Long-range and short-range mechanisms of hydrophobic attraction and hydrophilic repulsion in specific and aspecific interactions

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Among the three different non-covalent forces acting in aqueous media, i.e. Lifshitz–van der Waals (LW), Lewis acid–base (AB) and electrical double layer (EL) forces, the AB forces or electron–acceptor/electron–donor interactions are quantitatively by far the predominant ones. A subset of the AB forces acting in water causes the hydrophobic effect, which is the attraction caused by the hydrogen-bonding (AB) free energy of cohesion between the water molecules which surround all apolar as well as polar molecules and particles when they are immersed in water. As the polar energy of cohesion among water molecules is an innate property of water, the hydrophobic attraction (due to the hydrophobic effect) is unavoidably always present in aqueous media and has a value of $\Delta G^{\text{hydrophobic}} = -102 \text{ mJ/m}^2$, at 20°C, being equal to the AB free energy of cohesion between the water molecules at that temperature. The strong underlying hydrophobic attraction due to this effect can, however, be surmounted by very hydrophilic molecules and particles that attract water molecules more strongly than the free energy of attraction of these molecules or particles for one another, plus the hydrogen-bonding free energy of cohesion between the water molecules, thus resulting in a net non-electrical double layer repulsion. Each of the three non-covalent forces, LW, AB or EL, any of which can be independently attractive or repulsive, decays, dependent on the circumstances, as a function of distance according to different rules. These rules, following an extended DLVO (XDLVO) approach, are given, as well as the measurement methods for the LW, AB and EL surface thermodynamic properties, determined at 'contact'. The implications of the resulting hydrophobic attractive and hydrophilic repulsive free energies, as a function of distance, are discussed with respect to specific and aspecific interactions in biological systems. The discussion furnishes a description of the manner by which shorter-range specific attractions can surmount the usually much stronger long-range aspecific repulsion, and ends with examples of *in vitro* and *in vivo* effects of hydrophilization of biopolymers, particles or surfaces by linkage with polyethylene oxide (PEO; also called polyethylene glycol, PEG). Copyright © 2003 John Wiley & Sons, Ltd.

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INTRODUCTION

The classical non-covalent short- to long-range macroscopic-scale interactions between apolar and/or polar surfaces, immersed in a liquid, as described by the DLVO (approach of Derjaguin, Landau, Verwey and Overbeek) theory (Derjaguin and Landau, 1941; Verwey and Overbeek, 1948) traditionally deal with apolar Lifshitz–van der Waals (LW) attraction and electrical double layer (EL) repulsion, as a function of distance, ℓ. However, when the liquid in question is polar and especially when the liquid is water which is very polar, another category of non-covalent interactions is quantitatively more dominant than either LW or EL interactions, i.e. the polar Lewis acid–base (AB) or electron–acceptor/electron–donor interactions, first described by Lewis (1923), cf. van Oss *et al.* (1987, 1988) and van Oss (1993, 1994a, 1996). Thus specific as well as aspecific interactions between, for example, biopolymers, cells and/or particles occurring in a aqueous media, must always take these polar, Lewis acid–base (AB) interactions into account, as these typically represent about 90% of all the non-covalent interactions, in water, be they attractive or repulsive; see also Grasso *et al.* (2002).

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Nomenclature A, Hamaker constant; AB, (Lewis) acid–base; K_{aff}, affinity constant; $K_{\text{aff}}^{t \to 0}$, affinity constant extrapolated to zero time; k, Boltzmann's constant; $k = 1.38 \times 10^{-23}$ J per degree Kelvin; kT, free energy per molecule, or per molecule pair; $1 \text{ kT} = 4.04 \times 10^{-21}$ J at 293° K; L, liquid; ℓ, length, or distance; ℓ_{o}, minimum equilibrium distance; $\ell_{\text{o}} = 0.157$ nm; Q, elasticity modulus; R, radius; S, solid; T, absolute temperature, in degrees Kelvin; w, water; γ, surface or interfacial tension; ΔG, free energy of interaction; ζ, surface potential at the slipping plane; θ, contact angle, in degrees; λ, decay length of water; $\lambda \approx 1.0$ nm; ψ_{o}, surface potential at the very edge of particles or surfaces.

Table 1. Free energies of interaction as a function of distance, ℓ (ΔG_ℓ) for the interactions between two equal spheres of radius, R, and between one such sphere and a flat plate (cf. van Oss, 1994a, pp. 75–88)[a]

	ΔG_ℓ^{LW}	ΔG_ℓ^{AB}	ΔG_ℓ^{EL}
Two spheres of radius, R	$-AR/12\ell$[b]	$\pi R\lambda\Delta G_{\ell_0}^{AB_{11}}\exp[(\ell_0-\ell)/\lambda]$[c]	$0.5\epsilon R\psi_0^2\ln[1+\exp(-\kappa\ell)]$[d]
Sphere of radius, R, and a flat plate	$-AR/6\ell$[b]	$2\pi R\lambda\Delta G_{\ell_0}^{AB_{11}}\exp[(\ell_0-\ell)/\lambda]$[c]	$\epsilon R\psi_0^2\ln[1+\exp(-\kappa\ell)]$[d]

[a] It should be specially noted that, when spheres are involved, all three non-covalent free energies are proportional to the sphere radius, R.
[b] $A = 24\pi\ell_0^2\gamma^{LW} = -12\pi\ell_0^2\Delta G_{\ell_0}^{LW}$, where $\ell_0 = 0.157$ nm.
[c] λ is the characteristic decay length of water: $\lambda = 1.0$ nm at 20 °C. $\Delta G_{\ell_0}^{AB_{11}}$ is the Lewis acid–base free energy of interaction between two plane parallel plates, at molecular contact, i.e. at $\ell = \ell_0$.
[d] ϵ is the dielectric constant of the liquid medium (for water, $\epsilon \approx 80$). κ is the inverse thickness of the diffuse ionic double layer. ψ_0: cf. eq. (16).

Decay of non-covalent interactions in water, as a function of distance

Under physiological conditions, i.e. at an ionic strength, $\mu = 0.15$ M, EL forces decay more steeply as a function of distance than AB forces (whose rate of decay with distance is largely independent of the ionic strength). On the other hand, EL forces decay more gradually with distance than AB forces at $\mu \ll 0.15$. It is therefore difficult to make general rules as to which of these two interaction forces is the more long- or short-range: it all depends on the *in vivo* or *in vitro* conditions of the ambient liquid medium. Whilst both AB and EL forces decay exponentially as a function of distance, LW interactions, for instance the LW interactions between two spheres, decay quite gradually, i.e. proportionally to the distance, ℓ.

Decay with distance of LW forces. The LW force is the longest-range non-covalent force, for which the free energy of interaction between two spherical molecules or particles, or between a spherical molecule or particle and a flat surface, is proportional to the radius of the spherical entity. The LW free energy of interaction decays as a function of distance in proportion to the distance between the edges of the spherical entities, up to a distance of approximately 10 nm. Beyond about 10 nm retardation sets in, after which the decay becomes proportional to the square of the distance (Israelachvili, 1991). However, in biological systems (i.e. in aqueous media) LW interactions are rather feeble. To begin with, it should be noted that the combining rule for interaction between low energy entities (e.g. lipids) in water, depends on their Hamaker constants (A) (Hamaker, 1937; van Oss, 1994a, pp. 14, 154–160). As an example, let A_L stand for the Hamaker constant of a lipid and A_W for that of water, then their LW interaction energy is proportional to $(\sqrt{A_L} - \sqrt{A_W})^2$ so that, as A_L and A_W are similar in value, the value of the LW interaction energy is very small in this case. For proteins (P), interacting in water, A_P is slightly larger than A_W, but this does not suffice to give rise to an interaction energy, in water, amounting to more than about 2–3.5 kcal/M (i.e. about 3.5–6 kT), at closest approach. (EL energies of interaction between typical biological entities, immersed in water at physiological ionic strength, usually are no larger than those of LW interactions and they decay much more steeply as a function of distance; see Table 1.)

Decay with distance of Lewis AB forces. Lewis AB free energies of interaction between apolar or polar biopolymers or cells or particles, can, on the other hand, be easily about a decimal order of magnitude stronger than the above-mentioned exiguous values pertaining to LW and EL interactions. This is especially the case for some of the more hydrophobic entities, immersed in water.

AB forces are responsible for all non-electrostatic, non-covalent, polar interactions occurring in water. In aqueous media AB interactions can be attractive (hydrophobic attraction) or repulsive (hydrophilic repulsion), depending upon the degree of surface hydrophobicity or hydrophilicity of the entities involved. AB interaction energies between spherical entities immersed in water decay exponentially with distance as a function of the characteristic length of water, λ, see Table 1. Similar to LW forces, AB free energies of interaction between spherical entities are proportional to the spheres' radii.

Decay with distance of electrical double layer forces. EL interactions among spherical entities immersed in water decay exponentially with distance as a function of the thickness of the diffuse ionic double layer ($1/\kappa$, designated as the Debye length), see Table 1 and below. The Debye length itself is a function of the ionic strength of the liquid medium. At the physiological ionic strength of 0.15, the Debye length, $1/\kappa = 0.8$ nm, which is quite small and usually causes *in vivo* ΔG^{EL} values to be close to negligible, with the exception of nucleic acid interactions, as nucleic acids are quite strongly negatively charged. Electrical double layer potentials also cannot be neglected when using electrophoresis of biological cells or biopolymers as an analytical tool. Similar to LW and AB forces, EL free energies of interaction between spherical entities are proportional to the spheres' radii.

Hydrophobic attraction

Hydrophobic attraction is the strongest non-covalent, non-electrostatic binding force occurring between particles, molecules of polymers, immersed in water. The attraction between two apolar surfaces, or between one apolar and one polar surface, in water, is traditionally called the hydro-phobic effect. It is perhaps the designation 'hydrophobic'

which has, so far, prevented a more general understanding of the mechanism behind the hydrophobic effect. The name 'hydrophobic' is a misnomer. Hydrophobic surfaces (i.e. apolar surfaces) do not 'fear water', on the contrary, hydrophobic surfaces attract water (Hildebrand, 1979). For instance very hydrophobic materials, such as Teflon, or alkanes, when immersed in water attract water molecules with a free energy of about 40–50 mJ/m^2, which is not negligible.

Mechanism of the hydrophobic effect. Tanford (1980) recognized, in the last edition of his book (*The Hydrophobic Effect*) that hydrophobic attraction is driven by the free energy of cohesion of water. As mentioned above, LW forces do not contribute much to the total interaction energy between apolar entities, immersed in water. Thus the main, and often virtually the sole, driving force of hydrophobic attraction is the AB, or more precisely the hydrogen-bonding component of the free energy of cohesion of water.

One consequence of this is that the hydrophobic attraction component of the total free energy of interaction between any entities, i, immersed in water, w, is never absent. At 20°C it amounts to: $\Delta G_{iwi}^{\text{hydrophobic}} = \Delta G_{ww}^{\text{cohesion}} = -102$ mJ/m^2 (van Oss, 1994a, p. 44). Thus, in order for a net repulsion between hydrophilic entities, immersed in water, to prevail, the hydrophilic repulsion component of the total free energy of interaction has to be greater than the omnipresent hydrophobic interaction energy.

Van der Waals repulsions in organic media and in water

For a better understanding of the mechanism of non-electrostatic repulsion between hydrophilic entities such as biopolymers, immersed in water, it may be useful first to recall briefly the mechanism of net Lifshitz–van der Waals repulsions between different polymer molecules, dissolved in an organic solvent (between similar entities, immersed in a liquid, LW repulsions are not possible). In organic solvents, for instance, a net LW repulsion between two different polymers, 1 and 2, dissolved in the same solvent, s, occurs when the Hamaker constant of polymer 1 (A_1) is greater than that of the solvent (A_S), and that of polymer 2 (A_2) is smaller than A_S: ($A_1 > A_S > A_2$), or vice-versa: ($A_1 < A_S < A_2$). Under these conditions a phase separation occurs, where polymer 1 is mainly present in solution in one phase and polymer 2 in the other, and where the upper phase contains the polymer molecules with the lowest density (van Oss *et al.*, 1989).

LW repulsions can also contribute to the total free energy of interaction in water, albeit in a negative manner. This mainly happens at the water–air interface, when hydrophobic entities with a greater Hamaker constant than that of water (which is about as low as that of octane) undergo an LW repulsion by the water–air interface, which interface, however, is very hydrophobic (Docoslis *et al.*, 2000). Thus the hydrophobicity of the water–air interface simultaneously attracts such hydrophobic entities toward itself through a strong hydrophobic (AB) interaction, which overrides the relatively feeble local LW repulsion (van Oss, 1994a, p. 338).

Hydrophilic repulsion

Contrary to LW repulsions, non-electrostatic hydrophilic repulsions, occurring between hydrophilic entities immersed in aqueous media not only take place between different, but also between identical hydrophilic materials (macromolecules, cells or particles). This is because, contrary to LW interactions where materials have only one essential property, expressed as the Hamaker constant (A), hydrophilic materials have two (polar) properties, i.e. electron-acceptity and electron-donicity. Thus, in water, a net repulsion between identical hydrophilic surfaces (i) can occur when the electron-acceptity of i is smaller than that of water and its electron-donicity greater than that of water (van Oss, 1994a, pp. 38–43). In theory this would also be true when the electron-acceptity of i is greater and the electron-donicity smaller than that of water, but such cases have not, so far, been encountered in nature (van Oss *et al.*, 1997).

A further prerequisite for the occurrence of a net hydrophilic repulsion between hydrophilic entities immersed in water, is that the value of the polar (AB) free energy of repulsion, $|\Delta G_{iwi}^{\text{AB}}|$, is greater than the hydrophobic attraction energy due to the omnipresent hydrogen-bonding (AB) free energy of cohesion of water: $|\Delta G_{ww}^{\text{AB}}| = 102$ mJ/m^2, at 20°C (van Oss, 1994a, p. 44, 2000; see also Table 2).

Acid–base-driven stability vs steric stabilization. Upon the arrival on the scene of non-ionic surfactants and non-ionic but polar polymers (Schick, 1967), such as poly-ethylene oxide (PEO, also often called polyethylene glycol: PEG), the stability of hydrophobic (e.g. polystyrene) particles, coated with electrostatically neutral PEO molecules, created a dilemma, because this type of stabilization could not be explained via the classical DLVO theory (which only recognizes LW and EL forces, cf. above) without the action of electrostatic repulsion. To explain the stabilizing power of non-ionic surfactants and polymers, the still-popular but non-quantitative concept of 'steric' stabilization (see e.g. Napper, 1983) was introduced, which however does not explain what prevents non-ionic strands of PEO (PEG) adhering together when immersed in water. The real reason for this is the fact that at moderate temperatures (e.g. room temperature), in spite of being electrically neutral, PEO (PEG) molecules nevertheless repel one another in water, through a net hydrophilic (AB) repulsion. By measuring PEO's LW and AB surface properties (van Oss, 1994a, p. 176) one can demonstrate that PEO (PEG) molecules must indeed repel each other when immersed in water, with a precisely determined free energy of repulsion (van Oss 1994a, pp. 220–227). Direct measurements of the repulsive forces between PEO-coated surfaces have been done by Claesson (1986).

The only real 'steric' interaction that can actually take place between, for example, PEO strands, occurs in cases of a close encounter between particles which are stabilized by AB repulsions, in the guise of the relatively minor contribution furnished by the chain elasticity of strands of PEO (PEG) upon forced close approach between two such stabilized particles (Ottewill, 1967). The elastic modulus, Q, which determines this chain elasticity is, however, difficult

Table 2. Comparison between the interfacial mechanisms of 'hydrophobic' attraction and hydrophilic repulsion for typical hydrophobic and hydrophilic entities[a]

	I (LW adhesion between molecules of i, in water) $-2(\sqrt{\gamma_i^{LW}} - \sqrt{\gamma_w^{LW}})^2$	II (polar cohesion of i) $-4\sqrt{\gamma_i^+ \gamma_i^-}$	III (polar cohesion of water) $-4\sqrt{\gamma_w^+ \gamma_w^-}$	IV (Polar adhesion between electron acceptor of $1i$ and electron donor of water) $+4\sqrt{\gamma_i^+ \gamma_w^-}$	V (polar adhesion between electron donor of $1i$ and electron acceptor of water) $+4\sqrt{\gamma_i^- \gamma_w^+}$	$=\Delta G_{iwi}$
	LW	AB				
ΔG_{iwi} of 'hydrophobic' attraction between apolar octane molecules of $\gamma_i^{LW} \approx 22$ mJ/m² immersed in water, at 20°C (for water, $\gamma_w^{LW} = 21.8$ mJ/m²)	0	0	-102 mJ/m²	0	0	$= -102$ mJ/m²
ΔG_{iwi} of hydrophilic repulsion between typical hydrophilic (dry) dextran T 150 of $\gamma_i^{LW} \approx 42$ mJ/m², $\gamma_i^+ = 0$ and $\gamma_i^- \approx 55$ mJ/m², immersed in water, at 20°C[b]	-6.6	0	-102 mJ/m²	0	$+149.8$ mJ/m²	$= +41.2$ mJ/m²
ΔG_{iwi} of hydrophilic repulsion between hydrophilic PEO (PEG) 6000 molecules, of $\gamma_i^{LW} = 43$ mJ/m², $\gamma_i^+ = 0$ and $\gamma_i^- = 64$ mJ/m², dissolved in water, at 20°C[c]	-7.1	0	-102 mJ/m²	0	$+ 161.6$ mJ/m²	$= +52.5$ mJ/m²

[a] Taken in part from van Oss (1994a, p. 44).
[b] From van Oss (1994a, p. 180).
[c] From van Oss (1994a, p. 176).

to determine (Ottewill, 1967; van Oss, 1994a, p. 240), but it could play a role in determining the optimal PEO (PEG) chain length to be utilized, and/or the optimal density of the stabilizing polymer to be used per unit surface area.

Principally, however, the fundamental mechanism of stabilization of aqueous suspensions of particles by the attachment of hydrophilic non-ionic polymers lies in the latter's mutual hydrophilic (AB) repulsion in water, which can be accurately predicted and quantitatively expressed in SI units from simple measurements of the surface properties of the hydrophilic non-ionic polymer, and those of water [van Oss et al., 1987, 1988; van Oss, 1993, 1994; see eq. (13), Table 2, and below]. The enthalpic and entropic contributions to both hydrophobic attraction and hydrophilic repulsion energies have been treated by van Oss and Good (1991) and van Oss (1997); see also van Oss (1994a, 1996).

THEORY AND MEASUREMENT

Theory

Apolar, or van der Waals and Lifshitz–van der Waals interactions and their decay with distance. Van der Waals interactions between atoms or small molecules comprise, in the order of their discovery: dipole–dipole or orientation (van der Waals–Keesom) interactions; dipole-induced dipole or induction (van der Waals–Debye) interactions; and fluctuating dipole-induced dipole or dispersion (van der Waals–London) interactions (van Oss et al., 1988; van Oss, 1994a, pp. 7–17). The dispersion (van der Waals–London) interactions are quantitatively generally the most important. Among the non-covalent interactions the orientation (Keesom) and induction (Debye) forces have traditionally but confusingly elicited the impression that they have to be considered polar forces. For the longest time this illusion has made it difficult to form a clear distinction between the in reality apolar van der Waals interactions and the real polar (i.e. Lewis acid–base) interactions.

However, through Lifshitz's (1955) approach it became clear that, on a macroscopic scale, all three types of van der Waals interactions should be treated together, in the manner already outlined by Hamaker (1937) for macroscopic-scale van der Waals–London forces. Thus, on a macroscopic scale, non-retarded van der Waals–London interactions (up to a distance slightly under 10 nm) and van der Waals–Keesom and Debye interactions, all decay as the square of the distance for plane-parallel surfaces, and simply proportional to the distance between two spheres (as measured between the spheres' edges, not their centers) as well as between a sphere and a flat surface.

Chaudhury (1984) adapted Lifshitz's approach to colloidal and interfacial systems. The designation by Visser (1972) of LW interactions is followed here for the combined (Keesom + Debye + London)–van der Waals interactions. The Hamaker (also sometimes called van der Waals) constant of material, i (A_i) is proportional to the LW (or apolar) component of the surface tension of material, i, i.e. γ_i^{LW} (van Oss et al., 1988):

$$A_i = 24\,\pi\,\ell_o^2\,\gamma_i^{LW} \tag{1}$$

where ℓ_o is the minimum equilibrium distance to which two condensed-phase surfaces can approach one another. At 20°C, $\ell_o = 0.157 \pm 0.01$ nm (SD) (van Oss et al., 1988; van Oss, 1994, pp. 154–160), so that:

$$A_i = 1.8585 \times 10^{-14}\,\gamma_i^{LW} \tag{2}$$

where A_i is expressed in ergs (1 erg = 10^7 J), when γ_i^{LW} is expressed in units of ergs/cm^2 which are equivalent with units of mJ/m^2.

Two equal spheres, of radius, R, decay as a function of distance, ℓ, between the spheres' edges, as far as their LW interactions are concerned as (see also Table 1):

$$\Delta G_{iwi}^{LW}(\ell) = -AR/12\,\ell \tag{3}$$

For the LW interactions between a sphere with radius, R, and a flat plate, the decay as a function of distance, ℓ, is:

$$\Delta G_{iwi}^{LW}(\ell) = -AR/6\,\ell \tag{4}$$

and:

$$\Delta G_{iwi}^{LW}(\ell) = -2\,\gamma_{iw}^{LW} \tag{5}$$

where γ_{iw}^{LW} is the apolar (LW) interfacial tension between i and w:

$$\gamma_{iw}^{lw} = \left(\sqrt{\gamma_i^{LW}} - \sqrt{\gamma_w^{LW}}\right)^2 \tag{6}$$

so that γ_{iw}^{LW} is always positive. γ_i^{LW} is the apolar surface tension component of material, i, which is directly proportional to the Hamaker constant of material i (A_i), as indicated above, in eqs (1) and (2).

Polar, or Lewis AB (electron-acceptor/electron-donor) interactions and their decay with distance. In polar or Lewis AB interactions, the free energy of interaction between two parallel flat surfaces, immersed in water, is a major part of the total interfacial (IF) interaction energy (van Oss, 1994a, pp. 37–42):

$$\Delta G_{iwi}^{IF} = \Delta G_{iwi}^{LW} + \Delta G_{iwi}^{AB} \tag{7}$$

and

$$\Delta G_{iwi}^{IF} = -2\,\gamma_{iw} \tag{8}$$

where γ_{iw} is the total interfacial tension between the surface of material, i, and water, w. Furthermore, γ_{iw} consists of:

$$\gamma_{iw} = \gamma_{iw}^{LW} + \gamma_{iw}^{AB} \tag{9}$$

where

$$\gamma_{iw}^{AB} = 2\left(\sqrt{\gamma_i^+\gamma_i^-} + \sqrt{\gamma_w^+\gamma_w^-} - \sqrt{\gamma_i^+\gamma_w^-} - \sqrt{\gamma_i^-\gamma_w^+}\right) \tag{10}$$

and where γ_i^+ is the electron-acceptor parameter of the polar surface tension component of i, γ_i^{AB} and γ_i^- is its electron acceptor parameter, such that:

$$\sqrt{\gamma_i^{AB}} = 2\sqrt{\gamma_i^+\gamma_i^-} \tag{11}$$

and:

$$\gamma_i = \gamma_i^{LW} + \gamma_i^{AB} \tag{12}$$

The total interfacial free energy of interaction between

surfaces of i, immersed in water, w, then is [combining eqs (6), (8) and (10)]:

$$\Delta G_{iwi}^{IF} = \underset{I}{-2\left(\sqrt{\gamma_i^{LW}} - \sqrt{\gamma_w^{LW}}\right)^2} \underset{II}{-4\left(\sqrt{\gamma_i^+ \gamma_i^-}\right.} \\ + \underset{III}{\sqrt{\gamma_w^+ \gamma_w^-}} - \underset{IV}{\sqrt{\gamma_i^+ \gamma_w^-}} - \underset{V}{\sqrt{\gamma_i^- \gamma_w^+}}\left.\right) \quad (13)$$

(see also Table 2).

ΔG_{iwi}^{AB} (at contact), consisting of terms II, III, IV and V of eq. (13), decays for two equal spheres of radius, R, as a function of the distance, ℓ, between the spheres' edges as:

$$\Delta G_{iwi}^{AB}(\ell) = \pi R \lambda \Delta G_{\ell_0}^{AB^{11}} \ \exp\left[(\ell_o - \ell)/\lambda\right] \quad (14)$$

where $\Delta G_{\ell_0}^{AB^{11}}$ is the value of ΔG_{iwi}^{AB} indicated above in eq. (13), terms II, III, IV and V, for the AB free energy of interaction between two flat parallel surfaces of i, at closest approach ($\ell = \ell_o$), immersed in water, w. Similarly, for a sphere and a flat plate of material, i, in water:

$$\Delta G_{iwi}^{AB}(\ell) = 2\pi R \lambda \Delta G_{\ell}^{AB^{11}} \ \exp[(\ell_o - \ell)/\lambda] \quad (15)$$

where in both eqs. (14) and (15), the characteristic length of water $\lambda = 1.0$ nm for distances $\ell \geq 1.0$ nm.

Electrical double layer interactions and their decay with distance. Whilst ΔG^{LW} and ΔG^{AB} are measured together, via contact angle determinations, the free energy of electrostatic, or more precisely, electrical double layer interactions (ΔG^{EL}), is measured by entirely different, electrokinetic approaches, usually by measuring electrophoretic or electro-osmotic velocities, which yield the ζ-potential of particles or molecules when placed in an electric field (see e.g. Hunter, 1981; van Oss, 1994a, pp. 46–65, 128–153). The ζ-potential corresponds to the potential at the slipping plane, which is proportional to the velocity observed during dynamic measurements in a DC electric field. For conversion to free energies one needs to obtain the ψ_0-potential, which corresponds more closely to the potential at the very edge of charged particles or molecule (i.e. inside the slipping plane) according to:

$$\psi_o = \zeta \ (1 + z/R) \exp \kappa z \quad (16)$$

where z is the distance from the particle's surface to the slipping plane (usually around 0.3–0.5 nm), R is the radius of the charged particle or molecule and $1/\kappa$ is the thickness of the diffuse double layer ($1/\kappa = 0.8$ nm at physiological ionic strength, i.e. at $\mu = 0.15$; at $\mu = 0.10$, $1/\kappa = 1.0$ nm, and at $\mu = 0.01$, $1/\kappa = 3.16$ nm). Equation (16) is valid for ξ-potential values up to about 50 mV.

The EL interaction between two equal spheres with radius, R, decays as a function of distance, ℓ, as:

$$\Delta G_{iwi}^{EL}(\ell) = 0.5 \epsilon R \ \psi_0^2 \ln[1 + \exp(-\kappa\ell)] \quad (17)$$

where ε is the dielectric constant of the liquid medium. For a sphere of radius R and a flat plate:

$$\Delta G_{iwi}^{EL}(\ell) = \epsilon R \ \psi_0^2 \ln[1 + \exp(-\kappa\ell)] \quad (18)$$

Equations (3), (4), (14), (15), (17) and (18) are also grouped together in Table 1.

Proportionality of the radius of curvature of curved bodies with the free energies of all three classes of non-covalent interactions. In Table 1 it can be seen that the values of the LW, AB and EL energies of interaction involving spheres of radius R are all proportional to R, at contact, as well as at a distance. It is important to note that this also applies to distally protruding parts of particles or cells, such as in cases of cells with microscopic-scale pointy processes with a small radius of curvature which allows them readily to penetrate macroscopic-scale repulsion fields; see Fig. 1, below.

Long-range and short-range interactions. Aspecific macroscopic repulsions (which keep dispersed peripheral blood cells apart from one another and allow the aqueous solubility of, for example, plasma proteins) and specific microscopic attractions are both long-range in nature. For instance, the aspecific, macroscopic-scale mutual repulsion which keeps plasma proteins in aqueous solution typically still is at least of the order of $+0.5$ kT at an inter-protein (edge-to-edge) distance of about 5 nm. Similarly, in the absence of the above-mentioned aspecific repulsion, the interaction of an advantageously oriented antigen such as a biopolymer with an immunodominant epitope of a small radius of curvature directed toward a paratopic site of an antibody molecule, would give rise to a specific microscopic-scale mutual attraction which also would make itself felt with an energy of -0.5 kT, at a distance of about 5 nm. In most real cases, however, the macroscopic-scale aspecific repulsion, being operative between much larger bodies and/or surface areas than the microscopic-scale specific attraction between much smaller discrete sites, is quantitatively significantly stronger than the specific microscopic-scale attraction.

The mechanism of this locally prevailing microscopic attraction is further explained below. This mode of analysis is alluded to as the extended DLVO (XDLVO) approach. [DLVO is named after Derjaguin and Landau (1941) and Verwey and Overbeek (1948), who plotted the LW attraction and the EL repulsion as a function of distance, whereas XDLVO extends the approach to interactions taking place in water, by also incorporating the AB interactions (van Oss *et al.*, 1990; Wu *et al.*, 1999; Grasso *et al.*, 2002).] For an earlier brief (qualitative) introduction to the XDLVO approach, see also van Oss (1997).

It is of course at 'contact', i.e. at the minimum equilibrium distance for non-covalent interactions (van Oss and Good, 1984), at $\ell_0 = 0.157$ nm ($\pm$ 0.01 nm, SD; van Oss *et al.*, 1988; van Oss, 1994a, p. 157), that the specific, microscopic-scale attractive forces (if and when these prevail) are at a maximum, as the specific, truly short-range interactions take over. However, in cases where the long-range macroscopic-scale repulsion forces are so strong that they prevent specific microscopic-scale attraction forces achieving close-range contact at all, only a long-range equilibrium may exist, creating an unbridgeable zone between the two mutually repelling entities.

Quantitative expression of hydrophobicity and hydrophilicity. The quantitative degree of hydrophobicity or hydrophilicity of a material, i, is expressed as the IF free energy of interaction between two entities, i, immersed in

water, w, as ΔG_{iwi}^{IF} (van Oss and Giese, 1995), where:

$$\Delta G_{iwi}^{IF} = \Delta G_{iwi}^{LW} + \Delta G_{iwi}^{AB} \qquad (19)$$

see also eq. (13) and Table 2. When $\Delta G_{iwi}^{IF} < 0$, i, is hydrophobic and the (negative) value of ΔG_{iwi}^{IF} then is the quantitative measure of the degree of hydrophobicity of i. Conversely, when $\Delta G_{iwi}^{IF} => 0$, i is hydrophilic and the (positive) value of ΔG_{iwi}^{IF} then is the quantitative measure of the degree of hydrophilicity of i (van Oss and Giese, 1995).

In Table 2, eq. (13) is once more displayed, together with the numerical examples for a completely hydrophobic (apolar) compound (octane), and for two strongly polar hydrophilic materials (solid dextran, T150; and dissolved polyethylene oxide, 6000, also known as polyethylene glycol 6000). For both cases the large free energy of attraction of the hydrophobic effect is prominently obvious as term III, which represents the polar (AB) free energy of cohesion of water, of -102 mJ/m^2. For the apolar molecule octane, term III is the only term with a value other than zero, because even the usually attractive (i.e. negative) LW term is zero, as in this case the γ^{LW} of water is virtually identical to the γ^{LW} of octane, so that term I here is also zero. With molecules or materials, i, with a γ^{LW} that differs from that of water, there is an additional (usually feebly) attractive (i.e. negative) value for term I. This is the reason why both ΔG_{iwi}^{LW} and ΔG_{iwi}^{AB} [cf. eq. (19)] are incorporated in the quantitative definition of hydrophobicity and hydrophilicity. ΔG_{iwi}^{EL} is not taken into account in this definition because it is measured with a totally different (i.e. electrokinetic) methodology, it is often not measured at all, and its value, when measured, is strongly dependent on the ambient ionic strength, in contrast with ΔG_{iwi}^{LW} and ΔG_{iwi}^{AB}. Finally, especially in biological systems, the value of $|\Delta G_{iwi}^{EL}|$ is usually even lower than that of $|\Delta G_{iwi}^{LW}|$.

Concerning hydrophilic repulsion, the example given in Table 2 is that of solid dextran (e.g. as in Sephadex). It should be noted that solid, polar, non-ionic hydrophilic surfaces virtually always are monopolar (van Oss *et al.*, 1987). They are also, equally invariably, monopolar electron-donors (van Oss *et al.*, 1997). There is a strong repulsion between two dextran surfaces, immersed in water, caused by the polar free energy of adhesion between dextran and water, via the attraction between the strong electron-donor of dextran and the electron-acceptor of water [cf. term V of eq. (13)], see also Table 2), amounting to $+149.8$ mJ/m^2. However, after deducting the ever-present hydrophobic attraction term of -102 mJ/m^2 due to the polar cohesion of water (term IV), as well as a relatively small LW attraction of -6.6 mJ/m^2 (term I), there remains a smaller but still sizeable repulsion between two dextran surfaces, immersed in water, of $+41.2$ mJ/m^2 (Table 2). Roughly the same applies to polyethylene oxide (polyethylene glycol). (It should be noted that dextran molecules, when dissolved in water, are bipolar, with a small but non-neglible γ^+ of 2.0 mJ/m^2 and a high γ^-, of 57.0 mJ/m^2, cf. Docoslis *et al.*, 2000.)

Interactions between two different materials, immersed in water. The interfacial free energy of interaction between two **different** molecules or particles, 1 and 2, immersed in

water, w, is described as (van Oss, 1994, p. 23):

$$\Delta G_{iw2}^{IF} = \left(\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_2^{LW}}\right)^2 - \left(\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_W^{LW}}\right)^2$$
$$- \left(\sqrt{\gamma_2^{LW}} - \sqrt{\gamma_W^{LW}}\right)^2 + 2\left[\sqrt{\gamma_W^+}\left(\sqrt{\gamma_1^-} + \sqrt{\gamma_2^-} - \sqrt{\gamma_W^-}\right)\right.$$
$$\left. + \sqrt{\gamma_W^-}\left(\sqrt{\gamma_1^+} + \sqrt{\gamma_2^+} - \sqrt{\gamma_W^+}\right) - \sqrt{\gamma_l^+ \gamma_2^-} - \sqrt{\gamma_1^- \gamma_2^+}\right]$$
$$(20)$$

[in the case of solid polar entities, which most often are monopolar in a number of the terms in eq. (20), it can often be zero or positive].

It still comes as a surprise to many that hydrophobic attractions often occur between a hydrophobic and a hydrophilic entity, in aqueous media. It should be remembered, however, that this type of interaction between a hydrophobic and a hydrophilic material, in water, forms the basis of the mechanism of the separation of proteins in reversed-phase liquid chromatography (RPLC). In RPLC hydrophilic proteins adsorb to hydrophobic beads (e.g. coated with octadecyl groups). Elution subsequently takes place by decreasing the hydrogen-bonding free energy of cohesion of water, by gradual dilution of the aqueous medium with, for example, acetonitrile, thus diminishing the hydrophobizing capacity of the liquid phase.

Measurement

The Contact Angle Method. The measurement method of choice for the quantitative determination of all the applicable γ^{LW}, γ^+ and γ^- values is the contact angle method. To that effect one uses the Young–Dupré equation (van Oss, 1994, pp. 21–22, 89–107):

$$(1 + \cos\theta)\gamma_L = 2(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^+ \gamma_L^-} + \sqrt{\gamma_S^- \gamma_L^+})$$
$$(21)$$

where θ is the measured (advancing) contact angle, γ_L is the surface tension of the liquid with which the contact angle is measured, γ_S^{LW}, γ_S^+ and γ_S^- are, respectively, the apolar (LW) component of the surface tension of the solid surface, S, and the electron-acceptor and the electron-donor parameters of the polar (AB) component of S; cf. eqs (11) and (12). The surface thermodynamic properties (at 20 °C) of the liquids that are most frequently used for measuring contact angles are given in Table 3. It should be noted that, in order to obtain a finite, measurable contact angle, the surface tension of the liquid must be greater than that of the solid surface:

$$\gamma_L > \gamma_S \qquad (22)$$

As there are three fundamental properties of polar materials, $\gamma_S^{LW}, \gamma_S^+$ and γ_S^-, one must measure contact angles with three different liquids, e.g. one apolar liquid (diiodomethane, see van Oss *et al.*, 2001a), and two polar ones, of which one must be water. Thus the Young–Dupré equation [eq. (21)] must be used three times for the

Table 3. Surface tension components (γ_L^{LW} and γ_L^{AB}) and parameters of γ_L^{AB} (γ_L^+ and γ_L^-) (in mJ/m^2 for the most commonly used high-energy contact angle liquids (b) at 20 °C[a]

Liquid		γ_L	γ_L^{LW}	γ_L^{AB}	γ_L^+	γ_L^-
Polar	Water	72.8	21.8	51.0	25.5[b]	25.5[b]
	Glycerol	64.0	34.0	30.0	3.92	57.4
	Formamide	58.0	39.0	19.0	2.28	39.6
Apolar	Diiodomethane	50.8	50.8	0	≈0.01[c]	0

[a] cf. van Oss (1994a, p. 183).
[b] This is the standard assumption. Although the γ_L^{AB} value for water is well-established, the γ_L^+/γ_L^- ratio is not known at present. However for the expression of the values of interfacial tensions with water (γ_{iw}) and of all free interfacial energies of interaction (ΔG^{IF}) involving water, or in water as the liquid medium, the above assumption cancels out, γ^{AB}, γ_{iw} and ΔG^{IF} values are quantitatively expressed in absolute SI units (van Oss, 1994a, pp. 24–26).
[c] cf. van Oss *et al.* (2001a).

determination of these three surface thermodynamic properties, at 20 °C, for each solid material, *S*; see Table 4 for the surface thermodynamic properties of a number of biological and other organic material that may be of interest.

Macroscopic-scale interactions. It should be noted that the *γ*-values obtained via contact angle measurements and calculated from the contact angles by means of the Young–Dupré equation [eq. (21)], as outlined above, usually pertain

Table 4. Surface tension properties of some liquid molecules and of a number of solid (or dried) polymers and biopolymers, at 20 °C, in mJ/m^2 (van Oss, 1994a, Chap. XIII)

Material	γ^{LW}	γ^{AB}	γ^+	γ^-
Water (liquid)	72.8	51.0	25.5[a]	25.5[a]
Octane (liquid)	21.6	0	0	0
Hexadecane (liquid)	27.5	0	0	0
HSA, dry[b], pH 4.85	44.0	1.0	0.03	7.6
HSA, dry[b], pH 7.0	41.0	0.4	0.002	20.0
HSA, one layer of hydration, pH 7.0	26.6	1.03	0.003	87.5
HSA, two layers of hydration, pH 7.0	26.8	35.7	6.3	50.6
IgG, dry[b], pH 7.0	42.0	3.2	0.3	8.7
IgG, hydrated, pH 7.0	34.0	17.3	1.5	49.6
IgA, hydrated, pH 7.0	26.8	0	0	93.0[c]
Polyethylene	33.0	0	0	0
Poly(ethyleneoxide)	43.0	0	0	64
Cellulose	44.0	10.5	1.6	17.2
Dextran T-150, dry[b]	42.0	0	0	55

HSA, human serum albumin.
[a] These are the standard assumed values for water at 20 °C. All the other γ^+ and γ^- values in this table are relative with respect to these standard values for water (van Oss *et al.*, 1987, 1988). However, the γ^{LW} and γ^{AB} values are absolute and independent of the γ^+ and γ^- standard values for water.
[b] All 'dry' values pertain to air-dried surfaces of these materials.
[c] IgA is the most hydrophilic of human immunoglobulins; it cannot be easily purified by reversed-phase liquid chromatography but it can be purified by means of hydrophobic interaction chromatography.

only to the macroscopic-scale, or global (i.e. averaged) surface properties of a condensed-phase material. This is especially true of the surface properties of living cells, proteins, polysaccharides, etc. On a macroscopic level all such biological cells or macromolecules tend to repel each other when placed in an aqueous medium. As a consequence of this AB repulsion peripheral blood cells can form a stable suspension in the blood and lymph circulation and do not normally clump together (EL-driven repulsion plays a minor role in the stability of erythrocytes, and is virtually negligible in the case of leukocytes). Plasma proteins remain in aqueous solution due to their AB repulsion. Both stably suspended peripheral blood cells and dissolved plasma proteins have, distally, fairly homogeneously hydrophilic surfaces, to obviate aspecific mutual attraction of a hydrophobic nature. When any hydrophobic patches are present on the distal surface of either blood cells or plasma proteins, these have to be small and few in number. With blood cells, hydrophobic moieties are sited close to or on the cell membranes, well below the glycocalyx strands. With plasma proteins, hydrophobic peptides are either situated at the interior of the protein's tertiary structure, or in a concavity or cleft of a protein's surface, as in IgG where hydrophobic patches must not give rise to aspecific clumping, while remaining able to function as parts of receptor sites which can participate in specific, microscopic-scale ligand–receptor (epitope–paratope interactions).

The macroscopic-scale free energies of repulsion between cells and/or biopolymers, immersed in water, can be derived from the surface-thermodynamic properties of these entities, determined by means of contact angle measurements, obtaining ΔG_{iwi}^{mac} via eq. (13), or ΔG_{iw2}^{mac} via eq. (20).

Microscopic-scale interactions. The determination of the microscopic-scale surface properties of specifically attractive sites follows a different approach. It is usually feasible to determine the equilibrium, or rather affinity constant (K_{aff}), of the specific attraction and hence the value of the specific, microscopic level value of ΔG_{iw2}^{mic} (van Oss *et al.*, 2001b) according to:

$$\Delta G_{iw2}^{mic} = -kT \ln(55.56 K_{aff}^{t \to 0}) \qquad (23)$$

where the multiplication factor of 55.56 (corresponding to

the number of moles water per liter) is required to be able to express $K_{\text{aff}}^{t \to 0}$ in terms of liters/mol, instead of as inverse mol fractions [in eq. (23), k is Boltzmann's constant, i.e. $k = 1.38 \times 10^{-21}$ J per degree Kelvin, and T is the absolute temperature in degrees Kelvin), see van Oss, 1994b.

Influence of hysteresis. In order to avoid, or at least to minimize the influence of hysteresis, $K_{\text{aff}}^{t \to 0}$ is determined via Langmuir isotherms constructed after different times of contact between ligand, 1, and receptor, 2, in water; by extrapolation to zero time exposure, $K_{\text{aff}}^{t \to 0}$ is obtained for each Langmuir isotherm (plots of amount of soluble reagent bound vs its concentration) where the slope of the tangent to the Langmuir curve at zero concentration of the soluble reagent indicates $K_{\text{aff}}^{t \to 0}$). Briefly, when Langmuir plots are made at three or four different times of exposure of the same solute, adhering to a given specific ligand or surface site, a plot of the different K_{aff} values found, vs. the different exposure times, when extrapolated to $t = 0$, will yield $K_{\text{aff}}^{t \to 0}$ (Docoslis *et al.*, 2001). Then, estimating, for instance in the case of protein–protein interactions, the proportions of the LW, AB and EL contributions from the usual shares of LW + EL contributions in the interactions of such materials [where the share of the LW + EL contributions represents no more than between 6 and 12% of the whole (van Oss *et al.*, 2002) and knowing the total $\Delta G_{iw2}^{\text{mic}}$ value, the value of the usually rather dominant $\Delta G_{iw2}^{\text{ABmic}}$ (as compared with $\Delta G_{iw2}^{\text{LWmic}}$ and $\Delta G_{iw2}^{\text{Elmic}}$), follows as a fairly accurate first approximation. The attractive value of $|\Delta G_{iw2}^{\text{mic}}|$ is usually several times smaller than that of the repulsive $|\Delta G_{iw2}^{\text{mac}}|$. Examples of values of $\Delta G_{iw2}^{\text{mac}}$ and $\Delta G_{iw2}^{\text{mic}}$, and of an integrated combination of the two (all as a function of distance) are discussed above. The only known class of specific antigen–antibody (Ag–Ab) interactions which do not undergo hysteresis and where therefore $K_{\text{aff}} = K_{\text{eq}}$, are Ag–Ab interactions which are purely due to electrical double layer forces. For this and further discussion of the influence of hysteresis, see van Oss (1997).

DISCUSSION

How a specific short-range microscopic attraction can overcome a stronger, long-range, aspecific macroscopic repulsion field

As is clear from eqs (3), (4), (14), (15), (17) and (18), which are also shown grouped together in Table 1 as the equations governing the XDLVO rules for free energies of LW, AB and EL interactions as a function of distance (involving spherical entities), the free energies for all three classes of interaction are proportional to the radii, R, of the spheres, and/or of the curvatures of processes protruding distally from these spheres. This proportionality between free energies of interaction and R means, for instance, that a smooth hydrophilic particle or cell, with a radius, R, will undergo a macroscopic-level long-range repulsion from a flat hydrophilic surface (or from a bigger hydrophilic cell whose surface may, as a first approximation, be taken as flat) of about +57 kT at close range. This suffices to prevent such a cell from approaching that flat hydrophilic surface closely
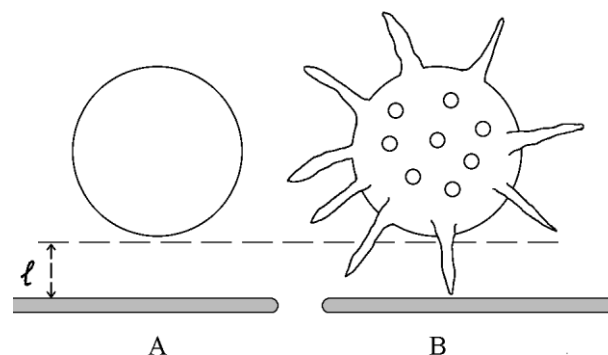
Figure 1. Schematic presentation of differences in accessibilty of round spherical bodies and a flat plate, for a smooth sphere with a relatively large radius (left) and a similar sphere with essentially the same main radius but which is, in addition, endowed with protruding long, thin processes, each with a small radius of curvature (right). (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 $\ell = 4$ or 5 nm. (B) A similar spherical cell or particle endowed with long thin spikey processes whose ends have a small radius of curvature which can readily penetrate the macroscopic-scale repulsion field and thus achieve microscopic-scale specific contact; see text, and Table 2. In both (A) and (B) the dotted line indicates the limit of closest approach (ℓ) for a smooth hydrophilic cell or particle with a relatively large radius of curvature.

enough to make any contact with it. On the other hand, if the same hydrophilic particle or cell is endowed with thin spiky protuberances with a distal radius of curvature of their tip of for instance $r = 1/100\ R$, such protuberances will only be repelled at close range with an energy of about +0.6 kT each, which would, on average, enable one or more such tips, when well-placed in proximity of a microscopic-scale specific attractive site, to make contact and bind with a multiple-kT bond strength to that receptor site. In this manner a specific microscopic-scale short-range attraction which is (even at close-range) weaker than the global, macroscopic-scale aspecific repulsion field, can nonetheless locally overcome that repulsion field.

This general mechanism of making specific contact with receptors on cells notwithstanding a general strong aspecific repulsion field, is sketched in Fig. 1. It is used by many viruses (e.g. influenza virus, adenoviruses, bacteriophages, etc.) which have adopted this strategy for binding to the cells they need to enter in order to survive and multiply. Phagocytic cells (e.g. polymorphonuclear neutrophils, or PMNs) also use this approach. PMNs extrude long thin pseudopodia with a small distal radius of curvature, for making contact with bacteria, which they then engulf and subsequently digest. Other phagocytic cells, e.g. macrophages, use a comparable approach, by means of thin, protruding folds. When a PMN's capacity for forming pseudopodia is diminished, its bacterial uptake is also significantly decreased; the same applies to macrophages, when they are forced to become more smoothly spherical (van Oss *et al.*, 1972, 1975; van Oss, 1978).

When platelets become 'sticky' under the influence of adenosine diphosphate (ADP), their surface tension proper-

ties do not change, but their shape does (White, 1968; van Oss *et al.*, 1972, 1975). In the presence of ADP, platelets become spiculated, with protruding microvilli with a small radius of curvature which allows them to make adhesive contact with other cells and tissues (White, 1968; van Oss *et al.*, 1972, 1975, pp. 108–124; van Oss, 1978).

Another example of the role of protruding processes with a small radius of curvature is the immunodominance of those surface sites of antigenic proteins with the smallest radii of curvature, which have aptly been called 'elbows' by Atassi (1975). These pointy surface epitopes owe their immunodominance to their favorable location on the native protein's tertiary configuration's outer surface as well as to their small radius of curvature which makes these epitopes the most likely to make contact with the appropriate B lymphocyte by being best fitted to pierce the latter's macroscopic repulsion field. It should also be noted that these elbow-shaped immunodominant surface epitopes of soluble proteins are overall hydrophilic as a *conditio sine qua non* of being situated at the protein–water interface. These hydrophilic epitopes can nevertheless readily combine specifically with a relatively hydrophobic paratope, which for protection against precipitation of dissolved immunoglobulin molecules is located inside a concavity, or cleft, of their Fab moiety. (With respect to the common occurrence of hydrophobic attractions between a hydrophilic and a hydrophobic entity, see above.)

How hydrophilic repulsions overcome the strong omnipresent hydrophobic attraction, to achieve a net repulsion

In interactions taking place in water, the free energy of hydrophobic attraction between two completely apolar molecules (e.g. octane molecules, cf. Table 2), $\Delta G_{iwi}^{AB} = -102$ mJ/m^2, being equal to the polar (AB) free energy of cohesion of water ($\Delta G_{ww}^{ABcoh} = -102$ mJ/m^2). This hydrophobic attraction energy does not even disappear in interactions between the most hydrophilic entities, *i* (for instance, dextran, cf. Table 2), when immersed in water. To achieve a net hydrophilic repulsion, the absolute value of the strong polar interaction between the hydrophilic entities and water molecules [due to the electron-donor of *i* (in this case, dextran) attracting the electron-acceptor of water, *w*, cf. term V of eq. (13), as exemplified in Table 2] has to be greater than the hydrophobic attraction energy [term III of eq. (13) of $|102|$ mJ/m^2, plus a small Lifshitz–van der Waals attraction, term I of eq. (13), of (in this case) $|6.6|$ mJ/m^2]. In this manner the difference of $+41.2$ mJ/m^2 is the net free energy of hydrophilic repulsion of the case in point, i.e. the difference between the gross free energy of repulsion caused by the strong polar interaction between dextran and water, and the hydrophobic plus Lifshitz–van der Waals attractions, in water. Thus, of the initial $+149.8$ mJ/m^2, only $+41.1$ mJ/m^2 represents the final, net free energy of hydrophilic repulsion between the dextran particles of the cited example, when immersed in water. One rarely encounters net free energies of hydrophilic repulsion significantly much exceeding $+50$ mJ/m^2.

Role of bacterial capsules as an anti-phagocytic stratagem

Circulating peripheral phagocytic cells such as polymorphonuclear leukocytes (PMNs, or neutrophils) as well as monocytes or macrophages, do not react with, but repel all circulating erythrocytes, always leaving a microscopically noticeable distance between the leukocytes' and the erythrocytes' surfaces (Anonymous, ca. 1952). This repulsion is mainly due to the net Lewis AB repulsion between the hydrophilic outer layers of the glycocalices of leukocytes and erythrocytes. Similarly, phagocytic cells (PMNs as well as monocytes and macrophages) repel encapsulated bacteria (Klainer and Geis, 1973; van Oss *et al.*, 1975), which prevents their phagocytic uptake as long as anti-capsular antibodies are absent. However, in the presence of (opsonizing) antibodies against capsular biopolymers, the aspecific macroscopic-scale repulsion between phagocytes and bacteria is locally diminished and specific, microscopic-scale contact is made between opsonized bacterial surfaces and the Fc receptors on the phagocytic cells, which then is followed by phagocytic engulfment (Anonymous, ca. 1952; Wilson *et al.*, 2002). In this process complement can also play a role (Wilson *et al.*, 2002). When, however, complement-fixing antibodies against non-capsular bacterial antigens of the bacterial cell wall are used, these, as well as complement factors, can penetrate inside the (fairly unsubstantial) capsule and bind to the bacterial cell walls, without inducing contact with phagocytes or causing phagocytic ingestion (van Oss and Gillman, 1973; Cunningham *et al.*, 1975; Wilson *et al.*, 2002). When the capsule of, for example, *Staphylococcus aureus*, strain Smith, is mechanically removed, the bacterial cell surface becomes less hydrophilic and its phagocytic uptake is strongly enhanced (van Oss *et al.*, 1975). The same behavior was noted with *Salmonella typhimurium*, where 'rough' strains were readily phagocytized whilst 'smooth' (i.e. encapsulated) strains resisted phagocytosis (Cunningham *et al.*, 1975).

Although both proteinaceous as well as polysaccharide bacterial capsules aspecifically repel phagocytic cells, erythrocytes, etc., bacterial capsular structures can also specifically attract and bind to mammalian cells such as squamous cells (Berkeley *et al.*, 1980).

Repulsive action of polyethylene oxide and its applications

PEO is one of the strongest monopolar electron-donating polymers known. Two strands of PEO 6000, dissolved in water, upon close approach repel one another with a free energy of repulsion of about $+2.8$ kT (or about $+1.6$ kcal/M; van Oss *et al.*, 1990; van Oss, 1994a, p. 222). Thus, strands of PEO (PEG), attached to a protein molecule, can significantly enhance the aspecific repulsive force exerted in water between such a 'pegylated' protein molecule and other proteins or cells. This is not because of any ill-defined so-called 'steric' repulsive force caused by the presence of attached PEO strands, but simply as a consequence of the net non-covalent Lewis AB repulsion between strongly electron-donating molecules when they are immersed in

water (van Oss *et al.*, 1987). When one knows the surface thermodynamic properties of PEO (van Oss, 1994a, p. 177) and of water (*ibid.*, p. 183), one can find the value of the free energy of repulsion in question, using eq. (13); see also Table 2. This mutual repulsion between two PEO macro-molecules, immersed in water, is also a *conditio sine qua non* for their aqueous solubility (van Oss and Good, 1996; Docoslis *et al.*, 2000).

By far the largest industrial application of PEO is in the stabilization of (e.g. polystyrene) latex particle suspensions, for water-based paints. For preparative laboratory- and larger-scale separation and purification purposes PEO is used, generally in conjunction with dextran, for aqueous partition separations. Here aqueous solutions of, for example, 3–8% each of PEO and dextran are mixed together and after a relatively short time interval the mixed solution separates into two phases, with most of the dissolved dextran in the bottom phase and most of the dissolved PEO (PEG) in solution in the top phase (Albertsson, 1986; Zaslavsky, 1995). When several different biopolymers are dissolved in the mixed PEO–dextran solution, the different biopolymers find themselves, after phase separation, partitioned in different proportions (characteristic to each biopolymer) in one or the other of the two phases. Proteins (e.g. enzymes), peptides, nucleic acids, viruses and even cells have been separated by this method (Albertsson, 1986). Aqueous partition has also been used analytically, e.g. to estimate the degree of hydrophobicity/hydrophilicity of amino acid side-chains (Zaslavsky, 1995).

PEO-coated glass surfaces at the inside of glass vessels have been used for the crystallization of proteins, to avoid attachment of protein molecules to the glass surface. In this manner pristine protein crystals could be obtained, for use in X-ray diffraction analysis of such protein crystals, to obtain their tertiary structure (Van Alstyne *et al.*, 1999). Before the application of PEO-coated glass crystallization vessels, undeformed and/or unadsorbed protein crystals were best obtained by performing the crystallization under conditions of microgravity, i.e. during Shuttle space flights. The tertiary configuration of human serum albumin was first derived from X-ray diffraction studies obtained during a Shuttle flight (He and Carter, 1992).

PEO (PEG) in drug delivery. Drugs attached to hydro-phobic latex particles, which are attached to antibodies provided for specific binding to the desired organ, are virtually completely eliminated upon injection *in vivo* by the lymphatic system (formerly alluded to as the reticulo-endothelial system) in less than 2 min. When, on the other hand, such particles or vesicles intended as aids to drug delivery also have PEO (PEG) strands attached to them, they can remain in circulation for days, or even up to about a week; (see e.g. Illum and Davis, 1984; Illum *et al.*, 1987; Gref *et al.*, 1994). The mode of action is much like the action of phagocytosis-evading encapsulated bacteria, i.e. such 'pegylated' particles and leukocytes, as well as such particles and tissue cells effectively repel one another. Nonetheless, the protruding specific Fab moieties which are also attached to them can overcome the repulsion caused by PEO and thus make contact with the desired cells or tissues.

PEO (PEG) used as stealth vectors for gene delivery. Adenoviruses are promising vectors for gene delivery, but the presence *in vivo* of existing antibodies to adenoviruses as well as of killer cells drastically decreases the number of these virus particles that actually succeed in reaching their target organ. However, adenovirus particles to which PEO (PEG) has been attached are transformed into 'stealth vectors' which can evade opsonizing antibodies, killer cells and phagocytes This treatment even appears to make such stealth viruses toleragenic (Croyle *et al.*, 2001).

PEO (PEG) used as a non-denaturing linkage spacer for protein attachment to solid surfaces or particles. When adsorbed or covalently bound to hydrophobic polymer surfaces, proteins tend to become altered or denatured through changes induced in their tertiary configuration (Naim *et al.*, 1998). This denaturation can be obviated by attaching to such a surface or particle via a hydrophilic spacer arm consisting of PEO (Holmberg *et al.*, 1992). This approach is useful in ELISA-type immunoassays (which frequently use hydrophobic polymer surfaces as carriers), as it helps to avoid altering the antigenicity of attached proteins. The PEO-tethering approach has also been used with advantage in extracorporeal anti-tumor immunother-apy with Interleukin-2, as the PEO-mediated tethering appears to increase its half-life. PEO-tethering of collagen (as a coating for bio-implantable polymer surfaces for purposes of cell culture surfaces) proved useful in allowing 2.5 times more HeLa cells to become attached to surfaces treated with PEO-linked collagen, as compared with directly adsorbed collagen (Holmberg *et al.*, 1992).

Use of PEO (PEG) attached to single dissolved proteins. Antibody fragments, injected singly or attached to a carrier for purposes of drug delivery, have a short half-life. That half-life can be considerably increased by attaching PEO strands to Fab′ moieties. Unfortunately, the binding capacity of such pegylated Fab′ fractions decreases as their half-life increases. However with only two PEO strands, of 25 kDa, attached to the hinge area terminus of the Fab′, an improved binding capacity can be combined with an increased half-life (Chapman *et al.*, 1999)].

Antigens, when attached to PEO strands, can convert to toleragens. Sehon *et al.* (1987) and Sehon (1989) could successfully and permanently suppress the antibody re-sponse to ovalbumin (OA) with PEO-conjugated OA. Earlier, Lee and Sehon (1977) reported that they could abrogate the formation of reaginic antibodies in pre-sensitized mice with PEO-conjugated allergens. This, however, does not appear to be the general rule. Even though PEO-conjugated antigens can act as toleragens *in vivo* when administered *prior* to exposure to the antigen in question, PEO-conjugated antigens do not as a rule appear to be able to *abrogate* the anamnestic immunity to that antigen in animals that had been previously immunized with it as easily as the prevention of immunity in naive animals (see e.g. Atassi *et al.*, 1992; Oshima and Atassi, 2000).

Mechanism of PEO-linked toleragenicity. The precise mechanism of PEO (PEG)-induced tolerance of proteins such as OA has not, to date, been cogently described. However, it has been shown by Chapman *et al.* (1999) that,

even though the PEO (PEG) attached to Fab′-fragments causes the Fab′–PEO complexes to remain in circulation much longer than untreated Fab′ molecules, Fab′–PEO complexes retain their full antigen-binding capacity. Conversely, antigenic molecules such as OA (Sehon *et al.*, 1987; Sehon, 1989), when linked to PEO (PEG) retain their antibody-binding capacity, for antibody molecules as well as for antibody fragments even when these are attached to larger entities such as lymphocytes. Thus, single OA-PEO complexes can bind to those B-lymphocytes that have anti-OA receptors on their surface. However once such OA–PEO complexes attach to B-lymphocytes carrying anti-OA specific reactors in sufficient numbers to occupy a significant portion of these cells' surfaces, the PEO-induced macroscopic repulsion between such B-lymphocytes and other blood cells (e.g. T-lymphocytes) becomes sufficiently predominant to prohibit any close contact between the two types of lymphocytes, which then prevents the occurrence of the usual sequel of events which normally gives rise to the formation of anti-OA antibodies. This ultimately results in the deletion of all B-lymphocytes with anti-OA specificity.

According to this mechanism, despite the fact that the abrogation of existing humoral immunity to a given antigen (Ag) through the administration of Ag–PEO complexes appears more difficult than the prevention of immunity to that Ag in naive animals, the abrogation of existing immunity by the administration of large amounts of PEO-linked Ag should be possible. This may still be a promising approach to the abrogation of humoral autoimmunity (see e.g. Atassi *et al.*, 1992; Oshima and Atassi, 2000).

CONCLUSIONS

The non-covalent interaction forces, operative in aqueous media comprise: (a) on an atomic scale, van der Waals forces and on a molecular and macromolecular scale, LW forces; (b) Lewis AB forces; and (c) EL forces. All three forces decay as a function of distance according to different rules.

These rules have been described by the DLVO theory. In the classical DLVO theory only LW and El forces were considered. However, in water the AB forces have to be included, as they represent around 90% of the total non-covalent interaction forces, be they attractive or repulsive. Therefore, whilst the classical DLVO (LW + EL) approach does not suffice in water, the extended DLVO (XDLVO) approach (LW + AB + EL) is applicable to interactions in aqueous media.

Quantitatively the strongest interaction in water is the hydrophobic effect, which is always attractive. The hydrophobic attraction between molecules or particles immersed in water is caused by the hydrogen-bonding (AB) free energy of cohesion of water, which is always present.

Hydrophilic entities, immersed in water, can repel one another through net AB forces. Hydrophilic repulsion occurs when hydrophilic molecules, particles or cells attract water molecules more strongly than the AB cohesive attraction between water molecules.

A consequence of the use of hydrophilic coatings of surfaces with, for example, proteins, polysaccharides (e.g. bovine serum albumin, streptavidin or dextran), or poly-ethylene oxide, to obviate aspecific attachment of specific reagents (e.g. antibodies, enzymes, other ligands) is that one creates a strong-macroscopic-scale repulsion field. Thus, whilst in so doing, the specific entity does not become aspecifically adsorbed (which one aimed at), it becomes instead aspecifically repelled, which many workers do not sufficiently realize. This aspecific hydrophilic repulsion can significantly decrease the amount bound in the desired specific attraction, and it will also underestimate an observed kinetic rate constant of the specific interaction by one or two orders of magnitude.

There are different rules for the decay as a function of distance of the three different non-covalent interaction forces between spherical entities, immersed in water, but all three are proportional to the radius of curvature of the spherical entities. Thus, smooth, round hydrophilic particles, biopolymers or cells, with a relatively large radius, undergo a large net macroscopic-scale (AB) repulsion, which precludes contact with other similar entities. However, similar particles, biopolymers or cells, endowed with thin spicules or processes with a small distal radius of curvature, can readily overcome the AB repulsion and on a microscopic level achieve contact with other particles, cells or surfaces. This phenomenon is operative, *inter alia*, in the phagocytosis of bacteria, in cell contact made by viruses, and in the activation of platelets.

The free energies of aspecific, repulsive, macroscopic-scale interactions among identical or different spherical or flat entities immersed in water can be quantitatively determined by means of contact angle measurements.

Attractive, specific, microscopic-scale interactions between small, discrete sites (e.g. epitope–paratope, or ligand-receptor site interactions) on the other hand, are best quantified by determining the specific affinity (equilibrium) binding constant between these attractive sites. From the affinity constant, the free energy of specific (microscopic-scale) interaction follows and can be subdivided into LW, AB and EL contributions by other means.

The use of PEO (PEG) is exemplified in a number of applications, showing how linkage to PEO can: obviate capture of drugs by the lymphatic system; act as a stealth factor in gene delivery; prevent the denaturation of proteins upon their attachment to hydrophobic surfaces; and transform antigens into toleragens. In all these cases PEO (PEG) functions by endowing the entities it is attached to with its AB-based repulsive energy, due to its strong monopolar electron-donicity.

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