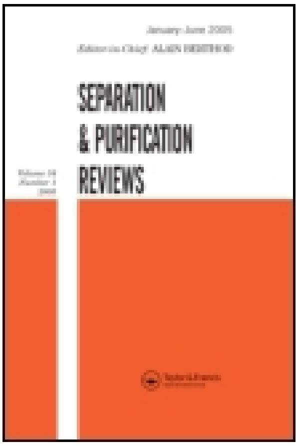

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Role of the Polar Properties of Water in Separation Methods

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The role of the polar properties of water in separation methods are presented in two parts:

Part I: Properties of Water
General principles are given of the three non-covalent energy types acting upon non-polar as well as polar entities when these are immersed in water: Lifshitz-van der Waals (LW), Lewis Acid-Base (AB) and Electrostatic (EL) energies. The dominant one of these three is the Lewis acid-base (AB) interaction, which in biochemical and other organic interactions are about an order of magnitude stronger than the van der Waals and electrostatic energies combined. Included are the most important equations, describing these interactions when occurring in water.

Part II: Forces Originating in Water and their Significance in Separation Methods

- *Hydrophobic attraction between particles.*
- *Hydrophobic interaction between solute molecules immersed in liquid water is caused by the hydrogen-bonding free energy of cohesion between the water molecules which surround these particles or solute molecules.*
- *The solubility equation is given, which links the free energy of interaction, ΔG_{iwi}, between the solute molecules (i) immersed in water (w), and the natural logarithm of the aqueous solubility (s)*

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of i, expressed in mol fractions. Insolubility and partial solubility in water are due to strong mutual attraction and moderate attraction among molecules, respectively. Total solubility in water is due to mutual repulsion between solute molecules.

- Discusses conditions of stability versus instability (flocculation) of aqueous particle suspensions.
- Discusses the hyper-hydrophobicity of the water-air interface and its interactions with: A) small water-soluble molecules (it repels them) and: (B) amphiphilic molecules such as surfactants, alcohols, proteins, etc. (it strongly attracts them).
- Treats adhesion and adsorption energies between two different entities, immersed in water, such as antigens and antibodies. These energies are commonly expressed as affinity constants (K_{aff}), the value of which is strongly influenced by the time spent during the initial adhesion or adsorption step, where the K_{aff} value steadily increases the longer that initial adsorption step lasts. This phenomenon is called hysteresis, or binding hysteresis. However the hysteresis effect can be avoided by reducing the initial exposure episode to time (t) approaching $t = 0$, to obtain $K_{aff}^{t\rightarrow 0}$.
- Two different aqueous phases can be formed by dissolving two different water-soluble polymers, e.g., poly-(ethylene oxide) and dextran, which repel one another in aqueous solution. Such aqueous two-phase systems can be used cause the preferential migration of two different solutes to two different phases and thus to effect their separation and purification. Results of a study of multiple sub-phases forming within the middle phase of micro-emulsions are also discussed.
- Advancing freezing fronts are discussed in this Section, i.e., the rejection or engulfment of certain solutes or cells by a vertical ice column which is made to advance further vertically by continuing to cool it from below.
- The size, or rather the radius of curvature (R) of blood cells, blood proteins, bacteria and viruses, can play an important role in, e.g., the in vivo phagocytic engulfment of bacteria by leucocytes, the transformation of platelets to become "sticky", the attachment of viruses to cells and of bacteriophages to bacteria.

KEYWORDS Hydrophobic attraction, flocculation, water-air interface, adhesion energies, antigens and antibodies, affinity constants, aqueous phase separation, advancing freezing fronts, size of cells, microbes and proteins

PART I: PROPERTIES OF WATER

Three Non-covalent Interaction Forces

Among the liquids employed in separation methods taking place in liquid media, water is the most frequently used. When undergoing separation processes in aqueous media the immersed molecules or particles are subjected to a combination of the three non-covalent interaction forces, namely Lifshitz-van der Waals (LW), electrostatic (EL) and Lewis acid-base (AB) forces. Until the late 1980's only the first two of these were widely recognized and utilized, i.e., the LW and EL forces. In aqueous media LW forces were held to be always attractive and whilst EL forces could be either repulsive or attractive, they most frequently appeared to occur in the repulsive mode.

A theory on the stability of electrically charged particles, suspended in water and making use of the attractive or repulsive energies between these particles as a function of distance was first proposed by Hamaker in the Netherlands (1, 2). In the Soviet Union, similar work was done by Derjaguin and Landau (3) and in the Netherlands, Verwey and Overbeek (4, 5) who worked at the same Institute as Hamaker, further elaborated upon Hamaker's approach. Verwey and Overbeek (4) did not actually mention the use of water as their liquid medium, but especially in their treatment of electrical double layer laws, they described a liquid whose electrochemical properties were remarkably reminiscent of those of water. The combined theories of Derjaguin and Landau (3) and Verwey and Overbeek (4) subsequently became famous as the DLVO theory (after the first letters of their surnames) and long remained popular, even for use with water. The DLVO theory still only treated the van der Waals and electrical double layer energies which, as is now known, represent significantly less than one third of the forces acting upon polar as well as apolar solutes or particles when immersed in water, in all aqueous colloidal and biological systems; see van Oss (6, pp. 34–35). Therefore, for all interactions in aqueous systems, the old DLVO approach should always be completed by adding the polar, Lewis acid-base interaction energies, thus extending it to form the *Extended DLVO Theory*.

Meanwhile, from an analysis by Girifalco and Good (7), Fowkes (8) had rightly concluded that, at 20°C, the non-polar (i.e., the Lifshitz-van der Waals) part of the surface tension of liquid water is 21.8 mJ/m^2. As the total surface tension of water, at 20°C, is 72.8 mJ/m^2, this leaves $72.8 - 21.8 = 51.0$ mJ/m^2 for the polar (Lewis acid-base) component of the surface tension of water.

From the above analysis it follows that, as the surface tension of water is proportional to the total free energy of cohesion between the water molecules in liquid water, the apolar Lifshitz-van der Waals part represents 30% of the free energy of cohesion of water and the polar Lewis acid-base part represents 70% of its free energy of cohesion. The precise relation between the total surface tension (γ_w) of a liquid such as water (w) and the free energy of cohesion (ΔG_{ww}) between two water molecules is expressed as:

$$2\gamma_w = -\Delta G_{ww} \qquad [1]$$

Apolar Properties of Liquid Water - Lifshitz-van der Waals (LW) Forces

Using equation 1, the apolar LW part of the free energy of cohesion of liquid water (at 20°C) then amounts to: $\Delta G_{ww}{}^{LW} = -2 \times 21.8 = -43.6$ mJ/m^2. Now, $\Delta G_{ww}{}^{LW}$ is directly proportional to what is called the Hamaker constant, A, which, for the Lifshitz-van der Waals component of the free energy of cohesion of liquid water shall be designated as A_{ww}, such that:

$$A_{ww} = 24\pi d_o^2 \times \gamma_w{}^{LW} = 1.8585 \times 10^{-14} \times \gamma_w{}^{LW} \qquad [2]$$

where the minimum equilibrium distance between two condensed-phase surfaces or molecules, $d_o = 0.157$ nm (9). Equation 2 is also valid for all other apolar materials *i*, so that, in general:

$$A_{ii} = 24\pi d_o^2 \times \gamma_i{}^{LW} \qquad [2A]$$

when $\gamma_i{}^{LW}$ is expressed in ergs/cm^2 (i.e. mJ/m^2), to obtain A_{ii} in ergs (or in units of 1×10^{-7} Joules); see van Oss (10, p.20). As the surface tensions and their components and parameters of solids and liquids are readily measured, the decay of interaction energies as a function of distance, as far as a material's Hamaker constant (A) is concerned, is easily determined with the help of equation 2A (when the $\gamma_{ii}{}^{LW}$ value of a material *i* is known), so that the decay vs. distance of interaction energies of apolar materials when immersed in water can be readily derived, using the rules given later in "Decay as a Function of Distance".

On a microscopic (atomic) scale and in the gaseous phase, there are three separate classes of van der Waals interaction forces, i.e., those caused by:

1. Dipole-dipole interactions (*orientation* or van der Waals-Keesom forces) first identified by Keesom (11–13);
2. Dipole-induced dipole interactions (*induction*, or van der Waals-Debye forces) first identified by Debye (14, 15) and:
3. Fluctuating dipole-induced dipole interactions (*dispersion*, or van der Waals-London forces) first identified by London (16),

where the van der Waals-London forces are the most prevalent. The energies exerted by each one of the above three microscopic (atomic) scale classes of van der Waals forces differ among each other (10, 17). However, on a macroscopic (molecular and macromolecular) scale, Lifshitz (18) showed that all three van der Waals interaction forces decay according to the same rules (e.g., as the square of the distance, in a parallel flat plates' conformation) and they manifest, together, a free energy which is proportional to the van der Waals, or Hamaker constant (A), defined here: Chaudhury (19) and equation 2A.

For the interaction between two *identical* apolar molecules, 1, immersed in an apolar liquid, L, one may use the following equation:

$$2\gamma_{IL}^{LW} = -\Delta G_{ILI}^{LW} = -2(\sqrt{\gamma_1}^{LW} - \sqrt{\gamma_L}^{LW})^2 \qquad [3]$$

which shows that as $2(\sqrt{\gamma_1}^{LW} - \sqrt{\gamma_L}^{LW})^2$ always must be positive, or zero, ΔG_{1L1}^{LW} always must be (zero) or negative, i.e., *attractive*. Therefore, when immersed in an apolar liquid, L, apolar solute molecules, 1, always will attract and can never repel one another. As first noted by Hamaker (20), with two *different* apolar molecules, 1 and 2, immersed in an apolar liquid, L:

$$\Delta G_{1L2}^{LW} = -2(\sqrt{\gamma_1}^{LW} - \sqrt{\gamma_L}^{LW}) \times (\sqrt{\gamma_2}^{LW} - \sqrt{\gamma_L}^{LW}) \qquad [4]$$

and according to equation 4, ΔG_{1L2}^{LW} will be positive, i.e., *repulsive*, when (10, 17):

$$\gamma_1^{LW} < \gamma_L^{LW} < \gamma_2^{LW}, \text{or when } \gamma_1^{LW} > \gamma_L^{LW} > \gamma_2^{LW} \qquad [5]$$

Thus, while in apolar systems two *similar* apolar molecules, when immersed in an apolar liquid, cannot repel one another, two *different* apolar molecules, when immersed in an apolar liquid can repel each other, under the conditions described in the inequalities [5], above. As the Hamaker constant, A, is proportional to γ^{LW}, the inequalities shown above, as conditions for van der Waals repulsions to be possible between two different materials, immersed in a liquid, these conditions can also be written as:

$$A_1 < A_L < A_2, \text{or} : A_1 > A_L > A_2 \qquad [5A]$$

One may therefore state that two *identical* apolar molecules with only one characteristic apolar property (their Hamaker constant, A) in common will not be able to repel each other when immersed in an apolar liquid, whereas two *different* apolar molecules, with different apolar properties (Hamaker constants, A_1 and A_2), will indeed be able to repel each other when immersed in an apolar liquid; see the inequality statements [5A], above. This only makes it possible for apolar macromolecules to be soluble in apolar solvents when the surface properties of these apolar macromolecules and those of the solvent molecules are fairly similar.

Now, for polar polymer molecules to be soluble in a polar solvent such as water, it generally is a requirement that such polymer molecules repel one another when immersed in water. As shown above, this is not possible in apolar systems. It turns out that identical polar polymer molecules, to be soluble in a polar liquid (such as water), need to be able to repel one another when immersed in such a liquid. These polar polymer molecules will only be able to achieve that if they have two different salient polar surface properties where one of these is larger than the first analogous polar property of water and the other one is smaller than the second analogous polar property of water (9). This led us to opt for the Lewis acid-base (AB) system as the most fitting dual property polar principle, consisting as it does, of the two independently variable properties of electron-acceptors and electron-donors whose importance is especially in aqueous systems; see “Polar Interactions in Liquid Water”.

Polar Properties of Liquid Water — Lewis Acid-Base (AB) Forces

The polar part of non-covalent interaction energies among condensed-phase materials when immersed in water is closely linked to their polar surface tension properties, as well as to the polar surface tension properties of water. When polar macromolecules manage to dissolve in a polar liquid such as water, a repulsion between identical polar molecules then must occur, which is stronger than the ambient Lifshitz-van der Waals attraction plus hydrophobic attraction energies.

Now, in contrast with the omnipresent LW forces, which are only due to one physical property (the Hamaker constant, A, which is proportional to the apolar component of the surface tension component, γ^{LW}), polar forces, which typically occur among hydrophilic compounds, are governed by *two separate properties*, (i) electron-accepticity (as in Lewis acids), indicated by γ^{+} and (ii) electron-donicity (as in Lewis bases), indicated by γ^{-}. These two interaction types, together, are designated as Lewis acid-base (AB) interactions (9, 21). The total surface tension (γ) of polar condensed-phase molecules or materials consists of:

$$\gamma = \gamma^{LW} + \gamma^{AB} \qquad [6]$$

$$\gamma^{AB} = 2\sqrt{\gamma^{+}\,\gamma^{-}} \qquad [7]$$

where:
and where, for water, at 20°C, $\gamma_{w}{}^{AB} = 51.0$ mJ/m^2.

It has been shown that when polar, hydrophilic macromolecules *i* are immersed in water (w), mutual repulsion between these macromolecules is favored when:

(A) $\gamma_i^+ < \gamma_w^+$ and $\gamma_i^- > \gamma_w^-$

or when:

(B) $\gamma_i^+ > \gamma_w^+$ and $\gamma_i^- < \gamma_w^-$

where the conditions shown under A) are by far the most common (10, 17). Thus the dual properties all identical polar molecules have in common, favor their mutual repulsion in water and thereby their aqueous solubility.

The polar as well as apolar properties of water and of various other compounds are treated in more detail in the following sub-Section.

Interactions Between Liquid Water and Other Apolar and Polar Compounds

Apolar condensed-phase materials, immersed in an apolar liquid undergo only apolar, i.e., Lifshitz-van der Waals interactions. However, when immersed in liquid water, *apolar* condensed-phase molecules, macromolecules as well as particles undergo a strong, polar-driven attraction to one another, caused by the surrounding water molecules and their strong hydrogen-bonding free energy of cohesion.

Liquid water is the most bipolar of all commonly occurring polar liquids, such as alcohols (e.g., glycerol, ethylene glycol), amides (e.g., formamide), ketones, etc., in that it exhibits strong electron-accepticity *as well as* strong electron-donicity, expressed as γ_w^+ and γ_w^-, respectively. The precise quantitative determination of both γ_w^+ and γ_w^-, at room temperature, has not thus far proved possible. However, the precise value of their product, at 20°C, is known, because γ_w^{AB} is exactly 51.0 mJ/m^2 at 20°C; equation [7]. Thus, although the exact ratio: γ_w^+/γ_w^- is as yet unknown, from manifold experimental data (10, 17) it is clear that at room temperature the γ_w^+ and γ_w^- values are each considerable and of a comparable order of magnitude. It was therefore decided to assume the values for both γ_w^+ and γ_w^- to be equal to $(0.5 \times 51.0) = 25.5$ mJ/m^2 each, thus establishing their ratio, $\gamma_\mathrm{w}^+/\gamma_\mathrm{w}^-$, at *unity* at 20°C (9, 21).

The values of the polar surface tension parameters γ_i^+ and γ_i^- of other polar compounds and molecules *i* can all be defined relative to the assumed values for water at 20°C, established above as 25.5 mJ/m^2 each. Nonetheless, although to some extent based on this assumption, the values for non-aqueous polar compounds or molecules, expressed as γ_i^{AB}, γ_{12}^{AB}, $\Delta G_{12}^{\mathrm{AB}}$ and $\Delta G_{1w2}^{\mathrm{AB}}$ are absolute and independent of the assumption: $\gamma_\mathrm{w}^+/\gamma_\mathrm{w}^- = 1$, as in the process of deriving these entities, the values of the above-mentioned assumption have been cancelled-out (10).

The Surface Tension Properties of a Number of Common Apolar and Polar Liquids and Solids

Table 1 shows the surface tension properties of five non-polar liquid alkanes. Being apolar, these liquids have only the Lifshitz-van der Waals (LW) surface tension components (γ^{LW}) of the total surface tension (γ), so that in their case, $\gamma_{\mathrm{L}} = \gamma_{\mathrm{L}}^{\mathrm{LW}}$ and both their γ_{L}^{+} and γ_{L}^{-} and therefore also their $\gamma_{\mathrm{L}}^{\mathrm{AB}}$ are zero. Table 1 shows the same data for four completely apolar solids, also with γ_{L}^{+} and γ_{L}^{-} as well as $\gamma_{\mathrm{L}}^{\mathrm{AB}}$ equal to zero.

Table 1 also shows the surface tension properties of six polar liquid compounds. The first four of these are dipolar and these four dipolar liquids are the ones which are the most commonly used in contact angle determinations on account of their high surface tensions (γ). The requirement for

TABLE 1 Surface Tension Properties of Some Compounds at 20°C (in mJ/m^2)

mJ/m^2	γ	γ^{LW}	γ^{AB}	γ^{+}	γ^{-}
Apolar Liquids					
Hexane	18.4	18.4	0	0	0
Octane	21.6	21.6	0	0	0
Decane	23.8	23.8	0	0	0
Dodecane	25.35	25.35	0	0	0
Hexadecane	27.5	27.5	0	0	0
Apolar Solids					
Teflon FEP	18.1	18.1	0	0	0
Poly-(isobutylene)	25.0	25.0	0	0	0
Poly-(propylene)	25.7	25.7	0	0	0
Poly-(ethylene)	33.0	33.0	0	0	0
Polar Liquids					
Water	72.8	21.8	51.0	25.5	25.5
Glycerol	64.0	34.0	30.0	3.92	57.4
Formamide	58.0	39.0	19.0	2.28	39.6
Ethylene glycol	48.0	29.0	19.0	3.0	30.1
Diiodo-methane	50.8	50.8	0	≈0.01	0
Ethyl ether	17.0	17.0	0	0	9.0
Polar solutes in the dry state versus dissolved in water					
Glucose (dry state)	42.2	42.2	0	0	51.1
Glucose (in water)	150.9	42.2	108.7	34.5	85.6
Sucrose (dry state)	41.6	41.6	0	0	59.5
Sucrose (in water)	141.8	41.6	100.2	28.5	88.0
NaCl (dry state)	42.6	42.6	0	0	58.1
NaCl (in water)	196.7	42.6	154.1	53.3	111.4
Dextran (dry state)	42.0	42.0	0	0	55.0
Dextran (in water)	63.4	42.0	21.4	2.0	57.0
Surface tension properties of water and ice					
Ice (Hydrated) at 0°C	70.6	28.5	42.05	10.5	42.1
Water at 0°C	75.8	22.8	53.0	19	37
Water at 20°C	72.8	21.8	51.0	25.5	25.5
Water at 38°C	70.0	21.0	49.0	32.4	18.5

Data from refs. 6, 10, 17, 75, 76.

contact angle measuring liquids to have an elevated surface tension themselves is important because one can only obtain finite contact angles on various solid surfaces (S) when the surface tension of the measuring liquid (L) is greater than that of the solid surface, i.e., $\gamma_L > \gamma_S$. When $\gamma_L < \gamma_S$, the liquid just spreads over the solid surface, which results in a zero contact angle. The fifth liquid (diiodomethane, also called methylene iodide) is almost completely apolar and on account of its high γ value (for an apolar liquid), it is often used for measuring contact angles on polar as well as on apolar surfaces to determine their γ^{LW} value (22). The sixth and last polar liquid listed in Table 1 (ethyl ether) is a monopolar compound (21). Monopolar compounds are polar in that they usually have a rather large γ^- value but they have no γ^+. With monopolar compounds their γ value is therefore equal to their γ^{LW} value because, being monopolar, their γ^{AB} equals zero (equation [6]).

Table 1 also shows the surface tension properties of four molecules which are monopolar electron-donors in the dried state, but when dissolved in water they are dipolar, i.e., they display γ^+ as well as γ^- values, where in the case of the top three, low molecular weight compounds, their γ^+ values have become fairly sizeable. In the case of the fourth compound, dextran (a polymer of glucose), the value of its γ^+ (2.0 in the liquid state) is much more modest in size. With all four compounds however, their dipolarity in the liquid state is such that $\gamma^- > \gamma^+$ (23). From Table 1, it may also be observed that the sugars, sucrose and glucose, as well as NaCl have a much higher surface tension when dissolved in water than the glucose polymer, dextran.

This corresponds closely to the high γ^+ and γ^- values of the first three, low molecular compounds in the liquid state, which gives them a high free energy of cohesion caused by the strong polar interaction between their electron-acceptors and electron-donors when dissolved in water. It is obvious however that with the macromolecular, poly-glucose (dextran) molecules this increase in surface tension, when dissolved in water, is much smaller. This is because upon polymerization of glucose to become dextran, most of the latter's free electron-acceptor sites disappear through covalent bonding during the polymerization process. Dextran molecules, once dissolved in water, show only a slight degree of electron-accepticity, in comparison with its monomer, glucose. The electron-donating monopolarity of dried polar compounds appears to be a generally occurring phenomenon, among organic as well as inorganic materials (Table 1 and (10, 24)).

Table 1 (bottom part) shows the surface tension properties of water at 0°C, 20°C and 38°C, as well as those of hydrated ice at 0°C. In the case of liquid water it should be noted that when it is significantly colder than 20°C it is a much stronger electron-donor than electron-acceptor, whereas when it is 18°C warmer than 20°C, it has a much stronger electron-accepticity than electron-donicity, which is further discussed in the light of equation [9].

Four Indispensable Equations

The first of the four indispensable equations in connection with polar interactions in a strongly polar liquid such as water is the Young-Dupré equation pertaining to contact angle measurements in order to determine the surface tension properties $\gamma_{\mathrm{i}}^{\mathrm{LW}}$, γ_{i}^{+} and γ_{i}^{-} of condensed-phase solid polar material *i* with the help of contact angle liquid (L) (6, 10, 17):

$$(1+\cos\theta)\,\gamma_L = 2\left[\sqrt{\gamma_i^{LW}\,\gamma_L^{LW}} + \sqrt{\gamma_i^{+}\,\gamma_L^{-}} + \sqrt{\gamma_i^{-}\,\gamma_L^{+}}\right] \qquad [8]$$

where: γ_L, γ_L^{LW}, γ_L^{+} and γ_L^{-} are the known values for contact angle liquid, L. For such a polar material, *i*, one needs to measure contact angle with three different liquids, L_1, L_2 and L_3, where two of these liquids must be significantly polar, and using equation [8] three times (i.e., once with each liquid) to solve for the unknowns, γ_i^{LW}, γ_i^{+} and γ_i^{-}.

The γ-values shown in Table 1 have all been determined using equation [8] three times. For the methodologies of γ-value determination see Refs. (10), Chapter VII and (17) Chapter XII. These methodologies include direct contact angle measurements on flat surfaces, as well as thin layer wicking for the determination of contact angles on the surfaces of small solid particles.

The second and possibly the most important one of these four equations describes the interfacial (superscript IF) interaction energy, $\Delta G_{\mathrm{iwi}}^{\mathrm{IF}}$, between two *identical* polar molecules or particles, immersed in a polar liquid, which is here taken to be water:

$$\begin{aligned}\Delta G_{\mathrm{iwi}}^{\mathrm{IF}} = -2\gamma_{\mathrm{iw}} = {} & \underset{A}{-2\left(\sqrt{\gamma_i^{LW}} - \sqrt{\gamma_w^{LW}}\right)^2} - 4\Big[\underset{B}{\sqrt{(\gamma_i^{+}.\gamma_i^{-})}} + \underset{C}{\sqrt{(\gamma_w^{+}.\gamma_w^{-})}} \\ & - \underset{D}{\sqrt{(\gamma_i^{+}.\gamma_w^{-})}} - \underset{E}{\sqrt{(\gamma_i^{-}.\gamma_w^{+})}}\Big]\end{aligned} \qquad [9]$$

where γ_{iw} is the interfacial tension between i and w. Term A describes the (*attractive*) Lifshitz-van der Waals interaction energy between two molecules or particles, i, when immersed in water. Term B is the (*attractive*) polar free energy of cohesion of compound, i. Term C is the large (*attractive*) hydrogen-bonding-driven free energy of cohesion between the water molecules, which is permanently active in liquid water and which amounts to −102 mJ/m^2, at 20°C. When non-polar hydrophobic molecules or particles are immersed in liquid water, they are clumped together because of term C, which is always operative: it is the driving force of hydrophobic attraction, also known as the "hydrophobic effect". Hydrophilic molecules or particles also undergo the influence of the hydrophobic effect but they can overcome this by exerting an even stronger hydrophilic repulsion in the guise of the hydration repulsion generated via the free energy described by

TABLE 2 Summary of the Influence of Terms A-E of Equation 9 on Hydrophobic and Hydrophilic Compounds, Immersed in Water

	Terms of equation 9					
	A (negative)	B (negative)	C* (negative)	D (positive)	E** (positive)	ΔG_{iwi}^{IF} (***)
Hydrophobic molecules immersed in water	small	zero	-102 mJ / m^2	zero	zero	strongly negative, i.e., attractive due to hydrophobic effect (term **C**)
Hydrophilic molecules immersed in water	small	between zero and relatively small	-102 mJ / m^2	usually between zero and small	$>> +102$ mJ/ m^2	quite positive, i.e., repulsive due to hydration pressure (term E)

*) Cause of hydrophobic attraction.

**) Cause of hydrophilic repulsion.

***) $\Delta G_{iwi}{}^{IF} = \Delta G_{iwi}{}^{LW} + \Delta G_{iwi}{}^{AB}$, where ΔG^{IF} is the total interfacial (IF) free energy of interaction between two entities (i), when immersed in water (w).

term E. Terms D and E represent the two possible (*repulsive*) polar energies exerted by the hydration of molecules or particles, i.

However, term E is most frequently the dominant term of the two, where a preponderantly electron-donating molecule or particle interacts with the electron-accepting moiety of the molecules of liquid water. As has been discussed earlier, most polar compounds are mainly or solely electron-donors. Thus it is usually term E, which is the major (*repulsive*) term representing the free energy of polar repulsion in water, also described as hydration energy but also often alluded to as "hydration pressure" (6, p. 66, 10, p. 260 and 17 pp. 218–228; see also Table 2).

The third crucial equation describes the interaction energy, $\Delta G_{1w2}{}^{IF}$, between two *different* molecules and/or particles, 1 and 2, immersed in water, w (6, 10, 17):

$$\Delta G_{1w2}^{IF} = 2[\surd(\gamma_1{}^{LW}.\gamma_w{}^{LW}) + \surd(\gamma_2{}^{LW}.\gamma_w{}^{LW}) - \surd(\gamma_1{}^{LW}.\gamma_2{}^{LW}) - \gamma_w{}^{LW} + (\surd\gamma_w^+).(\surd\gamma_1^- + \surd\gamma_2^- - \surd\gamma_w^-) + (\surd\gamma_w^-).(\surd\gamma_1^+ + \surd\gamma_2^+ - \surd\gamma_w^+) - \surd(\gamma_1^+.\gamma_2^-) - \surd(\gamma_1^-.\gamma_2^+)] \qquad [10]$$

Finally, the fourth indispensable equation links the aqueous solubility, s, in mol fractions, with the interfacial tension between molecules, *i*, when immersed in water, w:

$$\Delta G_{iwi}^{IF} \,.\, S_c = kT \,.\, \ln s \qquad [11]$$

where $\Delta G_{iwi} = -2\ \gamma_{iw}$, both of which terms being expressed in mJ/m^2 units and where S_c is the contactable surface area between two molecules at their closest approach, usually most conveniently expressed in Å^2, or in 10^{-16} cm^2 and k is Boltzmann's constant = 1.38×10^{-16} ergs/degree, while T is the absolute temperature in Kelvin. The solubility, s, of *i* should be expressed in dimensionless mol fractions; see Table 3.

Comparative Impacts of Lifshitz-van der Waals (LW), Lewis Acid-Base (AB) and Electrostatic (EL) Energies When Acting in Water

The relative degrees of influence of LW and AB forces when acting in water are both to be found in equation 10, while those of EL forces are briefly treated separately at the end of this section.

ΔG_{iwi}^{LW}

As far as ΔG_{iwi}^{LW} is concerned, term A of equation 9 fully describes the import of this term which describes the LW interaction energy between two molecules or particles, *i*, when immersed in water, w, where γ_i^{LW} and γ_w^{LW} are proportional with the Hamaker constant, A_1 and A_w, respectively. From the form of term A of equation 9 it is clear that the interaction energy between two identical entities, *i*, when immersed in a liquid such as water, becomes seriously attenuated by that immersion. For instance, when drops of octane ($\gamma_{oct}^{LW} = 21.6$ mJ/m^2) are immersed in water ($\gamma_w^{LW} = 21.8$ mJ/m^2), term A of equation 9 amounts to $\Delta G_{oct.w.oct}^{LW} = -0.00092$ mJ/m^2 which, for all practical purposes, is equal to zero. For other materials or compounds, immersed in water, using, e.g., biological or other colloidal systems, the absolute value of ΔG_{iwi}^{LW} will rarely be greater than $|-7|$ to $|-10|$ mJ/m^2.

ΔG_{iwi}^{AB}

With ΔG_{iwi}^{AB} it is an entirely different matter: here all four subsequent terms of equation 10: B, C, D and E, can play a role. However, it is clear from Table 1 that these and other similar apparently monopolar compounds are only monopolar when dry, while in aqueous solution they are definitely

TABLE 3 The Two Categories of Aqueous Solubility, Pertaining to the Solubility Equation

Solubility			
Mol/L	Mol fraction	ΔG_{iwi} (kT)	Example
*	244.7	+5.5	Human serum albumin with 2 hydration layers
*	148.4	+5.0	
*	68.7	+4.23	DEX T150[a]
*	15.6	+2.75	PEO 6,000[b]
*	7.4	+2	
*	4	+1.4	
*	3	+1.1	
*	2	+0.7	
55.56	1	0	
27.78	0.5	−0.69	
5.56	0.1	−2.30	
			Glucose[c]; Sucrose[d]
5.56×10^{-1}	0.01	−4.61	
5.56×10^{-2}	0.001	−6.91	
5.56×10^{-3}	0.0001	−9.21	Octanol[e]
5.56×10^{-4}	0.00001	−11.51	
5.56×10^{-5}	0.000001	−13.02	Octane[f]
5.56×10^{-6}	0.0000001	−16.12	

[a] s = 68.7 mol fr,; $\Delta G_{iwi} = +4.23$ kT (6)
[b] s = 15.6 mol fr.; $\Delta G_{iwi} = +2.75$ kT (6)
[c] s = 0.048 mol fr.; $\Delta G_{iwi} = -3.04$ kT (23)
[d] s = 0.035 mol fr,; $\Delta G_{iwi} = -3.35$ kT (23)
[e] s = 0.000,081 mol fr.; $\Delta G_{iwi} = -9.24$ kT (49)
[f] s = 0.000,002,2 mol fr.; $\Delta G_{iwi} = -13.02$ kT (49)

*This column is not extended further upward; the next entry above this one would indicate a Mol/L concentration greater than 55.56 (pure water itself). There are very few other compounds of which one would be able to confine 55.56 Mol, at ambient pressure and temperature, in 1L. The theoretical aqueous solubilities given for hydrated human serum albumin and for dextran and poly(ethylene oxide) (PEO) simply indicate that these polymers are theoretically infinitely soluble in water, in practice the main limitation is the excessive viscosity at high concentrations (6).

Top half: $s \geq 1$ theoretical mol fraction (complete solubility); bottom half: $s \leq 1$ mol fraction (partial solubility).

dipolar with often quite elevated AB values and it should be stressed that *equation 9 is only valid for molecules in the liquid state*, either naturally, or when dissolved in a liquid (e.g., water). In the case of polar *polymers* however (see, e.g., dissolved dextran in Table 1), the value of AB is still not very high, which in most cases leaves term B of equation 10 moderately low. Term C of equation 9 on the other hand pertains exclusively to the polar properties of water, i.e., to its hydrogen-bonding driven free energy of cohesion, which is *always* present and active, with a value of −102 mJ/m^2 (at 20°C).

This large permanent attraction in liquid water causes the hydrophobic attraction, also known as the "hydrophobic effect" which acts in a maximally unhindered manner on all completely hydrophobic molecules or particles once they are immersed in water. This is so because with completely apolar molecules or particles, when immersed in water, terms B, D and E are zero. Even with partly polar/partly apolar molecules or particles it is difficult completely to overcome the hydrophobic attraction term C of equation 10, of -102 mJ/m^2.

Such a feat is only possible with very hydrophilic molecules or particles (when immersed in water), as they need to surmount not only the hydrophobic attraction (term C, of -102 mJ/m^2), but also their own polar attractive contribution (term B of equation 10). In other words, very hydrophilic macromolecules can only be soluble in water when they repel one another in water. Doing so, they can give rise to a very large positive value of term E of equation 9, i.e., their *i*-value is large so that they can combine with the $\gamma_w{}^+$ of water (which is equal to 25.5 mJ/m^2 at 20°C), to allow it to surpass the -102 mJ/m^2 value due to the hydrophobic effect which is always present in water. As mentioned before (see the preceding sub-Section), at temperatures higher than 20°C, the value of $\gamma_w{}^+$ increases, which then results in an increase in the positive value of term E of equation 9 and concomitantly increases the positive value of $\Delta G_{iwi}{}^{AB}$, thus making macromolecules, *i*, more water-soluble.

$\Delta G_{iwi}{}^{IF} = \Delta G_{iwi}{}^{LW} + \Delta G_{iwi}{}^{AB}$

In Table 2 a summary is given of the relative influence of the five terms of equation 9 on the total interfacial free energy ($\Delta G_{iwi}{}^{IF}$, where IF stands for "interfacial") of the interaction between two molecules, compounds or particles (*i*) when immersed in water (w). $\Delta G_{iwi}{}^{LW}$ and $\Delta G_{iwi}{}^{AB}$ are treated together here and in Table 2, because they are habitually measured together, via contact angle determinations, using equation 8. However, $\Delta G_{iwi}{}^{EL}$, where EL stands for "electrostatic" and whose value also must be determined in order to acquire the total value of the interaction energy ($\Delta G_{iwi}{}^{TOT} = \Delta G_{iwi}{}^{IF} + \Delta G_{iwi}{}^{EL}$) between two identical particles, compounds or molecules, immersed in water is on account of its very nature, always measured separately.

$\Delta G_{iwi}{}^{EL}$

In most colloidal and in all biological systems, which tend to have a fairly elevated ionic salt content (in mammalian systems this is of the order of 0.15 M NaCl), $\Delta G_{iwi}{}^{EL}$ represents only between about 7% and 9% of $\Delta G_{iwi}{}^{TOT}$. See for instance regarding the roles of LW, AB and EL energies in the stability of human blood cells in whole blood in van Oss (17, p. 317), in which system in addition to $\Delta G_{iwi}{}^{EL}$, $\Delta G_{iwi}{}^{LW}$ interaction energies represent only between

2.3% and 7.0% of the whole, the rest being provided by ΔG_{iwi}^{AB} energies. Detailed electrostatic (EL) interaction mechanisms and equations are given in van Oss (17, pp. 46–65; pp. 81, 82 and 128–153) and (10, pp. 51–69; p. 84 and 179–201).

Decay with Distance of LW, AB and EL Energies – The Extended DLVO Theory

The Extended DLVO approach treats the decay of ΔG^{LW}, ΔG^{AB} and ΔG^{EL} interaction energies as a function of distance (d) according to different rules for each of the different types of forces. This means that for each of the three interaction types the ΔG values must first be determined at closest approach, at $d = d_o$ (i.e., at the minimum equilibrium distance, $d_o = 0.157$ nm) before one proceeds to determine each of the ΔG values separately at their decayed values at various distances, d, before adding them all together again at any given distance, d. However, especially for interactions occurring in water and as a consequence of the quantitatively predominant influence of the usually quite elevated values of ΔG^{AB} (regardless of whether ΔG^{AB} happens to be positive or negative), it is always preferable to carry out Extended DLVO analyses.

When this need does occur, methodologies and some equations can be found in van Oss (6, 10, 17). The original references regarding the need to use the Extended DLVO theory (comprising LW, EL as well as AB energies) and not the classical old DLVO approach (using only LW and EL energies) for use in aqueous systems are detailed in another work (25), whereas further confirmation of the necessity to include ΔG^{AB}, in addition to ΔG^{LW} and ΔG^{EL} for use in water, were proposed (26, 27).

PART II. FORCES ORIGINATING IN WATER AND THEIR SIGNIFICANCE IN SEPARATION METHODS

Precipitation is the first important separation method. It is essential to know by which mechanism e.g., an amphoteric macromolecule that was originally dissolved in water, becomes insoluble by, for instance, hydrophobic precipitation. This can be done:

A. by salting-out or increasing the surface tension of water by the admixture of, inter alia, neutral salts [which have a higher surface tension than pure water (23, 28, p. F-28), or:
B. by changing the pH of the aqueous solvent to a value equal to the isoelectric point of the amphoteric macromolecule, which causes that macromolecule to become hydrophobic, thus also leading to its

immobilization, but in this case because of a change in the surface properties of the macromolecules themselves (29).

These two different mechanisms of insolubilizing macromolecules by hydrophobic interactions are therefore discussed separately.

Increasing and Decreasing the Hydrophobic Attraction in Liquid Water

The hydrophobic attraction (also known as the "hydrophobic effect") is solely due to the hydrogen-bonding-driven free energy of cohesion between the water molecules in liquid water and is described by term C of equation 9, where it is expressed as:

$$\Delta G_{iwi}^{\text{Hydrophobic}} = -4\sqrt{(\gamma_w^+ . \gamma_w^-)} \qquad [12]$$

$\Delta G_{iwi}^{\text{Hydrophobic}}$ is clearly only function of the polar parameters of the surface tension of water (γ_w^+ and γ_w^-). $\Delta G_{iwi}^{\text{Hydrophobic}}$, at 20°C, amounts to -102 mJ/m^2, which is always present and active in liquid water. It is an exclusively polar (i.e., Lewis acid-base) driven free energy of attraction acting most strongly on completely non-polar molecules and particles when these are suspended or dissolved in liquid water.

There is one other major negative term (term A: the LW term) which also is always attractive. However, as a consequence of its mathematical form, representing the difference between the square roots of two LW items which often have values that are close to being of the same order of magnitude, the total value of term A of equation 9 is usually rather small (Table 2) and varies from case to case. Thus, although term A should not be neglected, it is not one of the more prominent water-driven forces such as hydrophobic attraction (term C) or hydrophilic repulsion (term E).

Finally, increasing or decreasing the temperature of liquid water has only a very small influence on increasing as well as on decreasing hydrophobic interactions in water (6, pp. 64, 65).

Increasing the hydrophobic attraction in liquid water

Increasing the hydrophobic attraction in water is most effectively done by the admixture of low molecular weight, polar water-soluble solutes such as neutral salts or simple sugars, at high concentrations. These polar solutes have a higher surface tension than liquid water so that when added to water, they raise its surface tension and with it, its "hydrophobizing" capacity (Table 1). It is also clear from Table 1 that when dissolved in water, NaCl has a significantly higher γ^{AB} than glucose or sucrose, so that the admixture of (e.g., one or two molar NaCl) to water or aqueous solutions is more effective than a comparable amount of sugar. The addition of pluri-molar amounts of salts in order to desolubilize, e.g., various proteins, has long been known as "salting-out", where the use of $(NH_4)_2SO_4$ has become one of the most popular choices.

DECREASING THE HYDROPHOBIC ATTRACTION IN LIQUID WATER

To decrease the hydrophobizing capacity of water one should add low surface tension polar solutes (usually in the liquid form) to the water, such as acetonitrile ($\gamma_L = 29.9$ mJ/m^2) or ethylene glycol ($\gamma_L = 48.4$ mJ/m^2) (30) for the surface tensions of these and other liquids. Both surface tensions of these two potential additives, which are well below the 72.8 mJ/m^2 of water at 20°C, can serve for the "resolubilization" of previously hydrophobically precipitated proteins, as well as for the elution of proteins or other biopolymers that were previously bound to the stationary phase of reversed-phase liquid chromatography (RPLC) columns (10, pp. 344–346; 17, pp. 357–358; 31).

In the case of some exceedingly hydrophilic proteins such as human immunoglobulin-A (IgA), this biopolymer is so hydrophilic that it will not bind to the octadecyl (C18) substituted beads of the stationary phase (normally used in RPLC). One then typically changes the RPLC purification approach by including a first attachment step in the guise of a salting-out step by adding about 1 M. $(NH_4)_2SO_4$, which causes the binding of hyper-hydrophilic IgA molecules, which can subsequently be eluted from the stationary phase hydrophobic C18 beads by gradually diminishing the ionic strength of the aqueous mobile phase, using a method called salt-mediated hydrophobic chromatography (10, p. 346; 17, p. 358).

Increasing and Decreasing the Hydrophobicity of Molecules and Particles, Immersed in Water

INCREASING THE HYDROPHOBICITY OF MOLECULES AND PARTICLES

Apart from salting-out proteins, which is solely due to a change in a property of the aqueous solvent, there are no other salient approaches involving only properties of water, without also causing changes in the surface properties of molecules or particles. In this sub-section on the other hand, the following approaches serve to raise the surface hydrophobicities of molecules and particles themselves, while immersed or dissolved in water.

A. *Hydrophobizing* amphiphilic macromolecules (i.e., mainly proteins) is possible by changing the pH of their aqueous medium to that of the iso-electric pH of the protein, at which pH the protein reaches its maximum surface hydrophobicity (32, 33).

B. *Dehydration* of a protein's surface, while still in aqueous solution, increases its surface hydrophobicity, e.g. by the admixture of ethanol (34). This method has classically been used for the fractionation of human blood serum proteins, in conjunction with the previously mentioned method of maximally increasing a protein's surface hydrophobicity by operating at its isoelectric pH, at low temperatures (35–37). Working at low temperatures does little to enhance the hydrophobizing procedure, but it helps in combating denaturation and in maintaining sterility.

C. *Adding cationic surfactants* to negatively charged particles render their surfaces hydrophobic. One of the simplest indications of hydrophobicity can be furnished by the particle γ^- value when it is (at low γ^+ values) approximately 29 mJ/m^2 or below (17, Table XV-1, p. 209). This is an exceedingly handy indicator when attempting to monitor the extent of particle hydrophobization by the admixture of various additives (Table 1). In Table 1, top part, all hydrophobic compounds have zero γ^- values, while the polar liquids diiodo-methane and ethyl ether are hydrophobic with very low γ^- values.

For treatment with cationic surfactants, initially negatively charged *hydrophilic* smectite clay particles from Southern Wyoming, entitled SWy-1, were used, with a γ^- value of about 30.5 mJ/m^2 and a low γ^+ value of about 1.1 mJ/m^2 (38). The cationic surfactants that were found most effective in hydrophobizing the surfaces of SWy-1 clay particles were primary alkyl ammonium bases (PAB) as well as quaternary ammonium bases (QAB). Using PAB's with alkyl groups of from 6 to 14 carbons chain length and with γ^- values obtained with these PAB's between 5.3 and 9.8 mJ/m^2, indicating significant hydrophobicity.

However, upon treatment with a PAB of 15 carbons chain length we reduced the γ^- value of SWy-1 to 0.1 mJ/m^2, thus achieving virtually total hydrophobicity. This indicated that, on average, the longer the carbon chain length of a PAB, the more effective such a cationic surfactant proved to be in hydrophobizing initially hydrophilic SWy-1 smectite clay particles. With various different QAB cationic surfactants, lowering of the γ^- values of SWy-1 occurred between 19.5 and 8.7 mJ/m^2 (24, p. 243). It is also interesting to compare the γ^- values of a few metal oxides. For TiO_2: $\gamma^- = 46.3$; SiO_2: $\gamma^- = 41.4$; ZrO_2: $\gamma^- = 3.6$; SnO_2: $\gamma^- = 8.5$, (all in mJ/m^2) (24, p. 245).

D. *Adding salts with plurivalent counterions* to oppositely charged particles makes them more hydrophobic. A century-old method for increasing the hydrophobicity of electrically charged particles, suspended in water, is the Schulze-Hardy approach, using the addition of plurivalent counterions to aqueous suspensions of charged particles (39–41). These Authors and some of their successors postulated that the flocculation of electrically charged particles, suspended in water, was due to the decrease in ζ-potential caused by the addition of salts with plurivalent

> counterions with the additional property that the higher the valency of the counterions of the added salts, the less one needed of these salts to achieve destabilization. See, e.g., Overbeek (42), who proposed that this phenomenon followed the laws outlined in the classical DLVO theory to the effect that the counterions of these salts caused a decrease in the particles' ζ-potential (which was of the opposite sign of the counterions), thus decreasing their electrostatic repulsion which then allowed the van der Waals attraction to prevail by causing the particles to attract one another and hence to flocculate.

However, due to the development of the thin layer wicking method, permitting the determination of surface tension components and parameters of powders by Costanzo et al. (43), Wu et al. (44) were able to measure the surface tension properties of small particles such as glass, calcite and SWy-1 clay particles. With these three different negatively charged particles the admixture of modest amounts of two different salts with, respectively, plurivalent cations Ca^{2+} or La^{3+} gave rise to a mild decrease in the particles' ζ-potential, as well as to a complete change from surface hydrophilicity to surface hydrophobicity. These results indicated that the destabilization of initially stable negatively charged particle suspensions in water by the admixture of salts with plurivalent cations was really solely due to the latter's hydrophobizing effect and not to a decrease in their (negative) ζ-potential, as was believed by Overbeek (42). It should however not be forgotten that Overbeek, in 1952, could not have foreseen the discovery of the crucial role played by polar (Lewis acid-base) interaction energies in water (9) as applied, e.g., to a Schulze-Hardy type of interaction (44).

Decreasing the Hydrophobicity of Molecules and Particles

By far the most effective way of decreasing the hydrophobicity of molecules or particles is to attach strongly hydrophilic molecules to their surfaces. This can be done with non-ionic surfactants having a hydrophobic chain which attaches automatically to the molecules' or particles' hydrophobic moieties, plus a very hydrophilic chain that orients itself toward and into the water phase. The most effective and most frequently used of these is polyethylene glycol (PEG), also known as poly-(ethylene oxide) (PEO), where the term PEO seems most popular among physical scientists and engineers while PEG appears to be more favored by biological scientists and physicians.

Single PEO or PEG molecules are non-ionic surfactants that are separately and individually insoluble in water. Once immersed in water however, PEO molecules immediately occur as very soluble small hydrophilic micelles which are very soluble in water, in the guise of PEO trimers or tetramers (van Oss and Giese, in preparation). Coating proteins such as enzymes or antibodies with PEO/PEG is often alluded to as "pegylation," which makes them not only more water-soluble but also prevents such "pegylated" biopolymers from being phagocytized or otherwise inactivated after *in vivo* injection in patients, due to their enhanced repulsive power *vis-à-vis* circulating macrophages and granulocytes.

Solubility and Other Interactions in Aqueous Media

THE SOLUBILITY EQUATION

The solubility equation 11 for water as solvent may be expressed as:

$$2\gamma_{\text{iw}} \times S_c = -kT \ln s \qquad [11A]$$

where γ_{iw} stands for the interracial tension between molecule, *i,* and water, w and where S_c stands for the contactable surface area between two identical molecules, *i*, when dissolved in liquid water, w. Equation 11 describes the free energy, ΔG_{iwi}, i.e., the interaction energy among two identical molecules, *i*, when immersed in water, w, while equation 11A describes the interfacial tension between two molecules, *i* and water, w, where both free energy and interfacial tension are directly proportional to the natural logarithm of the aqueous solubility of *i*, when the solubility s is expressed in mol fractions. It should be noted here that $\Delta G_{\text{iwi}} = -2\ \gamma_{\text{iw}}$ and it should also be stressed that:

$$\Delta G_{\text{iwi}}\,(\text{in mJ/m}^2)\,.\,S_c/kT = \Delta G_{\text{iwi}}{}^*(\text{in units kT}) \qquad [13]$$

where S_c, k and T have been defined with equation 11. The solubility equation can thus be simplified to:

$$\Delta G_{\text{iwi}}^* = \ln s \qquad [14]$$

so that the free energy of interaction between two identical molecules (*i*), dissolved in water, when expressed in kT units, is equal to the natural logarithm of the aqueous solubility (s) of molecules (*i*) where s is expressed in mol fractions.

Recently the aqueous solubility approach (equations 11, 11A, 12, 13 and 14) has gained more importance as one of the few remaining correct experimental pathways to the measurement of ΔG_{iwi} (and of γ_{iw}).

MEASUREMENT METHODS FOR γ_{IW} AND ΔG_{IWI}

After realizing that the drop-shape method for measuring γ_{iw} and ΔG_{iwi} values, using drops of liquids immersed in water (45, 46), yields incorrect results when the compound of which the immersed drop consists is polar, we proposed that the aqueous solubility approach could usefully replace the hanging drop shape and similar methodologies for the measurement (47). As a first approximation it is of course true that water-immiscible liquids, polar or otherwise, are supposed to be insoluble in water, but total aqueous insolubility of apolar as well as polar liquids is never completely zero, e.g., octane, which still has a small solubility at room temperature of 2.21×10^{-6} mol fractions (48), which suffices to use it in the solubility equation 11 and obtain accurate results (49).

The problem with methods like drop shape analysis is that a liquid drop of a polar compound which is immersed in water will, within a picosecond of its immersion, orient its polar moieties toward its interface with the surrounding water, which will influence the resulting drop shape to appear as one that reflects a much greater hydrophilicity than the *average* composition of the drop's liquid would warrant. In other words this orientation of many (if not most) of its hydrophilic constituents toward the liquid-water interface gives the false impression of much too great a hydrophilicity of the immersed drop.

In contrast, the solubility approach does not suffer from such erroneous results obtained as a consequence of the anisotropic drop-shape approach. That is because in a state of maximum aqueous dissolution, all the separate molecules of a polar solute that water can carry are, by definition, freely dispersed in actual solution, meaning that all of the dissolved polar molecules have each one of their apolar, as well as of their polar, moieties completely surrounded by water molecules, so that the entire polar as well as the apolar surface of each separate solute molecule fraction fully interfaces with water.

CONDITIONS FOR THE VALIDITY OF THE SOLUBILITY EQUATION

'Upon further exploration of the possibilities opened by the solubility equation (equations 11 and 11A) it became clear that for solutes such as glucose, sucrose and NaCl, the values for the surface tension components and parameters of ΔG_{iwi} and γ_{iw}, as employed when using the solubility equation, need to be those occurring in the liquid state, i.e., in the cases here under study, when dissolved in water. This is because once such solutes have been solidified (e.g., crystallized by air-drying), their surface tension components and parameters as measured on various sides of their solid surfaces, turned out to be significantly different from one another and also from the corresponding surface tension properties when measured in the liquid state (47).

It followed that for the aqueous solubility equation (equations 11 and 11A) to be applicable, the natural logarithm of the solubility, s, expressed in mol fractions, only correspond to ΔG_{iwi} or to γ_{iw} (both expressed in kT units) when these surface tension components and parameters had been determined, e.g., for various sugars and NaCl when measured when these were in the liquid state (i.e., when dissolved in water).

The γ_i^- second equation one may use is (23, 47):

$$\gamma_i^- \text{ (dried)} = \gamma_i^- \text{ (dissolved)} - \gamma_i^+ \text{ (dissolved)} \qquad [15]$$

In equations 8, 9 and 12, $\gamma_i^+{}_{\text{(dissolved)}}$ and $\gamma_i^-{}_{\text{(dissolved)}}$ are the only two unknowns, which can therefore be ascertained from the combined use of equations 9 and 12. Here $\gamma_{iw}^-{}_{\text{(dried)}}$ is easily determined from a flat exterior surface of an air-dried specimen of one of the solutes studied and γ_i^{LW} is as a first approximation held to be unchanged between the dissolved and the dried state.

On the other hand, in all cases where polar solutes, *i*, are normally present in the liquid state at room temperature [e.g., alkyl alcohols, phenol, even benzene (49)], their ΔG_{iwi} or γ_{iw} values are automatically valid as such when inserted in the solubility equation 11 or 11A.

TWO CATEGORIES OF AQUEOUS SOLUBILITY

To begin with, ΔG_{iwi}, as defined in equation 9, determines the hydrophilicity vs. hydrophobicity of a solute, *i*. When $\Delta G_{iwi} > 0$, *i* is hydrophilic and when $\Delta G_{iwi} < 0$, *i* is hydrophobic and the degree of hydrophilicity of *i* is expressed by the positive value of ΔG_{iwi}, whereas the degree of hydrophobicity of *i* is expressed by the negative value of ΔG_{iwi} (10, pp. 62–63). Table 3 shows the two categories of ΔG_{iwi} in terms of hydrophilicity (top part, $\Delta G_{iwi} > 0$) and hydrophobicity (lower part, $\Delta G_{iwi} < 0$). Thus hydrophilicity occurs when the aqueous solubility of *i* equals 1 mol fraction and higher and hydrophobicity occurs when the aqueous solubility of *i* equals less than 1 mol fraction.

A. *Solutes with infinite solubility* belong in the top part of Table 3, where $s > 1$ mol fraction and $\Delta G_{iwi} > 0$, making all solutes, *i*, in this state for all practical purposes infinitely soluble in water. This will become obvious when one considers that once a ΔG_{iwi} value of, e.g., + 0.7 kT is reached for a given solute, the aqueous solubility of that solute would amount to ≈ 2 mol fractions (see Table 3), corresponding to the at first sight unlikely aqueous theoretical solubility of more than 100 mol/L, but which in practice just signifies virtually infinite solubility; see the footnote (*) to Table 3.

Thus, in the solute category shown in the upper part of Table 3, where $\Delta G_{iwi} > 0$, one finds practically exclusively hydrophilic macromolecules with a large contactable surface area (S_c) which enhances the effect of mutual repulsion between their hydrophilic surfaces, thus favoring total aqueous solubility. It remains however possible to achieve insolubilization of various initially totally soluble biopolymers (such as certain proteins and polysaccharides) from their aqueous solutions by methodologies such as those already mentioned.

B. *Solutes with finite solubilities*, with, e.g., aqueous solubilities, $s < 0.1$ mol fraction (lower columns of Table 3), comprise all the organic and inorganic compounds whose solubilities one will be able to find quoted by Stephen and Stephen (48). All the many solutes listed therein would belong in the lower part of Table 3 because they all have finite solubilities that are significantly smaller than 0.1 mol fraction, so that they all have negative (i.e., attractive) ΔG_{iwi} values. Solutes with finite aqueous solubilities are therefore also technically speaking from very mildly to more severely hydrophobic.

All solute molecules with aqueous solubilities, s, smaller than 0.1 mol fraction attract each other with an energy, ΔG_{iwi}, more negative than -2.3 kT. These are listed in the lower part of Table 3, down to a value as low as –16.12 kT, which would often be considered to be insoluble in water. For instance whereas octane has an aqueous solubility, s, of 0.002 (vol. %), according to Stephen & Stephen (48), in the Merck Index octane is listed as "insoluble in water". However the lower part of Table 3 also depicts the minuscule but finite aqueous solubilities of a couple of solutes, in each case corresponding to equally finite (negative) ΔG_{iwi} values, expressed in units kT, i.e., indicating the values of the attractive energies between similar molecules, when immersed in water (49).

C. *The mechanism of partial aqueous solubility* of the solutes listed by Stephen & Stephen (48) is due to the fact that at least some of these molecules of each species listed, attract one another (while remaining dissolved) when all are immersed in water. This means that for each of these solutes their ΔG_{iwi} value (solubility equations 11 and 11A) has a negative value, given in the lower part of Table 3 where it can be seen that for all items with $\Delta G_{iwi} < 0$, the more negative ΔG_{iwi} is, the lower their aqueous solubility. The attraction between solute molecules of this class is clearly the mechanism by which such solute molecules bind to each other when immersed in water and the stronger their attraction the lower their solubility.

However, the crucial role played in that attraction by the physicochemical properties of the polar solvent, i.e., the water itself, is rarely enough emphasized. Nonetheless, referring to equation 9, which defines the various separate as well as the combined roles the solute, *i* and water, w, play in arriving at the total value of ΔG_{iwi} (equation 9), the role of liquid water in the dissolution process should not be overlooked. Actually, the role of liquid water in the dissolution process becomes preponderant when considering the influence of temperature changes in all cases of aqueous solubility.

EFFECT OF TEMPERATURE ON PARTIAL AQUEOUS SOLUBILITY

Much has been written about the rules for the aqueous solubility of various solutes (51, 52). However the use of the combined surface-thermodynamic properties of a polar solvent such as water and the polar as well as non-polar properties of the solutes to be dissolved in it were unknown in 1978 and not yet fully developed in 1992 and the precise non-polar plus polar solubility equation 11, above, was only published in one of its definitive forms (featuring γ_{iw}) by van Oss & Good in 1996 (49). Using equation 11, the value of the aqueous solubility (s, in mole fractions) of polar solute (*i*) can be derived from the free energy of interaction (G_{iwi}) between two molecules, *i*, when immersed in water (equation 9), where all γ-terms can be easily determined by means of contact angle measurements, using equation 8.

One notable property of the aqueous solubilities of many polar solutes is that these in many cases increase with an increase in temperature, see, e.g., many of the solubilities of polar organic solutes as a function of T, listed by Stephen and Stephen (48). Now, many such increases in solubility as a function of increases in T are preponderantly imputable to changes in the surface-thermodynamic properties as a function of increases in T of water itself. In contrast with most of the solutes that are soluble in water, liquid water embodies two significant but usually unnoticed temperature-dependent properties.

The first one of these is the formation of polymeric clusters of water molecules which are biggest at the lower temperatures of liquid water and which revert to the monomeric status when T is approaching the boiling point of water at 100°C and at normal atmospheric pressure. At room temperature (20°C) the cluster size of water has been determined to be approximately 4.5 water molecules per cluster, corresponding to an average molecular weight of 81. This was measured by means of a determinations of the contactable surface area (S_c) of water clusters at 20°C, via the solubilities found for water in a number of organic liquids (chloroform, benzene, toluene, xylene and cyclohexane), using the solubility equation 11 and yielding an S_c-value of 0.358 nm^2 $+/-$ 0.014 nm^2 (SD), corresponding to a cluster value of $\approx$ 4.5 molecules H_2O per cluster (47).

The second of the two temperature-dependent properties of liquid water is the increase in the ratio of the electron-accepticity ($\gamma_w{}^+$) to the electron-donicity ($\gamma_w{}^-$) of the water molecules in liquid water, with an increase in T (Table 4). Table 4 shows the changes in the surface tension components and parameters for water at 20°C and 95°C and similar data for glucose at 20°C are also shown. From these data, using equation 9, the ΔG_{iwi} values for glucose, dissolved in water, are calculated for 20°C and 95°C, yielding $\Delta G_{iwi} = -20.54$ mJ/m^2 (at 20°C) and –17.54 mJ/m^2 (at 95°C). Using the solubility equation 11 and S_c values for glucose, at 20°C: $S_{c\ (glucose)} = 0.58$ nm^2 and at 95°C: $S_{c\ (glucose)} = 0.68$ nm^2. The latter is slightly increased due to swelling in close-to-boiling water.

TABLE 4 Surface Tension Components and Parameters for Glucose (at 20°C) and for Water at 20° and 95°C (in mJ/m^2)

Temperature (°C)	$\gamma_{\mathrm{w}}^{+}/\gamma_{\mathrm{w}}^{-}$	$\gamma_{\mathrm{w}}^{\mathrm{LW}}$	$\gamma_{\mathrm{w}}^{\mathrm{AB}}$	γ_{w}^{+}	γ_{w}^{-}	$\gamma_{\mathrm{w}}^{\mathrm{TOT}}$
Glucose[a]	0.4	42.2	108.7	34.5	85.6	150.9
Water at 20°[b]	1.0	21.8	51.0	25.5	25.5	72.8
Water at 95°	2.0	20.0	41.8	28.6	14.8	61.8[c]

[a] Data from ref. 23.

[b] Data from ref. 6.

[c] Interpolated from data from ref. 30.

From these data and the S_c values, and using the solubility equation 11, the calculated solubility values for glucose were 0.05 mol fraction from $\Delta G_{\mathrm{iwi}} = -3.0$ kT at 20°C and 0.10 mol fraction from $\Delta G_{\mathrm{iwi}} = -2.3$ kT at 95°C. From the experimentally obtained aqueous solubilities (48), at 20°C, $s_{(\mathrm{glucose})} = 2.65$ mol/L (corresponding to 0.052 mol fraction), whereas at 95°C, $s_{(\mathrm{glucose})} = 4.81$ mol/L (corresponding to 0.087 mol fraction) equation 13. A good correspondence between calculated and experimental data was obtained for the aqueous solubility of glucose at 20°C, while a reasonable similarity between the calculated and experimentally obtained solubility was reached for glucose in almost boiling water (at 95°C).

Having thus shown a fair correlation between calculated and experimentally obtained aqueous solubilities for glucose at two different temperatures, it is relevant to draw attention to Table 4, depicting a large difference between the $\gamma^{+}{}_{\mathrm{w}}/\gamma_{\mathrm{w}}$ ratios, at 20° and 95°C. The doubling of this ratio in liquid water when heating it from room temperature to almost the boiling point plays an important role in bringing about a marked increase in the aqueous solubility of glucose and many other polar solutes. For the explanation of this solubility effect it is useful to return to equation 9, depicting the composition of ΔG_{iwi} at 20°C and how it becomes more positive upon heating. Upon further heating, ΔG_{iwi} may become so positive (i.e., repulsive) that it leads to total (virtually infinitely) aqueous solubility of solute, *i*, see Table 3. It is Term E of equation 9:

$$\text{Term E} = +\sqrt{(\gamma_i^{-}\,\gamma_w^{+})} \qquad [16]$$

which is the principal term that becomes more strongly positive with an increase in T, which in turn makes ΔG_{iwi} less negative, or with further increases in T, helps turn ΔG_{iwi} positive. Term E not only becomes more positive thanks to the increase in γ_{w}^{+} with a higher T value, but it should be stressed that polar, hydrophilic solutes (i) tend to be monopolar electron-donors, with a high value for γ_{i}^{-} of at least 28.3 mJ/m^2 which further contributes to a high positive value for Term E and thus also for ΔG_{iwi} (21).

The increase in the $\gamma_w{}^+/\gamma_w{}^-$ ratio when heating liquid water is closely linked to the decrease in the cluster size of water from a cluster molecular weight of 81 at 20°C, to the monomeric molecular weight of 18 when approaching its boiling point, at 95°C. This is a consequence of the fact that upon de-polymerization of the water clusters from 4.5 bound water molecules per cluster at 20°C, at 95°C practically all water molecules have become monomeric, which allows all of the earlier hydrogen-bound H atoms of the clusters to become freed from their bonds and thus to act as single electron-acceptors.

This results in the active presence of two free electron-acceptors per electron-donor (oxygen), i.e., in an acceptor-donor ratio, $\gamma_w{}^+/\gamma_w{}^- = 2$ near the boiling temperature, instead of the initial $\gamma_w{}^+/\gamma_w{}^-$ value equal to unity, at 20°C. This excess of $\gamma_w{}^+$ sites over $\gamma_w{}^-$ sites at 95°C helps in allowing more of the water molecules no longer bound in clusters to interact with polar solute molecules of which most tend to be monopolar electron-donors, so that they become more hydrated by the now much more strongly electron-accepting free water molecules. This is what enhances the aqueous solubility of these polar solutes at higher T.

Thus the rather commonly occurring increase in the aqueous solubility of polar solutes with an increase in T is due to a concomitant change in a physicochemical property of liquid water as a solvent and is not, or very little, due to a change in polar solutes such as glucose. The influence of T on the structure of liquid water is therefore always useful to take into account when the need arises to separate solutes as a function of their different aqueous solubilities at different temperatures.

Suspension Stability Versus Flocculation in Aqueous Media

NECESSITY TO USE THE EXTENDED DLVO APPROACH IN AQUEOUS MEDIA

In aqueous media, with immersed particles of similar size and density, but with different surface properties, the Extended DLVO approach can allow for a stable suspension of particles of one given set of surface properties to be separated from other particles that lack these surface properties. In all cases employing aqueous media the Extended DLVO approach [incorporating Lewis acid-base (AB) properties *in addition* to the classical Lifshitz-van der Waals (LW) attraction and the Electrostatic (EL) repulsion of the more simple and incomplete classical DLVO method] will allow one to discriminate much more accurately between two types of particles (of which at least one type must be polar), by selecting the conditions under which one type of particle will remain in stable suspension, while the other type of particle will flocculate.

An example has been published of the use of the simple, LW attraction plus EL repulsions only, as well as the Extended DLVO approach, including all three LW, EL and AB interactions, in the case of the Hectorite clay particles suspended in water (25). When only LW attractions and EL repulsions were considered, in the simple DLVO approach, complete particle stability was predicted to occur for all NaCl concentrations of the aqueous medium.

However, when in addition to just the LW attractions and EL repulsions the simple but incomplete DLVO approach was extended by including the AB attractions which surpassed the LW attraction by a further 66%, an outcome of flocculation was predicted for the hectorite particles suspended in an aqueous medium containing 0.1 molar NaCl or more. This prediction was confirmed by the experimental results. In the presence of 0.1 molar NaCl the positive (repulsive) value of ΔG^{EL} decreased to the point where the ΔG^{EL} repulsion energy became smaller than the combined $\Delta G^{LW} + \Delta G^{AB}$ attraction energies of the Hectorite particles that were immersed in the aqueous salt solutions, thus causing their flocculation. In other words, for the quantitative description of the stability or instability of suspensions of polar particles immersed in an aqueous medium, the simple, classical DLVO approach should never be used: for use in aqueous media the extension of the simple DLVO approach with the Lewis acid-base (AB) approach is an absolute requisite (25).

AQUEOUS SUSPENSIONS OF MICELLES

To avoid confusion with regular insolubilized solutes it should be noted that there is one class of solutes, namely the surfactants, that usually have a low aqueous solubility but that do not insolubilize by precipitating. Instead, they bind to one another when their solubility limit is reached forming hydrophilic particle-like complexes with each other. These associations are called micelles. In such cases the critical micelle concentration (CMC) is the concentration at which an aqueous surfactant solution reaches a maximum of *dissolved* surfactant concentration, a minimum surface tension and a minimum osmotic pressure (46).

In their *dissolved* form, these solutes act as surfactants, consisting of a relatively long hydrophobic chain plus a (usually) shorter hydrophilic moiety. The point where their equivalent of aqueous solubility is reached is called their critical micelle concentration which is the equivalent of the solubility of more conventional solutes. For use in the solubility equation (equations 11 and 11A) the CMC takes the place of the solubility (s), and is expressed in units of mol fractions (53, 54).

For rules of micellar stability see Part I of this paper under “Decay with Distance of LW, AB and EL Energies – The Extended DLVO Theory”. It should be noted that the outer structures of individual micelles are exceedingly hydrophilic as they each consist of multiple hydrophilic chains of many of the original surfactant molecules combined in the micelles’ outer spherical layer adjacent to the bulk aqueous medium.

Interactions with the Water-Air Interface by Molecules and Particles When Immersed in Water

It still is not universally realized that the water-air interface (situated at the upper surface of the water held in, e.g., a glass container partly filled with that liquid) is the most hydrophobic surface known to Man. Unbeknownst to most scientists working with aqueous solutions of polar solutes such as ordinary sugars or salts on the one hand, or with aqueous solutions of proteins or surfactants on the other hand, the measured surface tensions are much *less increased* than they should have been in the first case, and very much *more decreased* than would have been predicted in the second case. The cause of all this is the peculiar behavior of the water-air interface. Briefly: the water-air interface repels polar solute molecules that have a higher surface tension than water and it attracts polar molecules (and especially surface-active polar molecules) that have a lower surface tension than water.

One finds for example that with aqueous sucrose solutions (in the liquid state sucrose has almost twice the surface tension of water), with sucrose solutions of 10, 20, 30 and 40% in each case the surface tensions of the sucrose solutions thus obtained are about 14 times lower than the theoretical (calculated) surface tension (28); see also Table 5.

In contrast, with even tiny amounts of proteins or surfactants dissolved in water, the measured surface tensions of their aqueous solutions show values that are up to a hundred-fold and in some cases more than a thousand-fold lower than could have been expected from the amounts that had been dissolved (23); see also Table 6. It should be noted that almost a century ago, this phenomenon was identified and studied by Irving Langmuir (55).

TABLE 5 Comparison Between "Calculated" Increases in the Surface Tension of Water Due to the Admixture of Various Amounts of Sucrose, and "Measured" Surface Tension Increases

	Calculated		Measured		
Sucrose concentration (% w/w)	Bulk γ-sucrose solution (mJ/m^2)	Bulk (A) $\Delta\gamma$-sucrose solution (mJ/m^2)	γ-sucrose solution[a] (mJ/m^2)	(B) $\Delta\gamma$-sucrose solution (mJ/m^2)	(A)/(B)
0	72.0	0.0	72.0	0.0	–
10	79.0	7.0	72.5	0.5	14.0
20	86.0	14.0	73.0	1.0	14.0
30	92.9	20.9	73.4	1.4	14.9
40	99.9	27.9	74.1	2.1	13.3
55	110.4	38.4	75.7	3.7	10.4

[a] Data from ref. 28.

When dissolved in water, sucrose is repelled by the water-air interface to such a degree that less than 10% of it is measurable (at the water-air interface).

TABLE 6 Influence of Low Concentrations of Some Amphiphilic Compounds on the Measured Surface Tensions of their Aqueous Solutions

Amphiphilic compound	Solute concentration	"Measured" surface tension	Ref.
Dodecanol-PEO (C12E7; 7EO units)	10^{-5}M (≈ 0.0005% w/w)	From 72.8 mJ/m^2 down to 27 mJ/m^2 (= a 63% decrease)	22
n-propanol	0.1%	From 72.1 mJ/m^2 down to 67.1 mJ/m^2 (= a 6.9% decrease)	28
Human Serum Albumin	0.01%	From 72.1 mJ/m^2 down to 67 mJ/m^2 (= a 10.1% decrease)	23

THE CASSIE EQUATION

During the 1940s, among the first authors to investigate the water-air interface from the point of view of the vastly increased contact angles of water droplets deposited on or near the pores of woven tissues from which raincoats are manufactured, were Cassie and Baxter (56) and Cassie (57). Cassie proposed a simple equation permitting to determine the proportion of the cosines of water contact angles obtained on air (θ_{air}) to those obtained on the solid support (θ_{solid}) of woven or otherwise "rough" surfaces, with respect to the total ($\theta_{\text{aggregate}}$), as measured on the rough surface:

$$\cos\theta_{\text{aggregate}} = f_1 \cos\theta_{\text{solid}} + f_2 \cos\theta_{\text{air}} \qquad [17]$$

where f_1 is the proportion of the surface area occupied by the solid supporting material and f_2 the proportion of the surface area occupied by air and where f_1 and f_2 stand in the following relation to each other:

$$f_2 = 1 - f_1 \qquad [18]$$

The Cassie equation still is perfectly applicable to all rough or porous or woven surfaces (29). However, for the next 52 years after the first Cassie paper on the subject no great advances were published in this field until the appearance of an outstanding paper by Onda et al. in 1996 (58), who used a super-porous fractal foam of an alkyl-ketene dimmer, with a more than 99% contactable air surface upon which a water drop could form a 174° contact angle. This is most likely just about the closest one can approach the ideal theoretical value of 180° at the present state of the art. Onda et al. thus experimentally demonstrated with their close proximity to a 180° water contact angle on a surface consisting for more than 99% of air that such an air surface will approach total hydrophobicity and is also, paradoxically, entirely devoid of hydration (29, 58).

ROUGH SURFACES

The phenomena of impermeability for water drops deposited on woven rough surfaces as well as the highly elevated contact angles drops of water make when deposited on “rough” surfaces, described above, are clearly solely due to the fact that these drops are sitting for the major part of its total contacting water drop surface on the air part of the covered rough surface.

PROPERTIES OF DIFFERENT CLASSES OF SOLUTES WITH RESPECT TO THE WATER-AIR INTERFACE

Table 7 shows the properties of four classes of solutes of which the first three are soluble and the 4th is insoluble in water. The first two of these are repelled by the water-air interface, which causes a reduce increase of the measured surface tension of its aqueous solution being only 7% of the increase in surface tension that one would have calculated (e.g., sucrose dissolved in water) (23). The surface tension measurements were done by pendant drop shape analysis (45, 46). It should be recalled, however, that all of the five methodologies for measuring surface tensions of water as well as of aqueous solutions involve the water-air interface. These five methodologies are: (i) capillary rise, (ii) pendant drop, (iii) Wilhelmy plate, (iv) Du Nouy’s ring and (v) contact angle analysis (45, 46, 59).

Thus, in the light of the above-mentioned results, using any of these existing methods for measuring surface tensions of aqueous solutions of polar solutes of classes 1, 2, or 3 shown in Table 7 produces grossly inaccurate data. Class 4 compounds, non-polar substances, are not soluble in water, or have an exceedingly small aqueous solubility. For measurements of ΔG_{iwi} or γ_{iw}, however, the remaining, accurate method involves utilizing the solubility equation (eqs 11 or 11A).

Adhesion and Adsorption Energies Between Two Different Entities When Immersed or Dissolved in Water: ΔG_{1w2}

$\Delta G_{1w2}{}^{IF}$

The adhesion and adsorption free energies of interaction between two different entities while immersed or dissolved in water (ΔG_{1w2}) are composed of the two interfacial energy types, i.e., Lifshitz-van der Waals ($\Delta G_{1w2}{}^{LW}$) and Lewis Acid-Base ($\Delta G_{1w2}{}^{AB}$) energies, acting in various proportions. Each of these two interfacial free energy types can be either attractive or repulsive when operative in water (59) and are, together, represented by $\Delta G_{1w2}{}^{IF}$ (equation 10), such that:

$$\Delta G_{1w2}^{IF} = \Delta G_{1w2}^{LW} + \Delta G_{1w2}^{AB} \qquad [19]$$

TABLE 7 Solubilities and Other Properties of Different Classes of Solutes When in Close Vicinity of a Water-air (W-A) Interface

Property vis-à-vis W-A interface	Class of solute and properties	Examples of solutes	Aqueous solubility (mol fractions)	ΔG_{iwi} (in kT)
Strongly repelled by W-A interface	Polar; very hydrophilic; infinitely soluble in water	Neutral polysaccharides, such as Dextran	Theoretically: $s = 68.7$ or infinitely soluble	+4.23
Repelled by W-A interface	Polar; hydrophilic; finite solubility in water	Sucrose, glucose, NaCl	$s = 0.035$ $s = 0.048$ $s = 0.0814$	−3.35 −3.04 −2.51
Attracted to W-A interface via their hydrophobic chain, whereas their hydrophilic moiety stays orientated toward the bulk aqueous solvent	Partly polar; amphiphilic molecules. Orientate at the W-A interface with their hydrophilic moiety remaining dissolved in water. Form micelles above cmc	Surfactants and other amphiphillic molecules such as, e.g., organic alcohols; see, e.g., octanol	$s = 0.000,081$	−9.42
Strongly attracted to the W-A interface, but generally virtually insoluble in water, their particles or droplets may not reach the W-A interface	Non-polar; hydrophobic molecules of this class have such a low aqueous solubility that they are usually listed as "insoluble"	Octane	Exceedingly low aqueous solubility for octane $s = 2.2-10^{-6}$	−13.02

Data from 6, 10, 17, 28, 48, 50.

It should be noted that $\Delta G_{1w2}{}^{AB}$ can be negative (attractive) or positive (repulsive), whereas $\Delta G_{1w2}{}^{LW}$ is normally negative (attractive) but under special conditions it can be positive (repulsive). The latter happens when:

$$\gamma_1^{LW} < \gamma_w^{LW} < \gamma_2^{LW} \qquad [5\text{-B}]$$

or when:

$$\gamma_1^{LW} > \gamma_w^{LW} > \gamma_2^{LW} \qquad [5\text{-C}]$$

See also equation 5 in Part I of this paper.

Equation 10, expressing $\Delta G_{1w2}{}^{IF}$ pertains to the total interfacial free energy of interaction between two different molecules, particles, surfaces or other condensed-phase materials (1 and 2) when immersed or dissolved in water (w) and entities 1 and 2 may be either polar or non-polar. Of all modes of non-covalent interactions in water, equation 10 is utilized in the most variegated applications of which a number is discussed next. It should be added that of all equations mentioned in Part I of this paper, equation 10 is the one which is most germane to Separation Science in aqueous media.

The total $\Delta G_{1w2}{}^{IF}$ values can be attractive or repulsive, which can usually be modulated by means of additives administered to the aqueous medium. This can most often be done along similar lines as previously treated, see for instance the chromatographic separation method entitled Reversed-Phase Liquid Chromatography (RPLC).

Specific interactions – Antigen-antibody interactions

Specific interactions occur between various proteinaceous ligands and receptors, including antigens (Atg) and antibodies (Atb), lectins and carbohydrates, and enzymes and their substrates. Here Atg-Atb interactions are used as examples, which exclusively bind to one another in aqueous media and are driven by any or all of the three attractive non-covalent energies, Lifshitz-van der Waals (LW), Lewis Acid-Base (AB) and Electrostatic (EL) in any possible proportion, depending on the special characteristics of each of the many individual cases (6, 10, 17).

As it is the principal aim of potentially infectious agents such as bacteria, viruses, parasites, etc., to find their way into, e.g., mammalian (including human) organisms, via their blood and lymphatic circulations as well as into other intercellular aqueous media, these infectious agents tend to have a hydrophilic outer surface, on which their equally hydrophilic antigenic determinants (*epitopes*) are situated. In order to attach themselves to these hydrophilic epitopes, antibodies most often bind hydrophobically to these epitopes so that their antibody-active sites (*paratopes*) in most cases bind to them via Lewis Acid-Base (AB) forces. The paratopes of antibody molecules [i.e., immunoglobulins (Ig)] are placed near their amino-terminal sites on what are called the Ig's Fab chains (61), each placed inside the paratope's concavities. This allows them to avoid non-specific hydrophobic binding to other proteins or to cell surfaces which are also present in great numbers in their common aqueous medium.

In most cases each given antigenic specificity, on the exterior surface of a cell or a biopolymer, is expressed as a pattern of hydrophilic antigenic sites on the convex epitope. A complementary pattern of hydrophobic sites will be found in the concavity of the paratope of the corresponding antibody molecule. The epitope and paratope then will specifically bind hydrophobically to within the shortest possible distance, over the largest possible surface area. Here, especially the convex hydrophilic epitopic sites will, for the greater part, remain covered with a hydration layer, while the complementary paratopic moieties inside the paratopic concavity will at least in part also (albeit to a smaller extent) be covered with a somewhat thinner hydration coat. [Yes, hydrophobic surfaces also become, moderately but not negligibly, hydrated mainly by LW forces when immersed in water (6, 60)].

ATG-ATB BINDING HYSTERESIS AND THE AFFINITY BINDING CONSTANT

The fact that hydrophilic epitopes are rather strongly hydrated and that even the more hydrophobic paratopes often are also more modestly hydrated permits as a first approximation to describe their initial epitope-paratope bond as:

$$\text{epitope} - \text{n } H_2O - \text{paratope}$$

However, starting immediately upon formation of the initial bond shown above and as a function of time, the hydrophobic (AB) attraction continues to exert itself and in so doing squeezes out interstitial water of hydration, which increasingly causes the initial ΔG_{1w2}-mode of interaction to transform into a ΔG_{12} attraction, which is much stronger. Concomitantly, after the squeezing out of the water of hydration is completed, the initial hydrophobic (AB) attraction is no longer relevant in the absence of interstitial water and the continuing ΔG_{12} attraction forms an even stronger ΔG_{12}^{LW} bond (17, pp. 595–597). In most mammalian, including human species, the high ionic strength of the ambient aqueous medium makes ΔG_{EL} interactions feeble enough to be allowed to neglect them as a first approximation in Atg-Atb interactions *in vivo* as well as *in vitro*.

The hydrophobic/hydrophilic type of bond strength through the expulsion of water of hydration described above is one of the principal examples of bond strengthening called hysteresis.

A. *Hysteresis in aqueous media* (62, 63). This type of hysteresis refers to the bond strengthening as a function of time, which in this case pertains to Atg-Atb attractions but is not limited to specific Atg-Atb interaction as it also occurs in many other adsorption and adhesion phenomena taking place in water. In most cases it is therefore extremely useful to be aware of the (often unsuspected) strong quantitative importance of the effect of hysteresis on the measurement of affinity constants, $K_{affinity}$, abbreviated as K_{aff}.

B. *Affinity constants* are readily determined by using the mass action-type Atg-Atb reaction:

$$\mathrm{Atg} + \mathrm{Atb} \leftrightarrow \left[\mathrm{Atg} \cdot \mathrm{Atb}\right] \quad [20]$$

where:

$$K_{aff} = \left[\mathrm{Atg} \cdot \mathrm{Atb}\right] / \left[\mathrm{Atg}\right] \times \left[\mathrm{Atb}\right] \quad [21]$$

and where square brackets indicate the *concentrations* of the enclosed reagents (64).

It should be noted that as K_{aff} determinations according to equations 20 and 21 involve a prior attachment of Atg to Atb during a finite time span, e.g., of the order of one or several *hours*, Atg-Atb binding hysteresis causes the resulting measured K_{aff} value not only to be much too high but also furnishes no clue as to how much too high that may have been.

C. *Experimental data on Human Serum Albumin-Silica interaction hysteresis* were used in K_{aff} determinations instead of Atg-Atb binding experiments, due to the existence of more experimental data in the HSA-SiO_2 case than in the Atg-Atb case. The reason for this is due to the fact that available Atb's tend to be polyclonal (and thus quite varying in their properties) and monoclonal Atb's were expensive and hard to obtain in the required quantities. Instead a considerable amount of experimental data on a physicochemically similar system was at our disposal (63, 65). Another fundamental difficulty with many of the published data on adsorption is the fact that the initial adsorption time tends to vary arbitrarily between researchers and often varies from experiment to experiment with the same researchers.

D. *However, the use of* $K_{aff}{}^{t\rightarrow 0}$ solves most of these problems and furthermore allows the determination of the exact amount of hysteresis occurring as a function of the time delay caused during pre-adsorption (65) and Table 8. In fact, any published K_{aff} values that had not been determined by extrapolation to time zero ($K_{aff}{}^{t\rightarrow 0}$) (62, 65) should be regarded as unreliable. Briefly, the only reproducible exposure time for a protein to be adsorbed onto a solid surface when immersed in an aqueous solution of that protein, is a time span of less than one second, which is not readily measured but can be attained by extrapolation to time zero, from a few different adsorption observations done at short time intervals, yielding $K_{aff}{}^{t\rightarrow 0}$ (62).

Adsorption experiments have been done on an HSA-silica system, using different and much longer pre-adsorption times, i.e., after 1, 3, 6, 12 and 24 hours, where it was necessary also to know $K_{aff}{}^{t\rightarrow 0}$ (62,

TABLE 8 Hysteresis of a Typical Equilibrium Affinity Constant, K_{aff}, of the Adsorption of Human Serum Albumin (HSA) onto Polysized Silica Particles at 20°C

Pre-exposure time of silica particles to HSA	K_{aff} in L/mol	Hysteresis*
$K_{aff}^{t\rightarrow 0}$	4.11×10^7	reference value
1 hour	1.04×10^8	2.53×
3 hours	2.73×10^8	6.64×
6 hours	5.33×10^8	12.97×
12 hours	5.64×10^8	13.72×
24 hours	6.40×10^8	15.57×

*Hysteresis expressed as increases in K_{aff} values, as a function of elapsed time after $K_{aff}^{t\rightarrow 0}$

Data from ref. 62.

Table 8). Here one notes that using the adsorption from aqueous solutions of HSA onto SiO_2 particles, $K_{aff}^{t\rightarrow 0} = 4.11 \text{ x } 10^7$ L/mol and that via the increase in K_{aff} the hysteresis factor at 1 hour adsorption time was found to be a factor 2.53 times too high and after 24 hours it was 15.57 times too high. It should be noted that for the derivation of ΔG_{1w2}^{IF} from K_{aff} one uses:

$$\Delta G_{1w2}^{IF} \times S_c/kT = -\ln K_{aff}^{t\rightarrow 0} \qquad [22]$$

It should also be noted that, when using equation 22, the value of $K_{aff}^{t\rightarrow 0}$ (in L/mol) has to be multiplied by a factor 55.6 (i.e., the number of moles H_2O per liter water) in order to obtain $K_{aff}^{t\rightarrow 0}$ in the dimensionless unit of mol fractions. Then, for instance a $K_{aff}^{t\rightarrow 0}$ value of 4.11×10^7 L/mol (Table 8) corresponds to a value for ΔG_{1w2} of -21.6 kT (63, p. 286). However, the K_{aff} value of 1.04×10^8 L/mol, obtained after one hour's pre-adsorption (63) yields a value for ΔG_{1w2} of – 22.5 kT. Thus whilst the one hour's delay caused by the pre-adsorption of HSA (which showed a 2.53-fold increase in K_{aff} when expressed in L/mol units), in terms of ΔG_{1w2}, a much milder increase of only 4.3% due to the same hysteresis manifested itself when expressed in units kT. However this is only a consequence of the fact that real and significant differences between two large numbers are much greater than the differences between the logarithms of the same large numbers.

E. *Absence of hysteresis in purely electrostatic Atg-Atb systems.* It should be mentioned here that in the hydrophobic/hydrophilic type of adsorption one usually finds in Atg-Atb and many other aqueous adsorption systems, which act mainly via LW and AB forces, the type of hysteresis treated above is the more common one. However, in exclusively electrostatic

(EL) Atg-Atb systems, as in DNA-anti-DNA Atg-Atb complex formation, binding hysteresis does not occur (66).

AFFINITY CHROMATOGRAPHY

Affinity chromatography is a chromatography mode by which antibodies (Atb) to an antigenic ligand (Atg) have been attached as receptors to insoluble but hydrophilic particles (made, e.g., out of gelled agarose) and placed in a cylindrical column. The Fab parts of the bound Atb molecules, the parts with the paratopes, the receptors, are distally oriented on the receptor particles, with an aim to bind specifically to the surfaces of dissolved ligands (here the Atg) molecules, as a first step toward their isolation and purification. After a washing episode, more weakly and non-specifically bound contaminants from the column are removed first. The remaining, specifically bound Atg molecules are then eluted at high, or low pH, in a purified state. The usual Atg-Atb bond strengths are of the order of K_{aff} from 105 to 1010 L/mol, i.e., sufficiently strong for adequate receptor-ligand binding in the first step, but not too strong, to facilitate ready dissociation in the purified state as the final step (67).

Liquid Phase Separations

LIQUID PHASE SEPARATION IN NON-POLAR SYSTEMS

A. *Two identical solutes*. The free energy of interaction between two *identical* entities of non-polar materials or molecules (1), immersed or dissolved in a non-polar liquid (L) can be expressed as (10):

$$\Delta G_{1L1}^{LW} = -2(\sqrt{\gamma_1}^{\,LW} - \sqrt{\gamma_L}^{\,LW})^2 \qquad [23]$$

It follows from equation 23 that ΔG_{1L1}^{LW} always has a negative (attractive) or nil value, so that no phase separation can occur between different particles or molecules of non-polar material, 1, when immersed or dissolved in a non-polar liquid, L. It should be stressed that non-polar materials, solutes or solvents can only interact with one another via Lifshitz-van der Waals (LW) energies.

B. *Two different solutes*. The free energy of interaction between two *different* entities of non-polar materials or molecules (1 and 2), immersed or dissolved in a polar liquid (L) can be expressed as (10, 17):

$$\Delta G_{1L2}^{LW} = -2(\sqrt{\gamma_1}^{LW} - \sqrt{\gamma_L}^{LW})(\sqrt{\gamma_2}^{LW} - \sqrt{\gamma_L}^{LW}) \qquad [24]$$

It follows from equation 24 that ΔG_{1L2}^{LW} can be negative (attractive), zero, as well as positive (repulsive) (10, p. 38; 17, pp. 43–45), so that depending on the concentrations of the different solutes, separation into two phases may occur.

LIQUID PHASE SEPARATIONS IN POLAR SYSTEMS – TWO DIFFERENT SOLUTES

In polar systems, Lewis acid-base (AB) energies are operative in addition to Lifshitz-van der Waals (LW) energies. In other words, LW systems exist either by themselves or in addition to AB systems, but AB systems rarely exist just by themselves: they are virtually always accompanied by LW systems. Thus the system under this heading involves the complete interfacial (IF) combination, characterized by:

$$\Delta G_{1L2}^{IF} = \Delta G_{1L2}^{LW} + \Delta G_{1L2}^{AB} \qquad [25]$$

Equation 25 is an extended version of equation 10. The outcome of equations 10 and 25 can be positive (repulsive, so that phase separation between 1 and 2 is possible), or negative (attractive, which excludes phase separation between 1 and 2).

PHASE SEPARATION IN AQUEOUS SYSTEMS

Here equation 10 for ΔG_{1w2}^{IF} is applicable, and as two components (1 and 2) are dissolved in the same container with water as the continuous phase, component 1 as well as component 2 have to repel one another in water in the same container. However, one also has to contend with positive values of ΔG_{1w1}^{IF} as well as of ΔG_{2w2}^{IF}. If there are more than two components dissolved in the same aqueous medium (e.g., with ΔG_{3w3}^{IF}. . . . . .ΔG_{nwn}^{IF}), one can, in water, under the right conditions, have as many different separate phases as there are dissolved solutes, especially when each of the dissolved fractions, 1, 2, 3, ...n, cause their aqueous solutions to have different densities. Albertsson (68) reported on an aqueous phase separation with 18 separate phases where each phase comprises a solution of a differently substituted dextran.

AQUEOUS PARTITION

Phase separation systems between two water-soluble polymers have been used as an important separation and purification method for water-soluble polymers as well as for cells (68, 69). Two of the most frequently utilized polymers are dextran (DEX) and poly-(ethylene oxide) (PEO) (10, p. 256; 17, p. 285). For example, using an aqueous two-phase system formed with DEX and PEO, each dissolved at a concentration of at least 5 or 6% (w/w) and using hydrated human serum albumin (hHSA) as the protein to be isolated from a mixture:

$$\Delta G_{hHSA-w-PEO} = +34 mJ/m^2$$

and:

$$\Delta G_{hHSA-w-DEX} = +31 mJ/m^2$$

Thus, hHSA is more strongly repelled by PEO than by DEX. When hHSA is added as a protein to be isolated from an extract, to a two-phase aqueous DEX/PEO mixture, hHSA is preferentially attracted to the DEX-phase, from which it can be readily isolated (10, p. 285; 17, pp. 256–257).

MICROEMULSIONS

Microemulsions spontaneously form mixing appropriate proportions of surfactant, cosurfactant, oil and water (70, 71). An example could be:

1. 9.6 % w/w of a surfactant such as purified sodium dioctyl sulfosuccinate,
2. water (47.04% w/w),
3. NaCl (0.96% w/w),
4. ethanol (20% w/w),
5. n-octane (22.4% w/w).

These are mixed and kept at a constant 25°C for several days. Three transparent liquid phases can then be discerned:

1. Top phase: The hydrocarbon phase consisting mainly of octane and the anionic surfactant.
2. Middle phase: The microemulsion phase.
3. Brine phase consisting mainly of salt water.

It is the middle phase that is of interest. It is clear and transparent when viewed with the naked eye, but is known to contain many sub-microscopic (mainly oil) droplets, of a diameter of the order of 30 to 40 nm. We (and others) suspected however that the sub-micron droplets suspended in the middle phase sorted themselves out into different sub-phases, according to droplet size. As one of us (CJvO), in the 1980s had two Beckman instruments with Schlieren optics (a Beckman Model E analytical ultracentrifuge and a Beckman Model H analytical Tiselius electrophoresis apparatus), it became possible to determine whether the microemulsion's middle phase did indeed comprise sub-phases with different refractive indices that would be invisible with the naked eye. [Schlieren optics uses light passing through a transparent sample and then passes an oblique slit, followed by a cylindrical lens, so that a refractive index gradient in the transparent sample is projected on a photographic plate, or a viewing surface, showing a Gaussian curve representing the first derivative of the refractive index gradient (72).

The Schlieren optics revealed the presence of multiple (about a dozen) refractive index bands in the microemulsion middle phase, at 1 x G (standing after 5 days) as well as immediately visible under a 100,000G centrifugal field, thus proving that the clear transparent-looking middle phase with the naked eye indeed contained multiple sub-phases (10, 17, 71, 73).

Advancing Freezing Fronts

A slowly upward moving freezing front of ice formed in a large vertically placed test tube initially filled with water that is refrigerated by means of a cold source placed at the test tube's bottom can either engulf, or reject particles of molecules suspended or dissolved in the (upper) aqueous phase (10, pp. 122–124; 17, pp. 174–175; 24, pp. 166–67).

When particles or molecules (p) have a positive value for ΔG_{pwi}^{IF}, at 0°C (w is water and i stands for ice), an advancing freezing front will reject such particles or molecules and when ΔG_{pwi}^{IF} is negative, the advancing freezing front (at 0°C) will engulf them. For ice and water, at 0°C, their components and parameters (expressed in mJ/m^2) are as follows (6, p. 78; 10, p. 96):

$$\textbf{Ice}: \quad \gamma = 70.6;\ \gamma^{LW} = 28.5;\ \gamma^{AB} = 42.1;\ \gamma^{+} = 10.5;\ \gamma^{-} = 42.1$$

$$\textbf{Water}: \quad \gamma = 75.8;\ \gamma^{LW} = 22.8;\ \gamma^{AB} = 53.0;\ \gamma^{+} = 19.0;\ \gamma^{-} = 37.0$$

Hydrophilic particles of a montmorillonite clay from Southern Wyoming, called SWy-1, when in an aqueous suspension, are *rejected* by an (upwards) advancing ice front. However, when these SWy-1 particles are made *hydrophobic* by means of treatment with a quaternary ammonium salt, the advancing ice front *engulfs* them (75).

The advancing freezing front method has also been applied (*in vitro*) to human peripheral erythrocytes (i.e., red blood cells) and leukocytes (i.e., white blood cells). Human erythrocytes (glutaraldehyde-fixed, which does not change the surface properties of these cells) are quite hydrophilic ($\Delta G_{iwi}^{LW} > 0$), so that they are rejected into the upper, water-phase by upward advancing ice fronts. This happens in actual practice when normal red cells, intended for future transfusion, are frozen (because they keep longer in the frozen state).

However the drawback of red cell freezing is exactly the fact that while being rejected by the advancing ice front not only the cells but also the salts in their usual aqueous medium are rejected with the cells (plus most other solutes), so that both the cells and the salt ions become much more concentrated, which lyses the cells. On the other hand, when one causes an advancing freezing front to engulf the cells, subsequent freezing and thawing is not detrimental to red cells. To achieve this one needs to counteract the salting-out effect which enhances the hydrophobizing capacity of the aqueous medium caused by the increased salt concentration.

To diminish the salt-driven hydrophobizing effect of the aqueous solution in a somewhat milder manner than by the admixture of acetonitrile or ethylene glycol, in the case of fragile red blood cells one prefers to add fairly high concentrations (up to approximately 50%) of glycerol (10, pp. 368–371; 17, pp. 356–390; 76). These references also treat the fact the faster one allows the freezing ice front to advance, the more readily particles or cells, which are rejected by slow freezing fronts, become engulfed.

The Importance of Size

In the field of action at a distance, e.g., among phagocytic cells such as human neutrophils (whose main function is the removal and destruction of infectious agents from the blood stream (77), it is useful to know that for all cell-cell and cell-particle (e.g., bacteria) interactions between two spherical entities, with radius, R, or between one spherical entity and a flat surface, the three different non-covalent interaction types (LW, AB and EL) all decay as a function of distance (d) with an interaction energy, ΔG_{1w1} or ΔG_{1w2}, which is proportional to R as contained in the relevant Extended DLVO approach in aqueous media (6, pp. 31–48; 10, pp. 79–89; 17, pp. 75–88 and Part I of this paper).

For instance, human neutrophils in normal blood circulation are fairly smooth and approximately spherical (with a large R) and they are very hydrophilic, which allows them to maintain their (mainly AB-driven) distance from other leukocytes as well as from red cells. The function of neutrophils (also called polymorphonuclear leukocytes, or PMN's) is mainly to detect, approach, phagocytize and kill bacteria and other foreign particles that have found their way into a human's blood circulation, as that human's first line of defense. Normally big, smooth, round PMN's repel small, roughly spherical bacteria, mainly via AB repulsion, helped by their large R.

However, PMN's, activated by the presence of a microbe in their vicinity by means of chemical signals ("chemotaxis"), can extend long, thin pseudopodia with a small radius of curvature, i.e., with a small R. In this manner pseudopodia can pierce the repulsion field between PMN and the bacterium and will then not only touch the bacterium with the distal end of its pseudopod, with which it not only attaches to the microbe but then also engulfs it, transports it inside the pseudopod towards its (the PMN's) interior, where it encounters a phagosome by which it is enzymatically destroyed (77).

Another example of local decreases in the radii of curvature (R) among white blood cells is the change from smooth little blood platelets in normal

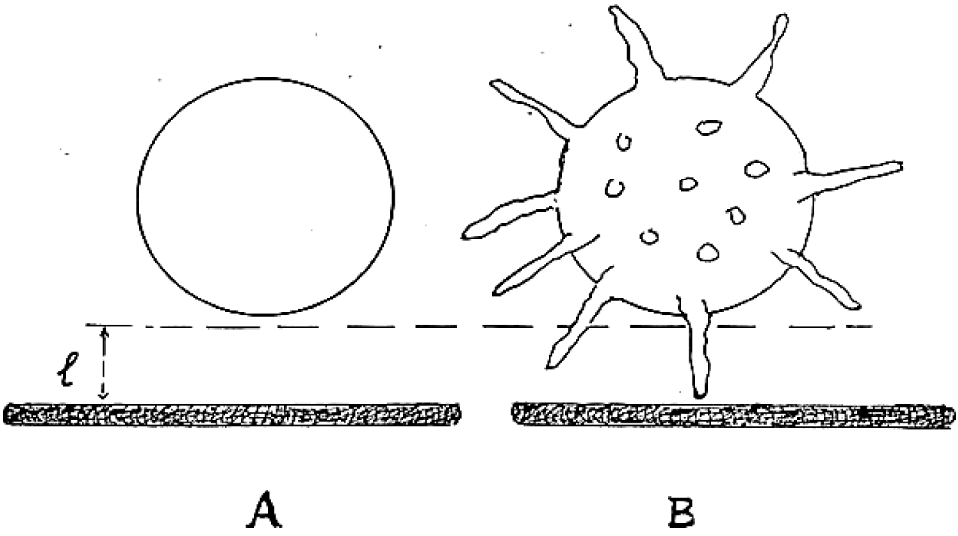

FIGURE 1 Particle shape and flat plate accessibility. (A) The smooth hydrophilic spherical cell or particle cannot make contact with a smooth flat hydrophilic surface because their mutual aspecific, macroscopic-scale repulsion prevents a closer approach than approximately l = 4 or 5 nm. (B) A similar spherical cell or particle endowed with long thin spiky processes whose ends with a small radius of curvature can readily penetrate the macroscopic-scale repulsion field and thus achieve microscopic-scale specific contact. In both (A) and (B) the dotted line indicates the limit of closest approach (l).

circulation to a strongly crenated (spiculated) form via a multiple pseudopod formation when reacting with adenosine tri-phosphate (ATP), which makes them “sticky”. However this “stickiness” is only due to the appearance of the platelets’ newly formed thin pseudopodia: neither the LW and AB-driven surface properties, nor the ζ-potential of the platelets changes upon ATP activation (77).

Finally, it is well known that most infectious viruses have multiple thin processes protruding distally from their surfaces such as the Human Immunodeficiency Virus or HIV (Figure 1). These thin protruding fiber-like processes of small radii of curvature help viruses make contact with cell surfaces prior to inserting themselves into a cell’s interior in order to infect them and without which they cannot reproduce (10, p. 363; 17, p. 350).

CONCLUSION

Interaction forces in aqueous media are critically important to know and to understand since they are responsible for the behavior of dissolved solutes. For interactions in water, the Derjaguin, Landau, Verwey and Overbeek (DLVO) theory needs to be the extended DLVO theory plus the added acid-base interactions. The four indispensable equations needed to make any calculations with interactions in solution chemistry were recalled and detailed. The different forms of the solubility equation were extensively presented.

These equations are needed to understand and predict solute behavior allowing for separation. Hydrophobic attraction can produce "insolubilization" leading to precipitation or solid flocculation or liquid phase separation. Antigen-antibody reactions are also governed by the same mechanisms. Differential affinity, adhesion and adsorption energies are critical in the understanding of chromatographic separations, in aqueous as well in other polar media.

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