Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Determination off the Hydrophobia Interaction Energy-Application to Separation Processes

C. J. Oss Van[a]; R. J. Good[b]; M. K. Chaudhury[c]
[a] DEPARTMENTS OF MICROBIOLOGY AND CHEMICAL ENGINEERING, STATE UNIVERSITY OF NEW YORK AT BUFFALO BUFFALO, NEW YORK [b] DEPARTMENT OF CHEMICAL ENGINEERING, STATE UNIVERSITY OF NEW YORK AT BUFFALO BUFFALO, NEW YORK [c] DOW CORNING CORPORATION, MIDLAND, MICHIGAN

To cite this Article Oss Van, C. J. , Good, R. J. and Chaudhury, M. K.(1987) 'Determination off the Hydrophobia Interaction Energy-Application to Separation Processes', Separation Science and Technology, 22: 1, 1 — 24
To link to this Article: DOI: 10.1080/01496398708056155
URL: http://dx.doi.org/10.1080/01496398708056155

SEPARATION SCIENCE AND TECHNOLOGY, **22**(1), pp. 1-24, 1987

Determination of the Hydrophobic Interaction Energy—Application to Separation Processes

C. J. VAN OSS

DEPARTMENTS OF MICROBIOLOGY AND CHEMICAL ENGINEERING
STATE UNIVERSITY OF NEW YORK AT BUFFALO
BUFFALO, NEW YORK 14214

R. J. GOOD

DEPARTMENT OF CHEMICAL ENGINEERING
STATE UNIVERSITY OF NEW YORK AT BUFFALO
BUFFALO, NEW YORK 14214

M. K. CHAUDHURY

DOW CORNING CORPORATION
MIDLAND, MICHIGAN 48640

Abstract

Surface tension measurement data are described that allow the determination of and the distinction between the long-range Lifshitz-van der Waals (LW) and the short-range (SR) forces that together constitute "hydrophobic interactions." A novel explicit formulation of the partial contributions of hydrogen bonds to surface tension and to free energy of adhesion is introduced. The different rules that apply to LW and to SR interactions are elaborated upon and the equations needed for the quantitative expression of these two interactions are given. The results obtained by this approach for energies of adhesion are compared with values derived from association and dissociation energies that have been determined earlier, showing an excellent agreement between these two different approaches. A number of applications of our surface-thermodynamic approach (treating LW and SR interactions separately) to various separation processes are discussed with regard to various modes of liquid chromatography, adsorption, membrane processes, blotting, zone melting, partition, precipitation, and other separation methods.

INTRODUCTION

Not only the strong attraction between apolar substances in water (*1*) but also the attraction between hydrophilic biopolymers and low-energy or apolar ("hydrophobic") surfaces immersed in water have been ascribed to "hydrophobic interactions" (*2*) or to a "hydrophobic effect" (3) and even to "hydrophobic bonds" (*4–7*). However, "hydrophobic interactions" are not "bonds" in the strict sense of the term, as van der Waals or hydrogen or electrostatic bonds are, but rather the resultant of the complex interactions between macromolecules and/or particles and the molecules of the liquid in which they are dissolved and/or immersed (*8*). The individual components of these complex interactions are van der Waals forces and hydrogen bonds; electrostatic forces do not usually play an important part in "hydrophobic" interactions, and are in any event better treated apart, as they also are more conveniently measured separately. Also, in certain cases, entropy has been reported to play an important role in the interaction (*9, 10*).

A most important step in the elucidation of the complex interaction between low- and high-energy compounds was made by Fowkes (*11–13*) in distinguishing between the dispersion and the polar contributions to the energies of cohesion. Recently it was pointed out that a somewhat more appropriate subdivision is one between long-range interactions (i.e., all van der Waals interactions of the London, Debye, and Keesom varieties combined), on the one hand, and short-range interactions (i.e., principally interactions involving hydrogen bonds) on the other hand (*14*). By this approach the energy components of "hydrophobic interactions" between hydrophilic macromolecules and/or particles and low-energy or apolar surfaces, immersed in water, can for the first time be quantitatively elucidated (*8, 15*). We propose to give some general guidelines on the methods that can be used to measure the parameters, and to predict the magnitude of these "hydrophobic interactions" occurring in the course of a variety of separation processes.

LONG-RANGE FORCES

Lifshitz-van der Waals (LW) Forces

The free energy of adhesion between Substances 1 and 2, at contact, *in vacuo,* is expressed by

$$\Delta G_{12} = \gamma_{12} - \gamma_1 - \gamma_2 \tag{1}$$

where γ_{12} is the interfacial tension between Substances 1 and 2, and γ_1 and γ_2 are the surface tensions of these compounds. The free energy of adhesion between the same two substances, immersed in Liquid 3, also at contact (*16*), is

$$\Delta G_{132} = \gamma_{12} - \gamma_{13} - \gamma_{23} \tag{2}$$

while

$$\Delta G_{131} = -2\gamma_{13} \tag{3}$$

Equations (1), (2), and (3) are valid for long-range *and* for short-range interactions, *at contact.* Interfacial tension components due to Lifshitz-van der Waals (LW) interactions (comprising dispersion, orientation, *and* induction forces (*8, 14, 16*)) can be obtained from LW surface tensions by the combining rule:

$$\gamma_{12}^{LW} = (\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_2^{LW}})^2 \tag{4}$$

The LW surface tension of Solid 1 can be measured by contact angle (θ) determinations (*15*) with LW Liquids 3 by using a variant of Young's equation:

$$1 + \cos\theta = 2\sqrt{\gamma_1^{LW}/\gamma_3} \tag{5}$$

The values of γ^{LW} of LW liquids are known for many liquids (*16*) or can easily be measured by various standard methods (*17*). The free energy of cohesion of a pure material is

$$\Delta G_{11}^{coh} = -2\gamma_1 \tag{6}$$

The form of Eq. (5) is also valid for long-range *and* short-range interactions, *at contact,* when the following expression is used:

$$\gamma_3^{TOT}(1 + \cos\theta) = 2\sqrt{\gamma_1^{LW}\gamma_3^{LW}} - \Delta G_{13}^{SR} \tag{7}$$

Here, the superscript SR refers to short-range interactions, see below. But the free energy expressions can only be connected rigorously to Hamaker coefficients in the case of LW interactions according to

$$\Delta G_{11}^{LW} = \frac{-A_{11}}{12\pi d^2} \tag{8}$$

where A_{11} is the Hamaker coefficient for Material 1, and d is the distance between two semi-infinite plane parallel slabs. In those terms, Eq. (8) also applies to ΔG_{132}^{LW} (Eq. 2) and to ΔG_{131}^{LW} (Eq. 3). The distance d is a variable, and this allows ΔG^{LW} to be calculated for all distances up to about 100 Å, after which retardation effects have to be taken into account (*14, 21*). These need not, however, be entered into here. At molecular contact, d becomes the minimum equilibrium distance d_0, which in all cases may be taken to equal about 1.5 to 1.6 Å (*22*), provided one neglects the effects of the Born repulsion (*16*).

Thus, by contact angle measurements on solids with apolar liquids, using Eq. (5), the γ^{LW} of such solids can be obtained, and the LW free energies of interaction between various solids (with each other or with different solids), while immersed in a given liquid, can be obtained with Eq. (3) or (2), using Eq. (4) to obtain the necessary values for γ^{LW}. It should be noted that ΔG_{132}^{LW} (Eq. 2) can have a positive as well as a negative value (*8*). For measuring the LW parameters of low-energy solids, hexadecane ($\gamma_3 \simeq 25.5$ mJ/m^2) is an excellent apolar liquid. For higher energy solids, diiodomethane ($\gamma_3 = 50.8$ mJ/m^2) can be used, as long as it is realized that it has also a small, but not quite negligible, polar component (see below).

Electrostatic Forces

As most of the low-energy moieties, with which higher energy compounds tend to interact in aqueous media, have a very low surface potential, long-range as well as short-range electrostatic interactions usually are negligible. However, if needed, the surface potential of most materials can be obtained by electrokinetic methods (e.g., electrophoresis) (*23*). Methods for obtaining the necessary surface potentials from electrokinetic measurements can be found in Refs. *23* and *24;* and equations developed by Verwey and Overbeek (*25*), for obtaining energies of electrostatic (ES) attraction (or repulsion) from these, have recently been reviewed elsewhere (*15*). At contact, i.e., at a distance of the order of 1.5 Å (see above), which usually is significantly smaller than the Debye length (*23-25*) (which varies from 8 Å in physiological saline water to 1000 Å in distilled water), ΔG^{EL} tends to be quantitatively overshadowed by the nonelectrostatic interfacial forces, discussed below.

It is only when electrostatic interactions play an important role in specific binding, as in many (but not all) antigen–antibody interactions (*5–7*), that they become quantitatively as important as the other interfacial forces. This is the case because with specific electrostatic interactions, negative charge points on one surface, upon closer approach, become precisely opposed to positive charge points on the other surface (and vice versa), thus giving rise to a moderate long-range attraction which, however, dramatically increases in strength at short distances due to the precise "lock-and-key" fit of locations of charge centers.

SHORT-RANGE (SR) FORCES

In aqueous or similarly polar liquid media, hydrogen bonds play a considerable role. For instance, 70% of the energy of cohesion of liquid water is due to hydrogen bonds (*8, 14*). Therefore, when a solid or a solute that has hydrogen donor and/or hydrogen acceptor capacities is immersed in water (which has strong hydrogen donor *and* hydrogen acceptor capacities), a stronger attraction will ensue than can be accounted for by just the LW attraction. The additional attraction, due to the formation of hydrogen bonds with the water molecules, has essentially a very short-range (SR) character, i.e., unlike LW interactions, its energy decays to zero within 3 to 4 Å *in vacuo* (*8*). However, at molecular contact, Eqs. (1), (2), and (3) are as valid for SR as for LW interactions. It is not necessarily true, however, that a relation in the form of Eq. (4) is valid for SR interactions (*26*). For highly hydrated substances (e.g., hydrated proteins) interacting with water, the form of Eq. (4) may be used as a first approximation to determine γ^{LW}, especially when θ is measured with water. However, the determination of γ^{SR} of a solid with the help of a liquid that does not have identical (or at least comparable) SR properties with the liquid medium may lead to erroneous results (*8*). Unlike the LW interactions, which are mathematically symmetrical, the short-range interactions (e.g., hydrogen bonds) are manifested as reciprocal, i.e., donor–acceptor (acid–base) responses.

We will now express γ^{SR} in a more rigorous manner (*22*) by first introducing the components of γ^{SR}, in anology with Drago's approach in solution thermodynamics (*27*):

$$\gamma_i^{SR} = 2\sqrt{\gamma_i^{\oplus}\gamma_i^{\ominus}} \tag{9}$$

Here, we let $\gamma^{\oplus}$ stand for the electron acceptor (Lewis acid) surface energy of Compound *i* and $\gamma^{\ominus}$ for its electron donor (Lewis base) energy;* $\gamma^{\oplus}$ and $\gamma^{\ominus}$ are not necessarily equal, see below. Since Compounds 1 and 2 may each have both electron donor and electron acceptor capabilities, the free energy SR interaction between Substances 1 and 2 may be described as

$$\Delta G_{12}^{SR} = -2(\sqrt{\gamma_1^{\oplus}\gamma_2^{\ominus}} + \sqrt{\gamma_1^{\ominus}\gamma_2^{\oplus}}) \tag{10}$$

However, Eq. (1) is also valid for ΔG_{12}^{SR}:

$$\Delta G_{12}^{SR} = \gamma_{12}^{SR} - \gamma_1^{SR} - \gamma_2^{SR} \tag{1A}$$

or, rearranged:

$$\gamma_{12}^{SR} = \Delta G_{12}^{SR} + \gamma_1^{SR} + \gamma_2^{SR} \tag{1B}$$

Combining Eqs. (1B), (9), and (10) (*22*):

$$\gamma_{12}^{SR} = 2(\sqrt{\gamma_1^{\oplus}\gamma_1^{\ominus}} + \sqrt{\gamma_2^{\oplus}\gamma_2^{\ominus}} - \sqrt{\gamma_1^{\oplus}\gamma_2^{\ominus}} - \sqrt{\gamma_1^{\ominus}\gamma_2^{\oplus}}) \tag{11}$$

From the Young-Dupré equation:

$$\Delta G_{SL} = -\gamma_L(1 + \cos\theta) \tag{12}$$

and using Eqs. (9) and (10), the complete Young equation in terms of (LW + SR) can now be established (see also Eq. 7):

$$1 + \cos\theta = \frac{-1}{\gamma_L^{TOT}}(\Delta G_{SL}^{LW} + \Delta G_{SL}^{SR}) \tag{13}$$

or

$$1 + \cos\theta = \frac{2}{\gamma_L^{TOT}}(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^{\oplus}\gamma_L^{\ominus}} + \sqrt{\gamma_S^{\ominus}\gamma_L^{\oplus}}) \tag{13A}$$

*Hydrogen bonds can be treated as Brönsted acid-base (hydrogen donor-hydrogen acceptor) interactions or, in the more general manner, of Lewis base-acid (electron acceptor-electron donor) interactions. Because the Brönsted theory is included in the Lewis theory (but not vice versa), we use the Lewis theory here.

where $\gamma_1^{TOT} \equiv \gamma_1^{SR} + \gamma_1^{LW}$.

If the $\oplus$ and $\ominus$ components of γ^{SR} are not determined separately, then only their product (via Eq. 9) can be reported.

In theory, using three different liquids with known γ_L^{LW}, $\gamma_L^{\oplus}$, and $\gamma_L^{\ominus}$ values, γ_S^{LW}, $\gamma_S^{\oplus}$, and $\gamma_S^{\ominus}$ should all be accessible by means of contact angle determinations. In practice, however, in many cases this may not be so easily done: for most polar liquids the $\gamma^{\oplus}$ and/or $\gamma^{\ominus}$ values are not yet known with any precision. $\gamma^{\oplus}$ and $\gamma^{\ominus}$ can be most easily determined in those cases where one of the interacting compounds lacks either a $\gamma^{\oplus}$ or a $\gamma^{\ominus}$ component (*28, 29*). The total γ^{SR} can be ascertained by contact angle measurement, preferably by using one apolar liquid for obtaining γ^{LW} (Eq. 5), followed by a polar liquid for determining γ^{SR}, using Eq. (25) given in Ref. *8*. It should be kept in mind, however, that the γ^{SR} thus obtained (i.e., *not* broken down into its + and − components) is only an approximate, empirical parameter, and can be used only with respect to interactions with the particular polar liquid L used in measuring the contact angle, as in Eq. (13).

When γ_{123}^{LW}, $\gamma_{123}^{\oplus}$, and $\gamma_{123}^{\ominus}$ are known (*22*), we may write,

$$\Delta G_{132}^{SR} = 2[\sqrt{\gamma_3^{\oplus}}(\sqrt{\gamma_1^{\ominus}} + \sqrt{\gamma_2^{\ominus}} - \sqrt{\gamma_3^{\ominus}}) + \sqrt{\gamma_3^{\ominus}}(\sqrt{\gamma_1^{\oplus}} + \sqrt{\gamma_2^{\oplus}} - \sqrt{\gamma_3^{\oplus}}) - \sqrt{\gamma_1^{\oplus}\gamma_2^{\ominus}} - \sqrt{\gamma_1^{\ominus}\gamma_2^{\oplus}}] \quad (14)$$

If the $\gamma^{\oplus}$ and $\gamma^{\ominus}$ data needed for use in Eq. (13A) are lacking, one must revert to (*8*)

$$\Delta G_{132}^{SR} = \gamma_{12}^{SR} - \gamma_{13}^{SR} - \gamma_{23}^{SR} \quad (2A)$$

using the approximation

$$\gamma_{ij}^{SR} = (\sqrt{\gamma_i^{SR}} - \sqrt{\gamma_j^{SR}})^2 \quad (4A)$$

to obtain γ_{ij}^{SR} (as a first approximation, with hydrated materials only).

Short-range (SR) surface properties of solids can also be estimated approximately by contact angle measurement, using one apolar (Eq. 5) and two polar liquids (Eq. 13A or Eq. 7), to obtain the necessary values for $\gamma_S^{\oplus}$ and $\gamma_S^{\ominus}$ (see above) or at least for γ_S^{SR}. For SR interfacial tensions, Eq. (11) can be used, or, if it is impossible to obtain values for $\gamma_S^{\oplus}$ and $\gamma_S^{\ominus}$, Eq. (4) may be reverted to as a first approximation. ΔG_{132}^{SR} can then be estimated via Eq. (14), or, as a first approximation, by using Eq. (2A).

Again, for ascertaining γ_S^{LW}, hexadecane, diiodomethane,* or α-bromonaphthalene can be used, while various polar liquids are available for γ_S^{SR}, including water. For determining $\gamma_S^{\oplus}$, dimethylsulfoxide may be used. This substance, with a fairly high value for its $\gamma_L^{\ominus}$ (approximately 30 mJ/m^2, see below) and no $\gamma_L^{\oplus}$, is a useful liquid (*28, 29*).

We have mentioned that entropy may play a direct role in "hydrophobic" interactions. An example sometimes cited is the entropy associated with the formation of a cluster (micelle) of amphiphilic molecules which has been ascribed to the existence of a cavity in the water phase to accommodate the hydrocarbon chain of a surfactant molecule (*9, 10*). No theoretical entropy relationships have been reported up to the present. We may estimate the component of interfacial entropy that is due to the water molecules at the surface of the cavity. Assuming that one out of the four possible hydrogen bonds of a water molecule is blocked off by the hydrocarbon molecule, the loss of entropy will be

$$\Delta S = k \ln \tfrac{4}{3} = 2.4 \text{ J/°K} \qquad (15)$$

per water molecule. Removal of the hydrocarbon chain will allow the cavity to collapse, and the water molecules at the cavity surface will return to fourfold hydrogen bonding. The fact that in liquid water there are less than four bonds per molecule actually existing at any instant does not affect this estimate. The cylindrical area of the cavity for, say, a 16-carbon chain, should be about 280 to 375 Å^2. Assuming an area of 10 Å^2 per water molecule, we may estimate the entropy of micelle formation for a C_{16} alkyl compound to be about 65 J/°K/mol of surfactant, and about 40 for a C_{10} chain. The observed values (*9, 10*) are in the range 55 to 142 J/°K/mol, in excellent agreement with the predictions.

THE TOTAL ADHESIVE OR INTERFACIAL FORCE AND "HYDROPHOBIC INTERACTIONS"

The Total Interfacial Interaction

The total adhesive or interfacial interaction at contact is composed of

*It should, however, be mentioned that diiodomethane (γ^{LW} = 50.8 mJ/m^2), being a relatively weak Lewis acid, has in addition a $\gamma^{\oplus}$ component of about 0.51 mJ/m^2 (*29*). α-Bromonaphthalene (γ^{LW} = 44.4 mJ/m^2) has a γ^{SR} component of about 0.8 mJ/m^2 (15); it has weak Lewis base as well as weak Lewis acid properties.

the electrostatic, the Lifshitz-van der Waals, and the short-range interactions:

$$\Delta G^{TOT} = \Delta G^{ES} + \Delta G^{LW} + \Delta G^{SR} \tag{16}$$

However, as in most cases in aqueous media the electrostatic potential of hydrophobic moieties tends to be very low, the ΔG^{ES} term for the interaction (at contact) between hydrophilic compounds with a hydrophobic surface, immersed in water, usually may be neglected (see above). The actual free energy of adhesive or interfacial (or "hydrophobic") interactions may thus be described as

$$\Delta G^{TOT} = \Delta G^{LW} + \Delta G^{SR} \tag{17}$$

in the same manner in which the total surface tension of any Compound i may be described as (*8, 14*)

$$\gamma_i^{TOT} = \gamma_i^{LW} + \gamma_i^{SR} \tag{18}$$

(where γ_i^{SR} is defined in Eq. 9), and the interfacial tension between Substances i and j as

$$\gamma_{ij}^{TOT} = \gamma_{ij}^{LW} + \gamma_{ij}^{SR} \tag{18A}$$

It should be noted, however, that Eq. (4) for obtaining γ_{ij}^{LW} may not be applied to Δ_{ij}^{TOT} except in those cases where $\gamma_{ij}^{SR} = 0$ (that is to say, Eq. 4 is only valid when both 1 and 2 are purely apilar, LW compounds). When one wishes to obtain γ_{ij}^{SR} (in those cases where insufficient $\gamma^{\oplus}$ and $\gamma^{\ominus}$ data are available for using Eq. 11), γ_{ij}^{LW} and γ_{ij}^{SR} must be determined separately. An example from Tables 1 and 2 will make very clear that sizeable errors tend to arise when ΔG_{132}^{TOT} values are calculated from ΔG_{132}^{TOT} values, using Eqs. (2) and (4), instead of from γ^{LW} and γ^{SR} separately: ΔG_{132}^{TOT}, when calculated for the interaction between hydrated albumin and Teflon in water, if done properly (Eq. 17), equals $\Delta G^{LW} + \Delta G^{SR} = +0.5 - 6.6 = -6.1$ mJ/m^2, while if ΔG_{132}^{TOT} is directly derived from the ΔG_{132}^{TOT} values via Eqs. (2) and (4), a value of only -0.6 mJ/m^2 would be arrived at. The experimental value obtained from the adsorption isotherm is -7.0 mJ/m^2, which is close to our value of -6.1 mJ/m^2, but a whole order of magnitude higher than -0.6 mJ/m^2.

TABLE 1
γ^{LW}, γ^{SR} and γ^{TOT} Values of Three Human Serum Proteins (hydrated as well as dry) of Two Low Energy Surfaces and of Water (in mJ/m^2) (*8*)

	γ^{LW}	γ^{SR}	γ^{TOT}
Proteins:			
HSA, hydrated	27.0	44.6	71.6
dry	31.4	15.7	47.1
IgG, hydrated	27.0	40.85	67.85
dry	37.0	4.0	41.0
IgA, hydrated	26.8	47.2	74.0
dry	36.3	3.5	39.8
Low-energy surfaces:			
PTFE	18.5	0	18.5
PST	42.0	0.5	42.0
Phenyl (on phenyl sepharose)	40.0	1.5	40.0
Octyl (on octyl sepharose)	27.0	0	27.0
H_2O	21.8	51	72.8

Measurement of γ^{LW} and γ^{SR} of Liquids

If the γ_L^{TOT} of liquids is known (*18*) or has been measured, the γ^{LW} component of a polar Liquid 1 can be determined by measuring the interfacial tension γ_{12}^{TOT} between that liquid and an apolar Liquid 2 and using (*30*)

$$\gamma_{12}^{TOT} = \gamma_1^{TOT} + \gamma_2^{TOT} - 2\sqrt{\gamma_1^{LW}\gamma_2^{LW}} \tag{19}$$

where, for strictly apolar liquids, $\gamma_2^{TOT} = \gamma_2^{LW}$. γ_{12}^{TOT} can be determined by a static drop or bubble method or by the spinning drop method (*31*). From γ_1^{LW} and γ_1^{TOT}, γ_1^{SR} can then usually be calculated directly with Eq. (18). However, with liquids with *only* a $\gamma^{\oplus}$ or *only* a $\gamma^{\ominus}$ in addition to their γ^{LW}, that $\gamma^{\oplus}$ or $\gamma^{\ominus}$ cannot contribute to their cohesive energy, so that in such cases $\gamma_1^{LW} = \gamma_1^{TOT}$. One such liquid is benzene with a $\gamma^{\ominus} \simeq 2$ mJ/m^2 due to its π-electrons. This value is obtained using Eqs. (11) and (18A) and the interfacial tension of benzene with water of 34 mJ/m^2 (*32*). For water, $\gamma^{LW} = 21.8$ and $\gamma^{SR} = 51$ mJ/m^2 (*8, 14*), in which the γ^{SR} might be taken to be composed of $\gamma^{\oplus} = \gamma^{\ominus} = 25.5$ mJ/m^2. However, this equal division of γ^{SR}

TABLE 2
Comparison between the Free Energies of Protein Adsorption Determined from Absorption Isotherms (ΔG^{ADS}) and Calculated from the γ^{LW} and γ^{SR} Values in mJ/m^2 (see Table 1) (*8*)

System	ΔG^{ADS}	ΔG_{132}^{TOT}
$HSA_{hydr}/H_2O/PTFE$	−7.0	−6.1
$HSA_{hydr}/H_2O/PST$	−6.3	−8.3
$IgG_{hydr}/H_2O/PTFE$	−9.0	−10.2
$IgG_{hydr}/H_2O/PST$	−10.7	−12.0

for water among $\gamma^{\oplus}$ and $\gamma^{\ominus}$ need not be correct: preliminary measurements indicate that more realistic values for water would be $\gamma^{\oplus} \simeq 36$ and $\gamma^{\ominus} \simeq 18$ mJ/m^2 (*29*). Dimethylsulfoxide (DMSO), which lacks a $\gamma^{\oplus}$ component, has a rather high $\gamma^{\ominus}$ value which is of the order of $\simeq 30$ mJ/m^2 (*33*).

If a liquid can be immobilized as a gel, contact angle measurements can be made with another liquid (even with a liquid that is miscible with the first one) on such a gel to arrive at γ^{LW} (*34*) using Eq. (5) or γ^{SR}, or $\gamma^{\oplus}$ and/or $\gamma^{\ominus}$ using Eq. (13) or (13A). Different gel concentrations must be used, and the value for γ of the gelled liquid must be arrived at by extrapolation to zero concentration of the gel material (*34*). In this manner the first estimation for $\gamma^{\ominus}$ of DMSO (see above) was obtained (*33*).

Measurement of γ^{LW} and γ^{SR} of Solids and Solutes

1. Hydrated Materials

Biopolymers (especially proteins) in aqueous solution are strongly hydrated, and their interaction with other bodies or substances immersed in water also takes place while in the hydrated state. Thus, for determining long-range as well as short-range interactions, contact angle measurements on such biopolymers also have to be made while they are in the hydrated state. To that end a protein in solution is best deposited as a thick hydrated layer on an ultrafilter membrane with a pore size smaller than the molecular dimensions of the protein. After a controlled drying period (the membrane having been deposited on a 1% agarose gel to control the drying process), contact angles are measured with the appropriate liquids (*8, 15*).

2. Dried Materials

While measurements on hydrated materials suffice for SR interactions of hydrated solutes, the inner (nonhydrated) core of, e.g., proteins, even though some distance away from interacting substances (i.e., from the liquid that is being used as a probe), will have some influence on the long-range (LW) interactions. That influence may typically amount to about 20% of the total LW interaction (*8*). To measure the LW properties of the inner core, the best approximation is to measure contact angles with the appropriate liquids on the dried material. In addition, contact angle measurements with polar liquids on dried biopolymers may yield data on some of their SR properties as well (*8, 15, 28, 29*).

Attractive and Repulsive Interactions

The individual ΔG_{132}^{LW} as well as ΔG_{132}^{SR} may have a negative *or* a positive value, connoting an attraction or a repulsion between 1 and 2 in Liquid 3 (*35, 36*). In a number of cases ΔG_{132}^{LW} may be positive while ΔG_{132}^{SR} is negative (*8*) or vice versa. The complete value of ΔG_{132}^{TOT} is constituted by the *sum* of these two interactions (Eq. 17).

When contact angles are measured at the hydrated surface of biopolymers as described above (as is extremely probable—see Table 2—this is the very same surface at which the interaction takes place with hydrophobic bodies immersed in water), repulsion effects due to hydration pressure (*37*) need not be taken into account (*15*). Such hydration pressure repulsions would tend to be entirely dissipated within the layer of hydration and should be negligible at even small distances outside that layer.

COMPARISON BETWEEN MEASURED ADHESIVE FORCES AND THE VALUES DERIVED FROM INTERFACIAL DATA

Direct Measurements

Israelachivili et al. developed a device for measuring long-range as well as short-range forces between two crossed cylindrical surfaces in liquids (*38*). With this force balance a number of workers in Canberra, Australia, succeeded in measuring directly the LW and SR forces in a number of instances. Pashley et al. recently measured the attraction between

hexadecyl groups coating the cylindrical surfaces in water (*39*). The cylindrical surfaces, made of mica, were coated with dihexamethyldimethylammonium acetate (DHDAA). At contact an adhesive energy $\Delta G_{131}^{TOT} = 56$ mJ/m^2 was found, which, as the authors remarked, is about two orders of magnitude higher than the value $\Delta G_{131}^{LW} = -0.67$ mJ/m^2 expected on the basis of $\gamma_1^{LW} = 27.5$ and $\gamma_3^{LW} = 21.8$ mJ/m^2. However, note that for water, $\gamma_3^{SR} = 51$ mJ/m^2, and (ideally) for a hydrocarbon, $\gamma_1^{SR} = 0$; so on this basis, $\Delta G_{131}^{SR} = -102$ mJ/m^2 (Eq. 3). This is somewhat higher than the measured ΔG_{131}^{TOT} but it is exceedingly likely that upon adsorption of DHDAA onto the mica surfaces the film will not be perfectly close packed. Thus a few of the polar groups of DHDAA or a few atoms of the mica surface still manifest their presence and give rise to a modest residual γ_1^{SR}, which would be in the form of $\gamma^{\ominus}$. If one then postulates this residual $\gamma^{\ominus} \simeq 5.34$ mJ/m^2, using Eqs. (3) and (11), one obtains $\Delta G_{131}^{SR} = -55.32$ mJ/m^2 which, with the addition of $\Delta G_{131}^{LW} = -0.67$ mJ/m^2, yields $\Delta G^{TOT} = -56$ mJ/m^2. Thus the adhesive energy found by Pashley et al. for protruding hexadecyl groups (originating from DHDAA) immersed in water (*39*) agrees remarkably well with the energy that can be predicted with our approach.

Also quite recently, Marra measured the attraction between two phospholipid bilayer surfaces (*40*) with the same device. He could distinguish between ΔG_{131}^{LW} and ΔG_{131}^{TOT} with this apparatus. Recalculated from his A_{131}^{LW} and A_{131}^{TOT} Hamaker constants (*22*), these values were $\Delta G_{131}^{LW} = -1.54$ mJ/m^2 and $\Delta G_{131}^{TOT} = -8.17$ mJ/m^2 (using Eq. 8), yielding $\Delta G_{131}^{SR} = -6.63$ mJ/m^2 by subtracting ΔG_{131}^{LW} from ΔG_{131}^{TOT} (Eq. 16). Such values would be obtained when $\gamma_1^{LW} = 30.7$ mJ/m^2 (Eq. 4) and $\gamma_1^{SR} = 28.3$ mJ/m^2. For hydrated phospholipids with the hydrophilic moieties protruding, that γ_1^{LW} value is slightly on the high side and the γ_1^{SR} value on the low side, at least in comparison with hydrated phospholipid vesicles deposited on a membrane (*41*). However, if the phospholipid double layers deposited on the mica become somewhat less than ideally organized, e.g., during deposition, after immersion in water, or during mutual compression and distortion at actual contact, the γ_1^{LW} and γ_1^{SR} values calculated above would be of the right order of magnitude.

Protein Adsorption

For a number of human serum proteins (in the hydrated as well as in the dry state) and for some of the more common polymer surfaces, the γ_1^{LW} and γ_1^{SR} values (and thus also the γ^{TOT} values) can be determined by contact angle measurement (Eq. 7) with a number of appropriate liquids

(*8, 15*); see Table 1. Using Eqs. (2), (2A), and (4), and Eqs. (1), (1A), and (18), values for the energies of adhesion ΔG_{132}^{TOT} and ΔG_{12}^{TOT} of these proteins onto these low energy polymer surfaces can be calculated. These calculated values can then be compared with the adsorption energies obtained from Langmuir isotherms. The values that correspond best to the actual adsorption energies are those of ΔG_{132}^{TOT}, derived from hydrated proteins (*8*); see Table 2.

Protein-Protein Interactions

Hydrated proteins (e.g., serum proteins) remain in stable solution due to the fact that, upon accidental close approach between two protein molecules, their energy of attraction is considerably smaller than the energy of their Brownian motion (1.5 kT) (*13*); see Table 3. Thus, with the exception of the relatively strongly negatively charged human serum albumin (HSA) molecules, which actually repeal each other, most other serum proteins achieve stability solely because their energy of attraction under physiological conditions is quite insufficient to overcome the thermal movement favoring their separation. However, when proteins become partially dehydrated, e.g., upon admixture of dehydrating agents such as $(NH_4)_2SO_4$, their γ^{SR} decreases (Table 1), leading to a strong increase in ΔG_{13}^{SR} and thus also in the negative value of ΔG_{131}^{SR} and of ΔG_{131}^{TOT} to values larger than $|-1.5\ kT|$, which favors complex formation, culminating in precipitation. Due to the much higher residual γ^{SR} of dehydrated HSA than of dehydrated immunoglobulins (IgG and IgA) (Table 1), HSA only precipitates at 2/3 saturated $(NH_4)_2SO_4$, while IgG and IgA already precipitate at 1/3 saturated $(NH_4)_2SO_4$ (*15*); see Fig. 1.

TABLE 3
Energies of Attraction between Protein Pairs, ΔG_{131}^{TOT}, in the Hydrated and the Dried State in mJ/m^2 and in kT (*15*)

	ΔG_{131}^{TOT} (hydr)		ΔG_{131}^{TOT} (dry)	
Protein	mJ/m^2	kT[a]	mJ/m^2	kT[a]
HSA	−1.0	−0.25	−22	−5.5
IgG	−1.7	−0.43	−57	−14.2
IgA	−0.7	−0.18	−59	−14.8

[a]To obtain kT, a surface area of contact of $\simeq 100\ Å^2$ was postulated.

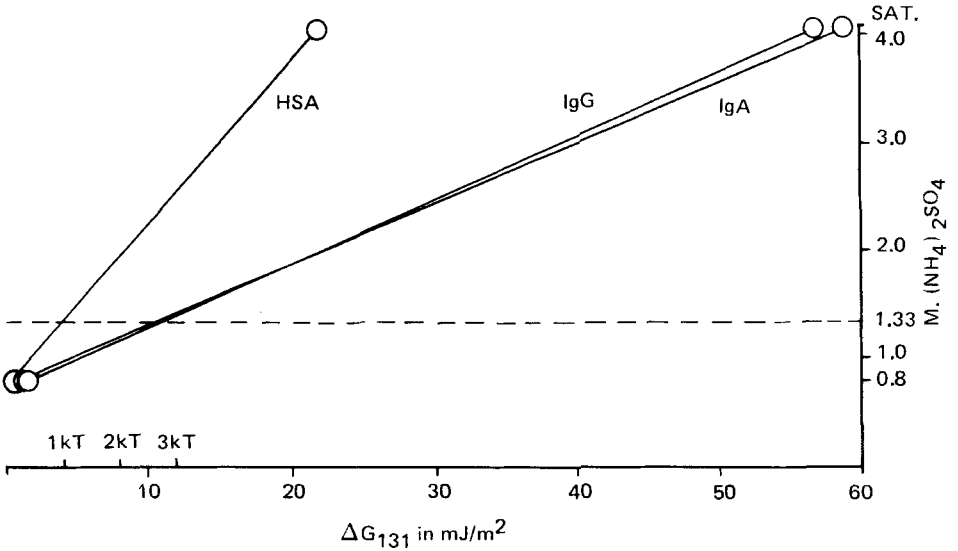

FIG. 1. Protein-protein interaction energy (ΔG_{131}) for HSA, IgG, and IgA as a function of the degree of dehydration, here taken as proportional to the $(NH_4)_2SO_4$ concentration. Total hydration is presumed to persist up to 0.8 *M* $(NH_4)_2SO_4$ and total dehydration must take place at 4.07 *M* $(NH_4)_2SO_4$ (saturation point for solutions in H_2O at 20°C). The dashed horizontal line indicates 1.33 *M* $(NH_4)_2SO_4$ at which IgG and IgA completely precipitate while HSA is still completely soluble. HSA only precipitates at 2.6 *M* $(NH_4)_2SO_4$. All of the protein precipitation occurs at ΔG_{131} values slightly above $|-1.5$ kT|. From Ref. *15*.

APPLICATIONS

Reversed Phase Liquid Chromatography

Proteins that spontaneously adsorb onto low-energy surfaces (see Table 2) can be *desorbed* from these surfaces by lowering the γ^{SR} of the liquid medium. This is what happens in the elution step of reversed phase liquid chromatography (RPLC). Table 4 shows the surface tension components corresponding to the desorption peak of the RPLC elution process of human IgG from an octyl sepharose column (*42*) at 33% (v/v) ethylene glycol (EG) in water. Taking into account an estimated 25% dehydration of the protein due to the presence of 33% EG, at the peak of elution one finds for the total free energy of interaction between IgG and the hydrophobic ligand that $\Delta G_{132}^{TOT} \simeq +1$ mJ/m^2, which would, of course, favor detachment.

TABLE 4
Protein Desorption. Elution of IgG from Octyl Sepharose Columns with 33% (v/v) Ethylene Glycol (EG) at Maximum Peak Height[a] (*8*). Surface Tensions in mJ/m^2

	γ^{LW}	γ^{SR}
1. IgG_{HYDR}	30.3	31.6[b]
2. Octyl sepharose	27.0	0
3. 33% EG	24.2	28.0

[a]Using Eqs. (2) and (4): $\Delta G_{132}^{TOT} = +3.2$ mJ/m^2, which favors elution.
[b]Taking into account partial dehydration (estimated at 25%).

Hydrophobic Interaction Chromatography

Some proteins are so hydrophilic (in the hydrated state) that they do not spontaneously attach to low-energy ligands in ordinary aqueous media; see immunoglobulin A (IgA) in Table 5. This is the only protein shown which does not reach a ΔG_{132}^{TOT} of -1.5 kT in its interaction with phenyl sepharose (*15*). However, upon partial dehydration under the influence of 1 *M* $(NH_4)_2SO_4$ (see Fig. 1), that ΔG_{132}^{TOT} now attains a value of -4.1 kT, thus favoring attachment (Table 5). In such a case it is clearly not necessary to lower the γ^{SR} of the liquid to achieve elution; it suffices to lower the $(NH_4)_2SO_4$ content (*41*) to rehydrate the IgA, which lowers the ΔG_{132}^{TOT} value again to less than -1.5 kT, giving rise to detachment.

In the accepted usage of the word at the present time, the distinction between RPLC and hydrophobic interaction chromatography (HIC) lies in the fact that in RPLC biopolymers spontaneously attach to a low-energy ligand in water and require lowering of the surface tension of water by the admixture of some organic solvent, while in HIC biopolymers only attach to such a ligand under the influence of a dehydrating agent, but readily detach upon removal of that agent (*44*).

Affinity Chromatography

It should be emphasized that the binding of antigens (AG) to antibodies (AB) (as in most other ligand-carrier, receptor-substrate, and enzyme-substrate systems) is almost always due to electrostatic forces (ΔG_{132}^{EL}) *as well as to* interfacial forces ($\Delta G_{132}^{LW} + \Delta G_{132}^{SR}$) (*45*). Only a few rare AG-AB systems are known that bind exclusively via *either* electrostatic (*46–48*) *or* interfacial forces (*49*). Even AG-AB systems that initially bind through electrostatic attractions only, such as bovine serum albumin-

TABLE 5
Hydrophobic versus Reversed Phase Liquid Chromatography (RPLC). Adsorption of Proteins onto Phenyl Sepharose (*15*)[a]

Protein	Solvent	ΔG_{132}^{TOT} mJ/m^2	ΔG_{132}^{TOT} kT
HSH	H_2O	−7.2	−1.8[b]
IgG	H_2O	−10.6	−2.7[b]
IgA	H_2O	−4.4	−1.1[c]
IgA	1 *M* $(NH_4)_2SO_4$	−16.2	−4.1[c]

[a]For the γ^{LW} and γ^{SR} values used, see Table 1; the surface area of likely contact is estimated at 100 Å^2 (*13*).
[b]RPLC possible.
[c]Hydrophobic chromatography required.

goat antibovine serum albumin (*50*), subsequently develop *secondary* interfacial bonds (*45*); and AG-AB systems that are solely interfacial also secondarily develop further interfacial bonds (*45, 49*), as do mixed AG-AB systems. This enhanced secondary interfacial bonding manifests itself by the higher energy needed for dissociation than is required for the prevention of association (*45*). This association-dissociation hysteresis [which appears to be absent only in purely electrostatic systems (*45–48*)] is an extremely important phenomenon in affinity chromatography because it plays a crucial role in the ease and/or completeness of the *elution step.* Here, as in other modes of liquid chromatography, it is important: (a) to limit the association energy to the lowest level compatible with acceptable binding, and (b) to keep the time lapse between attachment and elution as brief as possible to minimize the degree of secondary interfacial binding.

As most affinity (especially AG-AB) systems have electrostatic as well as interfacial components, it should be realized that complete elution can only be effected by *simultaneously* dissociating *both* types of bonds. This is usually best done by (*45–49*): (a) altering the pH (or in the case of rather weak bonds, increasing the ionic strength) of the medium, and by (b) lowering the γ^{SR} of the liquid *at the same time.*

Adhesion to Hexadecane/Water Interfaces

Fairly recently a clever method was developed for the adhesion of bacteria (*51*) or the adsorption of proteins (*52*) from aqueous media onto

the large surface area of the low-energy surface presented by the droplets of hexadecane emulsified in water. Elution is here effected through solidification of the alkane phase on cooling below 18°C, which breaks the emulsion and thus drastically reduces the low-energy surface area, causing the release of previously adhering bacteria or adsorbed proteins. This method is most promising both for analytical (e.g., to determine the relative hydrophilicity of bacteria) and preparative (e.g., for protein fractionation) purposes.

Membrane Fouling

Fouling (or "surface polarization") of membranes in reverse osmosis, hyperfiltration, and ultrafiltration can be attenuated (in aqueous systems) by making the entire membrane, or at least its upstream part, hydrophilic (*53*). For most solutes, ΔG_{132}^{SR} will have a rather low negative value (in water) if the γ^{SR} of the membrane is high (see Eq. 2A). If a polar polymer is used for the membrane, with a preponderant $\gamma^{\ominus}$ (and a very low $\gamma^{\oplus}$), or even better, an exclusive $\gamma^{\ominus}$ (and no $\gamma^{\oplus}$), ΔG_{132}^{SR} will then tend to be positive (see Eq. 15). Cellulose acetate appears to fulfill that requirement to a considerable extent (*54*). A pronounced electrical surface potential of the membrane, of the same sign of charge as the solute, also will cause a repulsion and strongly decrease fouling, but by a mechanism (*55*) that is somewhat different from that of what can, in any strict sense, be called interfacial repulsion. The drawback of the electrokinetic mechanism is that the application of a strong surface change usually decreases the membrane's mechanical strength while also tending to increase the hydration of the membrane surface at the same time.

Blotting

The transfer of DNA fractions from hydrophilic gels to nitrocellulose membranes by Southern (*56*) ("Southern blotting"), which was soon followed by the description of an analogous procedure for RNA (*57*) ("Northern blotting") and somewhat later for proteins (*58*) ("Western blotting"), has rapidly become an extremely important separation method in molecular biology, genetic engineering, and immunological detection (*59*). Surface tension measurements on nitrocellulose, nucleic acids, and proteins have shown that the interfacial attraction in aqueous media ($-\Delta G_{132}^{SR}$) is indeed considerable, especially in the case of proteins (*60*). Protein binding to nitrocellulose may be quantitatively likened to

reversed phase liquid chromatography (see above), while with nucleic acid blotting there are somewhat lower $|-\Delta G_{132}^{SR}|$ values, so that is is often necessary to "fix" nucleic acids onto nitrocellulose in the presence of high salt concentrations; this is analogous to hydrophobic interaction chromatography (see also above). It is therefore not surprising that cationized nylon membranes have been advocated more recently (*61, 62*), especially for the electrophoretic transfer of nucleic acids (in which process high salt concentrations cannot easily be used). With positively charged nylon membranes, the attachment mainly occurs through the highly negative ΔG_{132}^{EL} between the strongly negatively charged nucleic acids and the positively charged membrane.

Zone Melting

There is a strong analogy between zone melting (*63*) (by locally melting a band of the solid material and repeatedly moving the section that is being melted through the entire column) and other simple advancing solidification front processes (*64*). For any given system with solid material (1), impurities (2), and liquified material (3), ΔG_{132}^{TOT} must have either a positive or a negative value so that zone melting is essentially a no-lose situation. When $\Delta G_{132}^{TOT} < 0$, the impurities will be engulfed by the advancing solidification front (*64*), and thus, ultimately, after a sufficient number of passages, left behind. When $\Delta G_{132}^{TOT} > 0$, the impurities are pushed forward by the advancing solidification front (*64*), and thus accumulate in the front of the column. In either case the middle portion of the column eventually becomes depleted of impurities.

Partition

Partition, i.e., multistage countercurrent extraction in trains of immiscible solvents, which was pioneered by Lyman Craig (*65*), but which also has a strong analogy to partition chromatography [for which Martin and Synge (*66*) obtained a Nobel prize], was opened up to applicability to aqueous systems by Albertsson (*67*) who utilized phase separation systems of (typically) dextran and polyethylene glycol (PEG), both dissolved in water. We demonstrated earlier that the phase separation often encountered in solutions of two different polymers in organic solvents is generally due to a van der Waals repulsion (i.e., to a positive ΔG_{132}^{LW}; see Eq. 2) (*68*).

With water-soluble polymers the mechanism is less obvious. But one

new fact has recently become apparent: many carbohydrates are "monopolar" in the same way that dimethylsulfoxide is (see above) (*28*). Thus, dextrans are principally Lewis bases (i.e., they mainly have a $\gamma^{\ominus}$ and no significant $\gamma^{\oplus}$) (*29*) as are the polyethylene glycols. Then, aqueous solutions of dextrans and of PEG, when mixed, will manifest a strongly positive ΔG_{132}^{SR} (see Eq. 15), and thus usually also a positive ΔG_{132}^{TOT} (Eq. 18), which favors a repulsion (when the $\gamma^{\ominus}$ values of the two polymers are different), and thus a phase separation. In such systems, polymers (or particles) with a pronounced $\gamma^{\ominus}$, which is quantitatively different from the $\gamma^{\ominus}$ of, e.g., Phase A, will be repelled by it, and thus accumulate in Phase B (*69*).

Other Separation Methods Affected by Interfacial Phenomena

Many other separation methods are directly or indirectly affected by interfacial phenomena; only a few will be briefly mentioned here.

Among the separation methods that do not depend directly on interfacial phenomena, but which often are indirectly strongly affected by interfacial interactions, are virtually all liquid chromatography (LC) modes, including ion exchange, pore permeation, and metal chelation LC. Even if electrical charge and/or molecular size are the principal mechanisms of chromatographic separation, as long as the inert part of the stationary phase is not totally hydrophilic, some degree of additional (secondary) interfacial binding usually is unavoidable in aqueous media.

In electrokinetic methods, secondary interfacial adsorption onto the solid–liquid interfaces at all structural surfaces, as well as onto the interfaces with anticonvective materials (if present), must be taken into account.

Separation by foam flotation is, of course, fundamentally based on interfacial phenomena (*70*). For more recent developments, see Sebba (*71*).

Finally, separation by precipitation is intimately linked to interfacial phenomena in aqueous media. First, the precipitation of hydrophilic polymers by mild dehydration (see above, under Protein–Protein Interactions) can be entirely reduced to interfacial interactions (*15*). But also the precipitation of such polymers through electrical charge interactions becomes reinforced (as soon as the polymer molecules have approached each other to within the range of SR forces) by secondary interfacial forces. These may cause the reversal of the precipitation process to require quantitatively more enerlgy than was involved in the

initial precipitation step, and to involve measures that are qualitatively unrelated to the mechanism used to effect precipitation (*72*). Also, the solubility of, e.g., biopolymers such as proteins, is linked to their size, regardless of their relative surface hydrophilicity: if the size of the surface area of the planes of interaction at close approach becomes large enough, the total attraction will reach a value at which the interfacial interaction energy between two such polymers (ΔG_{131}^{SR}) becomes larger than $|-1.5$ kT|, from which point on attachment will be favored (provided the macromolecules' ζ-potential is small enough to obviate electrostatic repulsion) (*72*). Immune precipitation, which is analytically as well as preparatively a very important separation method (*73*), also usually is simply based on the initial formation of larger complexes (through immunochemical crosslinking) which then, as a second step (*74*), become insoluble through mutual interfacial interactions at $\Delta G_{131}^{SR} > |-1.5$ kT| without necessarily having undergone any change in surface tension or ζ-potential (*72*).

CONCLUSIONS

The "hydrophobic interaction energy" essentially consists of the total interfacial free energy ΔG_{132}^{TOT} between a (hydrophilic or a hydrophobic) Substance 1 reacting with a low energy (hydrophobic) Substance or Surface 2 while immersed or dissolved in an aqueous liquid Medium 3. That total interfacial free energy ΔG_{132}^{TOT} comprises the long-range Lifshitz-van der Waals interaction ΔG_{132}^{LW} *plus* the short-range interaction ΔG_{132}^{SR} which mainly originates in hydrogen bonds. The components of ΔG^{LW} and of ΔG^{SR} must be treated separately. They can be measured by means of contact angle determination with a number of liquids whose long-range and short-range surface tension components γ^{LW} and γ^{SR} are known. Comparisons between ΔG_{132}^{TOT} derived from γ^{LW} and γ^{SR} values and ΔG values measured directly agree closely.

"Hydrophobic interactions" seems to be somewhat of a misnomer, as they are entirely due to the sum of the long-range and the short-range interfacial forces acting in (very hydrophilic) aqueous media between a hydrophilic and a hydrophobic moiety. Thus "interfacial forces" or "interfacial interactions" would seem to be better terms for such interactions because they more aptly describe and define the underlying phenomena.

The role of interfacial forces in various separation methods (most modes of liquid chromatography, membrane separation methods, "blotting," zone melting, precipitation processes) is enormous.

In certain cases enough data are available to allow one to decompose

γ^{SR} into its electron acceptor, or Lewis acid ($\gamma^{\oplus}$), and its electron donor, or Lewis base ($\gamma^{\ominus}$), contributions. In some systems where the $\gamma^{\ominus}$ components are the sole or the dominant contributions to the γ^{SR} of both interacting Substances 1 and 2, a phase separation can occur between 1 and 2, even though both are dissolved or suspended in the same aqueous Medium 3. The same should, in principle, be true when the $\gamma^{\oplus}$ components are the sole or the dominant contributors to the γ^{SR} of Substances 1 and 2. Two-phase systems of this type are increasingly used in separation techniques.

Acknowledgment

This material was presented at the First International Conference on Separation Science and Technology under the aegis of the 191st National Meeting of the American Chemical Society, held at New York, April 1986 (*75*).

REFERENCES

1. C. Tanford, *Science* 200, 1012 (1978).
2. J. Rosengren, S. Påhlman, M. Glad, and S. Hjerten, *Biochim. Biophys. Acta, 412,* 51 (1975).
3. B. H. J. Hofstee, *J. Macromol. Sci.—Chem., A10,* 111 (1976).
4. B. H. J. Hofstee, *Anal. Biochem., 52,* 430 (1973).
5. D. Pressman and A. L. Grossberg, *The Structural Basis of Antibody Specificity,* Benjamin, Reading, Massachusetts, 1973, p. 8.
6. E. A. Kabat, *Structural Concepts of Immunology and Immunochemistry,* Holt, New York, 1976, p. 156.
7. M. W. Steward, in *Immunochemistry* (L. E. Glynn and M. W. Steward, eds.), Wiley, New York, 1977, p. 234.
8. C. J. van Oss, R. J. Good, and M. K. Chaudhury, *J. Colloid Interface Sci., 111,* 378 (1986).
9. M. J. Rosen, *Surfactants and Interfacial Phenomena,* Wiley, New York, 1978, pp. 116–117.
10. P. C. Hiemenz, *Principles of Colloid and Surface Chemistry,* 2nd ed., Dekker, New York, 1986, p. 445.
11. F. M. Fowkes, *J. Phys. Chem., 66,* 382 (1962).
12. F. M. Fowkes, *Ind. Eng. Chem., 56,* 40 (December 1964).
13. F. M. Fowkes and M. A. Mostafa, *Ind. Eng. Chem., Process Des. Dev., 17,* 3 (1978).
14. M. K. Chaudhury, "Short Range and Long Range Forces in Colloidal and Macroscopic Systems," PhD Dissertation, State University of New York at Buffalo, 1984.
15. C. J. van Oss, L. L. Moore, R. J. Good, and M. K. Chaudhury, *J. Protein Chem., 4,* 245 (1985).
16. C. J. van Oss and R. J. Good, *Colloids Surf., 8,* 373 (1984).
17. R. J. Good, *Colloid and Surface Science,* Vol. 11 (R. J. Good and R. R. Stromberg, eds.), Plenum, New York, 1979, p. 1.

18. J. J. Jasper, *J. Phys. Chem. Ref. Data, 1,* 841 (1972).
19. A. W. Neumann and R. J. Good, *Colloid and Surface Science,* Vol. 11 (R. J. Good and R. R. Stromberg, eds.), Plenum, New York, 1979, p. 31.
20. H. C. Hamaker, *Physica, 4,* 1058 (1937).
21. M. K. Chaudhury and R. J. Good, *J. Colloid Interface Sci., 94,* 292 (1983).
22. M. K. Chaudhury, R. J. Good, and C. J. van Oss, In Preparation.
23. P. G. Righetti, C. J. van Oss, and J. W. Vanderhoff (eds.), *Electrokinetic Separation Methods,* Elsevier, Amsterdam, 1979.
24. C. J. van Oss, *Sep. Purif. Methods, 4,* 167 (1975).
25. J. Th. G. Overbeek, in *Colloid Science,* Vol. 1 (H. R. Kruyt, ed.), Elsevier, Amsterdam, 1952, pp. 245–277.
26. F. M. Fowkes, in *Physicochemical Aspects of Polymer Surfaces,* Vol. 2 (K. L. Mittal, ed.), Plenum, New York, 1983, pp. 583–603.
27. R. S. Drago, G. C. Vogel, and T. E. Needham, *J. Am. Chem. Soc., 93*(23), 6014 (1971).
28. M. K. Chaudhury, C. J. van Oss, and R. J. Good, *Abstracts of the 60th Colloid Surface Science Symposium,* Atlanta, Georgia, June 1986.
29. M. K. Chaudhury, C. J. van Oss, and R. J. Good, In Preparation.
30. F. M. Fowkes, in *Surfaces and Interfaces,* Vol. 1 (J. J. Burke, N. L. Reed, and V. Weiss, eds.), Syracuse University Press, Syracuse, New York, 1982, pp. 27–36.
31. A. W. Adamson, *Physical Chemistry of Surfaces,* 4th ed., Wiley, New York, 1982, pp. 27–36.
32. R. J. Good and E. Elbing, *Ind. Eng. Chem., 62*(3), 54 (1970); *Chemistry and Physics of Interfaces 2,* American Chemical Society, Washington, D.C., 1971, p. 71.
33. C. J. van Oss, L. K. Ju, R. J. Good, and M. K. Chaudhury, In Preparation.
34. C. J. van oss, M. Roberts, R. J. Good, and M. K. Chaudhury, *Colloids Surf.,* Submitted.
35. J. Visser, *Adv. Colloid Interface Sci., 15,* 157 (1981).
36. C. J. van Oss, J. Visser, D. R. Absolom, S. N. Omenyi, and A. W. Neumann, *Adv. Colloid Interface Sci., 18,* 133 (1983).
37. V. A. Parsegian, N. Fuller, and R. P. Rand, *Proc. Natl. Acad. Sci. U. S. A., 76,* 2750 (1979).
38. J. N. Israelachvili, R. K. Tandon, and L. R. White, *J. Colloid Interface Sci., 78,* 430 (1980).
39. R. M. Pashley, P. M. McGuiggan, B. W. Ninham, and D. F. Evans, *Science, 229,* 1088 (1985).
40. J. Marra, *Colloid Interface Sci., 109,* 11 (1986).
41. C. J. van Oss and S. P. Ohki, In Preparation.
42. C. J. van Oss, D. R. Absolom, and A. W. Neumann, *Sep. Sci. Technol., 14,* 305 (1979).
43. G. J. Doellgast and A. G. Plaut, *Immunochemistry, 13,* 135 (1976).
44. Application Notes, *LKB News,* p. 5 (Spring 1985).
45. C. J. van Oss, R. J. Good, and M. K. Chaudhury, *J. Chromatogr., 376,* 111 (1986).
46. E. R. De Groot, M. C. Lamers, L. A. Aarden, R. J. T. Smeenk, and C. J. van Oss, *Immunol. Commun., 9,* 515 (1980).
47. R. J. T. Smeenk, L. A. Aarden, and C. J. van Oss, *Ibid., 12,* 177 (1983).
48. C. J. van Oss, R. J. T. Smeenk, and L. A. Aarden, *Immunol. Invest., 14,* 245 (1985).
49. C. J. van Oss, D. R. Absolom, A. L. Grossberg, and A. W. Neumann, *Immunol. Commun., 8,* 11 (1979).
50. C. J. van Oss, D. R. Absolom, and P. M. Bronson, *Ibid., 11,* 139 (1982).
51. M. Rosenberg and E. Rosenberg, *J. Bacteriol., 148,* 51 (1981).
52. M. Rosenberg, Z. Zosim, N. Reginov, and I. Ili, *Prep. Biochem.,* In Press.

53. H. Gregor, In Preparation.
54. C. J. van Oss, In Preparation.
55. C. J. van Oss, *Sep. Sci. Technol., 15,* 159 (1980).
56. E. M. Southern, *J. Mol. Biol., 98,* 503 (1975).
57. J. C. Alwine et al., *Methods Enzymol., 68,* 220 (1975).
58. J. Renart, *Proc. Natl. Acad. Sci. U. S. A., 16,* 346 (1979).
59. G. Bers and D. Garfin, *Biotechniques, 3,* 276 (1985).
60. C. J. van Oss, M. K. Chaudhury, and R. J. Good, *J. Chromatogr.,* In Press.
61. J. M. Gershoni and G. E. Palade, *Anal. Biochem., 124,* 396 (1982).
62. J. M. Gershoni and G. E. Palade, *Ibid., 131,* 1 (1983).
63. W. R. Willcox, in *An Introduction to Separation Science* (B. L. Karger, L. R. Snyder, and C. Horvath, eds.), Wiley, New York, 1973, p. 315.
64. A. W. Neumann, S. N. Omenyi, and C. J. van Oss, *Colloid Polym. Sci., 257,* 13 (1979).
65. L. C. Craig and D. Craig, in *Techniques of Organic Chemistry,* Vol. 3 (A. Weissberger, ed.), Interscience, New York, 1956, p. 149.
66. A. J. P. Martin and R. L. M. Synge, *Biochem., J., 35,* 91 (1941).
67. P.-Å. Albertsson, *Partition of Cell Particles and Macromolecules,* Wiley-Interscience, New York, 1960, p. 1971.
68. C. J. van Oss, S. N. Omenyi, and A. W. Neumann, *Colloid. Polym. Sci., 257,* 737 (1979).
69. C. J. van Oss, R. J. Good, and M. K. Chaudhury, *Sep. Sci. Technol.,* In Press.
70. R. L. Lemlich (ed.), *Adsorptive Bubble Separation Techniques,* Academic, New York, 1971.
71. F. Sebba, *Sep. Purif. Methods, 14,* 127 (1985).
72. C. J. van Oss, M. K. Chaudhury, and R. J. Good, *J. Protein Chem.,* In Press.
73. C. J. van Oss, in *Molecular Immunology* (M. Z. Atassi, C. J. van Oss, and D. R. Absolom, eds.), Dekker, New York, 1984, p. 361.
74. J. Steengaard, *Immunol. Today, 5,* 7 (1984).
75. C. J. van Oss, R. J. Good, and M. K. Chaudhury, Abstracts of the 191st American Chemical Society Meeting, 1986, p. 176.

Received by editor May 5, 1986