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C. J. Van Oss[a]; A. W. Neumann[b]; S. N. Omenyi[b]; D. R. Absolom[a]
[a] Immunochemistry Laboratory, Department of Microbiology, State University of New York at Buffalo, Buffalo, New York [b] Department of Mechanical Engineering, University of Toronto, Toronto, Ontario, Canada

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REPULSIVE VAN DER WAALS INTERACTIONS: THEIR ROLE IN VARIOUS SEPARATION METHODS

C.J. van Oss,[1] A.W. Neumann,[2] S.N. Omenyi[2] and D.R. Absolom[1]

Immunochemistry Laboratory,[1] Department of Microbiology,
State University of New York at Buffalo
Buffalo, New York 14214
and
Department of Mechanical Engineering,[2] University of Toronto,
Toronto, Ontario, Canada M5S 1A4.

I. INTRODUCTION

It has long been surmised, as a theoretical curiosity, that conditions could arise under which the sign of the van der Waals interaction between two different uncharged bodies, surrounded by a liquid, might be negative, i.e., that such bodies would repel each other. This possibility is implicit in Hamaker's classical paper on van der Waals-London interactions, although, remarkably enough, Hamaker somehow became persuaded that this view was erroneous, and wrote his paper with the apparent purpose to gainsay it: "If two particles are embedded in a fluid and the London-van der Waals force between particles and fluid is greater than between the particles themselves, it might be thought that the resultant action will be a repulsion rather than an attraction. As has been pointed out to the author by Dr. J.H. de Boer, this conclusion does not hold. Owing to a peculiar property of the London-van der Waals forces, the resultant force is generally attractive even when the particles are surrounded by fluid. This is a matter of considerable interest which warrants a detailed discussion."[1] However, in a more recent review on Hamaker constants, Visser stated explicitly: "When two materials are immersed in a liquid medium, and the interactions of each of these materials with that of the liquid medium is larger than the interaction between these materials themselves, spontaneous separation can occur due to dispersion forces only."[2] Fowkes demonstrated such a repulsive interaction with poly-(tetrafluoroethylene)-glycol-iron oxide.[3]

More recently, we have shown theoretically and experimentally that the sign of the net van der Waals interaction between two different solid bodies,[4] or between two different dissolved macromolecules,[5] in liquids, often is negative, i.e., they repel one another, even when they are electrically neutral, and when they are immersed in apolar liquids. Having elucidated the conditions under which the sign of the net van der Waals interaction between two different materials in liquids tends to become negative,[4,5] we proceeded to test the methodology arising from these considerations on the dissociation

of antigen-antibody bonds of the van der Waals-type,[6] and on the elution of proteins from hydrophobic chromatography columns,[7] the results of both of which confirmed the validity of the theory and the entire practicality of the ensuing experimental procedures.

Clearly the new capability to change the attraction between different (even neutral) solids and submerged in liquids, and/or dissolved macromolecules into a repulsion, has considerable implications for a variety of novel as well as traditional separation methods. A number of these implications are discussed here.

II. THEORY

For simplicity's sake we shall assume that the interaction between two different solid (or dissolved) bodies 1 and 2 in a liquid 3 may be represented as an interaction between semi-infinite slabs. Considering the Hamaker expression for the free energy for that case:[1]

$$\Delta F(d) = - A_{132}/12 \pi d^2 \qquad [1]$$

where A_{132} is the Hamaker coefficient of the interaction between bodies 1 and 2 in liquid medium 3 and d their separation distance. For the minimum separation distance d_o (and assuming that equation [1] is still valid at such small separation distances), the Hamaker coefficient can then be expressed as:

$$A_{132} = - 12 \pi d_o^2 \Delta F(d_o) \qquad [2]$$

The Hamaker coefficient A_{132} for the interaction between the two different bodies in a liquid can thus be calculated by means of equation [2], once one knows the free energy of adhesion between the two bodies, for which:

$$\Delta F_{132}^{adh} = \gamma_{12} - \gamma_{13} - \gamma_{23} \qquad [3]$$

where γ_{12} is the interfacial tension between bodies 1 and 2, and γ_{13} and γ_{23} are the interfacial tensions between, respectively, bodies 1 and 2 and liquid 3. The values for γ_{12}, etc., can be obtained from, e.g. those of γ_{1v} and γ_{2v}, via the equation of state approach,[8] while the values of γ_{1v}, etc. (v stands

for vapor) are easily determined by, e.g., the contact angle method.[9]

A_{132} can also be obtained in another way, by making use of:

$$A_{132} = A_{12} + A_{33} - A_{13} - A_{23} \quad [4]$$

For this approach A_{12}, A_{13} and A_{23} are obtained from:

$$A_{ij} = 12 \pi d_o^2 \Delta F_{ij}(d_o) \quad [5]$$

A_{33} can be derived from the free energy of cohesion:

$$\Delta F_{ii}^{coh} = -2\gamma_{iv} \quad [6]$$

In the case of liquid 3, γ_{3v} may be measured, e.g., with the pendant drop method,[10] or with the Wilhelmy method.[11]

In any case, a positive value for A_{132} implies that the net van der Waals interaction between particles (and/or macromolecules 1 and 2 will be attractive, and a negative value for A_{132} implies that the net van der Waals interaction between particles (and/or macromolecules) 1 and 2 immersed in liquid 3 will be repulsive. However, in certain cases, when the absolute value for A_{132} becomes closer to zero than $\approx \pm 3.5 \times 10^{-15}$ ergs, an exact prediction about attraction or repulsion, according to whether A_{132} is positive or negative, may no longer be reliable.[4, 5]

III. EXPERIMENTALLY TESTED APPLICATIONS

A. Separation of Particles by Advancing Solidification Fronts

The engulfing, or the rejection, of solid particles suspended in a melt, by an advancing solidification front can be of great importance in metallurgy, in soil science and civil engineering, in biology and, potentially, as a separation method. In process metallurgy, e.g., in the processing of molten lead, the distribution of various metal oxide particles, and/or other impurities between the melt and the ingot can be of considerable importance.[12, 13] In soils, ice lenses tend to form in winter, which can be almost devoid of soil

particles. When this phenomenon occurs under a road-bed, upon thawing pockets of water remain trapped between the road-bed and the underlying soil. This is an important cause of cracking of road surfaces by traffic, after thaw sets in.[14] In biology the engulfment of bacteria by phagocytes ultimately is the organism's major defense mechanism against infective invaders, whilst the ability of bacteria to be rejected by phagocytes is the principal mechanism by which many pathogenic bacteria achieve virulence.[9, 12, 15, 16] The advancing solidification front method has also been proposed as a procedure for separating particles according to size.[17]

Various surface thermodynamic,[12, 18, 19] as well as fluid dynamic,[17, 20, 21] treatments of particle engulfment and/or rejection phenomena at solid-liquid interfaces have been published. For relatively slowly advancing solidification fronts, where a state of equilibrium may be assumed to exist, and where surface thermodynamic considerations are therefore applicable, there may be certain advantages in converting the surface thermodynamic data into values pertaining to van der Waals interactions (see above).[4,23] One of the advantages is that values for the van der Waals interaction energies thus obtained should remain valid even under non-equilibrium conditions.

Various matrix materials have been studied.[19, 23] One of the most thoroughly investigated melt materials is naphthalene;[4, 12, 17, 18] we shall utilize that system here as the principal example of the influence of the sign of the net van der Waals interaction on the rejection or engulfment of various particles, at an advancing solidification front of naphthalene.[4, 23]

Particles with diameters varying between 0.2 and 0.01 mm, consisting of acetal, nylon-6, nylon-6, 6, nylon-12, nylon-6, 10, nylon-6, 12, polystyrene, teflon, and siliconized glass were used. Only the glass beads and the polystyrene particles were spherical; particles of the other materials were irregularly shaped. Via the surface tensions (determined by means of the advancing contact angle method, for the solids, and with the Wilhelmy plate technique, for the melt,[4, 23] and by making use of the equation of state),[8]

the combined Hamaker coefficients A_{132} (for particle 1, suspended in liquid naphthalene 3, and with solidified naphthalene 2) could be derived. This was done with the help of equations [2] and [3] , and by assuming a separation distance d_o of 1.82 A, from Hamaker coefficients assembled in the literature[2] and those found from our own data.[4] Table I compares the combined Hamaker coefficient A_{132} found for the various particles, with an advancing naphthalene solidification front, with their engulfment, or rejection by the solidifying melt.

Clearly, except for the very small value of 3.5×10^{-15} ergs found for Nylon-6, 12 in this system, in all other cases, where the combined Hamaker coefficient A_{132} is indubitably positive, a van der Waals attraction exists, and the particles become engulfed. Equally clearly, in all cases where A_{132} is distinctly

TABLE I

Hamaker Coefficients A_{132} at 80°C, Compared with Rejection or Engulfment of Various Particles by an Advancing Solidification Front of Naphthalene, from [4, 23]

Nature of particle	A_{132} in 10^{-14} ergs	Particle behavior
Acetal	− 3.27	Rejection
Nylon-6	− 2.81	Rejection
Nylon-6, 6	− 2.67	Rejection
Nylon-12	− 1.97	Rejection
Nylon-6, 10	− 0.92	Rejection
Nylon-6, 12	+ 0.35	Rejection
Polystyrene	+ 2.01	Engulfment
Teflon	+ 6.43	Engulfment
Siliconed glass	+ 8.28	Engulfment

negative (so that a van der Waals repulsion prevails), the particles become indeed rejected.

Actually, it can easily be shown[2] that A_{132} (considering equation [4]) always is negative when:

$$A_{11} > A_{33} > A_{22} \qquad [7]$$

or when:

$$A_{11} < A_{33} < A_{22} \qquad [8]$$

which (compare equation [1]) is the same as stating that A_{132} will always be negative when:

$$\Delta F_{11} < \Delta F_{33} < \Delta F_{22} \qquad [9]$$

or when:

$$\Delta F_{11} > \Delta F_{33} > \Delta F_{22} \qquad [10]$$

or (see equation [6]), when:

$$\gamma_{1v} > \gamma_{3v} > \gamma_{2v} \qquad [11]$$

or when:

$$\gamma_{1v} < \gamma_{3v} < \gamma_{2v} \qquad [12]$$

It thus is instructive simply to compare the surface tensions (γ_{1v}) of the various particles used, with the surface tensions of liquid and solid naphthalene; see Table II.

Nylon-6, 10, nylon-12, nylon-6, 6, nylon-6 and acetal all clearly obey equation [11] (i.e., their $\gamma_{1v} > \gamma_{3v}$, which again $> \gamma_{2v}$), whilst polystyrene, teflon and siliconed glass equally clearly do not obey that equation. The former five should thus be rejected (as they are) and the latter three engulfed (as is indeed the case). The exception is nylon-6, 12, which with $\gamma_{1v} = 31.8 < \gamma_{3v} = 32.8$, just barely disobeys equation [11] and therefore ought to be (but is not) engulfed. This slight discrepancy is without much doubt due to the experimental uncertainty inherent in the insignificance of the difference between 32.8 and 31.8 dynes/cm ($\gamma_{3v} - \gamma_{1v}$).

TABLE II

Surface Tensions (γ_{1v}) of Various Particles, Compared with the Surface Tensions γ_{3v} of Liquid and γ_{2v} of Solid Naphthalene at 80°C, and with their Rejection or Engulfment by an Advancing Solidification Front of Naphthalene, from [4, 23]

γ_{2v} of solid naphthalene in dynes/cm	γ_{3v} of liquid naphthalene in dynes/cm	Nature of particle	γ_{1v} of particle in dynes/cm	Particle behavior
26.4	32.8	Acetal	42.2	Rejection
		Nylon-6	40.0	Rejection
		Nylon-6, 6	40.5	Rejection
		Nylon-12	38.5	Rejection
		Nylon-6, 10	35.4	Rejection
		Nylon-6, 12	31.8	Rejection
		Polystyrene	27.7	Engulfment
		Teflon	15.7	Engulfment
		Siliconed glass	11.3	Engulfment

Thus particle engulfment or rejection by an advancing solidification front under equilibrium conditions appears to conform quite well to the theory developed above: van der Waals attraction gives rise to engulfment, whilst a negative (repulsive) net van der Waals interaction results in particle rejection.

B. Separation of Polymers in Solution by Phase Separation

When two different polymers 1 and 2 are both soluble in the same solvent 3, the two polymers (each separately dissolved in that solvent) upon being mixed together, either will stay mixed in one homogeneous solution, or they will spontaneously segregate into two separate phases. This behavior

bears a strong analogy with the engulfment or rejection of particles by solidification fronts, treated above.

Many studies have been published on the phase separation of polymer solutions.[24-28] Most theoretical studies have been preoccupied with the enthalpy and entropy of mixing, generally making use of the Flory-Huggins formulation of the combinatorial entropy.[29] Contrary to Scott's contention that if two polymers are incompatible (i.e., they separate) in one solvent, they will tend to be incompatible in other solvents,[30] in actuality polymer pairs that are incompatible in some solvents frequently are compatible in others.[31,32] Thus the properties of the solvent play an important role, in addition to those of the polymers, in determining the compatibility or separation of such ternary sustems. Most of the methods hitherto available for the prediction of polymer-polymer compatibility in various solvents are tedious (involving, e.g., the elaboration of phase diagrams), and frequently not very reliable, especially when the polymer samples are not monodisperse .[28] The formulation of a simple and unifying approach seemed therefore desirable. The same treatment used in the interpretation of engulfment versus rejection of particles by a solidification front (see above), was applied to the prediction of compatibility or separation of polymer pairs in solution.[5, 23] A negative Hamaker coefficient A_{132} for the interaction of two different polymers 1 and 2 in the solvent 3 implies repulsion between the two types of polymer molecules. As A_{131} and A_{232} are always positive, like molecules will always attract each other. Thus, a negative A_{132} favors phase separation. To verify this possibility experimentally, thirty one pairs of polymers dissolved in various solvents were studied and the Hamaker coefficient A_{132} of each ternary system was calculated from surface tension data in the same manner as described above. The results[5, 23] are summarized in Table III.

Evidently, in all cases where the combined Hamaker coefficient A_{132} is unmistakably positive, the polymer pairs exert a van der Waals attraction on each other, in their respective solvents, and are compatible. For some systems

TABLE III

Comparison Between the Sign of the Hamaker 2% (w/v) Solutions of Coefficient A_{132} and Compatibility or Separation of 31 Polymer Pairs in Various Solvents[5, 23]

Ternary System	A_{132} (in 10^{-14} ergs)	Visual observation*
Polymethyl methacrylate/methyl ethyl ketone/ Cellulose acetate	+ 7.51	C
Polyvinyl chloride/methyl ethyl ketone/Cellulose acetate	+ 4.97	C
Polymethyl methacrylate/tetrahydrofuran/ Cellulose acetate	+ 4.83	C
Polyvinyl chloride/methyl ethyl ketone/Polymethyl methacrylate	+ 4.65	C
Polystyrene/methyl ethyl ketone/Polymethyl methacrylate	+ 3.79	C
Polyvinyl chloride/tetrahydrofuran/ Cellulose acetate	+ 2.80	C
Polyvinyl chloride/tetrahydrofuran/Polymethyl methacrylate	+ 2.57	C
Polystyrene/methyl ethyl ketone/Polyvinyl chloride	+ 2.50	C
Polyisobutylene/chlorobenzene/Polypropylene	+ 2.17	C
Polystyrene/tetrahydrofuran/Polymethyl methacrylate	+ 1.88	C
Polyisobutylene.dichlorobenzene/Polystyrene	+ 1.88	S**
Polystyrene/nitrobenzene/Polymethyl methacrylate	+ 1.84	C
Polystyrene/benzene/Polymethyl methacrylate	+ 1.12	C
Polystyrene/tetrahydrofuran/Polyvinyl chloride	+ 1.07	C
Polystyrene/cyclohexanone/Polyvinylidene fluoride	+ 0.66	C
Cellulose acetate/methyl ethyl ketone/ Polystyrene	+ 0.40	C
Cellulose acetate/tetrahydrofuran/Polystyrene	+ 0.21	S
Polyisobutylene/chlorobenzene/Polystyrene	+ 0.18	C
Polyvinyl chloride/chclohexanone/Polyvinylidene fluoride	+ 0.09	C
Polystyrene/chlorobenzene/Polymethyl methacrylate	− 0.09	C
Polystyrene/dichlorobenzene/Polymethyl methacrylate	− 0.37	S
Polyisobutylene/chlorobenzene/Polyvinyl chloride	− 0.67	S
Polyisobutylene/benzene/Polystyrene	− 1.12	S
Polyisobutylene/toluene/Polystyrene	− 1.27	S
Polyisobutylene/tetrahydrofuran/Polystyrene	− 1.45	S
Polyisobutylene/carbon tetrachloride/Polystyrene	− 1.53	S
Polyisobutylene/cyclohexanone/Polystyrene	− 1.61	S
Polyisobutylene/dichlorobenzene/Polymethyl methacrylate	− 1.98	S
Polyisobutylene/tetrahydrofuran/Polymethyl methacrylate	− 3.48	S
Polyisobutylene/benzene/Polymethyl methacrylate	− 3.49	S
Polyisobutylene/tetrahydrofuran/Cellulose acetate	− 3.76	S

* C = compatible; S = separation.

** Compatible at lower concentrations.

2% solutions are somewhat too concentrated, but at 0.9% the Polyisobutylene/dichlorobenzene/Polystyrene system also is compatible, as emerged from a closer study of possible concentration effects.[5, 23] Also, with decidedly negative values for A_{132}, the polymer pairs clearly undergo a net van der Waals repulsion, and segregate out (see Table III). Only at very small absolute values of A_{132}, when they become closer to zero than $\approx 3 \times 10^{-15}$ ergs (precisely as was the case with particle engulfment or rejection, see above), it is no longer always possible to predict polymer compatibility or separation (e.g., with Cellulose acetate/tetrahydrofuran/Polystyrene, and with Polyvinyl chloride/cyclohexanone/Polyvinylidene fluoride, see Table III), even at low concentrations.[5, 23] In the case of the dissolved polymer pairs it is also possible to predict compatibility or separation by comparing the surface tensions of the polymers with those of the solvents, with the help of equations [11] and [12], in the same manner as was done with particle engulfment or rejection by solidification fronts, see Table II above. By that method it is superfluous to calculate the values of A_{132} and a simple comparison of surface tensions (γ_{1v}, γ_{3v}, and γ_{2v}) already permits a prediction of polymer compatibility or separation in all cases were clear-cut differences between all three values exist. A few examples are given in Table IV. Only the four last systems given in Table IV obey equation [12] and thus segregate out, whilst the first four systems, that do not obey either equation [11] or [12], are compatible.

Clearly, polymer compatibility or separation in ternary systems also conforms well to the theory developed above; van der Waals attraction allows compatibility of polymer pairs dissolved in a given common solvent, whilst a negative (repulsive) net van der Waals interaction results in polymer separation.

C. Separation of Proteins by Hydrophobic Chromatography

Virtually all polymeric biological substances, such as proteins and polysaccharides, have a surface tension that is lower than that of water, which is

TABLE IV

Surface Tensions (γ_{1v} and γ_{2v}) of Various Polymer Pairs, Compared with the Surface Tensions (γ_{3v}) of Solvents, with Respect to their Compatibility or Separation after Mixing

	Surface tensions in dynes/cm			Compatibility (C) or Separation (S)
	γ_{1v}	γ_{3v}	γ_{2v}	
Polyisobutylene/chlorobenzene/Polypropylene	18.9	32.6	28.0	C
Polystyrene/nitrobenzene/Polymethyl methacrylate	32.2	43.9	39.0	C
Polystyrene/cyclohexanone/Polyvinylidene fluoride	32.2	34.3	25.0	C
Cellulose acetate/methyl ethyl ketone/Polystyrene	40.0	24.6	32.2	C
Polystyrene/dichlorobenzene/Polymethyl methacrylate	32.2	35.5	39.0	S*
Polyisobutylene/chlorobenzene/Polyvinyl chloride	18.9	32.6	34.0	S*
Polyisobutylene/benzene/Polymethyl methacrylate	18.9	28.9	39.0	S*
Polyisobutylene/tetrahydrofuran/Cellulose acetate	18.9	27.4	40.0	S*

* These systems obey equation [12]: $\gamma_{1v} < \gamma_{3v} < \gamma_{2v}$

their natural solvent. They thus will all be more or less strongly attracted to hydrophobic surfaces (with a low surface tension), in water, due to a net positive van der Waals interaction (see above). The van der Waals attraction that causes biopolymers to adhere to hydrophobic surfaces (in aqueous media) can, as shown above, be made repulsive by lowering the surface tension of the water to a value below that of the biopolymer, (although still above that of the hydrophobic surface) thus causing it to desorb, or elute. This is the mechanism of the separation method called hydrophobic chromatography.[7] Hydrophobic chromatography is a separation method that grew empirically, as a useful but less specific variant, out of affinity chromatography. The method was described, almost simultaneously, by a number of different authors,[33-37] of whom the priority seems to rest with Hofstee, who at first called the method "hydrophobic affinity chromatography".[33] In the beginning great importance was attached to "salt effects",[34-38] but it became apparent that the elution of proteins from hydrophobic surfaces could mainly be enhanced by the addition of organic solvents such as ethylene glycol,[33, 39] and of detergents.[38] Hofstee stressed that, on the other hand, hydrophobic "bonding" is enhanced by increasing salt concentrations.[40] It is now increasingly recognized that elution of proteins from hydrophobic adsorbents is best done with detergents,[41] or with other surface-tension lowering additives such as ethylene glycol.[42] For a recent review, see Ochoa.[43]

Most inert hydrophobic polymers have little or no electrostatic charge, and any charge they have is likely to be slightly negative; e.g., phenyl sepharose has a ζ-potential of - 2 mV at low (μ = 0.04) ionic strength.[44] Thus the addition of salt may be of some help in the adsorption of (also usually negatively charged) proteins, but should be avoided in the elution step (see below).

Phenyl-sepharose as well as octyl-sepharose[39] (Pharmacia, Piscataway, NJ), have been used in hydrophobic chromatography of proteins.[7, 41] A study was made of the elution of proteins from whole human serum, after ad-

sorption onto phenyl-sepharose, and from the concentrations (and thus the surface tensions) of ethylene glycol corresponding to the maximum concentration of each of the eluted protein fractions,[7] the free energy of detachment ΔF_{132} could be calculated for each eluted protein, as well as the values of the combined Hamaker coefficients A_{132}, see Table V.

The six proteins came off the column in the order of their decreasing surface tension (γ_{1v}), as the surface tension (γ_{3v}) of the eluant solution decreased. The free energy of detachment ΔF_{132} for each protein was positive (favoring detachment), and of a remarkably constant value. The Hamaker coefficients A_{132} derived from these ΔF_{132} values were of course all negative (corresponding to a net van der Waals repulsion), and of an order of magnitude corresponding to the higher values among those observed in the van der Waals repulsion phenomena noted earlier[4, 5] (see also Tables I and III).

It thus seems evident that hydrophobic chromatography in which attachment of solutes (and/or particles) to the adsorbent surface occurs principally by van der Waals attraction, when the surface tension of the liquid medium is higher than the surface tensions of both the solutes (and/or particles) and the absorbent. Elution occurs by changing the van der Waals attraction into a repulsion by lowering the surface tension of the liquid to a value intermediate between that of the solutes (and/or particles) and the adsorbent. (Of course, for attaching the solutes to the adsorbent the surface tension of the liquid may also be lower than that of both solutes and adsorbent; elution then occurs by increasing the surface tension of the liquid to a value in between the surface tensions of the solutes and the absorbent).

In polar liquids, such as water, electrostatic interactions can of course never be quite ruled out. These can be partly obviated by the addition of salt, but too high a salt concentration in the elution step should be avoided, on account of the increase in surface tension most salts bring about. When electrostatic attraction between solutes and adsorbent prevails, changes in pH in the eluant may be more effective than increases in ionic strength (see

TABLE V

Hydrophobic Chromatography on Phenyl-sepharose of Whole Human Serum.[7] Proteins are given in the order (1 to 6) in which they eluted from the column. Also given are the surface tensions of the proteins (γ_{1v}), the surface tension of the eluant at the peak of the concentration at elution for each protein (γ_{3v}), the free energy of detachment for each protein (ΔF_{132}) and the combined Hamaker coefficient (A_{132}) for each protein (See equations [2] and [3]) *

	Eluted protein	γ_{1v} of the protein (in dynes/cm)	γ_{3v} of the eluant (in dynes/cm)	ΔF_{132} for the protein (in ergs/cm^2)	A_{132} for ** the protein (in 10^{-14} ergs)
1	α_2 macro-globulin	70.6	67.3	+ 3.5	– 4.3
2	serum albumin	70.2	64.0	+ 5.4	– 6.6
3	α_2 HS glyco-protein	68.1	59.0	+ 5.6	– 6.8
4	β_{1C}-β_{1A} (C3)	67.8	56.0	+ 5.8	– 7.1
5	Immuno-globulin G	67.2	54.0	+ 5.6	– 6.8
6	Transferrin	66.8	53.0	+ 5.3	– 6.5

* The γ_{2v} of the adsorbent (phenyl-sepharose) is taken to be 40.9 dynes/cm.

** Assuming the separation distance d_o to be $\approx$ 1.8 Å.[4, 5]

below). When an electrostatic repulsion between solutes and adsorbent exists, increased ionic strengths may be of some advantage in the coupling stage (because of the shielding as well as the surface tension raising effects), but should be completely avoided in the elution step, as more repulsion can then only be helpful.

D. Separation of Antigens and Antibodies

Antigen-antibody bonds are principally Coulombic (electrostatic) and/or van der Waals-London bonds.[45] Some antigen-antibody systems interact solely by van der Waals interactions,[46, 47] others through a combination of Coulombic and van der Waals bonds; the latter always are operative, due to the small separation distances between antigenic determinant and antibody-active site.[47]

The existence of antigen-antibody systems that virtually solely undergo van der Waals-London interactions[46, 47] make it possible to verify some of the theories mentioned above. The dextran-anti-dextran system[47] unfortunately does not readily give rise to precipitate formation; however the system involving the hapten 3-azopyridine (P_3), when coupled to rabbit serum albumin (P_3A), will precipitate quite well with rabbit anti-P_3 (elicited with P_3 coupled to bovine gamma globulin).[46] Thus the precipitating P_3A-anti-P_3 system was extensively studied, as an exclusively van der Waals-London interaction system, and compared with a typical combined van der Waals and Coulombic precipitating system, bovine serum albumin-goat anti-bovine serum albumin (BSA-anti-BSA).[6]

P_3A-anti-P_3 precipitates can at neutral pH be dissociated at $\gamma \approx 50$ dynes/cm. The completeness of the dissociation could be demonstrated by analytical ultracentrifugation. Prevention of P_3A-anti-P_3 precipitate formation is attained at ≈ 62 dynes/cm. The reason for the lower surface tension needed for the complete dissociation of P_3A-anti-P_3 precipitates than for the prevention of their formation must be sought in the fact that once an antigen-antibody precipitate is formed, part of the interstitial liquid between antigenic determinant and antibody-active site becomes expelled, which tends to strengthen the antigen-antibody bond,[47] so that it requires more energy to dissociate such a bond, once formed, than to prevent its formation.[6]

Precipitates of the combined Coulombic-van der Waals-London system BSA-anti-BSA could not be dissociated at neutral pH at surface tensions of the

liquid medium below even 48 dynes/cm, nor could at neutral pH BSA-anti-BSA precipitation be prevented at surface tensions of the medium below 52 dynes/cm. Nor could, at the surface tension of water, pH values as low as 3 or as high as 9.5, dissociate BSA-anti-BSA precipitates; see Table VI. Only lowering the surface tension of the liquid to ≈ 50 dynes and lowering the pH to 4.0, or raising it to 9.5, would result in dissociation of BSA-anti-BSA;[6] see Table VII. By electrophoresis it could be demonstrated that in mixed systems such as these, the dissociation of antigen-antibody complexes by this method also is complete.[48]

The surface tension of the liquid medium can conveniently be lowered by means of the addition to the buffer of, e.g., ethylene glycol,[6] dimethyl

TABLE VI

Influence of the Liquid Medium's Surface Tension (γ_{3v}) on the Dissociation of P_3A-anti-P_3 and BSA-anti-BSA Precipitates, and on the Prevention of the Formation of these Precipitates[6]

γ_{3v} in dynes/cm	Dissociation of precipitates of		Prevention of precipitation of	
	P_3A-anti-P_3	BSA-anti-BSA	P_3A-anti-P_3	BSA-antiBSA
73.8	+*	+	+	+
70.0	+	+	+	+
65.0	+	+	±	+
62.0	+	+	−	+
59.8	+	+	−	+
50.8	±	+	−	+
49.8	− *	+	−	+
48.8	−	+	−	+
48.0	−	+	−	+

* + signifies precipitates; − stands for disappearance or lack of formation of precipitate.

TABLE VII

Influence of the Liquid Medium's Surface Tension (γ_{3v}) and of its pH on the Dissociation of BSA-anti-BSA Precipitates [6]

γ_{3v} in dynes/cm	pH	Presence of precipitates of BSA-anti-BSA
73.8	9.5	+
50.3	9.5	–
50.3	9.0	+
50.3	8.5	+
73.8	7.2	+
50.3	7.0	+
50.3	4.5	+
51.4	4.0	–
73.8	4.0	+
73.8	3.0	+

sulfoxide,[6] or propanol. Of the latter, concentrations of ≈ 0.25 - 0.50% (lowering the surface tension of water to respectively 59 and 52 dynes/cm) [49] generally suffice. The popularity and efficacy of, e.g., propionic acid in helping to dissociate antigen-antibody bonds is easily understood when one realizes that low concentrations readily lower the pH of water to below 4, and its surface tension below 44 dynes/cm. Citric acid, acetic acid and acid glycine act in the same manner.

P_3A-anti-P_3 complexes dissociated with ethylene glycol solutions will re-precipitate upon removal of the ethylene glycol by dialysis.[6] With the P_3A-anti-P_3 system the γ_{1v} of the antibody-active site probably is close to ≈ 65 dynes/cm and the γ_{2v} of the antigenic determinant as low as ≈ 40 dynes/cm.

Thus in immunochemical systems also, van der Waals interactions can be given a net negative value (i.e. they can be changed from attractive to repulsive interactions) by lowering the surface tension of the liquid medium to a value intermediate between those of the two different interacting sites. Dissociation of antigen-antibody complexes via this approach is reversible and, contrary to the dissociation procedures hitherto at our disposal,[50] complete.[6, 48]

This approach to the dissociation of antigen-antibody bonds is bound to have considerable impact on a variety of analytical and preparative immunochemical procedures; it opens new possibilities in, e.g.: the determination of antigen-antibody ratios in circulating antigen-antibody complexes from animals or patients with immune complex disease,[48] the quantitative elution of blood group antibodies from erythrocytes,[51] the study of the influence of various salts on the dissociation of Coulombic antigen-antibody bonds under conditions of zero (or slightly negative) van der Waals attraction,[52] and finally, in the improvement of the methods used in immuno-adsorption (see Affinity Chromatography, below).

IV. OTHER GERMANE APPLICATIONS

A. Hydrophobic Chromatography of Cells

Hydrophobic chromatography of cells actually has been applied for several years in a number of instances. Since the early 1960's the property of polymorphonuclear granulocytes (PMN's) to adhere preferentially to nylon fibers has been utilized for the removal of these particular leukocytes from human blood, prior to transfusion of the blood to patients with anti-leukocyte antibodies, who are prone to manifest a transfusion reaction.[53] The same property of PMN's has been used by Dierassi et al.[54] to adsorb these cells from whole blood, and subsequently to elute them and to transfuse the isolated and concentrated PMN's into leukopenic patients. PMN's will only adhere well to hydrophobic surfaces when they remain endowed with multiple pseudo-

podia with a small radius of curvature, which allows them to overcome the electrostatic repulsion that would otherwise prevent a sufficiently close approach between cells and adsorbent to permit adhesion to ensue.[9, 16, 55, 56] Elution of PMN's from hydrophobic surfaces without damaging the cells still is a difficult problem.[57]

Lymphoid cells contain subgroups of cells that adhere preferentially to hydrophobic surfaces.[58] As long however as phagocytic cells (macrophages, monocytes or PMN's) may be intermixed with the lymphocytes, there is a likelihood that these will prove to be the adherent cells.[59]

The elution of adherent cells can be much facilitated by lowering the surface tension of the liquid medium. The least toxic surface tension lowering agent appears to be dimethyl sulfoxide (DMSO), which has been successfully used as a cryoprotectant with a variety of blood cells, e.g., erythrocytes and platelets,[60] lymphocytes,[61] and PMN's.[62] It is however essential to add the DMSO to the cells in an extremely gradual manner, in order to prevent osmotic damage.[63]

B. Affinity Chromatography

1. Immunoadsorption. From the results obtained in dissociating van der Waals bonds of antigen-antibody precipitates (discussed above), it is clear that in immunoadsorption, where either the antigenic determinant or the antibody-active site is the solid ligand, elution can be made much more efficient after determination of the optimal surface tension of the eluant at which the van der Waals attraction is converted into a repulsion.

Specifically bound material (e.g., antibodies) will eventually dissociate from the solid ligand without altering the buffer, if the interactive system is of relatively low affinity.[64] However, in most cases elution necessitates changing the pH, the ionic strength and/or the temperature of the buffer.[64] In the light of the total empiricism of that type of approach, it is not surprising that whenever possible a solvent containing an excess of specific hapten (or antigenic determinant) was hitherto preferred for elution.[65, 66]

If however a specific hapten is not available, and affinity elution thus not possible, the best method then is to lower the surface tension of the eluant to the desired level, and, if necessary (in the case of strong superimposed Coulombic interactions simultaneously to lower, or (what was hitherto rarely possible) to raise the pH (see above).

2. Affinity chromatography with immobilized enzymes. With this method, affinity elution is the rule. However, non-affinity methods of elution can be used in many cases.[64] Lowering the surface tension of the medium has not so far been applied on purpose, although high concentrations of guanidine HCl has been used successfully;[64] it is not generally realized that 5 M guanidine HCl lowers the surface tension of water to 50.1 dynes/cm.[52]

C. Reversed-Phase Chromatography

Horvath and Melander[67] have recently pointed out the analogy between hydrophobic chromatography (see above) and reversed-phase chromatography. The latter process employs a non-polar stationary phase and a polar (aqueous or organic) eluant.[67, 68] This system, like antigen-antibody interactions and, to a smaller degree, hydrophobic chromatography, depends on electrostatic as well as on van der Waals bonding. As far as van der Waals interactions are concerned, van der Waals repulsions in the eluting step clearly play an important role, as witnesses the correlation found between low surface tension and high eluant strength,[67] and it can be a considerable advantage in this type of chromatography to determine the sign and the value of the van der Waals interaction at various concentrations of eluant, to optimize the results, and to put their interpretation on a more solid basis.

D. Partition

Countercurrent distribution, or partition, is a powerful separation method based upon differences in the relative solubilities of solutes in two immiscible solvents, developed a third of a century ago by Craig.[69, 70] On a small scale, the technique is best used in the guise of partition chromatog-

raphy.[71] For medium-scale preparative purifications, however, the process is still quite viable and versatile;[72] continuous versions of the method have been developed by Ito et al.[73]

Albertsson has made possible the use of the method with immiscible aqueous systems.[74] At first sight it appears paradoxical that in a solvent such as water (with an exceptionally high surface tension), different polymers (each with a lower surface tension than water) would tend to repel one another, and segregate into different phases (see above, under Separation of Polymers in Solution by Phase Segregation). However, it can be shown that under the influence of at least one of the polymers (with detergent properties), the surface tension of the aqueous solvent actually is significantly lowered,[75] so that the rules established above still hold true. Albertsson's work has not only opened the possibility to separate cells by countercurrent distribution[74] but, due to the potentially relatively small difference in surface tensions between the polymers that can be used in aqueous systems, extremely narrow differences between cells can be distinguished with the aqueous phase method.[76,77]

It would thus seem logical to suggest that the partition method with immiscible organic solvents can be extended and refined by making use of segregating systems, using only one solvent, and two polymers (see Table III), so as to allow a discrimination between compounds that differ only slightly, such as e.g., optical isomers.

E. Separation of Various Materials from Solid Carriers

1. Soil removal from fabrics. This general process, comprising washing, dry cleaning and many other related procedures, although well understood and often also impeccably expressed,[78] is much more readily conceptualized when explained in terms of van der Waals interactions, rather than, e.g., in terms of detergency.

For the removal of hydrophobic material (1) from a fabric (2), when immersed in an aqueous or non-aqueous liquid medium (3), the free energy of adhesion ΔF_{132}^{adh} in that system can be derived from the various

surface tensions via equation [3] (see also, e.g., Adámson).[78] Equation [1] illustrates that when ΔF_{132}^{adh} becomes positive, the Hamaker coefficient A_{132} acquires a negative value, so that in liquid 3, materials 1 and 2 will repel each other, which is all that is needed for the removal of hydrophobic material from a fabric. This holds true regardless whether detergents are used to lower the surface tension of water to a value below that of the fabric, or whether one employs organic solvents that have a surface tension below that of the fabric. The efficiency of these processes clearly can be optimized by determining the surface tension of each fabric and/or other material, and selecting the detergent or organic solvent with which the most appropriate liquid surface tension can be attained, to achieve the desired separation.

2. Petroleum separations. Detergents have long been used in the field of secondary oil recovery.[79] In other applications involving the recovery of oil from oil shales or, especially, from tar (or oil-) sands,[80] exact determinations of the surface tensions of the oil as well as of the rocks or sands in question can be most helpful in determining the optimal conditions for detaching the petroleum components.

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