm ⁻³ urea	6 mol dm ⁻³ GuCl
HCONH ₂	-115	29	55
HCONHCH ₃	273	314	174
HCON(CH ₃) ₂	739	723	681
CH ₃ COHN ₂	1	140	158
CH ₃ CONHCH ₃	237	480	445
CH ₃ CON(CH ₂ CH ₃) ₂	2365	—	1188
CH ₃ CONHCH ₂ CONH ₂	-221	717	523

repulsions between the differently solvated apolar and amidic groups. The overall impression one gets from the solution studies of amides and small peptides is that the observed enthalpic effects are generally a compromise between the solvent (water)-solute interactions and the solute-solute interactions. Solvent reorganization around small non-polar molecules has been comprehensively discussed by Lee³⁶¹ who attributes hydrophobicity mainly to differences in solvent reorganization which produces compensating changes in enthalpy and entropy.

Turning now to studies of aqueous solutions of saccharides, the enthalpies of dilution of the binary and ternary aqueous solutions of the isomeric disaccharides cellobiose, maltose, and trehalose have been used by Gaffney *et al.*³⁶² to obtain the homo- and heterotactic enthalpic virial coefficients in Equation 17, where

$$\Delta H = n[h_2(m' - m) + h_3(m'^2 - m^2) + \dots] \quad (17)$$

where ΔH is the enthalpy change on diluting a solution of initial molality m containing n moles of solute to a final molality m' . For a ternary mixture (two disaccharides A and B plus water) $m = m_A + m_B$. The enthalpic virial coefficients h_2 and h_3 are given for binary and ternary mixtures by:

$$h_2 = h_{AA}(\text{or } h_{BB}); \quad h_3 = h_{AAA}(\text{or } h_{BBB}) \quad (18)$$

$$h_2 = (h_{AA} + 2h_{AB} + h_{BB})/4 \quad (19)$$

$$h_3 = (h_{AAA} + 3h_{AAB} + 3h_{ABB} + h_{BBB})/8 \quad (20)$$

To evaluate the heterotactic terms it is necessary to have the homotactic terms which are obtained from the enthalpies of dilution of the binary solutions. Table 9 shows pairwise and triplet interaction coefficients for disaccharides derived from Equation (17). The pairwise interaction coefficients are all positive (as they are for all monosaccharides) showing that there is a net repulsion between hydrated saccharides. For isomeric sugars, the group additivity approach of Savage and Wood³⁶³ to interaction enthalpies cannot be used to explain the enthalpies, since it would predict that the virial coefficients for isomers would all be the same. It is clear that the hydration of sugars depends not only on the number of hydroxy groups but

³⁶¹ B. Lee, *Biopolymers*, 1991, **31**, 993.

³⁶² S. H. Gaffney, E. Haslam, T. H. Lilley, and T. R. Ward, *J. Chem. Soc., Faraday Trans.*, 1988, **84**, 2542.

³⁶³ J. J. Savage and R. H. Wood, *J. Solution Chem.*, 1976, **5**, 733.

Table 9 Enthalpic virial coefficients for disaccharides in aqueous solution at 25°C (Ref. 362)

Solute	$h_2/\text{J kg mol}^{-2}$	$h_3/\text{J kg}^2 \text{mol}^{-3}$
Maltose	571.1	-57.0
Trehalose	794.9	
Maltose-cellobiose	682.4	-69.4
Maltose-trehalose	615.3	-77.1
Cellobiose-trehalose	722.5	-56.1

also on their position and orientation. Kabayama and Patterson³⁶⁴ proposed that equatorial hydroxy groups have a greater ability to hydrogen bond to an expanded tridymite water lattice. Developing this concept further, Gaffney *et al.*³⁶² used model-building to incorporate the saccharides into an ice lattice, and to optimize hydrogen bonding, while maintaining sterically and electronically acceptable conformations. The larger the number of hydrogen bonds formed with the lattice, *i.e.* the greater the solvation, the greater the saccharide–saccharide repulsion and hence the larger the second virial coefficient. The second virial coefficient for homotactic and heterotactic interactions can thus be expressed as follows:

$$h_{\text{AA}} = n_{\text{A}}^{h2} H_{\text{nh}} \quad (21)$$

$$h_{\text{BB}} = n_{\text{B}}^{h2} H_{\text{hh}} \quad (22)$$

$$h_{\text{AB}} = n_{\text{A}}^h n_{\text{B}}^h H_{\text{hh}} \quad (23)$$

where n_{A}^h and n_{B}^h are the numbers of hydrogen bonds formed between solutes A and B with solvent. It was shown that for monosaccharides and disaccharides the second virial coefficients were a linear function of the hydration number products ($n_{\text{A}}^h \cdot n_{\text{B}}^h$) or (n_{A}^{h2}) with a common slope (H_{hh}) equal to $4.0 \pm 1.6 \text{ J kg mol}^{-2}$.

Microcalorimetry has been used to good effect in combination with high pressure liquid chromatography to study the thermodynamics of enzyme-catalysed hydrolysis of some disaccharides³⁶⁵ and oligosaccharides.³⁶⁶ The first paper³⁶⁵ also summarizes thermodynamic information (ΔG° , K , ΔH° , ΔS° , and ΔC_p°) for hydrolysis of disaccharides from the literature. On the basis of this study, which includes data for 12 disaccharides involving the following linkages: galactose–fructose (1 → 4), galactose–glucose (1 → 6), glucose–fructose (1 → 6), glucose–glucose (1–1'), glucose–fructose (1 → 3) and galactose–arabinose (1 → 3), it is found that at 298 K the entropies of hydrolysis are remarkably constant at $40 \pm 7 \text{ J mol}^{-1} \text{ K}^{-1}$ and the enthalpies of hydrolysis are small and in the range -5 to $+6 \text{ kJ mol}^{-1}$, with the interesting exception of sucrose which is $-15.0 \text{ kJ mol}^{-1}$. The abnormally high enthalpy of hydrolysis of sucrose is attributed to the fact that on hydrolysis it forms D-glucose and D-fructofuranose, but the fructofuranose then converts to the equilibrium mixture of pyranose and furanose forms; a process which occurs with an entropy change of $-15.2 \text{ kJ mol}^{-1}$ (at 298 K). At equilibrium the proportions of fructopyranose and fructofuranose are 72% and 28% respectively, and thus the contribution to the enthalpy of hydrolysis is $-15.2 \times 0.72 = -10.9 \text{ kJ mol}^{-1}$. Using

³⁶⁴ M. A. Kabayana and D. Patterson, *Can. J. Chem.*, 1958, **36**, 563.

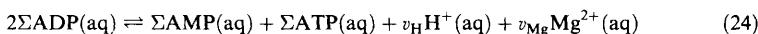
³⁶⁵ Y. B. Tewari and R. N. Goldberg, *Biophys. Chem.*, 1991, **40**, 59.

³⁶⁶ R. N. Goldberg, D. Bell, Y. B. Tewari, and M. A. McLaughlin, *Biophys. Chem.*, 1991, **40**, 69.

the latter figure, the enthalpy of hydrolysis to D-glucose and D-fructofuranose would be $-15.0 - (-10.9) = -4.1 \text{ kJ mol}^{-1}$ in line with the other disaccharides. The enthalpies of hydrolysis of oligosaccharides in the series maltose to maltoheptose, catalysed by 1,4- α -glucosidase, are consistent with an average enthalpy of $-4.53 \pm 0.04 \text{ kJ mol}^{-1}$ for the hydrolysis of the α -1,4 linkage.

The thermodynamics of the isomerization of sugar phosphates and the hydrolysis of sugar phosphates have been reported.^{367,368} This is the first time calorimetric data have been reported for these processes although previous studies have produced equilibrium data. The papers also contain summaries of previously published work and hence are a particularly useful contribution to the sugar phosphate field. The heat capacity changes for the isomerizations are given (or estimated) so that the variation of equilibrium constants with temperature can be calculated from the data. At 298 K the equilibrium constants for the isomerizations of 6-phosphates of glucose and mannose, and the 5-phosphates of ribose and arabinose have equilibrium constants in the range 0.2 to 1.6, and the enthalpic and entropic contributions to ΔG° are of comparable magnitude. The concentrations of the isomers are similar at equilibrium; in contrast the isomerization of glucose-1-phosphate to glucose-6-phosphate has an equilibrium constant of 17 at 298 K, this is the only sugar phosphate isomerization which has an 'unbalanced' concentration ratio at equilibrium. The enthalpies of hydrolysis of the 6-phosphates of glucose, mannose, fructose, and the 5-phosphates of ribose and ribulose, catalysed by alkaline phosphatase (EC 3.1.3.1), have been measured by microcalorimetry. The hydrolysis enthalpies for the 6-phosphates of glucose and fructose are slightly pH dependent and approach a constant value at high pH (> 9); they also depend on magnesium ion concentration, increasing in a range up to 7 mmol dm^{-3} . Some buffer concentration dependence was also observed when Tris buffer was used, suggesting an interaction between Tris and one or more of the species in the reaction.

One of the more important reactions involved in the conversion of chemical energy to mechanical energy and in the control of metabolic reactions is the disproportionation of adenosine 5'-diphosphate (ADP) to adenosine 5'-triphosphate (ATP) and adenosine 5'-monophosphate (AMP). The reaction is catalysed by the enzyme adenylate kinase (also called myokinase or ATP:AMP phosphotransferase (EC 2.7.4.3)). Both metal ions (*e.g.* Mg^{2+}) and protons are involved so that the reaction can be written as Equation 24.



where the summation signs denote the total amounts of each species which may exist in numerous proton or metal bound forms, and v_H and v_{Mg} denote the stoichiometric numbers of protons and magnesium ions involved, both of which can be either positive or negative depending on the pH and ionic strength. The charges on the ADP, ATP, and AMP also depend on the solution conditions; in the fully ionized state they are ADP^{3-} , ATP^{4-} , and AMP^{2-} . The complexities of the disproportionation become apparent when we consider all the possible equilibria which can exist between the many species covered by the summations in Equation (24). A

³⁶⁷ Y. B. Tewari, D. K. Steckler, and R. N. Goldberg, *J. Biol. Chem.*, 1988, **263**, 3664.

³⁶⁸ Y. B. Tewari, D. K. Steckler, and R. N. Goldberg, *J. Biol. Chem.*, 1988, **263**, 3670.

thermodynamic treatment of the reaction has been set up in terms of the multitude of equilibria which includes the effects of metal ion and proton binding by Goldberg and Tewari.³⁶⁹ A mass of data for the equilibria has been collected together and a flow chart produced describing how to go about calculating the thermodynamic parameters for the reaction under specific conditions of temperature, pH, pMg and ionic strength, which includes accounting for activity coefficients in terms of an extended Debye-Hückel equation.³⁶⁹ In a following study a combination of HPLC and microcalorimetry has been used to determine equilibrium constants and enthalpies for the disproportionation over the temperature range 286 to 311 K, ionic strength range 0.06 to 0.33 mol kg⁻¹, pH range 6.04 to 8.87 and pMg range 2.22 to 7.16.³⁷⁰ Under approximately physiological conditions the equilibrium constant for the overall disproportionation is 0.93 ± 0.02 . For the reference reaction involving the fully ionized species at 298.15 K:



the thermodynamic parameters are as follows: $\Delta G^\circ = 3.70 \pm 0.11 \text{ kJ mol}^{-1}$, $\Delta H^\circ = -1.5 \text{ kJ mol}^{-1}$, $\Delta S^\circ = -17 \pm 5 \text{ J mol}^{-1} \text{ K}^{-1}$ and $\Delta C_p^\circ \sim -46 \text{ J mol}^{-1} \text{ K}^{-1}$. The study is without doubt the most comprehensive to date, and the two papers contain an impressive compilation of data on this important complex biochemical reaction.

The existence of life forms at high temperatures opens up a whole new area of study of protein stability which will eventually require thermodynamic measurements on amino acids at high temperatures and pressures. Izatt *et al.* have made a start at a very appropriate place: the effect of temperature and pressure on the protonation of glycine,³⁷¹ extending flow calorimetric measurements up to 75°C and 12.5 MPa. The protonation is exothermic at all temperatures and pressures and the enthalpy is only weakly dependent on pressure. The results are compared with previous data on alkanolamines where the trends of the enthalpy and entropy of protonation with temperature are opposite to those found for glycine.

Studies on Biological Macromolecules. – The stability of the native state of globular proteins continues to be a central theme in both the theoretical and experimental study of biological macromolecules. In this context the hydrophobic interaction is dominant and the development of methods of assessing the magnitude of the hydrophobic interaction for a given protein has occupied many researchers and given rise to a proliferation of hydrophobicity scales,^{372,373} the most commonly used one being that of Kyte and Doolittle.³⁷⁴ Hydrophobicity scales are also used extensively to construct hydropathy plots for membrane proteins.³⁷⁵ Given the whole protein sequence, the hydropathy plot is constructed by calculating the Gibbs energy of transfer of a sequence of amino acid residues (generally a 20 amino acid 'window' which can span the membrane bilayer) from the aqueous phase to an α -helical conformation

³⁶⁹ R. N. Goldberg and Y. B. Tewari, *Biophys. Chem.*, 1991, **40**, 241.

³⁷⁰ Y. B. Tewari, R. N. Goldberg, and J. V. Advani, *Biophys. Chem.*, 1991, **40**, 263.

³⁷¹ R. M. Izatt, J. L. Oscarson, S. E. Gillespie, H. Grimsrud, J. A. R. Renuncio, and C. Prendo, *Biophys. J.*, 1992, **61**, 1394.

³⁷² Y. Nozaki and C. Tanford, *J. Biol. Chem.*, 1971, **246**, 2211.

³⁷³ H. R. Guy, *Biophys. J.*, 1985, **47**, 61.

³⁷⁴ J. Kyte and R. F. Doolittle, *J. Mol. Biol.*, 1982, **157**, 105.

³⁷⁵ D. M. Engleman, T. A. Stertz, and A. Goldman, *Ann. Rev. Biophys. Biophys. Chem.*, 1986, **15**, 321.

in a hydrophobic environment, starting with each amino acid in the protein sequence *i.e.* residue numbers 1–20, 2–21, 3–22 *etc.* The average Gibbs energy per amino acid in the 20 amino acid ‘window’ is then plotted as a function of sequence position. Such plots show peaks and troughs. In general, a trough of approximately 80 kJ mol^{-1} ($\Delta G_{\text{tra}} = -80 \text{ kJ mol}^{-1}$) correlates with the stable insertion of a sequence into the membrane bilayer. The potential use of such plots is extensive, since the expansion of the number of known protein sequences now available in the protein sequence bank which has resulted from the determination of sequences from the complementary DNA. One of the most commonly used ways of obtaining hydrophobicity scales is from the measurement of the solubilities of amino acids (aa) in water (w) and an organic solvent (org) chosen to represent the interior of a protein (*i.e.* a hydrophobic environment). The Gibbs energies of transfer (δG_{trs}) are then calculated from an equation of the form (26)

$$\delta G_{\text{trs}} = RT \ln \left(\frac{x_{\text{aa}}^{\text{w}}}{x_{\text{aa}}^{\text{org}}} \right) \quad (26)$$

where x_{aa}^{w} and $x_{\text{aa}}^{\text{org}}$ are the mole fractions at saturation (solubilities). By doing this for all the amino acids, and subtracting the value of δG_{trs} for glycine from each to eliminate the contributions of the amino and carboxylic groups, the transfer Gibbs energies for each amino acid side chain are obtained. The values of δG_{trs} are a measure of the strength of hydrophobic interactions, *e.g.* δG_{trs} for the phenylalanine side chain is approximately -7 kJ mol^{-1} , but for the polar glutamate side chain it is approximately 3 kJ mol^{-1} .

A significant anomaly in the assessment of the hydrophobic effect arises when the transfer Gibbs are correlated with the accessible surface areas of solutes.³⁷⁶ From the solubilities of hydrocarbons in water and their accessible area, the hydrophobic effect corresponds to $80\text{--}130 \text{ J mol}^{-1} \text{ \AA}^{-2}$. If this ‘microscopic’ value is compared with the ‘macroscopic’ value that can be obtained from the surface tension of the hydrocarbon–water interface, which is approximately $300 \text{ J mol}^{-1} \text{ \AA}^{-2}$, it is clear that the Gibbs energies of transfer based on Equation (26) are significantly underestimating the hydrophobic effect. Following the work of De Young and Dill,³⁷⁷ Sharp *et al.*³⁷⁸ have reconsidered the solution thermodynamics pertaining to transfer processes between solvents. They argue that when a solute partitions between two phases, since the composition of the phases is different, the intermolecular interactions the solute makes are different. Hence, for the net transfer Gibbs energy to be zero, as required by the equivalence of the chemical potentials in both phases, the difference in intermolecular interactions must be counterbalanced by differences in Gibbs energy arising from the different concentrations of the components. The concentration dependence is referred to as the ‘volume entropy’ (ΔS^{ve}) and the condition of equilibrium on partitioning a solute between two phases requires that the Gibbs energy change due to differences in intermolecular interactions, the unitary Gibbs energy, must be compensated by the volume entropy *i.e.* in terms of partial molar quantities $\overline{\Delta G}$ (unitary) = $T\overline{\Delta S}^{\text{ve}}$. The consequence of this treatment is that because interactions are concentration dependent, and the volume entropy depends on the sizes of solute and solvent species, Equation (26) should be written in terms of

³⁷⁶ K. A. Sharp, A. Nicholls, R. F. Fine, and B. Honig, *Science*, 1991, **252**, 106.

³⁷⁷ L. R. De Young and K. A. Dill, *J. Phys. Chem.*, 1990, **94**, 801.

³⁷⁸ K. A. Sharp, A. Nicholls, R. Friedman, and B. Honig, *Biochemistry*, 1991, **30**, 9686.

volume fractions (ϕ_w and ϕ_{org}). Furthermore it should also contain an additional term dependent on the molar volumes of solute (V_s), water (V_w), and the chosen organic solvent (V_{org}), as follows:^{377,378}

$$\delta G_{trs} = RT \ln \frac{\phi_w}{\phi_{org}} + RTV_s \left(\frac{1}{V_w} - \frac{1}{V_{org}} \right) \quad (27)$$

Using this approach Sharp *et al.*³⁷⁸ have recalculated transfer Gibbs energies for the *N*-acetyl amino acids from partitioning data between water and octanol, and water and cyclohexane. The water–octanol revised scales were dramatically different to those based on mole fractions, and changed sign; when used to calculate the change in stability of several proteins arising from single-residue mutations they gave much better agreement with the experimental measurements. A further consequence was that the hydrophobic surface energies for n-alkanes were increased to approximately $200 \text{ J mol}^{-1} \text{ \AA}^{-2}$, considerably closer to the ‘macroscopic’ value, although the actual values are strongly dependent on molecular sizes and hence curvature.³⁷⁶

There have been numerous other theoretical studies on the various factors which are implicated in protein unfolding, including the effects of hydration of accessible surface areas of peptides^{379,380} and globular proteins,^{381–385} an analysis of 67 proteins to assess the importance of pairwise interactions on packing density, in which it was concluded that packing does not determine the native fold of proteins;³⁸⁶ a thermodynamic scale for helix-forming tendencies of amino acids;³⁸⁷ the application of a many-state spin glass model;³⁸⁸ and the use of molecular dynamics to determine the thermodynamics of hydration of cavities in proteins.³⁸⁹

It was found some years ago by Privalov³⁹⁰ that when the specific enthalpy of denaturation (enthalpy per gram) is plotted as a function of temperature for a range of globular proteins, the plots converge to the same point ($\sim 110^\circ\text{C}$). In re-addressing this issue Lee³⁹¹ shows that both for the enthalpies and entropies of transfer of solutes from a non-aqueous phase to water there exists temperatures, defined as the isoenthalpic and isoentropic temperatures, at which the enthalpies and entropies become equal for all solute species of a given class. For a number of hydrocarbon solutes the isoentropic temperature for their dissolution in water is $\sim 110^\circ\text{C}$, the convergence temperature for protein denaturation. It is shown that the existence of these temperatures can be explained by a common formalism based on a linear relationship between the thermodynamic quantity and the surface area, or the number of groups, brought into contact with water. For proteins this property measures the polar/non-polar mix of internal interaction within the protein interior. It is concluded that the

³⁷⁹ T. Ooi, M. Oobatake, G. Nemethy, and H. A. Scheraga, *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 3086.

³⁸⁰ K. P. Murphy and S. J. Gill, *J. Mol. Biol.*, 1991, **222**, 699.

³⁸¹ T. Ooi and M. Oobatake, *J. Biochem.*, 1988, **103**, 114.

³⁸² J. R. Livingstone, R. S. Spolar, and M. T. Record, *Biochemistry*, 1991, **30**, 4237.

³⁸³ P. L. Privalov and G. I. Makhadze, *J. Mol. Biol.*, 1992, **224**, 715.

³⁸⁴ C. N. Pace, *J. Mol. Biol.*, 1992, **226**, 29.

³⁸⁵ A. Nicholls, K. A. Sharp, and B. Honig, *Proteins: Structure, Function and Genetics*, 1991, **11**, 281.

³⁸⁶ M. Behe, E. E. Lattman, and G. D. Rose, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 4195.

³⁸⁷ K. T. O’Neil and W. F. DeGrado, *Science*, 1990, **250**, 646.

³⁸⁸ J. D. Bryngelson and P. G. Wolynes, *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 7524.

³⁸⁹ R. C. Wade, M. H. Mazor, J. A. McCammon, and F. A. Quiocho, *Biopolymers*, 1991, **31**, 919.

³⁹⁰ P. L. Privalov, *Adv. Protein Chem.*, 1979, **33**, 167.

³⁹¹ B. Lee, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 5154.

contributions of the non-polar and polar groups to protein stability are approximately equal and that the solvent-accessible surface area of the denatured state is no more than two thirds the fully extended state. Spolar *et al.*³⁹² have also addressed the importance of the transfer of polar surfaces on protein folding, making use of transfer data on organic amides from water to the pure liquid phase. This approach led to the following expression for the change in heat capacity on protein folding ($\Delta C_{\text{fold}}^{\circ}$)

$$\Delta C_{\text{fold}}^{\circ} (\text{J mol}^{-1} \text{K}^{-1}) = -(1.34 \pm 0.17)\Delta A_{\text{np}} + (0.59 \pm 0.17)\Delta A_{\text{p}} \quad (28)$$

where ΔA_{np} and ΔA_{p} are the areas (\AA^2) removed from exposure to water. For a wide range of globular proteins $\Delta C_{\text{fold}}^{\circ}$ is in reasonable agreement with the experimental values for the heat capacity changes on folding.

A somewhat different approach has been used by Khechinashvili³⁹³ to estimate the contributions to stability for ribonuclease S, lysozyme, and myoglobin. Here the buried surface areas of amino acid residues were estimated from the difference between the accessible surface area for each atom or group in the protein and the accessible surface area of the same atom or group for the respective amino acid residue X in the model compound ala-X-ala (where X is in the β -conformation) based on the data of Lee and Richards.³⁹⁴ Using this approach, the thermodynamic parameters for the hydration of non-polar and polar groups were calculated and used in conjunction with the experimental parameters for unfolding to estimate the conformational contributions. Table 10 shows the 'balance sheet' for ribonuclease S at 298 K. Both the non-polar and polar groups hydrate exothermically and with a decrease in entropy. The overall enthalpy of hydration ($-2653 \text{ kJ mol}^{-1}$) in combination with

Table 10 Contributions to the thermodynamic parameters of unfolding of ribonuclease A at 298 K (Ref. 393)

$\Delta_N^u G$ (experimental)/kJ mol ⁻¹	44
$\Delta_N^u H$ (experimental)/kJ mol ⁻¹	302
$\Delta_N^u S$ (experimental)/kJ K ⁻¹ mol ⁻¹	0.866
ΔG_h^{NP} (non-polar groups)/kJ mol ⁻¹	128
ΔG_h^{P} (polar groups)/kJ mol ⁻¹	-1478
ΔG_h (total)/kJ mol ⁻¹	-1350
ΔH_h^{NP} (non-polar groups)/kJ mol ⁻¹	-664
ΔH_h^{P} (polar groups)/kJ mol ⁻¹	-1989
ΔH_h (total)/kJ mol ⁻¹	-2653
ΔS_h^{NP} (non-polar groups)/J K ⁻¹ mol ⁻¹	-2.66
ΔS_h^{P} (polar groups)/J K ⁻¹ mol ⁻¹	-1.71
ΔS_h (total)/J K ⁻¹ mol ⁻¹	-4.37
$\Delta G_c = \Delta_N^u G - \Delta G_h^{\text{NP}} - \Delta G_h^{\text{P}}$	1394
$\Delta H_c = \Delta_N^u H - \Delta H_h^{\text{NP}} - \Delta H_h^{\text{P}}$	2955
$\Delta S_c = \Delta_N^u S - \Delta S_h^{\text{NP}} - \Delta S_h^{\text{P}}$	5.24

³⁹² R. S. Spolar, J. R. Livingstone, and M. T. Record, Jr, *Biochemistry*, 1992, **31**, 3947.

³⁹³ N. N. Khechinashvili, *Biophys. Biochim. Acta*, 1990, **1040**, 346.

³⁹⁴ B. Lee and F. M. Richards, *J. Mol. Biol.*, 1971, **55**, 379.

the entropic term ($T\Delta S_h = -1302 \text{ kJ mol}^{-1}$) leads to a negative Gibbs energy of hydration, which on subtraction from the experimental Gibbs energy of denaturation (unfolding) gives a positive contribution for the conformational change. The enthalpy and entropy changes arising from the conformational change are similarly estimated. The heat capacity change associated with the polar and non-polar groups were used to obtain the effects of temperature on the thermodynamic parameters. For the non-polar groups the enthalpy of hydration becomes less exothermic with increasing temperature and the entropy less negative, whereas for the polar groups the hydration enthalpy becomes less exothermic and the entropy more negative. The overall Gibbs energies of unfolding pass through a maximum positive value corresponding to a temperature of maximum stability. Both extremes of heat and cold make the native state less stable.

The process of 'cold denaturation' of proteins as a general phenomenon has been considered by Franks *et al.*³⁹⁵ who point out that a model of unfolding based on a positive partial molal heat capacity change, independent of temperature, results in a negative change in heat capacity on denaturation at low temperatures (*i.e.* on cold denaturation) and hence they propose a physically more realistic model, which allows a temperature-dependent heat capacity change that changes sign at some temperature within the range of stability of the native protein. A comprehensive review of cold denaturation by Privalov³⁹⁶ lists the conditions of cold inactivation of approximately 30 enzymes, most of which are multimeric, and many reversibly dissociate into subunits on cooling although the tertiary structure of the subunits remain intact. It is thus important to distinguish between 'cold inactivation' and cold denaturation, which occurs when the tertiary structure unfolds. Cold denaturation is often characterized by a high degree of reversibility. It is also clear that the denatured state at low temperatures, where hydrogen bonding is strong, is not the same as the high temperature denatured state where hydrogen bonding is weaker. Cold denaturation also features in a study of cytochrome c at low pH³⁹⁷ and in the model of protein denaturation proposed by Dzakula *et al.*^{398,399}

Studies on the thermal properties of polypeptides can be useful in obtaining data which relate to secondary structural features in proteins; despite the many possible biologically relevant polypeptides which might be studied, data from relatively few polypeptides have been reported. Calorimetric data exist for the enthalpy of α -helix formation of poly(L-glutamic acid)⁴⁰⁰ and poly(L-lysine),⁴⁰¹ and heat capacity studies on polyglycine, poly(L-alanine) and poly(L-valine) in the solid state have now been reported.⁴⁰² The enthalpies of the α -helix to coil transition for the solvent-induced conformational change of the sodium and caesium salts of poly(L-glutamic acid) in aqueous media, as measured by microcalorimetry, have revealed the importance of cation size,⁴⁰³ and DSC methods have been used to determine the enthalpy change

³⁹⁵ F. Franks, R. H. M. Hatley, and H. L. Friedman, *Biophys. Chem.*, 1988, **31**, 307.

³⁹⁶ P. L. Privalov, *Crit. Rev. Biochem. Mol. Biol.*, 1990, **25**, 281.

³⁹⁷ Y. Kuroda, S.-I. Kidokoro, and A. Wada, *J. Mol. Biol.*, 1992, **223**, 1139.

³⁹⁸ Z. Dzakula and R. K. Andjus, *J. Theor. Biol.*, 1991, **153**, 41.

³⁹⁹ Z. Dzakula and R. K. Andjus, and M. Bozic, *J. Theor. Biol.*, 1991, **153**, 61.

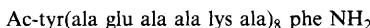
⁴⁰⁰ G. Rialdi and J. Hermans, *J. Am. Chem. Soc.*, 1966, **88**, 5719.

⁴⁰¹ P. Y. Chou and H. A. Scheraga, *Biopolymers*, 1971, **10**, 657.

⁴⁰² K. A. Roles and B. Wunderlich, *Biopolymers*, 1991, **31**, 477.

⁴⁰³ H. Daoust and D. St-Cyr, *Biopolymers*, 1988, **27**, 1267.

for the temperature-induced transition in an alanine-containing 50-residue peptide (I).⁴⁰⁴



(1)

Compound (I) represents a compromise to overcome the problems of the broadness of the thermal unfolding transitions of short polypeptides and the difficulty of synthesis of pure long polypeptides. The repeating sequence, when more than three repeats long, forms stable α helices. Alanine has one of the highest helix propensities of the amino acids found in proteins. Circular dichroism and DSC were combined to determine the van't Hoff and calorimetric enthalpies of the helix-to-coil transition, which occurs in the temperature range 10° to 75°C with a DSC peak at approximately 42°C. Even for this compound the transition is relatively broad, and it was not possible to determine the pre- and post-transitional baselines, which has to be deduced from estimates of the lowest excess heat capacities and fitting procedure. The results of this study gave a calorimetric enthalpy of 4.5 kJ mol⁻¹ per residue and a van't Hoff enthalpy of 1.1 kJ mol⁻¹ per residue for the helix to coil transition of poly(L-alanine). The difference between the two values suggests that α -helix formation is not a two-state process. The magnitude of the calorimetric enthalpy change, when compared with the value of 4.6 kJ mol⁻¹ per residue for poly(L-lysine) and poly(L-glutamate), suggests that the amino acid side chains have relatively little effect on the enthalpy, with any change coming from the hydrogen bonding of the peptide backbone.

Numerous applications of DSC measurements to protein unfolding have been reported including the denaturation of bacteriorhodopsin,⁴⁰⁵ taka-amylase A,⁴⁰⁶ cytochrome c peroxidase,⁴⁰⁷ rhodopsin and opsin,⁴⁰⁸ lobster haemocyanin,⁴⁰⁹ thermolysin,⁴¹⁰ pancreatic pro and carboxypeptidase,⁴¹¹ tropomyosin,⁴¹² glutamine synthetase,⁴¹³ ribonuclease T1,⁴¹⁴ Δ -crystallins of lens fibre cells,⁴¹⁵ *Escherichia coli* thioredoxin,⁴¹⁶ the α -amylase inhibitor tendamistat,⁴¹⁷ phosphoglycerate kinase,⁴¹⁸ phaseolin,⁴¹⁹ and lysozyme in which one of the four disulfide bridges was removed.⁴²⁰ Thermodynamic studies have also been carried out on the unfolding of

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- ⁴¹² J. M. Sturtevant, M. E. Holtzer, and A. Holtzer, *Biopolymers*, 1991, **31**, 489.
- ⁴¹³ A. Ginsburg and M. Zolkiewski, *Biochemistry*, 1991, **30**, 9421.
- ⁴¹⁴ C.-Q. Hu, J. M. Sturtevant, J. A. Thomson, R. E. Erickson, and C. N. Pace, *Biochemistry*, 1992, **31**, 4876.
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- ⁴²⁰ A. Cooper, S. J. Egles, S. E. Radford, and C. M. Dobson, *J. Mol. Biol.*, 1992, **225**, 939.

alligator metmyoglobin,⁴²¹ α -amylase,⁴²² phage T4 lysozyme,^{423,424} $\beta\beta$ -tropomyosin,⁴²⁵ the monomer–dimer association of tubulin,⁴²⁶ the interactions between the subunits of bovine pancreatic procarboxypeptidase A-56 complex,⁴²⁷ the tetramerization of melittin,⁴²⁸ and the thermally induced conformational transition of double stranded xanthan.⁴²⁹ From these studies we highlight some of the more interesting features.

The study of cytochrome c peroxidase (CcP)⁴⁰⁷ showed that at pH 7 the DSC curves for thermal denaturation have two endotherms with mid-point transition temperatures of $43.9 \pm 1.4^\circ\text{C}$ and $63.3 \pm 1.6^\circ\text{C}$, with associated enthalpies of $285 \pm 63 \text{ kJ mol}^{-1}$ and $402 \pm 79 \text{ kJ mol}^{-1}$, respectively. The enzyme (molecular mass 34 168) consists of a single polypeptide chain of 294 residues and a single non-covalently bound heme group. Removal of the heme to give the apoenzyme has a profound effect on the thermal behaviour in that the low-temperature transition is lost, leaving only the transition with mid-point $59.7 \pm 0.6^\circ\text{C}$ and an enthalpy of $448 \pm 121 \text{ kJ mol}^{-1}$. The loss of the low-temperature transition, which might suggest that the domain associated with the heme group has become unfolded, does not, however, effect the transport properties (sedimentation and diffusion coefficients) of the enzyme: the apoenzyme has only a slightly larger radius (2.8 nm) than the holoenzyme (2.4 nm). In terms of specific enthalpy, the binding of heme to the apoenzyme causes an increase from $12.9 \pm 1.7 \text{ J g}^{-1}$ to $23.4 \pm 4.6 \text{ J g}^{-1}$. This effect is very similar to that found on binding glucose to hexokinase, which approximately doubles the specific enthalpy of unfolding,⁴³⁰ although in this case the hexokinase unfolds in two thermal transitions, at 41°C and 48°C , in the absence of glucose and in a single transition, at 51°C , when glucose is bound. The crystallographic structure of CcP shows that about 50% of the 294 residues are in the α -helical conformation and the protein is folded in two well-defined domains with the heme bound in a cleft between them. Domain I contains the N- and C-terminal residues 1–145 and 266–294, and domain II contains residues 146–265. Domain I is the more flexible domain and unfolds in the low-temperature transition; it is not, however, obvious as to why, on removing the heme, domain I becomes stabilized by the more rigid domain II so that only the single high temperature transition is found.

The unfolding of many small globular proteins can be described by a two-state model in which the native state (N) unfolds cooperatively to the denatured state (D) with no significantly stable intermediate states; the unfolding of larger proteins may not be so easily described. A key enzyme in nitrogen metabolism, which catalyses the synthesis of L-glutamine from L-glutamate, ammonia and ATP, is glutamine synthetase (GS). This is a large metalloenzyme (molecular mass 622 000) with 12 identical subunits arranged in 2 layers of 6. The 12 active sites are formed at the

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⁴²² M. Violet and J.-C. Meunier, *Biochem. J.*, 1989, **263**, 665.

⁴²³ J. D. Klem, J. A. Wozniak, T. Alber, and D. P. Goldenberg, *Biochemistry*, 1991, **30**, 589.

⁴²⁴ J. A. Bell, W. J. Bechtel, U. Sauer, W. A. Baase, and B. W. Matthews, *Biochemistry*, 1992, **31**, 3590.

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⁴²⁶ D. L. Sackett and R. E. Lippoldt, *Biochemistry*, 1991, **30**, 3511.

⁴²⁷ T. Michon, J.-C. Sari, S. Granon, B. Kerfelec, and C. Chapus, *Eur. J. Biochem.*, 1991, **201**, 217.

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⁴³⁰ K. Takahashi, J. L. Carey, and J. M. Sturtevant, *Biochemistry*, 1981, **20**, 4693.

interfaces between adjacent subunits which bind two metal ions (Mn^{2+}), L-glutamate and ATP. The dodecameric enzyme partially unfolds on heating, and the process is reversible. DSC scans show a single endothermic transition with a peak at approximately 44° to 52°C depending on the free Mn^{2+} concentration:⁴¹³ at a Mn^{2+} concentration of 1.0 mM the transition temperature is $51.6 \pm 0.1^\circ\text{C}$ and the enthalpy change is $883 \pm 17 \text{ kJ mol}^{-1}$. The DSC scans were deconvoluted on the basis of two models: a random model for multiple independent two-state transitions, and a sequential model for two sequential two-state transitions. The van't Hoff enthalpy ΔH_{vH} , calculated from the equation

$$\Delta H_{vH} = 4RT_m^2 C_{p_m}/\Delta H_{cal} \quad (29)$$

where C_{p_m} is the maximum heat capacity of the transition at T_m , and ΔH_{cal} is the calorimetric enthalpy, was used to calculate the cooperativity ratio, $CR = \Delta H_{cal}/\Delta H_{vH}$. For a simple two-state process $CR = 1$ while a $CR > 1$ suggests overlapping independent two-state transitions or sequential two-state transitions for the thermal unfolding of a multidomain protein. It was found that $CR = 1.6$ for glutamine synthetase suggesting two or more domains. A minimum of two two-state transitions was required to fit the DSC data suggesting that there are two cooperative units per dodecamer with slightly different thermal stabilities. Spectroscopic methods were used to obtain a measure of the exposure of tryptophan and tyrosine residues on thermal unfolding and indicated partial unfolding of the 12 active-site structures in the dodecamer. For all subunits to partially unfold in a concerted fashion without dissociation, at least one of the two thermodynamic domains must be composed of both N- and C-domain structures. It is interesting that this large enzyme can partially, but reversibly, unfold with a specific enthalpy of 1.4 J g^{-1} , which is less than 10% of that for the complete unfolding of a small protein such as lysozyme (31 J g^{-1}).

The extensive use of urea and guanidinium hydrochloride as protein denaturants continues to promote thermodynamic studies concerned with the nature of their interactions with proteins.^{396,431–435} Pfeil *et al.*⁴³³ have investigated the interaction of guanidinium chloride (GuHCl) with apocytochrome c in acid solution by isothermal titration calorimetry. The value of this protein is that in acid solution (pH 2) it has no significant secondary or tertiary structure and hence behaves as a random coil so that DSC reveals no thermally induced transitions. The transfer enthalpy from water to guanidinium chloride was found to be well described by Equation 30,

$$Q = \Delta H \frac{Ka}{1 + Ka} \quad (30)$$

where ΔH is the total enthalpy of binding ($\sum \Delta H_i n_i$) to n_i non-interacting binding sites with a binding constant K , and a is the activity of GuHCl. For binding to apocytochrome c, $K = 1.16 \pm 0.06$ and $\Delta H = 549 \pm 9 \text{ kJ mol}^{-1}$ at 25° (pH 2.3). The interesting feature of this study is that it shows that the species bound to the protein is GuHCl and not the cation GuH^+ . Had the latter been the case, the activity in Equation (30) would have had to be replaced by $a^{1/2}$. Double reciprocal plots of $1/Q$ vs. $1/a$ and $1/Q$ vs. $1/a^{1/2}$

⁴³¹ C. N. Pace and D. V. Laurents, *Biochemistry*, 1989, **28**, 2520.

⁴³² F. Ahmad and C. C. Bigelow, *Biopolymers*, 1990, **29**, 1593.

⁴³³ W. Pfeil, W. Welfse, and V. E. Bychkova, *Stud. Biophys.*, 1991, **140**, 5.

⁴³⁴ B. A. Shirley, P. Stanssens, U. Hahn, and C. N. Pace, *Biochemistry*, 1992, **31**, 726.

⁴³⁵ C. N. Pace, D. V. Laurents, and R. E. Erickson, *Biochemistry*, 1992, **31**, 2728.

were constructed, and the former plot was found to be linear whilst the latter was highly curved, ruling out the binding of the cation in preference to the molecule GuHCl. At low denaturant concentrations, Equation (30) approximates to Equation 31,

$$Q \approx \Delta HKc \quad (31)$$

where c is the molar concentration of denaturant, and hence Q/c is constant and its value is characteristic for the thermodynamic state of the protein. For cytochrome c and α -lactalbumin in acid, *i.e.* denatured states, Q/c has values of approximately 130 kJ mol^{-1} whereas for proteins in their native state, where the number of binding sites is lower, the values are in a range $50\text{--}110 \text{ kJ mol}^{-1}$ depending on temperature.

The development of the technique of site-directed mutagenesis, in which a single amino acid in a protein sequence can be replaced by another amino acid of choice, opens up the possibility of investigating the contribution made by a particular amino acid residue to the energetics of binding to specific sites in proteins. A study of this type has been carried out by Kelley *et al.*⁴³⁶ to investigate ligand binding to the kringle-2 domain of tissue plasminogen activator (t-PA). 'Kringles' are small domains in proteins containing *ca.* 80 amino acid residues with a characteristic three disulfide bonded structure. Both plasminogen and t-PA have kringles which have binding sites for L-lysine. *In vivo* the lysine side chains in the fibrin molecule bind to plasminogen. Kringle-2 of t-PA binds to both lysine and lysine analogues, and the interaction was believed to require both the ϵ -amino and the α -carboxylate groups of the ligand, the carboxylate group binding to histidine (his) 64. To test this possibility mutants having the his 64 replaced by tyrosine and phenylalanine were produced and the enthalpy of interaction was measured by microcalorimetry. The results showed that his 64 does not in fact participate in ligand binding by ion-pair formation since replacing it by tyrosine had a relatively small effect on the enthalpy of interaction with L-lysine. The enthalpy of interaction with the wild type protein (his 64) was $-23 \pm 2 \text{ kJ mol}^{-1}$ and for the protein with his 64 replaced by tyrosine the enthalpy was -18 kJ mol^{-1} , the Gibbs energies of interaction were -24 kJ mol^{-1} and -21 kJ mol^{-1} respectively. The interaction is thus dominated by the enthalpy. A further observation was that the kringle-2 domain was stabilized to thermal denaturation on replacing his 64 with either tyrosine or phenylalanine.

In the polynucleotide and nucleic acid field, thermodynamic studies have been reported on cation-induced DNA condensation,⁴³⁷ protein complexation,⁴³⁸ drug binding,⁴³⁹ ethidium and propidium intercalation,⁴⁴⁰⁻⁴⁴² structure-energetic correlations⁴⁴³⁻⁴⁴⁵ and the effect of magnesium ions on the thermal denaturation of calf

⁴³⁶ R. F. Kelley, A. M. DeVos, and S. Cleary, *Proteins: Structure, Function and Genetics*, 1991, **11**, 35.

⁴³⁷ R. Marquet and C. Houssier, *J. Biomolecular Structure and Dynamics*, 1991, **9**, 159.

⁴³⁸ M. H. P. Van Genderen and H. M. Buck, *Biopolymers*, 1989, **28**, 1653.

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⁴⁴⁰ H. P. Hopkins, Jr. and W. D. Wilson, *Biopolymers*, 1987, **26**, 1347.

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⁴⁴² L. A. Marky and R. B. Macgregor, Jr., *Biochemistry*, 1990, **29**, 4805.

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⁴⁴⁵ H. H. Klump and D. L. Maeder, *Pure Appl. Chem.*, 1991, **63**, 1357.

thymus DNA.^{446,447} The variety of structural forms of the DNA double helix naturally leads to questions concerning the relative stability of the structures. Circular DNA, as exists in plasmids, can have several secondary structures including supercoiled, relaxed, and open-circular and it is interesting to consider the energetics of base-pair dissociation of DNA in these forms relative to linear DNA. Thumm *et al.*⁴⁴³ have investigated this problem using a combination of DSC, UV absorption, and circular dichroism to determine the thermodynamic stability of DNA from several *E. coli* derived plasmids. In order to promote a decrease in melting temperature of the DNAs the experiments were carried out in a chaotropic agent (7.2M sodium perchlorate). Table 11 summarizes some of the results for the Gibbs energy of stabilization (ΔG_{st}) calculated from the melting temperature (T_m) and the calorimetrically measured enthalpy of dissociation ($-\Delta H_{st}$) *i.e.* $\Delta G_{st} = \Delta H_{st} (1 - T/T_m)$. Inspection of the parameters in Table 11 shows that the supercoiled and relaxed circular DNAs are more stable than the linear and open-circular forms, the enthalpies of association (ΔH_{st}) are on average more exothermic by *ca* 4.5 kJ(mol bp)⁻¹ (bp = base pair) and the entropies on association by *ca* 8 J(mol bp)⁻¹ K⁻¹. In molecular terms, Thumm *et al.*⁴⁴³ argue that the intrinsic stabilization enthalpy per base-pair in 7.2M perchlorate is the same for all configurations of the plasmids, and any increase in temperature will result in removal of the supercoils (which in perchlorate have the negative configuration), such an energy input will not be required in the case of linear or open-circular DNA.

Other thermodynamic studies on nucleic acids include antisense oligonucleotide hybridization,⁴⁴⁸ the thermodynamics of RNA and DNA hairpin loop forma-

Table 11 Thermodynamic stability parameters for different secondary structures of DNA in 7.2M sodium perchlorate

DNA secondary structure (species)	T_m °C	$\Delta G_{st}(298K)/$ kJ (mol bp) ⁻¹	$\Delta H_{st}/$ kJ (mol bp) ⁻¹	$\Delta S_{st}/$ J (mol bp) ⁻¹ K ⁻¹
Linear ^a	50.5	-1.7	-22.1 ± 0.9	-68
Linear ^b	51.5	-1.9	-22.7 ± 0.3	-70
Linear ^c	52.8	-2.0	-24.0 ± 0.2	-74
Open-circular ^a	51	-1.7	-21.8 ± 0.7	-67
Open-circular ^b	51.5	-1.9	-22.7 ± 0.3	-70
Supercoiled ^a	75	-3.8	-26.8 ± 0.8	-77
Supercoiled ^b	79.7	-4.1	-26.3 ± 0.5	-75
Supercoiled ^c	79.7	-4.2	-27.2 ± 0.7	-77
Relaxed ^a	75.6	-4.1	-27.9 ± 0.9	-80
Relaxed ^b	80.2	-4.3	-27.4 ± 0.9	-78
Relaxed ^c	80.4	-4.5	-28.4 ± 0.9	-80

^a *E. coli* plasmid (*Col* E1 amp) 11 000 base-pairs (bp)

^b *E. coli* plasmid (pUC 19) 2686 bp

^c *E. coli* plasmid (pWH 931) 3823 bp

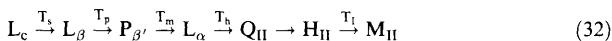
⁴⁴⁶ A. P. Vlasov and V. T. Andranov, *Biophysica*, 1991, **36**, 31.

⁴⁴⁷ A. P. Vlasov, L. I. Yakhontova, and V. T. Andrianov, *Biophysica*, 1991, **36**, 437.

⁴⁴⁸ S. M. Freier, W. F. Lima, Y. S. Sanghvi, T. Vickers, M. Zouves, P. D. Cook, and D. J. Ecker, in 'Gene Regulation: Biology of Antisense RNA and DNA', ed. R. P. Erickson and J. G. Izant, Rowen Press, N.Y., 1992, pg 95.

tion,^{449,450} and an extensive study of the stability of DNA dumbbells.^{451–453} DNA dumbbells consist of a linear duplex of complementary base-pairs linked at the ends by identical bases of variable loop size. These structures have considerable advantages for the study of nearest-neighbour base stacks compared with linear synthetic DNAs, DNA restriction fragments or long repeating copolymers, where various complicating features can obscure the interpretation of melting curves in terms of nearest-neighbour interactions. Dumbbells with a 16 base-pair duplex stem and end loops of from 2–14 thymidine bases were studied. The melting transitions of the larger looped dumbbells deviate considerably from two-state behaviour and the results are compared with the melting behaviour of much larger dumbbells and small hairpin loops.

Thermotropic Behaviour in Lipid-Aqueous Systems. – *Pure Lipid Systems.* The thermal transitions of hydrated phospholipids are continuing to be studied and increasing numbers of papers are appearing. A special issue of *Chemistry and Physics of Lipids*⁴⁵⁴ was devoted to phospholipid phase transitions in 1991 and constitutes a valuable addition to the literature and an excellent starting point for new workers in the field who need to assess the emphasis of current research. The general features of phospholipid phase transitions are reviewed by Marsh,⁴⁵⁵ a review of the principles and methods of investigating them by Laggner and Kriegbaum⁴⁵⁶ and the reversibility of phase transitions is comprehensively discussed by Tenchov.⁴⁵⁷ The generalized sequence of thermotropic transitions for phospholipids that exhibit limiting hydration can be written as follows,⁴⁵⁵



where the phases from the low temperature end are; L_c (crystalline), L_β (hydrated lamellar gel phase); $L_{\beta'}$ denotes tilted chains and L_β chains not tilted with respect to the bilayer normal), $P_{\beta'}$ (intermediate rippled gel phase), L_α (fluid lamellar phase), Q_{II} and H_{II} are non-lamellar phases based on inverted micelles arranged on cubic (Q_{II}) or hexagonal (H_{II}) lattices. At the highest temperature, an isotropic melt may be formed consisting of random inverted micelles (M_{II}).

The process represented by Equation (32) shows several transition temperatures, the most important of which is the transition, T_m , from the rippled gel phase ($P_{\beta'}$) to the fluid or liquid crystalline phase (L_α). The subtransition temperature (T_s) was reported in 1980⁴⁵⁸ and is only observed on slowly cooling the $L_{\beta'}$ phase. For dipalmitoylphosphatidylcholine (DPPC) T_s is 18°C, T_p (the pre-transition temperature) is 35°C and T_m is 41°C. In molecular terms, the subtransition is believed to be associated with the loss of head group hydration and conversion of the acyl-chain packing from hexagonally disordered to the more ordered orthohombic, the pre-transition with rippling of the gel state bilayer, and the main transition with acyl-chain

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⁴⁵³ T. M. Paner, M. Amarasingha, and A. S. Benight, *Biopolymers*, 1992, **32**, 881.

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⁴⁵⁵ D. Marsh, *Chem. Phys. Lipids*, 1991, **57**, 109.

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⁴⁵⁷ B. Tenchov, *Chem. Phys. Lipids*, 1991, **57**, 165.

⁴⁵⁸ S. C. Chen, J. M. Sturtevant, and B. J. Gaffney, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 5060.

melting, in which *trans*-gauche conformational changes occur in the acyl chains, which are in the all *trans* conformation in the gel state. The enthalpy changes associated with the transitions at T_s , T_p and T_m for DPPC are approximately 14 kJ mol⁻¹, 6 kJ mol⁻¹, and 33 kJ mol⁻¹ respectively. Slater and Huang⁴⁵⁹ have now found a new subgel phase which, in contrast to the subgel (SGI) which forms slowly at T_s on cooling the L _{β} phase, forms immediately upon cooling. This new subgel they call SGII and is associated with a 'sub-subtransition' which for DPPC occurs at 6.8°C with an enthalpy change of 1 kJ mol⁻¹. The very low enthalpy and the fact that the SGII phase can only be observed in a limited time window has probably precluded previous detection. It is suggested that it may be involved with a re-orientation of the acyl chains from hexagonally disordered packing to orthorhombic packing similar, but presumably not identical, to that occurring at the subtransition temperature after slow cooling the gel phase (L _{β}).

The relationships between chain-melting temperatures (T_m) and pre-transition temperatures (T_p) and acyl-chain length and acyl-chain length asymmetry continue to be addressed.^{460–466} In the approach of Marsh^{464,465} the transition enthalpies (ΔH_t) and entropies (ΔS_t) are assumed to depend linearly on acyl chain length (n) for sufficiently high n , thus

$$\Delta H_t = n\Delta H_{inc} + \Delta H_o \quad (33)$$

$$\Delta S_t = n\Delta S_{inc} + \Delta S_o \quad (34)$$

where ΔH_{inc} and ΔS_{inc} are the incremental values per CH₂ group, and ΔH_o and ΔS_o are constant end contributions. For symmetrical disaturated phosphatidylcholines, chain length $n = 14$ to 22, equations (33) and (34) become:

$$\Delta H_t (\text{kJ mol}^{-1}) = 3.89(\pm 0.08)n - 29.7(\pm 1.3) \quad (35)$$

$$\Delta S_t (\text{J mol}^{-1} \text{ K}^{-1}) = 9.62(\pm 0.25)n - 51.0(\pm 4.2) \quad (36)$$

for the enthalpy and entropy of the main transition (T_m). To obtain an expression for T_m equations (33) and (34) are written as:

$$\Delta H_t = \Delta H_{inc}(n - n_o) \quad (37)$$

$$\Delta S_t = \Delta S_{inc}(n - n'_o) \quad (38)$$

and since for a first-order transition $\Delta H_t = T_m \Delta S_t$ the relationship between T_m and n becomes:

$$T_m = \frac{\Delta H_{inc}}{\Delta S_{inc}} \left(1 - \frac{(n_o - n'_o)}{(n - n'_o)} \right) \quad (39)$$

It follows from equation (39) that T_m is a linear function of $(n - n'_o)^{-1}$. Application of

⁴⁵⁹ J. L. Slater and C. Huang, *Biophys. J.*, 1987, **52**, 667.

⁴⁶⁰ J. T. Mason and C. Huang, *Lipids*, 1981, **16**, 604.

⁴⁶¹ C. Huang, *Klin. Wochenschrift*, 1990, **68**, 149.

⁴⁶² C. Huang, *Biochemistry*, 1991, **30**, 26.

⁴⁶³ G. Cevc and D. Marsh, 'Phospholipid Bilayers. Physical Principles and Models', Wiley-Interscience, New York, 1987, pg 442.

⁴⁶⁴ D. Marsh, *Biochim. Biophys. Acta*, 1991, **1062**, 1.

⁴⁶⁵ D. Marsh, *Biophys. J.*, 1992, **61**, 1036.

⁴⁶⁶ H. Z. Wang and C. Huang, *Biochim. Biophys. Acta*, 1991, **1067**, 17.

this equation to the main transition of the saturated diacylphosphatidylcholines gives values of $\Delta H_{\text{inc}}/\Delta S_{\text{inc}} = 399.8 \pm 1.6 \text{ K}$ and $n_o - n'_o = 2.29 \pm 0.05$, which compare with $403.4 \pm 17.4 \text{ K}$ and 2.33 ± 1.06 , respectively, as determined by calorimetry. The same form of equation can be used to describe the pretransition for the cholines giving $\Delta H_{\text{inc}}/\Delta S_{\text{inc}} = 409.7 \pm 1.8 \text{ K}$ and $n_o - n'_o = 2.71 \pm 0.06$, so that the chain length dependence of both transitions can be explained in the same way. It also follows that for a sufficiently long chain length both the main and pretransitions coincide, the value of n being approximately 26. Marsh⁴⁶⁴ records data for a range of other phospholipids and discusses the use of an equation of the form of (37) and (38) for the transition from the lamellar to the inverted hexagonal (H_{II}) phase for saturated diacylphosphatidylethanolamines.

The relationship between T_m and chain length asymmetry for saturated diacylphosphatidylcholines has been examined by Wang and Huang⁴⁶⁶ in a study of two series of compounds (with molecular masses 678 and 706) covering a wide range of chain length asymmetry. The study was directed to testing the empirical equation:

$$T_m = 154.2 + 2.0(\Delta C) - 142.8(\Delta C/CL) - 1512.5(1/CL) \quad (40)$$

suggested by Huang⁴⁶² where ΔC is the effective chain length difference in C—C bond lengths between the two acyl chains for a phosphatidylcholine molecule in the gel state and CL is the effective length of the longer of the two chains, also in C—C bond lengths. The study covers 25 compounds, $\Delta C/CL$ 0.04 to 0.639 (molar mass 678) and $\Delta C/CL$ 0.037 to 0.675 (molar mass 706). In the range of $\Delta C/CL$ upto 0.41 Equation (40) can be used to predict T_m to within *ca.* 0.8°C, in this range T_m decreases approximately linearly with $\Delta C/CL$, however, above 0.4, T_m increases and passes through a maximum at approximately 0.57 due to changes in packing constraints.

The relationship between the phospholipid head-group structure and chain melting temperature (T_m) is complex, in that numerous factors contribute to shifts in $T_m(\Delta T_m)$ with head group structure^{467,468} and the composition of the aqueous medium.⁴⁶⁹ Cevc⁴⁶⁷ has discussed shifts in T_m and described them in the following of Equation 41,

$$\Delta T_m = \Delta T_{m,\text{el}} + (\Delta T_{m,h}^{\text{PO}_4} + \Delta T_{m,h}^H) + \Delta T_{m,\text{bond}} + \Delta T_{m,\text{vdw}} \dots \quad (41)$$

where the subscripts el, h, bond, and vdw refer to electrostatic ion-lipid and ion-ion interactions, hydration (arising from phosphate (de)protonation and water binding sites on polar residues), interlipid bonds and van der Waals forces respectively. The changes in T_m arising from changes in some of these factors are shown in Table 12, for dipalmitoylphosphatidylethanolamine (DPPE) at various pH and states of methylation. The transition temperature is shifted down by increasing alkalinity and methylation. Protonation of the phosphate group at pH 0 increases T_m while methylation reduces hydration and hence T_m . Changes of pH can thus lead to isothermal chain melting which suggests a sensitive mechanism for the regulation of biological processes since such isothermal transitions can occur very rapidly.⁴⁶⁹ The

⁴⁶⁷ G. Cevc, *Biochemistry*, 1987, **26**, 6305.

⁴⁶⁸ H. Hauser, *Chem. Phys. Lipids*, 1991, **57**, 309.

⁴⁶⁹ G. Cevc, *Chem. Phys. Lipids*, 1991, **57**, 293.

Table 12 Effect of phospholipid head group structure on chain melting temperature for dipalmitoylphosphatidylethanolamine (DPPE) (Ref. 467)

Lipid	pH		
	0	8	13
DPPE	67	63.5	42
DPPE (CH_3)	61.5	58	43
DPPE ($\text{CH}_3)_2$	56	48	43
DPPE ($\text{CH}_3)_3$ *	50	42	42

* Trimethylated DPPE is DPPC.

importance of surface polarity and interfacial hydration in membrane systems is thus as important in regulating T_m as changes in acyl chain length.

Numerous studies reporting the application of DSC to the study of the thermotropic phases of lipid systems have been reported including dimyristoylphosphatidic acid (pH-induced phase changes),⁴⁷⁰ phosphatidylcholines containing *dl*-methyl-anteiso-branched fatty acids,⁴⁷¹ phosphatidylcholines with linear saturated acyl chains with chain lengths (10–22 carbon atoms),⁴⁷² long acyl chain (16–26 carbon atoms) cerebroside sulfates,^{473,474} *N*-stearyl sphingomyelin,⁴⁷⁵ polymerizable phospholipids,⁴⁷⁶ the transfer of phosphatidylcholines between small unilamellar vesicles,⁴⁷⁷ the relationship between chain melting temperature and hydration pressure in DPPE ($\text{CH}_3)_2$ bilayers at low water contents,⁴⁷⁸ and phase transitions of DPPE in fused salt (*N*-ethylammonium nitrate).⁴⁷⁹

The study of Simon *et al.*⁴⁷⁸ describes an interesting application of DSC to test the relationship between chain melting temperature and the decay of length (λ) of the hydration pressure (P_h) between apposing bilayer surfaces which is given by the equation $P_h = P_o \exp(-d_f/\lambda)$ where d_f is the distance between the bilayers. P_h Can be measured as a function of d_f by equilibrating bilayers with solutions of known osmotic pressure in combination with X-ray diffraction measurements of d_f . Hydration forces between bilayers are short-range, and hence the decay length is of the order of 0.1–0.2 nm. Cevc and March⁴⁸⁰ derived Equation 42 for the change in transition temperature (ΔT_m) on hydration,

$$\Delta T_m = T_m - T_{\text{anhdyd}} = \Delta T_m(\text{O}) \tanh \frac{nV}{\lambda A} \quad (42)$$

where n is the number of water molecules per lipid molecule, V_m is the molar volume of water and A the area per lipid molecule, T_{anhdyd} is the transition temperature for the anhydrous lipid, and $\Delta T_m(\text{O})$ the difference in transition temperature at full hydration. For DPPE($\text{CH}_3)_2$, T_m measured by DSC at various degrees of hydration ($n = 0$ to 30) was fitted to Equation (42) to give a decay length of 0.67 nm, how-

⁴⁷⁰ A. Blume and J. Tuchtenhagen, *Biochemistry*, 1992, **31**, 4636.

⁴⁷¹ R. N. A. H. Lewis, B. D. Sykes, and R. N. McElhaney, *Biochemistry*, 1987, **26**, 4036.

⁴⁷² R. N. A. H. Lewis, N. Mak, and R. N. McElhaney, *Biochemistry*, 1987, **26**, 6118.

⁴⁷³ J. M. Boggs, K. M. Koshy, and G. Rangaraj, *Biochim. Biophys. Acta*, 1988, **938**, 361.

⁴⁷⁴ J. M. Boggs, K. M. Koshy, and G. Rangaraj, *Biochim. Biophys. Acta*, 1988, **938**, 373.

⁴⁷⁵ P. R. Maulik, P. K. Sripada, and G. G. Shipley, *Biochim. Biophys. Acta*, 1991, **1062**, 211.

⁴⁷⁶ A. Blume, *Chem. Phys. Lipids*, 1991, **57**, 253.

⁴⁷⁷ T. M. Bayerl, C. F. Schmidt, and E. Sackmann, *Biochemistry*, 1988, **27**, 6078.

⁴⁷⁸ S. A. Simon, C. A. Fink, A. K. Kenworthy, and T. J. McIntosh, *Biophys. J.*, 1991, **59**, 538.

⁴⁷⁹ W. Tamura-Lis, L. J. Lis, and P. J. Quinn, *Biophys. J.*, 1988, **53**, 489.

⁴⁸⁰ G. Cevc and D. Marsh, *Biophys. J.*, 1985, **47**, 21.

ever, the decay length from X-ray diffraction measurements was 0.13 nm, although both methods were in good agreement at higher degrees of hydration ($n = 30$ to 80). The reason for the difference between the values of λ at low hydration was attributed to the fact that the first water molecules are located primarily in the head group region of the bilayer, and not between adjacent bilayers, so that the dominant interbilayer interaction is not hydration pressure but largely steric repulsion between apposing bilayers.

Binary Systems of Phospholipids and Other Molecules. Small molecules and ions can frequently have significant effects on the thermotropic properties of phospholipid systems and the study of such effects continuously gives rise to reports in the literature, many of which contain DSC measurements. Table 13 summarizes a selection of some of the systems that have been investigated by DSC^{481–496}. Some of the additives are drugs; for example, amphotericin B is used in liposomal formulations for the treatment of systemic fungal infections, the anthracycline duanomycin is a potent anti-tumour drug, and verapamil is a calcium-channel blocker which increases the sensitivity of multi-drug-resistant tumour cells to duanomycin. Azone (1-dodecylazacycloheptan-2-one) and C12-thiamorpholine-3-one are skin penetration enhancers which have potential use in topical drug delivery. DSC has become an important tool in the study of drug-membrane interactions⁴⁹⁰ and studies using various forms of bilayer assemblies such as either multilamellar or unilamellar vesicles are valuable model systems for studying drug interactions with bilayers. DSC is also being used in the study of the cryopreservation of liposomes, which is important in the development of liposomally based drug delivery systems.⁴⁹¹

In a study of the effects of salts on the phase behaviour of 1-palmitoyl-2-oleylphosphatidylethanolamine (POPE), Sanderson *et al.*⁴⁸⁸ found that they could correlate transition temperatures with ions in relation to their increasing chaotropic ability. In water, POPE undergoes two thermal transitions associated with a high enthalpy phase change at T_m ($L_\beta \rightarrow L_\alpha$ state) and a low enthalpy change at T_h , when the L_α state passes to the hexagonal inverted micellar phase (H_{II}). The effects of salts on T_m and T_h depend on whether the ions are water-structure makers (kosmotropic ions) or water-structure breakers (chaotropic ions). For example, in Na_2SO_4 (a kosmotrope) T_m is slightly increased and T_h decreased, whilst in NaI (a chaotrope), T_m is decreased and T_h increased. Ordering salts in terms of their effectiveness in lowering the $L_\alpha \rightarrow H_{II}$ transition temperature (T_h) yields the series: $Na_2SO_4 > NaF = NaCl > NaBr > NaI > NaSCN$, which is the same order as the Hofmeister series for the salting-in of proteins. The effects of the anions on the enthalpy and entropy of the $L_\beta \rightarrow L_\alpha$ phase change follow no clear pattern, but for the $L_\alpha \rightarrow H_{II}$ transition the enthalpy and entropy change increase with salt concentration for the early members of the Hofmeister series (Na_2SO_4 , NaF , and $NaCl$) and decrease with salt concentration for the later members (NaI and $NaSCN$).

The study of daunomycin (DNM) and verapamil (VRP) on the thermotropic behaviour of DPPC vesicles has exposed some unusual effects which may have significance in relation to the action of these drugs.⁴⁸⁷ For the binary system DPPC + DNM it is found that DNM abolishes the pre-transition (T_p) broadens and

⁴⁹⁰ J. K. Seydel, *Trends. Pharmac. Sci.*, 1991, **12**, 368.

⁴⁹¹ H. Talsma, M. J. van Steenberger, P. J. M. Salemink, and D. J. A. Crommelin, *Pharm. Res.*, 1991, **8**, 1021.

Table 13 DSC studies on phospholipid bilayer-small molecule systems

<i>Phospholipid (bilayer type)</i>	<i>Additive</i>	<i>Ref.</i>
DHPC (vortexed dispersion)	cholesterol	481
DMPC (multilamellar dispersion)	myotoxin a	482
DMPG (multilamellar dispersion)	stearic/arachic acid	482
DPMC (multilamellar dispersion)	stearic/arachic acid	483
DSPC (multilamellar dispersion)	oleic (OA)/linoleic (LA) acid	483
DMPC (multilamellar dispersion)	oleic (OA)/linoleic (LA) acid	483
DSPC (small unilamellar vesicles)	myristic/palmitic/stearic acid	484
DPPC (small unilamellar vesicles)	myristic/palmitic/stearic acid	484
DSPC (small unilamellar vesicles)	myristic/palmitic/stearic acid	484
DPPC-d ₅₂ (multilamellar vesicles)	gramicidin	485
DMPC-d ₅₄ (multilamellar vesicles)	gramicidin	485
DMPC/DMPG (7:3 mole ratio multilamellar vesicles)	amphotericin B	486
DPPC (multilamellar vesicles)	verapamil	487
DPPC (multilamellar vesicles)	daunomycin	487
POPE (hydrated solid)	sodium salts (SO ₄ ²⁻ , F ⁻ , Cl ⁻ , Br ⁻ , I ⁻ , SCN ⁻)	488
DMPG (multilamellar dispersion)	NH ₄ ⁺ , Li ⁺ , Na ⁺ , Ca ²⁺	468
POPS (multilamellar dispersion)	NH ₄ ⁺ , Li ⁺ , Na ⁺ , Ca ²⁺	468
DPPC (multilamellar vesicles)	1-dodecylazacycloheptan-2-one	489
DPPC (multilamellar vesicles)	C12-thiomorpholine-3-one	489
DEPE (multilamellar dispersion)	mono and disaccharides	492
DEPE (multilamellar dispersion)	acylcarnitines (C12 → C24)	493
DPPC (multilamellar vesicles)	retinol, retinoic acid	494
DPPE (multilamellar vesicles)	retinol, retinoic acid	494

Table 13 *continued*

<i>Phospholipid (bilayer type)</i>	<i>Additive</i>	<i>Effects</i>	<i>Ref.</i>
DPPC (multilamellar dispersions)	nucleosides and nucleotides	Increase T_m , decrease ΔH ($L_\beta \rightarrow L_\alpha$)	495
DPE (multilamellar dispersions)	nucleosides and nucleotides	Increase T_m , decrease ΔH ($L_\beta \rightarrow L_\alpha$)	495
DPPA (multilamellar dispersions)	nucleosides and nucleotides	Increase T_m , decrease ΔH ($L_\beta \rightarrow L_\alpha$)	495
DMPC (multilamellar dispersions)	cannabinoids	Complex behaviour depending on additive/lipid ratio	496
DPPC (multilamellar dispersions)	cannabinoids	Complex behaviour depending on additive/lipid ratio	496

DHPC, dihexadecylphosphatidylcholine; DMPC, 1,2-dimyristoylphosphatidylcholine; DMPS, 1,2-dimyristoylphosphatidylserine; DPPC, 1,2-dipalmitoylphosphatidylcholine; DSPC, 1,2-distearoylphosphatidylcholine; DMPG, 1,2-dimyristoylphosphatidylglycerol; POPS, 1-palmitoyl-2-oleoylphosphatidylserine; POPE 1-palmitoyl-2-oleoylphosphatidylethanolamine; DPPC-d₂ and DMPC-d₃₄ have deuterated acyl chains; DEPE, 1,2-dilauroylphosphatidylethanolamine.

- 481 P. Laggner, K. Lohner, R. Koyanova, and B. T. Tenchov, *Chem. Phys. Lipids*, 1991, **60**, 153.
 482 W. K. Liddle, C. R. Middaugh, and A. T. Tu, *Chem. Phys. Lipids*, 1987, **45**, 93.
 483 A. Ortiz and J. C. Gómez-Fernandez, *Chem. Phys. Lipids*, 1987, **45**, 75.
 484 G. Ceve, J. M. Seddon, R. Hartung, and W. Eggert, *Biochim. Biophys. Acta*, 1988, **940**, 219.
 485 M. R. Morrow and J. H. Davis, *Biochemistry*, 1988, **27**, 2024.
 486 K. S. Hamilton, K. R. Barber, J. H. Davis, K. Neil, and C. W. M. Grant, *Biochim. Biophys. Acta*, 1991, **1062**, 220.
 487 J. M. Canaves, J. A. Ferrazat, and J. M. Gonzalez-Ros, *Biochem. J.*, 1991, **279**, 413.
 488 P. W. Sanderson, L. J. Lis, P. J. Quinn, and W. P. Williams, *Biochim. Biophys. Acta*, 1991, **1067**, 43.
 489 A. Rolland, A. Brzozkiewicz, B. Shroot, and J.-C. Jamouille, *Int. J. Pharm.*, 1991, **76**, 217.
 490 J. K. Seydel, *Trends Pharmacol. Sci.*, 1991, **12**, 368.
 491 H. Talsma, M. J. van Steenberger, P. J. M. Salermink, and D. J. A. Crommelynck, *Pharm. Res.*, 1991, **8**, 1021.
 492 M. Bryszewska and R. M. Epand, *Biochim. Biophys. Acta*, 1988, **943**, 485.
 493 R. M. Epand, K. S. Robinson, M. E. Andrews, and R. F. Epand, *Biochemistry*, 1989, **28**, 9398.
 494 A. Ortiz, F. J. Aranda, and J. Gómez-Fernandez, *Biochim. Biophys. Acta*, 1992, **1106**, 282.
 495 M. Szögyi and T. Cserháti, *Biochem. Int.*, 1992, **26**, 499.
 496 D.-P. Ying, T. Mavromoustakos, K. Besthal, and A. Matryannis, *Biochim. Biophys. Acta*, 1992, **1103**, 25.

reduces the temperature and enthalpy (ΔH_m) of the chain melting temperature (T_m). From the decrease in the enthalpy with DNM concentration it is deduced that each DNM molecule prevents 35 DPPC molecules from participation in chain-melting. In contrast VRP has little effect on either T_m or ΔH_m but for the ternary system DPPC–DNM–VRP the presence of VRP almost eliminates the effect of DNM on ΔH_m . Fluorescence–polarization and circular dichroism experiments show that the elimination of the effects of DNM by VRP cannot be accounted for either by the displacement of DNM from the bilayer or by the formation of a complex between the two drugs so the origin of this intriguing effect remains obscure.

Lipid Mixtures. The impetus behind the study of lipid mixtures is the desire to get a better understanding of the behaviour of lipids in biological membranes. In this context the number of possibilities for experimentation on even binary and ternary mixtures is vast. The onset and completion temperatures for transitions seen in DSC thermograms can be used to construct phase diagrams. The series of papers by Dörfler *et al.*^{497–499} exemplifies this approach. Their works cover binary mixtures of phosphatidylethanolamines with differing acyl chain lengths in the range C₁₂ to C₁₈,⁴⁹⁷ mixtures of branched and unbranched phosphatidylcholines⁴⁹⁸ and mixtures of methylated phosphatidylethanolamines with phosphatidylcholines and ethanolamines.⁴⁹⁹ For the phosphatidylethanolamines differing in acyl chain length, the enthalpies of the phase transition at T_m ($L_\beta \rightarrow L_\alpha$) show interesting deviations from ideality. The enthalpy of the transition ΔH_u may be written as Equation 43,

$$\Delta H_u = x_1 \Delta H_1 + x_2 \Delta H_2 + \Delta H_m^1 + \Delta H_m^s \quad (43)$$

where x_1 and x_2 are the mole fractions of lipid components 1 and 2 with enthalpies of chain melting ΔH_1 and ΔH_2 respectively, and ΔH_m^1 and ΔH_m^s are mixing enthalpies of the high and low temperature phase. For ideal mixing ΔH_m^1 and ΔH_m^s would be zero and hence ΔH_u would be a linear function of x_1 (or x_2). For mixtures of PEs differing in acyl chain length by two CH₂ units ΔH_u shows a negative deviation from ideality, since the PE's are completely miscible in both phases it is deduced that $\Delta H_m^1 \geq \Delta H_m^s$ where ΔH_m^1 must be negative. In contrast, for PE's with a chain length difference of six CH₂ units a miscibility gap occurs and ΔH_u deviates positively from ideality, which is caused by a positive ΔH_m^1 with $\Delta H_m^s = 0$. Other binary systems studied by DSC include mixtures of DPPC with a range of gangliosides,⁵⁰⁰ binary mixtures of galactocerebrosides, with and without α -hydroxy substituents on the acyl chains, a range of zwitterionic and anionic phospholipids with double-chain cationic amphiphiles,⁵⁰¹ and mixtures of diheptadecanoylphosphatidylcholine with 1-behenoyl-2-lauroylphosphatidylcholine.⁵⁰² Amongst the ternary mixtures that have been studies are cerebrosides with sphingomyelin/cholesterol and phosphatidylcholine/cholesterol,⁵⁰³ ternary mixtures of phosphatidic acid, phosphatidylcho-

⁴⁹⁷ H.-D. Dörfler, G. Brezesinski, and P. Miethe, *Chem. Phys. Lipid.*, 1988, **48**, 245.

⁴⁹⁸ H.-D. Dörfler and P. Miethe, *Chem. Phys. Lipids.*, 1990, **54**, 61.

⁴⁹⁹ H.-D. Dörfler, P. Miethe, and A. Mops, *Chem. Phys. Lipids.*, 1990, **54**, 171.

⁵⁰⁰ H. Kojima, K. Hanada-Yoshikawa, A. Katagiri, and Y. Tamai, *J. Biochem.*, 1988, **103**, 126.

⁵⁰¹ J. R. Silvius, *Biochim. Biophys. Acta*, 1991, **1070**, 51.

⁵⁰² R. B. Sisk and C. Huang, *Biophys. J.*, 1992, **61**, 593.

⁵⁰³ D. S. Johnston and D. Chapman, *Biochim. Biophys. Acta*, 1988, **939**, 603.

line and phosphatidylethanolamine,⁵⁰⁴ and a range of phospholipid mixtures with fatty acid ethyl esters.⁵⁰⁵

Rodham and Chapman⁵⁰⁶ have advocated the use of derivative analysis of digitized DSC thermograms for phospholipid mixtures. DSC studies of lipid dispersions are usually scanned at rates between $0.1^{\circ}\text{C min}^{-1}$ and $5^{\circ}\text{C min}^{-1}$ although static heat capacity measurements of pure lipid dispersions indicate that rates of $0.1^{\circ}\text{C min}^{-1}$ may be too fast for complete equilibration across some phase transitions (*e.g.* the $\text{P}_{\beta'} \rightarrow \text{L}_\alpha$ transition of DPPC). By taking the second derivatives of thermograms it is found that the onset temperatures of transitions (*e.g.* the melting of indium, the $\text{P}_{\beta'} \rightarrow \text{L}_\alpha$ transition of DPPC) are less dependent on scan rate and is therefore a better method of studying perturbations of transitions. When applied to mixtures of DMPC and DSPC 'metastable' phases are detected dependent on the thermal history of the samples.

The effect of Ca^{2+} ions on the thermotropic properties of an equimolar ternary mixture of dipalmitoylphosphatidic acid (DPPA), DPPC, and DMPE is found to depend significantly on the molar ratio of $\text{Ca}^{2+} : \text{DPPA}$.⁵⁰⁴ In the absence of Ca^{2+} ions the thermogram of the ternary lipid system shows a peak characteristic of merged transitions of the three randomly mixed lipids. Addition of Ca^{2+} ions at a molar ratio $\text{Ca}^{2+} : \text{DPPA}$ less than 1 shifts the peak to a higher temperature, but above a molar ratio of 1 a major peak appears, which is similar to the transition for a binary mixture of DPPC and DMPE. These features were interpreted in terms of the formation of small aggregates of DPPA in a random mixture of DPPC and DMPE at low $\text{Ca}^{2+} : \text{DPPA}$ molar ratios, whereas at high molar ratios Ca^{2+} ions induce lateral phase separation between a $\text{Ca}^{2+}-\text{DPPA}$ phase and a DPPC/DMPE phase.

Interaction with Macromolecules. – In this section we consider studies in which thermochemical methods have been used to investigate interactions between biochemically important macromolecules (proteins, nucleic acids, and polysaccharides) with ligands. This is a somewhat heterogeneous area of study, but nevertheless important, particularly where biochemical specificity is involved. In biochemical processes involving non-covalent interactions in aqueous media a favourable Gibbs energy of binding may arise as a consequence of either enthalpically or entropically driven processes, and in this respect it is important to our understanding of biochemical interactions to establish which of these mechanisms prevails. This information can be obtained by the measurement of binding and association constants in combination with calorimetric data, and/or from the temperature coefficient of binding constants. Table 14 summarizes some types of system that have been reported and for each the modulus of $\Delta H/T\Delta S$ is given, which indicates the relative magnitudes of the enthalpic and entropy contributions to the Gibbs energies of interaction.

With the development of peptide synthesizers, and the technique of site-directed mutagenesis, it is now possible to change specific amino acid residues in peptides and proteins and examine the effects of such specific changes on interactions. The work of Connelly *et al.*⁵⁰⁸ on the interaction of the two fragments of pancreatic ribonuclease A exemplifies this approach. Ribonuclease A can be cleaved with

⁵⁰⁴ K. Ohki, K. Takahashi, S. Kato, and A. Maesono, *Chem. Phys. Lipids*, 1989, **50**, 109.

⁵⁰⁵ M. F. Omudeo-Sale, P. Palestini, and M. Masserini, *Chem. Phys. Lipids*, 1992, **61**, 149.

⁵⁰⁶ D. K. Rodham and D. Chapman, *Biochim. Biophys. Acta*, 1988, **959**, 84.

Table 14 Thermodynamic studies on biological macromolecule-ligand interactions

<i>Macromolecule</i>	<i>Ligand</i>	<i>Experimental Method</i>	$ \Delta H/TAS $ (Temp K)	<i>Ref.</i>
Human Serum Albumin Ribonuclease S (RNase A minus residues 1–20)	laurate and myristate various S-peptides (residues 1–20)	Equilibrium dialysis as function of <i>T</i> Titration calorimetry	7–20 (298) 1.30 ± 0.06 (298)	507 508
Winged bean agglutinin II	various sugars	Spectroscopy, DSC and titration calorimetry	2.74 ± 1.11 (298)	509,
Winged bean agglutinin II Monoclonal antibody Se 155·4	H-disaccharide/2' fucosyllactose trisaccharide O-antigen epitope	Spectroscopy Microcalorimetry	13/12 2.05 (298)	510 509 510,
Monoclonal antibody Se 155·4	(tetrasaccharide O-antigen) _{8–20}	Microcalorimetry	$20 (n = 8),$ 1.8 (<i>n</i> = 20)	511
Poly[(dA-T).poly(dA-T)] Poly[(dA-T).poly(dA-T)] Poly(dA).poly(dT) Poly(dA).poly(dT) DNA DNA DNA	distamycin A/neotropsin duanomycin/ethidium bromide distamycin A/neotropsin duanomycin/ethidium bromide elliptines ethidium bromide distamycin A	Spectroscopy & microcalorimetry Spectroscopy & microcalorimetry Spectroscopy & microcalorimetry Spectroscopy & microcalorimetry Spectroscopy & microcalorimetry Spectroscopy & microcalorimetry Spectroscopy & microcalorimetry	3.1/7.5 (298) 15/11 (298) 0.59/0.22 (298) 0.33/0.22 (298) 0.7 (298) 4.2 (298) 3.1 (298)	513 513 513 513 513 514 514 514

507 A. O. Pedersen, B. Honore, and R. Brodersen, *Eur. J. Biochemistry*, 1990, **190**, 497.508 P. R. Connelly, R. Varadarajan, J. M. Sturtevant, and F. M. Richards, *Biochem.*, 1990, **29**, 6108.509 S. Acharga, S. R. Patanjali, S. U. Sajjan, B. Gopalakrishnan, and A. Surolia, *J. Biol. Chem.*, 1990, **265**, 11586.510 F. P. Schwarz, K. Puri, and A. Surolia, *J. Biol. Chem.*, 1991, **266**, 24344.511 B. W. Sigurskjold, E. Altman, and D. R. Bundle, *Eur. J. Biochem.*, 1991, **197**, 239.512 K. J. Breslauer, D. P. Remeta, W.-Y. Chou, R. Ferrante, J. Curry, D. Zanezkowski, J. G. Snyder, and L. A. Marky, *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 8922.
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subtilisin at the peptide bond between residues 20 and 21 to give the S-protein (residues 21–124) and a peptide (residues 1–20). However, it has been shown that residues 16–20 are not important in binding the peptide to the protein and a truncated peptide, the S-peptide (residues 1–15) interacts to give a structure which is identical with ribonuclease-S. The hydrophobic residues in the S-peptide which are believed to be particularly important are Met-13 and Phe-8. To investigate the importance of Met-13 Connelly *et al.*⁵⁰⁸ prepared a range of S-peptides in which Met-13 was replaced by seven other amino acid residues (glycine, alanine, α -amino-n-butyric acid (ANB), valine, leucine, isoleucine, and phenylalanine). The interaction of these S-peptides with the S-protein was investigated by titration calorimetry. The consequence of these changes was in every case to weaken the interaction. The Gibbs energies of interaction (ΔG°) for the range of peptides at pH 6 were found to be as follows: -40 kJ mol^{-1} (native S-peptide), -39 kJ mol^{-1} (Met-13 → Ile), -38.5 kJ mol^{-1} (Met-13 → Val), -33 kJ mol^{-1} (Met-13 → ANB), -37 kJ mol^{-1} (Met-13 → leu), -28 kJ mol^{-1} (Met-13 → Phe), -22.5 kJ mol^{-1} (Met-13 → Ala), and -19 kJ mol^{-1} (Met-13 → Gly). Thus some of the changes in ΔG° caused by the substitutions are relatively small. All seven complexes produced crystals of identical morphology and X-ray analysis gave very similar unit cell parameters. It was deduced that the relative stabilities of the complexes are governed by differences in the energetics of taking the residue in the isolated peptide, which is α -helical, from the aqueous solution and placing it in contact with the hydrophobic core of the protein, and that the major effect arises not from the differences in hydration of the residues in the S-peptide but from packing and/or hydration of residue-13 in the complexes.

Sugar and sugar-derivative interactions with plant lectins are generally highly specific and of considerable interest in relation to the effects of plant lectins on cell agglutination and growth. The basic lectin isolated from the winged bean *Psophocarpus tetragonolobus*, winged bean agglutinin (WBA 1), consists of two identical subunits of molecular mass 29 000 with one carbohydrate binding site per subunit. The thermodynamics of the binding of D-galactopyranoside derivatives have been studied by titration calorimetry and DSC.⁵¹⁰ The titration calorimetry gave single binding constants ranging from $0.56 \pm 0.14 \times 10^3\text{ M}^{-1}$ for D-galactopyranoside (Gal) at 323 K to $7.2 \pm 0.5 \times 10^3\text{ M}^{-1}$ for 2-acetamido-2-deoxygalactopyranoside (GalNAc) at 298 K. The binding enthalpies ranged from $-28.0 \pm 2.0\text{ kJ mol}^{-1}$ for GalNAc at 298 K to $-14.3 \pm 0.1\text{ kJ mol}^{-1}$ for methyl- β -D-galactopyranoside at 322 K. The binding reactions are enthalpically driven and occur with little change in the heat capacity. WBA 1 denatures on heating in the pH range 5.6–7.4, with two transitions occurring; one at 341.6 K, in which the two domains of the protein unfold as a single entity, and a higher temperature transition at 347.8 K, in which the remaining WBA 1 dimer unfolds to two monomers. On binding carbohydrate ligands both these transitions are shifted to higher temperatures. It is found that one ligand binds to the two domains unfolding at the lower temperature and the other to the domain unfolding at the higher temperature.

The studies of Sigurskjold *et al.*^{511,512} also address protein–carbohydrate interactions; here, the interactions between *Salmonella* O-antigen oligosaccharides and a

⁵¹² B. W. Sigurskjold and D. R. Bundle, *J. Biol. Chem.*, 1992, **267**, 8371.

monoclonal antibody Se155-4 have been investigated by titration microcalorimetry. The thermodynamics of binding of a trisaccharide (methyl 2-*O*-(α -D-galactopyranosyl)-3-*O*-(α -D-3,6-dideoxy-*xylo*-hexopyranosyl)- α -D-mannopyranoside, abbreviated to Gal[Abe]Man), which represents the epitope of a *Salmonella* O-antigen, are particularly interesting in that the temperature dependencies of the enthalpy and entropy of binding are large in the temperature range 9–37°C and show compensation. The heat capacity for binding changes sign from 1470 J mol⁻¹ K⁻¹ at 9°C to -1470 J mol⁻¹ K⁻¹ at 37°C. A large negative heat capacity change is usually indicative of a hydrophobic interaction, which is consistent with the structure of the antibody binding site known to be dominated by aromatic amino acid residues. The interaction between the binding site and Gal[Abe]Man, as well as involving hydrogen bonding and van der Waals interactions, appears to involve a substantial hydrophobic contribution, since only the latter would give rise to a large negative heat capacity change. There is a good correlation between the magnitude of heat capacity (ΔC_p°) changes and the area ΔA_{np} of non-polar surfaces exposed to water at 25°C.⁵¹⁵ For the transfer of hydrocarbons in water at infinite dilution to the pure liquid state $\Delta C_p^\circ / \Delta A_{np} = -1.38 \pm 0.38 \text{ J mol}^{-1} \text{ K}^{-1} \text{ Å}^{-2}$, and for the folding of proteins $\Delta C_p^\circ / \Delta A_{np} = -1.05 \pm 0.13 \text{ J mol}^{-1} \text{ K}^{-1} \text{ Å}^{-2}$. From a review of the literature, Sigurskjold and Bundle⁵¹² use the difference between the heat capacity of solid state sugars and the partial molar heat capacity of infinitely diluted aqueous solutions together with values of ΔA_{np} , to show that $\Delta C_p^\circ / \Delta A_{np} = -1.02 \pm 0.14 \text{ J mol}^{-1} \text{ K}^{-1} \text{ Å}^{-2}$ for a range of sugars. In this respect the hydrophobic effects involved in antibody–carbohydrate binding resemble hydrocarbon–water and protein folding interactions, although it is stressed that the latter processes have more modest temperature dependencies.

Enthalpy–entropy compensations feature in the studies of DNA–drug binding studies.^{513,514,516} The drugs netropsin and distamycin bind in the minor grooves of DNA, daunomycin is both an intercalator and groove binder and ethidium bromide is an intercalator. The thermodynamic parameters (ΔG° , ΔH° , and ΔS°) for the binding of these drugs to the homopolymer duplex poly(dA)–poly(dT) and the copolymer duplex poly[d(A-T)]–poly [(dA-T)] were measured by a combination of spectroscopy and batch microcalorimetry.⁵¹³ All the drugs exhibit similar binding affinities (ΔG°) at 25°C for the two polymers. However, the enthalpies and entropies are significantly different. At 25°C, binding of the drugs to the alternating copolymer is entropy driven whereas binding to the homopolymer is enthalpy driven. Thus the similar binding affinities result from compensating changes in the enthalpy and entropy. A difficulty in making microcalorimetry measurements in drug systems arises if the drugs self-associate in solution; in such cases it is necessary to make corrections for the enthalpies of self-association. In a detailed study of the interaction between daunomycin and DNA duplexes this problem has been overcome by the development of a stopped-flow microcalorimeter capable of measuring heats of interaction of the order of microjoules.⁵¹⁶ With this instrument it has been possible to determine the enthalpies of interaction at very low drug concentrations (10–20 μM) where the drug is monomeric. The enthalpies of binding of daunomycin to a range of DNA

⁵¹⁵ J. R. Livingstone, R. S. Spolar, and M. T. Record, Jr., *Biochemistry*, 1991, **30**, 4237.

⁵¹⁶ D. P. Remeta, C. P. Mudd, R. L. Berger, and K. J. Breslauer, *Biochemistry*, 1991, **30**, 9799.

host polymers were measured as a function of drug to phosphate group ratios. For the range of 10 DNA host polymers studied, a broad range of binding enthalpies were found reflecting sequence-dependent differences that included both exothermic and endothermic interaction heats. Together with measurements of the extent of drug association using NMR, the data were used to correct previously measured batch microcalorimetric data obtained at higher drug concentrations (0.5 to 1.0 M), for the enthalpy contribution arising from binding-induced dissociation of drug aggregates. Very good agreement was found between the net binding enthalpies derived from the corrected batch microcalorimetry and the stopped-flow microcalorimetry. This is the most direct model-independent determination of the binding enthalpies of the duanomycin–DNA interaction.

Amongst the other biological macromolecule interaction studies which report thermodynamic data may be noted studies on phospholipase A₂–ligand interaction,⁵¹⁷ substrate binding to α -thrombin as a function of pH,⁵¹⁸ the interaction of amine drugs with diphenylmethyl functionality to 2-hydroxypropyl- β -cyclodextrin,⁵¹⁹ cytidine-2-monophosphate binding to ribonuclease A,⁵²⁰ biotin binding streptavidin,⁵²¹ various substrate and inhibitor interactions with the enzyme 5-enolpyruvylshikimate-3-phosphate synthase,⁵²² the interaction of a homologous series of *n*-alkylsulfates with insulin and acid and alkaline solutions,⁵²³ and a study of ternary systems of two surfactants of differing hydrophobicity and insulin, in which it is shown that insulin–surfactant complexes can exhibit retrograde dissociation due to the formation of mixed micelles.⁵²⁴

⁵¹⁷ R. L. Biltonen, B. K. Lathrop, and J. D. Bell, *Methods in Enzymology*, 1991, **197**, 234.

⁵¹⁸ E. DiCera, R. De Cristofaro, D. J. Albright, and J. W. Fenton, *Biochemistry*, 1991, **30**, 7913.

⁵¹⁹ W.-Q. Tong, J. L. Lach, T.-F. Chin, and J. K. Guillory, *J. Pharm. Biochem. Anal.*, 1991, **9**, 1139.

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⁵²² J. E. Ream, H. K. Yuen, R. B. Frazier, and J. A. Sikorski, *Biochemistry*, 1992, **31**, 5528.

⁵²³ F. Samiento, G. Prieto, and M. N. Jones, *J. Chem. Soc., Faraday Trans.*, 1992, **88**, 1003.

⁵²⁴ M. N. Jones, E. M. Gilhooley, and A. R. Nicholas, *J. Chem. Soc., Faraday Trans.*, 1992, **88**, 2733.