International Journal of Pharmaceutics, 13 (1983) 115–144
Elsevier Biomedical Press

Review Article

Enthalpy–entropy compensation analysis of pharmaceutical, biochemical and biological systems

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(Received June 21st, 1982)
(Accepted July 5th, 1982)

Summary

Although linear free-energy relationships are familiar research tools in pharmaceutical and biochemical science, the analysis of data in terms of a further extrathermodynamic analysis, namely *enthalpy–entropy compensation*, is little used. This review attempts to draw attention to this latter procedure and describes the advantages and disadvantages of the method by using examples taken from the recent literature. Consideration is first given to the manner in which the analysis should be performed, and to the significance of found compensation relationships. Secondly, the analytical procedure is applied to data derived from a number of phenomena of pharmaceutical and biochemical interest, including, liquid–liquid distribution, chromatographic retention, aqueous solubility, complexation, film spreading, liposome–water partitioning, biological membrane permeation and receptor/enzyme–small molecule interactions. Results indicate that enthalpy–entropy compensation analysis, when performed using the statistically correct coordinate plane of enthalpy versus free-energy, can be a most valuable tool for examining the effect of physicochemical structure on various phenomena, for verification of similarity in behaviour, and for indicating outliers from general relationships.

1. Perspective

'Science owes more to the steam engine than the steam engine owes to Science
L.J. Henderson (1917)

Though Henderson was correct when reviewing how the concepts of heat engines and Carnot's cycle, etc., had aided the understanding of the energetics of systems,

0378-5173/83/0000-0000/$03.00

others have illustrated that the closed box approach to examining behaviour using classical thermodynamics can be limited. Pharmaceutical science is involved in examining extremely complex systems—a necessary activity if drug action is to be understood and improved upon. These complexities have suggested to many workers a research approach based on the use of stochastic modelling. Such efforts make use of a macroscopic (or thermodynamic) analysis, modified by the addition of sufficient microscopic detail to permit structural and medium effects to be considered. Although the meanings of the laws of classical thermodynamics are still subject to some debate, this present contribution takes the reader into the realm of *extrathermodynamics* to attempt to show how a specific extrathermodynamic analysis, *i.e. enthalpy–entropy compensation*, can be used in pharmaceutical science to appreciate and rationalize experimental findings. The study is intended to be illustrative rather than comprehensive, and shall incorporate some results from our own programme.

II. Extrathermodynamics

Prausnitz (1979) has written, 'classical thermodynamics is revered, honored, and admired, but in practice is inadequate'. He suggests further, a 3-step procedure necessary for the application of classical thermodynamics. First, the projection of a problem into an abstract (mathematical) world using abstract functions (such as chemical potential), which can be then used in the second step to solve the problem in the abstract domain. Although classical thermodynamics accomplishes these two steps easily, the reprojection of the solution back into the real world is handled insufficiently by the science. To return to physical reality it is necessary to relate the abstract terms and concepts to real physical properties. Prausnitz has suggested the use of molecular thermodynamics for solving real problems; with molecular thermodynamics being seen as a synthesis of classical approaches, statistical thermodynamics, molecular physics and physical thermodynamics. One way in which we can achieve such results is to employ *extrathermodynamics*. Unlike extra mathematics, etc., this does not imply additional effort; rather the Latin prefix is present to show that the science lies outside the formal structure of thermodynamics, although the approach is similar to that of thermodynamics, in that related microscopic mechanisms do not have to be identified during use. *Extrathermodynamic relations* have thus not risen directly from the principles of classical thermodynamics but rather are relationships found (or examined for), between (and amongst), thermodynamic quantities.

By modelling of complex systems the effects of changes in solute structure and/or environment are given in terms of 'similar', (by assumption), effects in model systems. Although pharmaceutical science abounds with such examples, they are almost entirely founded using free-energy-based terms. For example Eqn. 1 is the generalized form of the Hansch equation (1964), where the effect of bioactive molecule structure changes on activity and various physicochemical properties is given by:

$$\delta_x \Delta G^0_{BR} = \delta_x \Delta G^0_{hyd} + \delta_x \Delta G^0_{elec} + \delta_x \Delta G^0_{steric} \propto \delta_x \log K_{BR} \qquad (1)$$

where ΔG^0 terms are the standard free-energy changes in biological response (BR), hydrophobicity (hyd), electronic character (elec), and steric characteristics; the operator δ denotes the difference between an arbitrary and a standard process (in this case the effect of molecular substitution by group x). K is the measured constant response. Writing this equation in its more applicable form, using linear terms only, gives

$$\log C^{-1} = f \cdot (\log K_d) + g \cdot \log K_e + h \cdot S \tag{2}$$

where f–h are constants for any model, K_d, K_e and S are the physicochemical models of hydrophobic–hydrophilic balance (oil–water distribution constant), electronic character (ionization constant) and steric character (various terms available), respectively, and C refers to the concentration of compound necessary to elicit a defined biological response.

Eqns. 1 and 2 are seen to relate only the *extents* of properties to one another and are hence forms of *linear free-energy relationships*.

Although the first recognized linear free-energy relationship (LFER) is that due to Brønsted, it is as a result of the studies of Hammett (1940) that LFERs have become part of the infrastructure of physical organic chemistry. The Hammett equation (sic) relates changes in rate (k) or equilibrium (K) constants to changes in solute structure, such that

$$\log K = \log K_o + \rho\sigma \tag{3}$$

or

$$\log k = \log k_o + \rho\sigma \tag{4}$$

where subscript o denotes the unsubstituted or parent compound; the substituent constant σ quantifies the change in effect (relative to hydrogen), and is in principle independent of the nature of a process; and the process constant ρ depends on the nature of that process. For a chemical reaction correlated by the Hammett equation it can be given that

$$\log \frac{k_x}{k_y} = \rho \log \frac{K_x}{K_y} \tag{5}$$

where x and y are substituents. Combination of Eqn. 5 with the van 't Hoff isotherm gives

$$\frac{\Delta G_x^*}{RT} - \frac{\Delta G_y^*}{RT} = \rho \frac{\Delta G_x^0}{RT} - \rho \frac{\Delta G_y^0}{RT} \tag{6}$$

where ΔG^* is the free-energy of activation, and R and T are the gas constant and

temperature, respectively. Use of a δ operator (Eqn. 1) results in Eqn. 6 becoming

$$\delta_x \Delta G^* = \rho \delta_x \Delta G^0 \tag{7}$$

which is the fundamental Hammett linear free-energy relationship.

III. Isokinetic–isoequilibrium relationships [1]

The validity of the Hammett equation has been much discussed (e.g. Wells, 1968). To be able to examine what may be the cause of a linear relationship between the free-energy values (i.e. log k and log K) of two series we first need to recall the Gibbs equation, i.e.

$$\Delta G = \Delta H - T\Delta S \tag{8}$$

where ΔH and ΔS are the changes in enthalpy and entropy occurring upon a process. For a given series, substituents may affect both the enthalpic and/or entropic terms which contribute to the free-energies of activation or reaction. Describing the effect of substituents by

$$\delta\Delta G = \delta\Delta H - T\delta\Delta S \tag{9}$$

then it is seen that a linear Hammett relationship can only exist if each series shows either one of the following characteristics: (a) ΔS is constant; (b) ΔH is constant; or (c) that ΔH is linearly related to ΔS. For the cases where $\delta_x \Delta S = 0$, or $\delta_x \Delta H = 0$, such series are said to be *isoentropic* or *isoenthalpic*. However, the great majority of studied systems show the third type of behaviour, a phenomenon which has been much studied since it was brought to general attention by Leffler and Grunwald (1963). These workers proposed for such behaviour the general relationship:

$$\delta_x \Delta H = \beta \delta_x \Delta S \tag{10}$$

β is a proportionality constant [2], but since it has dimensions of absolute temperature, it is termed variously the *isokinetic*, the *isoequilibrium* or the *compensation temperature* (we shall return to this function later). From Eqns. 9 and 10 it follows that

$$\delta_x \Delta G = \delta_x \Delta H - (T/\beta)(\delta_x \Delta H) \tag{11}$$

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[1] The compensation effect is also referred to the θ rule in heterogeneous catalysis (Galwey, 1977).

[2] Leffler and Grunwald (1963) derived Eqn. 10 from phenomenological models using two approximations, and have shown that when ΔS and ΔH are constant and $\Delta C_p = 0$, (C_p = heat capacity) then β is a constant, but that for the case of ΔC_p = constant and $d\Delta C_p/dT = 0$, then β is temperature dependent.

or

$$\delta_x \Delta G = \delta_x \Delta H(1 - T/\beta) \tag{12}$$

(From this can it be seen that isoenthalpic and isoentropic series are unique cases of Eqn. 12, where β is zero and infinity, respectively.)

Unfortunately, although a great number of reactions and equilibria give linear relationships between changes in enthalpies and entropies, (e.g. Leffler and Grunwald, 1963), many recent workers have pointed out that *such correlations are practically meaningless* since the manner of their production is prone to large statistical error. This can be illustrated quite easily by first recalling that Eqn. 10. by proposing proportionality between enthalpy and entropy changes, implies (see Eqn. 12), a proportionality between free-energy and enthalpy changes. As discussed by, for example, Petersen (1964) and Ritchie and Sager (1964), a linear enthalpy change–entropy change plot can arise from the errors in measurement of ΔH being proportional to those in measurement of ΔS. Indeed ΔS is obtained using ΔH and ΔG values, with the result that a non-compensated series (i.e. where there is no relationship between ΔG and ΔH) has a high correlation between ΔH and ΔS. Thus there exists the paradox, that *the less accurately ΔH and ΔS are measured, the greater is the likelihood that they will be proportional to one another* (Ritchie and Sager, 1964; Exner, 1973; Krug et al., 1976a and b), and hence the corollary that *linear plots of ΔH and ΔS are not evidence for relationships between ΔH and ΔS.*

To attempt to circumvent this problem Exner (1964) has proposed that a criterion for the existence of a compensation effect is the linear relationship between kinetic (or equilibrium) constants at two temperatures, i.e.

$$\log k_{(T=1)} = e \cdot \log k_{(T=2)} + \text{constant} \tag{13}$$

with the term e being related to β (Eqn. 10) by

$$\beta = T_1 T_2 \left(\frac{e - 1}{e\, T_1 - T_2} \right) \qquad (T_1 > T_2) \tag{14}$$

Although Exner has also proposed a graphical method linked to a refined statistical treatment of the data (Exner, 1973), Leffler (1965) has warned that such techniques are inadequate since multiple interaction mechanisms per se will produce non-linear compensation effects, and that Exner's treatments (as well as that due to Wold and Exner, 1973), assume a linear functionality of this extrathermodynamic relationship.

(a) Testing for linear ΔH–ΔS compensation

Fortunately, it now is clear that a method to examine for ΔH–ΔS compensation behaviour is available, due to the extensive thermodynamic and statistical examination of the problem by Krug et al. (1976a, b and c; 1977) and Krug (1980). Krug and his group have shown that although a least-squares analysis of enthalpy–entropy values gives a β value that is closer to the *least* probable number rather than the

most probable number, an analysis of enthalpy–free-energy estimates is able to be non-biased.

This is best considered by examining Krug et al.'s statistical analysis of the

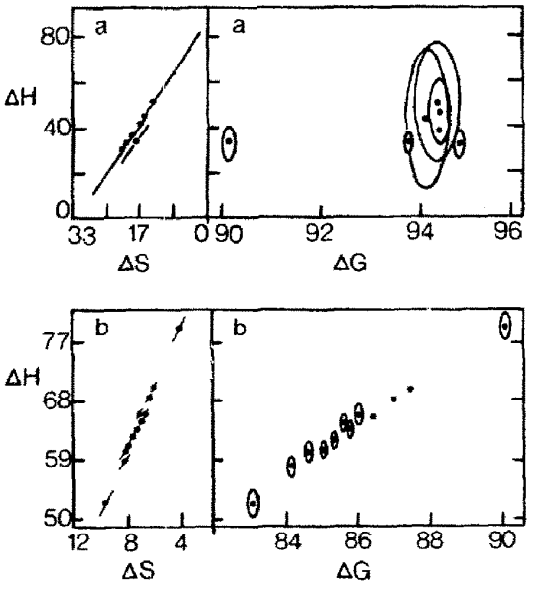

Fig. 1. Plots of thermodynamic parameter estimates and their respective 50% confidence regions for (a) the oximation of thymylketones and (b) the hydrolysis of ethyl benzoate. The lack of structure in the ΔH–ΔG plot for (a) indicates that the apparent linear distribution of estimates in the ΔH–ΔS plot is due to a realization of the propagation of experimental errors, and that unlike (b) no linear chemical compensation effect can be detected (ΔH, ΔG and ΔS have units of $kJ \cdot mol^{-1}$, $kJ \cdot mol^{-1}$ and $J \cdot mol \cdot K^{-1} \times 10^{-1}$, respectively). (Reproduced in modified form with permission from the authors and the Journal of Physical Chemistry, Vol. 80 page 2347. Copyright 1976 American Chemical Society.)

problem, by which they found that enthalpy and entropy estimates are distributed by experimental and measurement errors in elliptical probability regions that are very elongated and appear as lines (see Fig. 1), with slopes being the harmonic mean of the experimental temperatures (Thm). ΔH, ΔS and ΔG_{Thm} can be determined from a plot of log K (or log k) versus T^{-1}, with ΔH being estimated from the slope, ΔS from the intercept at $T^{-1} = 0$, and ΔG_{Thm} from the intercept at $T = Thm$. However, the only intercept estimate that is uncorrelated with the slope estimate is at $T = Thm$, (Krug et al., 1976a), and further, ΔS estimates are commonly so far removed from the data (Krug et al., 1976b) that errors in K (or k) propagate into an approximately straight line on a ΔH–ΔS plot, but propagate independently in a ΔH–ΔG plot (at Thm). These workers have recommended (Krug et al., 1976c) that regressions of functionalities between thermodynamic variables be performed in the ΔH_{Thm}–ΔG_{Thm} plane.

(b) β: meaning and measurement

Using ΔH values obtained with van 't Hoff operators, then a linear compensation

can be demonstrated by a visual inspection of the van 't Hoff plots, since all lines should intersect at a given point, β^{-1}. One can then plot [3] ΔH against ΔG_{Thm}, giving a line of slope γ. A linear compensation in ΔH–ΔG_{Thm} coordinates is related (Krug et al., 1976b and c; Krug, 1980) to that in ΔH–ΔS coordinates by

$$\Delta H = \gamma \Delta G + (1 - \gamma)\Delta G_{\beta} \tag{15}$$

where

$$\gamma = 1/(1 - Thm/\beta) \tag{16}$$

The physical meaning of β (Eqns. 10, 12 and 16) has been the subject of much debate (e.g. Leffler and Grunwald, 1963; Exner, 1963). It has been concluded (Exner, 1973; Krug et al., 1976b and c) that for chemical reactivity, practically all reported ΔH–ΔS correlations were incorrectly analyzed and that nearly all reported values of β have been artifacts due to incorrect data-handling procedures. Eqn. 16 has a number of important features in this respect. Apart from showing how β is easily calculated [4] from the ΔH–ΔG_{Thm} slope, it reveals that β may be not only positive but also negative, and as $\gamma \rightarrow 1$, β is subject to very large error and becomes very dependent upon the value of γ, such that a very small difference in slope will make a large difference in β (Tomlinson et al., 1981). Although this suggests to the present author and others (e.g. Exner, 1973) that the estimation of β and its discussion should not be the main aim of this extrathermodynamic analysis, it has been demonstrated (Krug et al., 1976c) that either least-squares regression of ΔH on ΔG_{Thm} estimates or a more exact technique (M-regression) [5], which takes into account the ratio of variances of both ΔH and ΔG, may be used to obtain β. [Krug et al.'s (1976a–c) statistical methods for analyzing ΔH vs ΔS data are based on the null hypothesis of

$$H_0: \beta = Thm \tag{17}$$

such that if the harmonic mean temperature, Thm, does not fall within the 95% confidence intervals of the estimated value, then it can be asserted, following rejection of the null hypothesis, that the observed ΔH–ΔS pattern is due to chemical (and not statistical) factors.]

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[3] Equivalent to a plot of $(1/Thm)(d\ln K/dT^{-1})$ versus $\ln K_{Thm}$ (for equilibrium constants).

[4] β cannot be calculated from the van 't Hoff plot ordinate intercept estimate by, for example, least-squares regression, since although a small error in the slope makes no difference for the intercept at the middle of the data (Section IIIa), the further from the mass of data one is the greater will be the error in calculating the ordinate intercept value.

[5] Krug et al. (1976c) point out that ordinary linear regression is a special case of M-linear regression for the case of no uncertainty in one of the variables, and hence strictly should not be used for regressions of ΔH against ΔG_{Thm}. Although, as discussed by Exner (1973), ΔG can be regarded as an independent variable since errors in its determination are generally very small.

(c) Non-linear compensation

Although visual inspection of data in the ΔH–ΔG_{Thm} plane will indicate a linear compensation, functionalities other than the linear one can exist between these two estimates, and, after a review of chemical theory and plots of ΔH_{Thm} versus ΔG_{Thm} estimates, it has been argued (Krug et al., 1976c) that linear chemical compensation between ΔH and ΔS may be considered a local linearity of a non-linear function (see Krug (1980) for a further discussion). Although few of the examples given in this contribution will show non-linear effects, further discussion on these can be found in Krug et al. (1976c) and Krug (1980).

(d) Calorimetric determination of ΔH

Although calorimetric methods will give ΔH values directly, such ΔH values still should not be correlated with ΔS values, nor should subsequently derived ΔS values be correlated with ΔG estimates. Both correlations will lead to an overestimation of the fit (Exner, 1973), and hence give totally inaccurate values of β.

(e) Significance of linear ΔH–ΔS compensation

Since estimates of ΔH and ΔG at the harmonic mean of the experimental temperatures are not statistically correlated, any observed linear (or indeed non-linear) relation between them probably has a chemical causality. One can deduce that if, for a series of compounds, all points fall on the same compensation line, that all changes in behaviour caused by alterations in solute structure and/or environment have a common physicochemical basis. The corollary to this is that occasional points which are removed from the compensation line indicate either a compound or environment acting by a different mechanism, or a suspected experimental or computational error, and point towards their re-examination. Thus, use of such an analytical function helps to classify, understand and perhaps even to predict behaviour. In the following section examples, relevant to pharmaceutical science and which illustrate these 3 functions of ΔH–ΔS extrathermodynamic analysis, are given.

IV. Compensation effects in pharmaceutical science

(a) Liquid–liquid distribution

Eqn. 1 illustrates the importance attached to the *hydrophobicity* of molecules with respect to their biological action. Particular utility has been made of liquid–liquid distribution coefficients (i.e. oil–water partition coefficients), as descriptors of solute hydrophobicity. Such information may be used not only in drug design (Eqn. 2), but in preformulation and analysis (separation techniques). In addition, distribution coefficients can give information on the solution thermodynamics of molecules of pharmaceutical interest, which can enable thermodynamic data to be derived for use in prediction of behaviour in such phenomena as solubility, compatibility, membrane permeability, etc.

Although solute oil–water distribution has been studied since the last century, and a large collection of distribution coefficients exist (Hansch and Leo, 1979), most

work has concentrated on determination of the free-energy-based coefficient itself, with few workers even reporting the temperature of measurement. Such information indicates the extent of distribution, but yields little information on why the process occurs. This can be afforded by a more complete thermodynamic description of the system to include, for example, the enthalpic and entropic contributions towards the distribution. Although it has recently been demonstrated by us (Kinkel et al., 1981) that enthalpy–entropy linear compensation can exist during organic solute partitioning, we have also indicated that this finding is: (1) dependent upon the nature of the two phases used; and (b) the method of obtaining estimates of ΔH and ΔS. Thus, Fig. 2 gives the enthalpy–entropy linear compensation plot [$(\Delta H - \Delta G)_{Thm}$ plane] found (Kinkel et al., 1981) for the distribution of some aromatic solutes between water and 2,2,4-trimethylpentane. The origin of the linear compensation behaviour (Table 1 in that article), can be postulated as due to the unique properties of water (Némethy et al., 1963; Lumry and Rajender, 1970; Tanford, 1973), suggesting that 2,2,4-trimethylpentane behaves almost as an ideal 'inert' hydrocarbon. Other examples of solute transfer to 'inert' solvents are given in Fig. 2 and described by Table 1 of Kinkel et al. (1981); all show linear enthalpy–entropy compensation. However,

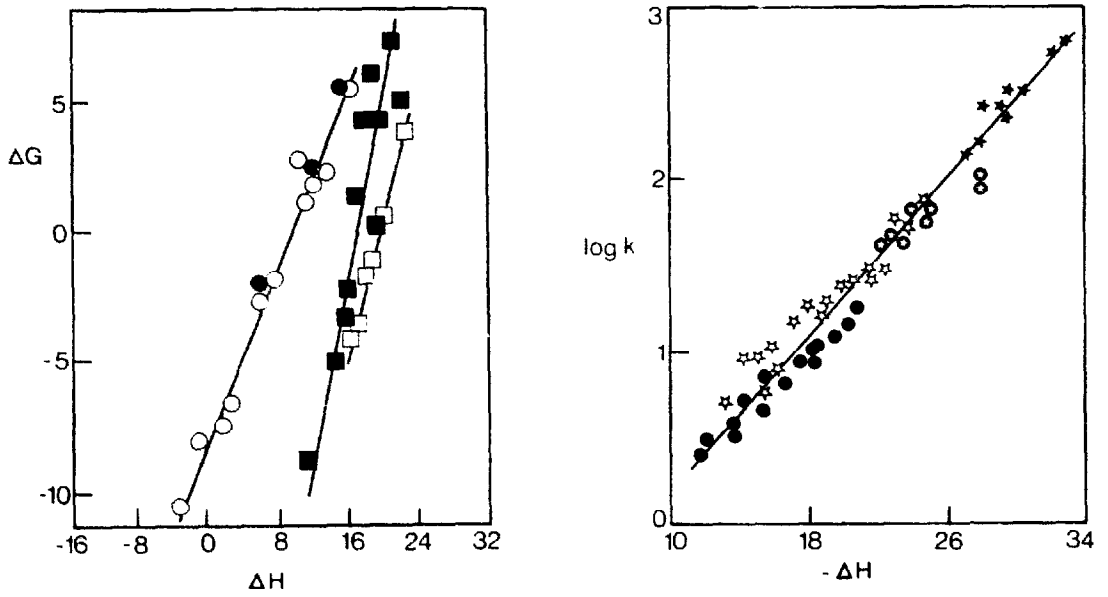

Fig. 2. Relationships between free-energies and enthalpies of solute distribution between solvent pairs having little mutual solubility. Thermodynamic quantities are in $kJ \cdot mol^{-1}$ units based on a molar concentration scale at 298°K (Thm). Key: circles are for water–2,2,4-trimethylpentane (closed points for microcalorimetric data); open and closed squares are for water–cyclohexane systems with different classes of solutes. (Modified from Kinkel et al., 1981.)

Fig. 3. Relationship between the logarithm of the chromatographic capacity factor, k', and enthalpies of retention, ΔH, ($kJ \cdot mol^{-1}$) at Thm (35°C) for the retention of substituted alkylbenzoates in various reversed-phase high-performance liquid–solid systems. Symbols denote different phase systems. (Tomlinson et al., 1981.)

partitioning systems where there is a high mutual solubility between the oil and the water phases (e.g. water–octan-1-ol) do not exhibit linear compensation when ΔH is determined from van 't Hoff plots, even though this has been claimed (Rogers and Wong, 1980) to be the case. Rogers and Wong presented data for the distribution of phenols between 0.15 mol · dm^{-3} aqueous NaCl and octan-1-ol and although they reported an excellent correlation between ΔH and ΔS (correlation coefficient, $r = 0.942$; $n = 18$), when the correct $(\Delta H - \Delta G)_{Thm}$ plane is used, no linear compensation ($r = 0.402$) is found. This does not mean that in the water–octan-1-ol system there is no true linear ΔH–ΔS compensation. As discussed for the micellization process, (Holtzer and Holtzer, 1974), macroscopic operational van 't Hoff relationships fail if the system itself changes with temperature, (in the case of micellization, the micellar number may change), and for water–octan-1-ol, temperature changes will alter the various secondary equilibria occurring—so causing an alteration in the standard state of this system. We are of the opinion (Kinkel et al., 1981) that only direct calorimetric determinations of ΔH can be used to examine for compensation for such solvent pairs, and our current researches (Riebesehl and Tomlinson, 1981) are directed towards this (Fig. 2).

It also appears that compensation patterns exist for the *kinetics* of liquid–liquid distribution (Schumacher and Nagwekar, 1974; Kinkel and Tomlinson, 1982), and their finding may well lead to a more complete description of the distribution effect being possible.

(b) Chromatographic retention

Although most interest in high-performance liquid chromatography (HPLC) is for analytical purposes, the technique is also useful for providing physicochemical information on molecules of pharmaceutical interest (e.g. Hafkenscheid and Tomlinson, 1981; Tomlinson, 1981).

Thus research in this area deals with optimizing and predicting solute retention and in describing retention in mechanistic terms. Though a thermodynamic approach to retention in chromatography can be argued as being an essential beginning to any constructive attempt at understanding and predicting retention (Snyder and Poppe, 1979), extrathermodynamics, in the form of linear free-energy relationships, has long been used to successfully reduce the amount of data required for practical predictions of retention.

Recent attention (Melander et al., 1978; Riley et al., 1979; Riley et al., 1981; Tomlinson et al., 1981) has been given to the phenomenon of linear enthalpy–entropy compensation occurring in liquid chromatographic systems. Over the normally accessible temperature range, retention (in the form of the capacity factor, k'), can be related to temperature by a modified van 't Hoff equation, viz.

$$\ln k' = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln\psi \qquad (18)$$

where ψ is the phase ratio, ΔH and ΔS are, here, the enthalpy and entropy changes upon solute transfer to the stationary phase; and the free-energy change, ΔG, during

solute retention can be related to both the thermodynamic distribution constant for the process and the capacity factor by

$$\Delta G = -RT \ln K_d = -RT \ln(k' \cdot \psi^{-1}) \quad (19)$$

By combining Eqns. 12 and 18 it can be shown (Melander et al., 1978), for reversed-phase HPLC systems (i.e. where the stationary phase is apolar) that

$$\ln k'_T = -(\Delta H/R) \cdot (1/T - 1/\beta) - \Delta G_\beta/R\beta + \ln \psi \quad (20)$$

where, to perform a ΔH–ΔS compensation analysis, T = Thm. In reversed-phase LC solute distribution from a mobile to a stationary phase is generally exothermic, that is, an increase in temperature causes a fall in retention. This is a particularly intriguing observation since in these systems retention is often presumed to be effected by the same physicochemical process responsible for the hydrophobic process, which has been regarded (Scheraga, 1979) as being entropically driven and endothermic. Thus there is major inconsistency between theoretical descriptions of the hydrophobic effect and experimental findings. This holds true also for liquid–liquid distribution behaviour (Section IV(a)) and for other supposedly hydrophobic-effect-mediated phenomena (see, for example, Section IV(d)).

For HPLC, however, some findings (Melander et al., 1978; Riley et al., 1979; Vigh and Varga-Puchony, 1980; Tomlinson et al., 1981), suggest that for reversed-phase systems a single compensation relationship can be used to describe retention of various solutes with a variety of eluents and stationary phases (see, for example, Fig. 3). Theory (Horváth et al., 1976; Krug et al., 1976a, b and c) suggests that a similar mechanism of retention (i.e. solvophobic chromatography) can be operating in these systems, such that the stationary material is acting as an 'inert' environment with column selectivity being due to mobile phase effects.

Although the solvophobic effect (sic) dominates in reversed-phase LC, the use of hydrophobic pairing ions which ion-pair with solute ions (Tomlinson et al., 1978) permits the ready retention of weak acids and bases in the LC systems. Much attention has been focussed (e.g. Horváth et al., 1977; Bidlingmeyer, 1979) on the mechanism of retention in ion-pair HPLC. That is, either an ion-exchange process (following pairing-ion adsorption onto the stationary support), or ion pairing in the mobile phase prior to ion-pair distribution to the stationary phase. Recently, we have described a number of linear enthalpy–entropy compensations found with various ion-pair reversed-phase HPLC arrangements (Fig. 4). The remarkable feature of these plots is that all arrangements have slopes which are similar to each other and to that described (Fig. 3) for non-ion-pair HPLC arrangements—although each plot is discrete from the others. According to Section III(e) this indicates that either all ion-pair and non-ion-pair systems are behaving similarly, and that displacement of the plots is due to an additional factor, or that the systems are not similar in their behaviour. We have considered that these displacements could be due to the differences in phase ratio (Eqns. 18–20) in the various phase systems studied. This has been investigated by recasting Eqn. 20 in terms of functional group

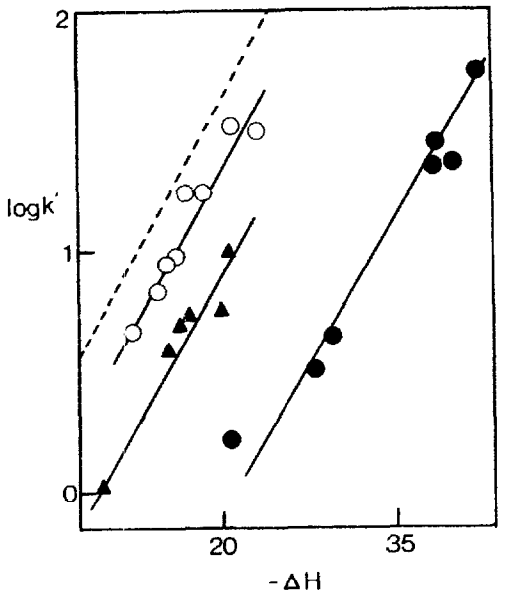

Fig. 4. Relationship between chromatographic capacity factor, k', at 40°C and enthalpies of retention, ΔH ($kJ \cdot mol^{-1}$), in ion-pair and non-ion-pair HPLC arrangements, Key: the dashed line is the regression line for the non-ion-pair system (analyzed at 40°C) given in Fig. 3; different data points refer to ion-pair reversed-phase HPLC systems constructed with differing phases and pairing ions. (Reprinted in modified form with permission of the copyright holder from Riley et al., 1981.)

behaviour (Eqn. 10). Thus, defining an extrathermodynamic chromatographic group contribution term, τ, as

$$\tau_x = \log(k'_j/k'_i) \tag{21}$$

where j and i refer to solutes which differ by a functional group x; since

$$\tau = (\Delta(\Delta G)_x)/(2.3RT) \tag{22}$$

combination of Eqns. 18, 20 and 22 gives

$$\tau_T = -[\Delta(\Delta H)/2.3R] \cdot (1/T - 1/\beta) - \Delta(\Delta G_\beta)/2.3R\beta \tag{23}$$

For the displacements shown in Fig. 4 to be due to differences in phase ratio, the use of Eqn. 23 to analyze these data in group contribution terms should give similar slope and intercept coefficients for all systems. Table I of Riley et al. (1981) gives such an analysis, and indeed shows that both slope coefficients are similar, and that in all cases intercept values (τ ordinate) approximate to zero.

In reversed-phase HPLC the hydrophobic effect is tempered by the presence in the aqueous mobile phase of an organic modifier. In both non-ion-pair and ion-pair LC both temperature and organic modifier content have been reported as having

similar effects on solute retention (Melander et al., 1979; Riley et al., 1981). Such concomitant (sic) phenomena enable a further feature of ΔH–ΔS compensation analysis to be identified, that is, the existence of a non-compensated ΔH residue. For the enthalpy of retention to be exclusively compensated (ΔH_c) then it follows that

$$\Delta H(\phi) = \Delta H_c(0)\, f(\phi) \tag{24}$$

where $\Delta H(\phi)$ and $\Delta H_c(0)$ are the enthalpy changes at mobile phase modifier volume fraction ϕ, and with no organic component in the mobile phase, respectively, and where the solvent composition function, $f(\phi)$, relates the two enthalpies (and is unity for solely water-mobile phases).

For ΔH to be comprised of both a compensated and a non-compensated (ΔH_{nc}) portion at $\phi = 0$ then (Melander et al., 1979)

$$\Delta H(\phi) = \Delta H_{nc}(0) + \Delta H_c(0)\, f(\phi) \tag{25}$$

such that

$$\ln k' = \frac{-\Delta H_c(0)\, f(\phi) - \Delta H_{nc}(0)}{RT} + \frac{\Delta H_c(0)[f(\phi) - 1]}{R\beta} + \frac{\Delta S(0)}{R} + \ln \psi \tag{26}$$

and where, when $f(\phi) = 1 + \chi\phi$ (where χ is a constant), the dependence is given by (Melander et al., 1979)

$$\ln k' = A_1\phi(1 + \beta/T) + A_2/T + A_3 \tag{27}$$

where the meanings of A_1–A_3 depend on whether either Eqn. 24 or Eqn. 25 is valid. To discriminate between these two models for the former case then A_2 is related to A_1 by

$$A_2 = -\beta A_1/\chi \tag{28}$$

and for the latter

$$A_2 = \Delta H_{nc}(0)/2.3R - \beta A_1/\chi \tag{29}$$

For both non-ion-pair LC (Melander et al., 1979) and for ion-pair LC using surface-active ions as pairing ions (Riley et al., 1981) the ratio of A_1 to A_2 is non-constant, indicating that the enthalpies of retention are not exclusively compensated by ΔS and that A_1, A_2 and A_3 are given by:

$$(\chi\Delta H_c(0)/2.3R\beta);\ (-[\Delta H_c(0) + \Delta H_{nc}(0)]/2.3R) \text{ and } (\Delta S(0)/2.3R + \ln\psi/2.3)$$

respectively. ΔH_{nc} can now be computed.

For alkylbenzenes in a non-ion-pair reversed-phase HPLC system a ΔH_{nc} of 15.9 $kJ \cdot mol^{-1}$ is reported, whereas for ion-pair systems we have given, for 3- and

4-monosubstituted benzoic acids a non-compensated enthalpy change residue of 15.4 $kJ \cdot mol^{-1}$, and for 2-substituted benzoic acids a residue of 7.6 $kJ \cdot mol^{-1}$. The finding of this structurally dependent non-compensated residue appears to explain previous findings (Table I of Riley et al., 1981) that *ortho* substituents perturb the general relationship between τ and $\Delta(\Delta H)$, and may be a significant result in elucidating retention mechanisms in reversed-phase liquid chromatography.

Melander et al. (1979) have carried this extrathermodynamic analysis further and have shown Eqn. 27 to be able to predict (and hence optimize) solute retention under a set of temperature and mobile phase composition conditions from retention data at other conditions. Naturally this relationship could be an extrathermodynamic basis for a retention index system to catalogue retention data—for use in, for example, describing drug hydrophobicity (Eqn. 1).

Chromatography provides at least one more example of the use of ΔH–ΔS compensation analysis. The gel chromatographic behaviour of tetraalkylammonium ions on Sephadex has been examined using ΔH–ΔS and ΔH–ΔG coordinate plots (Fig. 5a and b). The results given by these figures have enabled the authors (Ujimoto et al., 1981) to state, following Leffler and Grunwald (1963) and Krug et al., (1976a and b), that the chromatographic behaviour of the tetraalkylammonium ions do not obey a common physicochemical mechanism. However, these workers did not discuss the possibility of non-linear ΔH–ΔS compensation (Section III(c)), which may have been useful since the pattern described by Fig. 5b follows the increase in size of these ions.

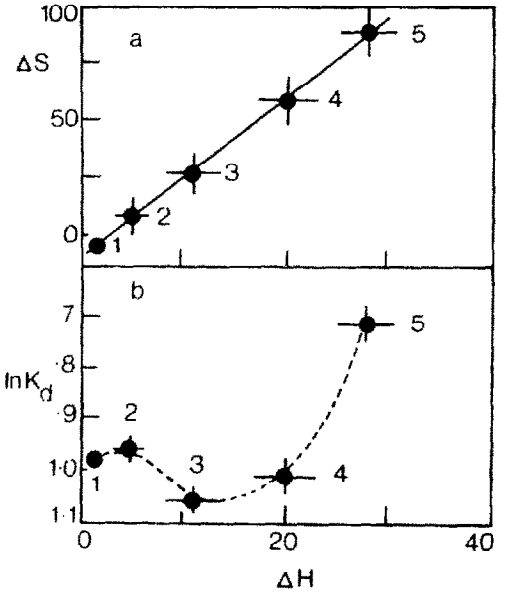

Fig. 5. Compensation analysis using (a) entropy ($J \cdot K^{-1} \cdot mol^{-1}$) and enthalpy ($kJ \cdot mol^{-1}$) coordinates and (b) free-energy/enthalpy coordinates for the gel chromatographic behaviour of tetraalkylammonium ions; where K_d are solute chromatographic distribution constants at 20°C (Thm), the numbers refer to increasing ion alkyl chain number, and the vertical and horizontal lines indicate the 95% confidence intervals of the parameter estimates. (Reprinted with permission of the authors, Ujimoto et al. (1981), and copyright holder.)

(c) Aqueous solubility

The previous examples illustrate how ΔH–ΔS analysis can be used to identify common mechanisms of behaviour and to assist in reinforcing theory. Further, the procedure can be used to identify inconsistencies between theory and practice. A recent example dealing with the aqueous solubilities of organic non-electrolytes (Rogers, 1982) illustrates this.

Since aqueous solubility is a prime characteristic of a drug with regard to delivery, much effort has been given to estimation and appreciation of the property. Although difficult to measure, attempts have been made to obtain the thermodynamic description of solubility using either direct microcalorimetry or indirect approaches using van 't Hoff operators. One significant study is that due to Rogers (1982) who has determined the aqueous solubilities of some phenols over a general 10–40°C temperature range. Although presenting this data in the form of regression analysis of $\ln X_2$ versus T^{-1}—where X_2 is the mole fraction solubility, since these systems may be non-ideal (and hence invalidate the use of van 't Hoff operators to obtain $\Delta\bar{H}_w$—the heat of solution), this worker has followed the treatment of Hollenbeck (1980) for calculating $\Delta\bar{H}_w$ in real solutions. Hollenbeck has argued that for a real, non-ideal solution

$$\Delta\bar{H}_w = \Delta H_F + \Delta\bar{H}_M \tag{30}$$

where ΔH_F is the heat of fusion and $\Delta\bar{H}_M$, the partial molal heat of mixing, is given by

$$\Delta\bar{H}_M = RT \ln \gamma_2 \tag{31}$$

where γ_2, the activity coefficient, is related to the activity, a, by $\gamma_2 = a_2/X_2$ and

$$\ln a_2 = \left(\frac{\Delta H_F^M}{RTm}\right) \ln\left(\frac{T}{Tm}\right) \tag{32}$$

Here, ΔH_F^M is the heat of fusion at the melting point Tm. Assuming, (Schwartz and Paruta, 1976), that the heat capacity is equal to the entropy of fusion, (ΔS_F^M), at the melting point and that for rigid molecules this is 13.5 e.u. (Yalkowsky and Valvani, 1980), then it follows from Eqns. 30–32 that

$$\Delta\bar{H}_w = 13.5[Tm + T \cdot \ln(T/Tm)] - RT \cdot \ln X_2 \tag{33}$$

Fig. 6 gives the ΔH–ΔS compensation plots ($\Delta\bar{H}_w$–$\Delta\bar{G}_w$ and $\Delta(\Delta\bar{H}_w)$–$\Delta(\Delta\bar{G}_w)$ coordinates) for phenols and phenol substituents' aqueous solubilities, constructed with the values calculated by Rogers using the one-temperature X_2 data approach of Eqn. 33. A number of features of the compensation plot require comment. First, both appear to be near perfect examples of linear ΔH–ΔS compensation. Second, for both, the slope of the enthalpy-free energy plot is unity, (see Eqn. 33), and for the functional group plot the intercept is zero with all changes in the free-energy of

solubility being almost entirely due to changes in the enthalpy change of the process. What appears to be happening is that the assumptions and approximations used to develop Eqn. 33 lead to a model which has a 'variable' (i.e. the first term on the

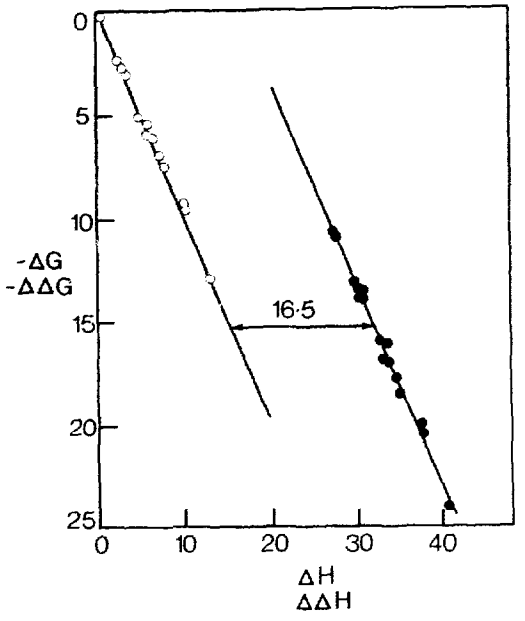

Fig. 6. Relation between free-energies (ΔG and Δ(ΔG)) and enthalpies (ΔH and Δ(ΔH)) of phenol (closed symbols) and phenol substituents' (open symbols) aqueous solubility (kJ·mol^{-1}) at 20°C, calculated by Rogers (1982) according to Eqn. 33. The horizontal difference betwen the shown regression lines is 16.5 kJ·mol^{-1}, which approximates to 13.5×4.12 (Tm + T ln(T/Tm).

right-hand side of the equation), which is almost invariant, (for example, an increase in Tm from 40.6°C for phenol to 115°C for *p*-nitrophenol alters the value of this term by approximately 400 J or 2.5%). This explains why the functional group plot has its characteristics, and shows that the model will generally force the data to yield an a priori 'correlation' between $\Delta\overline{H}_w$ and $\Delta\overline{G}_w$.

(d) Complexation

Frequently the normal van 't Hoff relationship is non-linear and its usual differentiation to obtain ΔH is not possible. What is possible, however, is to obtain higher-order relationships which describe the effect of temperature on the equilibrium (or rate) constant, and to differentiate this at fixed temperatures. Data obtained in this manner can then be used in any ΔH–ΔS analysis. Such behaviour exists for the complex coacervate interaction of large organic ions in water (Tomlinson et al., 1979a), and provides an example of the use of compensation analysis in identifying a common mechanism of behaviour and outliers from this.

Large organic ion interactions have implications for drug dosage form design, especially with regard to drug compatibility, stability and biological availability.

Charged ions here refer to chemicals often used as, for example, antimicrobial preservatives (e.g. benzalkonium chloride), taste-makers (e.g. saccharin sodium), solubilizers (e.g. sodium dodecylsulphate) and colours (e.g. amaranth). Although extrathermodynamic relationships between ion structure and complexation constants are common in the literature, they are unique for only a structurally very similar interacting set of ions (for example, see Fig. 9 of Tomlinson and Davis, 1978). In an attempt to provide a more complete and self-consistent description of such interactions, use has been made (Tomlinson and Davis, 1980) of compensation analysis. For both monovalent–monovalent and divalent–monovalent ion interactions the effect of temperature on the thermodynamic solubility product, K_s, describing the formation of a complex coacervate, can be given by

$$\log K_s = A - BT + CT^2 - DT^3 \qquad (34)$$

where A–D are the respective polynomial regression coefficients. Enthalpies for the interaction can be determined at the harmonic mean of the experimental temperatures (30°C) by differentiation of Eqn. 34 with respect to temperature. Fig. 7, gives the enthalpy–entropy compensation plot for the interaction between various large organic (and hydrophobic) ions. This shows that for the systems studied there can be described a general linear enthalpy–entropy compensation except for the interaction of an alkylsulphate with phenothiazine molecules (Tomlinson et al., 1979b). Dealing first with the compensation line: considering the variation in structures, molecular weights, charge distributions and number this is a finding which has potential use in

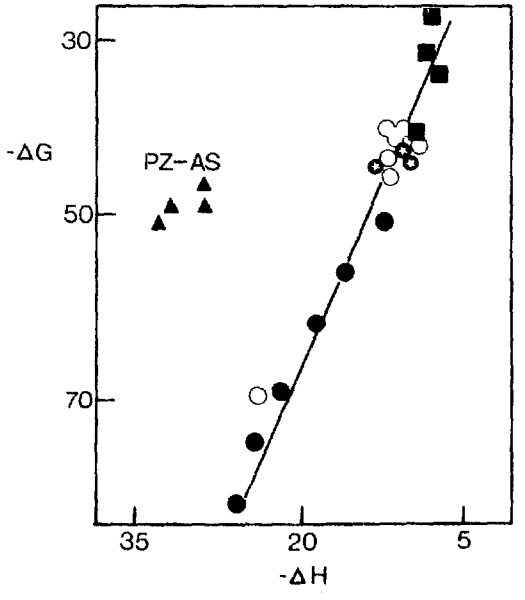

Fig. 7. Enthalpy–entropy compensation plot for large hydrophobic ion interaction showing the enthalpies and free-energies of complexation at Thm (35°C) (kJ·mol^{-1}). Each type of symbol denotes a different groups of interacting ions; phenothiazines (PZ) are complexing with an alkylsulphate (AS). (Tomlinson and Davis, 1980.)

predicting complexation effects, as well as in indicating that for all these systems the mechanism of interaction is essentially the same, that is, ion–ion electrostatic interaction reinforced by a strong hydrophobic effect.

As discussed earlier (Section III(e)), outliers from a general compensation relationship should be examined with respect to both experiment and theory. For the interaction of substituted phenothiazines with dodecylsulphate, their displacement from the compensation line (Fig. 7), can be rationalized in terms of reduction in the self-association of phenothiazines upon ion interaction, such that due to either entropy change or activity coefficient effects a displacement results.

Further examples of the possibilities for the use of ΔH–ΔS analysis for examining complexation phenomena include the study of 1 : 1 complex formation between β-cyclodextrin and a diverse range of drugs (Hardee et al., 1978), in which a flow microcalorimeter has been used to obtain the thermodynamic parameters. Analysis of these given parameters indicates no correlation betwen ΔH and ΔG of complexation, which reinforces the comments of the authors based on linear free-energy relationships, that these interactions are both non-specific and that for differing drug types different mechanisms of binding are involved. An additional use of ΔH–ΔS analysis has been given by Donbrow and Sax (1982) who, in reviewing the thermodynamics of the complexation reaction in aqueous solution of xanthines with various ligands, have shown for the interaction with caffeine that, although with the whole group of ligands studied approximately 36% of the variance between ΔH and ΔG is unaccounted for by the relationship ($r = 0.808$), there can be described a compensation plot for a limited number of aromatic ligands.

The examples given above have been chosen to illustrate the various features pertaining to ΔH–ΔS analysis. In the following examples this present contribution shall deal in a limited way with other phenomena of pharmaceutical interest where sufficient data on the energetics exist to attempt ΔH–ΔS analysis. Cases are only given where either ΔH–ΔS compensation exists (according to theory given in Section III), or where it has been reported in the original work as existing.

(e) Surface chemistry

As alluded to in the previous section, many compounds of pharmaceutical interest exhibit a surface activity which may be of relevance not only in the pharmaceutical phase but also during the pharmacodynamic activity of a compound. One means of studying such activity is to examine the monolayer spreading on various liquid and solid surfaces. However, even the extent of this easily conceptualized process is known to vary considerably with slight changes in solute structure. Arguing that determination of the thermodynamics of spreading on an aqueous surface should give much insight into the nature and prediction of this behaviour, Jalal et al. (1980) have determined the equilibrium monolayer spreading pressures of a wide range of structurally related fatty acids. In order to compare the spreading of solute from a bulk phase to the same area per molecule, these workers first estimated the enthalpies (and entropies) of spreading at various constrained monolayer areas per molecule (using a van 't Hoff operator approach). These data could then be extrapolated to some desired value of area per molecule. Having obtained ΔG, ΔH

and ΔS values in this manner, knowledge of the heat capacities of the monolayer and bulk phase fatty acid were used to yield extrapolated thermodynamic values at chosen temperatures and monolayer areas per molecule.

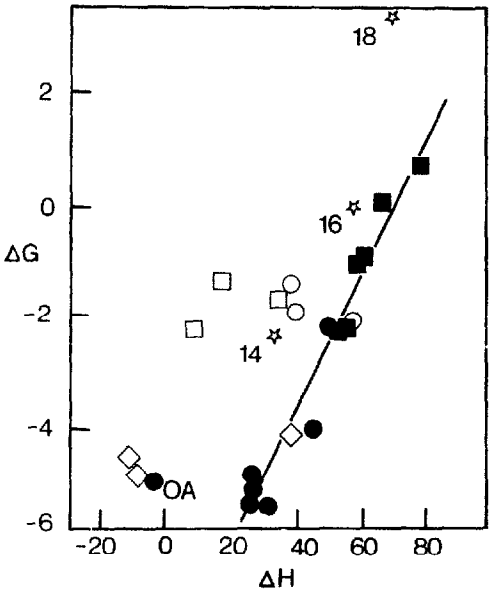

Fig. 8. Relation between enthalpies ($kJ \cdot mol^{-1}$) and free-energies ($kJ \cdot mol^{-1}$) of spreading of some fatty acid monolayers on an aqueous surface at 25°C (Thm) at 30 $Å^2$/molecule. (Values recalculated from data given by Jalal et al., 1981.) Key: closed datum points: *cis*- (circles) and *trans*- unsubstituted (squares) fatty acids; open points: acetylenic (circles), hydroxy (squares), di- and tri- unsaturated (diamonds) fatty acids, and saturated fatty acids, (stars, with homologue number); OA is *cis*-9-octadecanoic acid.

Jalal et al. studied a series of saturated, unsaturated and hydroxy fatty acids (including various *cis* and *trans* configurations). Although these workers concluded from a found single relationship existing between the enthalpies and entropies of spreading for all studied fatty acids, that the mechanism of solute spreading is the same regardless of chain structure, construction of a compensation plot in ΔH–ΔG coordinates (Section III(a) and III(e)) reveals that for the process of spreading of fatty acids there are structurally dependent ΔH–ΔS linear composition effects. Thus Fig. 8 shows that with one exception (*cis*-9-octadecanoic acid) for *cis* and *trans* unsubstituted unsaturated fatty acids, a single linear compensation line exists—indicating that indeed for these, the mechanism of solute spreading is the same. However, nearly all other acids studied are displaced significantly from this relationship, and although distinct compensation lines are not able to be assigned to these, they do appear to be spreading in a somewhat dissimilar manner (*cis*-9-octadecanoic acid's behaviour appears anomalous). These structural features confirm many of the other conclusions reached by Jalal et al. on the qualitative and quantitative aspects of spreading, and hence could form a basis for a predictive model of the phenomenon.

(f) Liposome–water partitioning

Although thermodynamics generally applies to small physical systems, extrathermodynamics are often applied to biochemical and biological problems (see Eqn. 1). One such example is for the distribution of small organic solutes between aqueous and liposome (or phospholipid vesicle) environments. Liposomes have been considered for (amongst others) increasing drug stability, as models of biological membranes, and as drug delivery carriers, and a description of the extent and rate of distribution of solutes between liposomes and water is of significance.

A number of groups have reported thermodynamics for the distribution and rates of permeation from water to liposomes, (Johnson and Bangham, 1969; Klein et al., 1971; Katz and Diamond, 1974a; Cohen, 1975a; Rogers and Davis, 1980; Ahmed et al., 1981). Although linearity (and hence compensation) between the ΔH and ΔS terms has been described in 3 of these studies (Katz and Diamond, 1974; Cohen, 1975; Ahmed et al., 1981), use of ΔH–ΔG coordinates reveals somewhat more complex behaviour (Fig. 9). Liposomes have a unique structure that alters with temperature, such that below a certain point (the transition temperature, Tc), these vesicles exist in a crystalline or 'frozen' state whereas above this temperature they exist in a liquid crystalline or 'melted' state. This phase transition effect is not reflected in the free-energies of distribution but rather in the enthalpic and entropic terms, such that below the transition temperature enthalpies and entropies are often

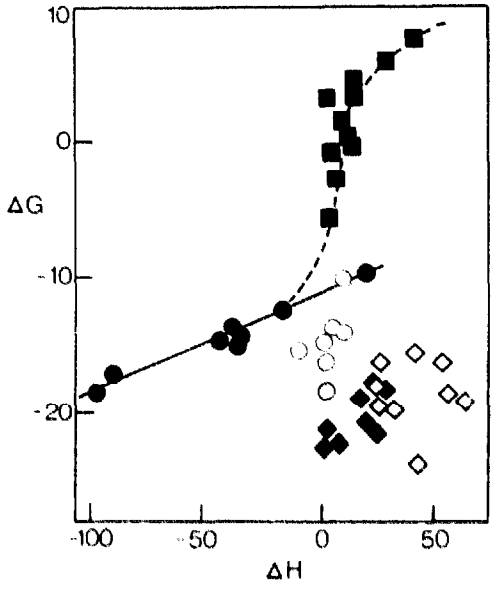

Fig. 9. Relation between free-energies and enthalpies (kJ·mol^{-1}) of solute partition between liposomes and water. Circles: phenols between dimyristoyl phosphatidylcholine liposomes (DPL) and 0.15 M NaCl above (closed points, 30°C) and below (open points, 15°C) the transition temperature (Tc) (Rogers and Davis, 1981); squares: non-electrolytes between dimyristoyl lecithin liposomes and 0.15 M KCl at 30°C (above Tc) (Katz and Diamond, 1974a); diamonds: phenothiazines between DPL and 0.15 M NaCl above (closed points, 30°C) and below Tc (open points, 15°C) (Ahmed et al., 1981). (Values have been recalculated from the original data for comparison purposes.)

reported as being at least a magnitude more positive. Katz and Diamond interpreted this in terms of the fact that in the crystalline state the hydrocarbon tails of lecithin pack more closely so that subsequent insertion of a solute into the membrane requires breaking of much stronger hydrocarbon–hydrocarbon intermolecular forces (compared to that required in the liquid crystalline state). In addition, the fraction of lipid bilayer volume occupied by solute may increase with temperature.

Fig. 9 gives the ΔH–ΔG plots for solute distribution between aqueous and dimyristoyl lecithin liposomes above and below the transition temperature (using published values or those calculated from the original). Below the Tc there is no evidence of true ΔH–ΔS compensation. However, above the Tc, linear enthalpy–entropy compensation is demonstrated for the distribution of some simple substituted phenols (Rogers and Davis, 1980); and further, considering the data of Katz and Diamond, this linear case could be part of an overall non-linear compensation pattern (see Section III(c)). Data for phenothiazine distribution (Ahmed et al., 1981) clearly show no compensation. A number of reasons for this can be proposed: (a) there is indeed no compensation; (b) since phenothiazines are hydrophobic ions and can undergo both self-association and ion pairing in water (Tomlinson et al., 1979b), both of which are temperature-dependent phenomena, these secondary equilibria and their meaning for the thermodynamic standard state need to be considered; or (c) that a much larger spread in ΔH and ΔG values needs to be considered.

Relative to previously described examples, determination of the energetics of distribution in liposomes is most difficult. Liposome systems given in Fig. 9 are multilamellar entities and sufficient time needs to be allowed in distribution studies for added solutes to equilibrate with all lamellae. It is perhaps relevant therefore to note that although the studies of Katz and Diamond and of Ahmed et al. used 24 h and 28 h equilibration times, respectively, the study using phenols where linear compensation is demonstrated, found much longer (40 h) equilibration periods to be necessary. A further comment here is that multilamellar liposomes contain a quantity of non-solvent water (Katz and Diamond, 1974b) which increases with temperature. As discussed earlier (Section III), this indicates that the standard state of the liposome is altering with temperature, which has the previously described implications for the use of van 't Hoff operators for obtaining ΔH values. The use of batch microcalorimetry in any further studies on liposome energetics is seemingly highly indicated.

Cohen (1975a and b) has considered the effect of temperature on the permeation rates of egg lecithin and dimyristoyl lecithin liposomes to non-electrolytes above and below the transition temperature, in the absence and presence within the liposomes of aqueous pore-forming antibiotics (gramicidin-A and nystatin), as well as cholesterol. Cohen (1975a) describes linear relationships between the activation energies for permeation across liposomes and a function of the corresponding entropy changes for both egg lecithin liposomes and these liposomes containing 30% cholesterol. Examination of these data using ΔH–ΔG coordinates reveals a somewhat different pattern (Fig. 10a and b). First, it is apparent that for egg lecithin liposomes studied at 10–35°C, linear compensation behaviour is shown for the rates of non-electrolyte permeation. However, incorporation of either cholesterol or, in

dimyristoyl lecithin vesicles, gramicidin-A, results in, for the solutes studied, true physicochemical linear ΔH–ΔS compensation becoming not demonstrable. Cohen has shown that there are regular relationships between the activation energies and

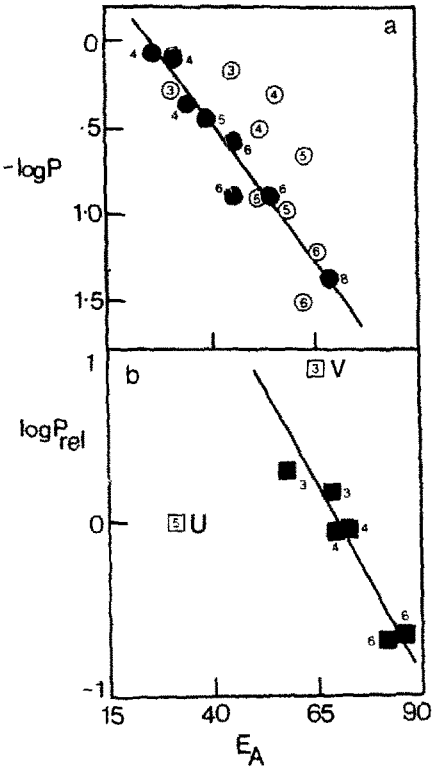

Fig. 10. Permeation rates, P (s^{-1}), versus apparent activation energies, E_A, ($kJ \cdot mol^{-1}$) at 10°C for non-electrolyte permeation across liposomal membranes below their transition temperature. Key: (a) closed and open datum points are for egg lecithin–phosphatidic acid (96:4) liposomes and egg lecithin–cholesterol (70:30) liposomes, respectively; (b) dimyristoyl-lecithin–dicetylphosphate (96:4) with gramicidin-A (100 μg/20 μmol lipid). U and V are urea and valeramide. Numbers refer to the maximum number of hydrogen bonds the solutes may form with water. (Values recalculated from Cohen, 1975a and b.)

the capacity of the solutes to form hydrogen bonds in water. It is noteworthy that the linear compensation pattern demonstrated for egg lecithin liposomes follows the rank ordering of solute hydrogen bonding potential (Fig. 10a). The role of cholesterol in cell membranes is of interest with respect to drug absorption, hence its incorporation into liposomes used in model studies. Evidence (Mabrey-Gaud, 1981) suggests that cholesterol-rich and cholesterol-poor phases exist in the phospholipid bilayer. Fig. 10a indicates that an effect of cholesterol is to selectively reduce the permeability of egg lecithin membranes to non-electrolytes. Certainly when solely permeation rates are examined (Cohen, 1975b) for an increase in liposome cholesterol content, there is a consistently greater selectivity for some non-electrolytes compared to

others. Although cholesterol incorporation will alter membrane thickness, Cohen (1975b) argues that for the processes of solute adsorption at the water–membrane interface and of solute dehydration, cholesterol largely affects the latter.

Liposomes prepared with saturated phospholipids treated with gramicidin-A are osmotically sensitive structures below their transition temperature. In addition, it is considered that gramicidin-A molecules form a transmembrane channel across lipid bilayers, which can result in an increase in permeability for those solutes with a diameter to fit into the pore. Fig. 10b shows that urea and valeramide are displaced from the other points. The behaviour of urea can be explained on the basis that this molecule, (which Cohen showed does not substantially pass through the lipid part of gramicidin-A-free liposomes), combines a small molecular size with a high hydrogen-bonding capacity. Incorporation of gramicidin-A processes liposomes with a substantial discrimination for branched solutes, such as valeramide, as shown in Fig. 13b. Although activation energy data for valeramide in an egg lecithin system (Fig. 13a) are not available for comparison, it is of particular interest to note from another study (Bindslev and Wright, 1976), that the permeation of branched chain solutes such as valeramide, across the toad urinary bladder membranes is much lower than for their unbranched isomers.

(g) Biological membrane permeation

Although as systems become more complicated the use of van 't Hoff operators to obtain ΔH data becomes less valid (Section IV(a)), sometimes it can be possible with such data to attempt a ΔH–ΔS analysis, as exemplified by this and the following sections.

Previously (Section IV(f)) we have seen that drug permeation through liposomal model membranes describes ΔH–ΔS compensation behaviour, with various small solutes exhibiting a different (pore transport?) mechanism of flux. It can be thus hypothesized that for liposomes to act as models of true biological membranes that ΔH–ΔS compensation patterns should exist also with the latter. Few reliable data exist to examine this hypothesis, but those which can be analyzed suggest that the hypothesis holds. Examine first the effect of temperature on non-electrolyte permeation across red cell membranes (Good, 1967; Galey et al., 1973). Galey et al. studied the effect for a series of hydrophilic and lipophilic solutes, including urea and some straight-chain amides as well as *iso*-butyramide and *iso*-valeramide. Fig. 11 is the free-energy–enthalpy plot of their results, which shows clearly a compensation effect, with small solutes (urea, formamide, acetamide and methylurea), displaced from the line (vide Fig. 10). As found for liposome systems, *iso*-valeramide (but not *iso*-butyramide) is displaced from the compensation line.

With respect to this present study, perhaps the results of Good (1967) are the most significant. Using ΔH–ΔS coordinates, Good has argued that for the haemolysis of human erythrocytes in a variety of hypertonic solutions of varying osmolarity there exists a (biological) example of the compensation law. By examining the original studies of Good (references in Good, 1967) a compensation plot in free-energy–enthalpy coordinates can be constructed (Fig. 12). Undoubtedly there is true linear compensation occurring; however, (and not revealed by Good's original plot),

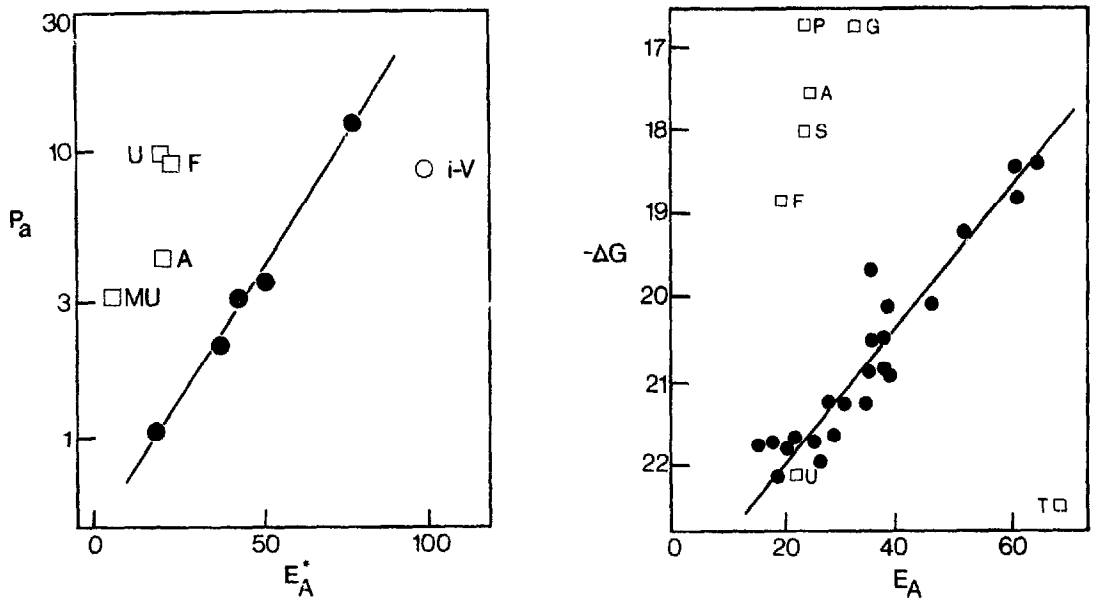

Fig. 11. Permeability coefficients, Pa, at Thm (25°C) ($cm \cdot s^{-1} \times 10^{15}$) on a log scale versus van 't Hoff temperature coefficient, E_A^*, ($kJ \cdot mol^{-1}$) for hydrophilic and lipophilic amide permeation across red cell membranes. Key: closed points, higher-order amides; U, MU, F, A and iV are urea, methylurea, formamide, acetamide and *iso*-valeramide, respectively (Values recalculated from Galey et al., 1973.)

Fig. 12. Free energies ($kJ \cdot mol^{-1}$) at 20°C versus apparent activation energies ($kJ \cdot mol^{-1}$), E_A, for haemolysis of red blood cells by non-electrolytes at a solution osmotic pressure of 6.5 at m. Key: as for Fig. 11 and with P, G, S and T representing *n*-propanol, glycerol, succinimide and thiourea, respectively. (Values recalculated from Good (1962), and references therein.)

it is seen that some compounds are displaced from the main relationship, and a group of these may be forming their own compensation relationship. Thiourea is a major outlier, and it is noteworthy that of the many compounds studied, thiourea alone was found by Good to markedly reduce the surface free-energy of water. In contradistinction to previous data, urea now falls on the general compensation line; however, these data are now for permeation through the membrane wall followed by haemolysis. Good (1967) has suggested that extracellular, membrane cell surface and intracellular hydration effects contribute to the overall observed haemolysis effect and the observed pattern in the compensation plot (Fig. 12) could be reflecting these different effects.

The displacement of urea and other small molecules from a general compensation relationship for membrane permeation can be also shown (Eqns. 35 and 36) using the data of Bindslev and Wright (1976) for non-electrolyte flux through the toad urinary bladder, i.e.

small molecules

$$E_A = 0.21 \log Pa - 0.58 \quad n = 5 \quad r = 0.968 \tag{35}$$

large polar molecules

$$E_A = 0.16 \log Pa - 1.20 \quad n = 10 \quad r = 0.879 \tag{36}$$

where E_A and Pa are the apparent activation energies (kcal · mol^{-1}) and permeability coefficients (cm · s^{-1} · 10^7) at 22°C, respectively.

Receptor and enzyme interactions

Solute interactions with biopolymers attract much effort, and include aspecific and specific drug–receptor and drug–protein interactions as well as enzyme binding and enzyme–substrate reactions. There is a growing amount of thermodynamic data for such processes, much of it realized because of the seminal study of Lumry and Rajender (1970) who, based on found ΔH vs ΔS relationships (and a very limited number of ΔH vs ΔG relationships) for a variety of processes of small solutes in water solution (including ion and non-electrolyte solvation, hydrolysis, oxidation–reduction, weak electrolyte ionization), as well as for water solutions of proteins, suggested that such 'compensation' behaviour was an ubiquitous property of water, and as such was a 'thermodynamic manifestation' of 'structure-making' and 'structure-breaking' properties. Analysis of many of these data using the treatment according to Krug and co-workers reveals that true linear ΔH–ΔS compensation is difficult to show; although those analyses are outside the scope of this present study, recent data do exist which permit linear ΔH–ΔS compensation to be demonstrated for drug–biopolymer processes. In particular, there are the deliberate and methodical studies of Gelb and Laufler (Gelb et al., 1981, and references therein), on the steric and electronic structural factors involved during binding of various organic non-electrolytes and anions in aqueous solution to cyclohexaamylose. (The importance of these complexes and their properties is related to their mimickry of biological enzyme systems.) For 15 aromatic non-electrolytes and 8 related anions, Gelb and Laufer have reported a linear correlation between ΔH and ΔS for 1 : 1 cyclohexaamylose–substrate complex formation, as well as correlation between this ΔH term and the intrinsic displacements of the C_1 of unbound substrate ^{13}C resonance in the various complexes. Although it was argued that the ΔH vs ΔS plot is due to chemical effects, it is not until the ΔH vs ΔG domain is used (Fig. 13) that this is seen to be true only for unionized species (with one outlier—cyclohexanecarboxylic acid, CA), with ionized compounds being outliers from a general linear compensation relationship. Gelb et al. (1981) argue that cyclohexanecarboxylic acid (CA) and 1-adamantanecarboxylate ion (AC^-) should be expected to fall outside any general compensation line (due to, respectively, restricted substrate conformational motion within the formed complex, and partial contribution of hydrophobic bonding to complex formation), although their thermodynamic values approximate to the given ΔH vs ΔS relationship. Fig. 13 reveals that the above arguments indeed hold for CA, but that the scatter pattern for ions around the compensation line makes the argument for AC^- inconclusive.

A recently reported study by Dorovska-Taran et al. (1980) on the temperature dependence of the hydrolysis of *p*-nitrophenylalkylcarboxylates catalyzed by alkaline

mesentericopeptidase (a bacterial proteinase), has shown that there exists chemical enthalpy–entropy compensation behaviour for this reaction. Eqns. 37 and 38 are the relevant relationships, and show that, for this study at least, both ΔG and ΔH, and

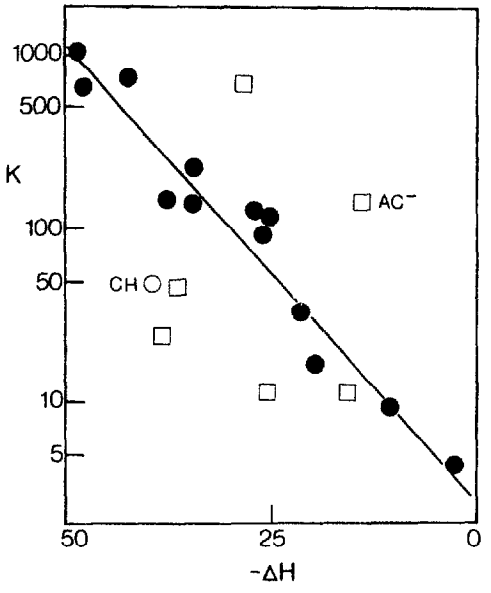

Fig. 13. Relation between observed cyclohexaamylose–substrate binary formation constant at 25°C, K, and the enthalpies of complexation (kJ·mol^{-1}) for uncharged (circles) and charged (squares) substrates. Key: CH and AC$^-$ are cyclohexanecarboxylic acid and adamantanecarboxylate ion, respectively. (Values taken or calculated from Gelb et al., 1981, and references therein.)

ΔS and ΔH are correlated, as follows from Eqns. 10 and 15 (Section III).

$$\Delta G^{\ddagger} = 0.36\Delta H^{\ddagger} + 12.9 \quad n = 6 \quad r = 0.991 \tag{37}$$

$$\Delta S^{\ddagger} = 2.1\,\Delta H^{\ddagger} - 44.2 \quad n = 6 \quad r = 0.996 \tag{38}$$

(where the activation parameters are in kcal·mol^{-1} and electrostatic units). Dorovska-Taran et al. have argued, based on compensation theory, that for enzyme reactions water is not the unique source of ΔH–ΔS compensation (as is often argued, e.g. Lumry and Rajender, 1970; Melander, 1974), and further that, since their results are in good agreement with those reported for mammalian proteinases, this implies a common mechanism of action of the enzymes derived from mammalian and bacterial species. Whether data for 6 compounds justifies this last argument remains to be seen, though perhaps it is noteworthy that analysis of the data of Belleau and co-workers (Belleau and DiTullio, 1970, and references therein), on the binding of some 35 quaternary ammonium salt ions to the enzyme acetylcholinesterase, and the kinetic effects of these salts on the methanesulphonylation of the acetylcholinesterase catalytic centre, shows no evidence of chemical compensation behaviour according to the theory given in Section III(a).

V. Final comments

In 1947 Hinshelwood described the compensation effect as one of the *essential* phenomena in chemical kinetics 'which are still mysterious'. Care has been taken in this present communication not to attribute found compensation to any particular phenomena. Lumry and Rajender (1970) argued for the unique properties of water as being responsible for compensation, but the number of examples of specific processes exhibiting compensation shown in this and other reports suggests otherwise. What is clear is that according to theory (Leffler and Grunwald, 1963), and treatment (Krug et al., 1976), a number of systems and processes of pharmaceutical interest exhibit linear and non-linear enthalpy–entropy compensation behaviour.

Due to the reporting of data in the literature it has not been possible to apply the suggested M-regression analysis to follow the Krug-Hunter-Grieger treatment rigorously. With data being analyzed as close as possible to the harmonic mean temperature, what has been carried out, where possible, has been to: (a) examine the van 't Hoff plots for a convergence point; and attendent upon this (b) to use free-energy and enthalpy coordinates to visually inspect the data for evidence of linear or non-linear compensation behaviour.

Four factors emerge from this study. First, although chemical compensation is suggested as occurring, it is seen to provide only partial confirmation of a hypothesis, and does point to outliers from general relationships as well as weaknesses in experimental design. Second, there is a paucity of reliable thermodynamic data in the pharmaceutical literature, and because of the complicated nature of pharmaceutical systems it is often preferable to collect such data calorimetrically rather than using van 't Hoff operators. Third, the compensation proportionality factor (β) is shown to be error-prone, and a characteristic parameter of a compensation plot could be the slope coefficient of the ΔH–ΔG plot (although comparison of values could be possible only if the same sampling temperature was used). Fourth, it is often reported that a process is entropically or enthalpically controlled. Considering the linearity found between (in theory at least) enthalpies and entropies for a process, such descriptions should be modified to give the proportion of the overall free-energy change for the process that is comprised of enthalpic or entropic effects.

Acknowledgements

It is a pleasure to acknowledge the discussion on this work, and the contributions to the studies contained within, with Professor S.S. Davis (Nottingham University, U.K.), Dr. C.M. Riley (University of Florida, U.S.A.), Dr. M.H. Abraham (Surrey University, U.K.) and Drs. J.F.M. Kinkel, W. Riebesehl, H.J.A. Wynne, and Ir. Th.L. Hafkenscheid of this Department.

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