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# CELL SURFACE ENERGY, CONTACT ANGLES AND PHASE PARTITION

## I. LYMPHOCYTIC CELL LINES IN BIPHASIC AQUEOUS MIXTURES

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## Summary

The surface energy of cells is the quantity which dominates certain physical interactions of cells such as adhesion to hydrophobic surfaces and phagocytosis. A linear relationship is derived relating the equilibrium constant obtained from phase partition in liquid-liquid systems with the surface energy difference obtained from contact-angle measurements. Using biphasic mixtures of Dextran and poly(ethylene glycol) in a medium of constant salt composition the expression is confirmed for transformed lymphocytic cell lines. The results demonstrate the importance of van der Waals' interactions in the phase-partition process, that phase partition can be used as a direct measure of cell surface hydrophobicity, and that the equilibrium constant of phase partition is directly related to the difference in the surface energy of the partitioned particle between the two phases.

## Introduction

Cell surfaces are now known to determine important aspects of many physiological and biophysical phenomena as diversely related as cellular immunity, motility, cell-cell and cell-substrate adhesion, phagocytosis, agglutination and morphogenesis. The magnitudes of the non-covalent interactions at the cell surfaces are critical to these processes. Qualitatively, these properties of the cell surface are referred to as cell surface hydrophobicity [1—8], but this term is ill defined and poorly describes the forces and their origin.

The hydrophobic effect, the aggregation of hydrophobic entities in an aqueous environment, results from the net free energy of interaction of the van

der Waals' forces at the surface of interacting proteins or other macromolecules, or of cells. If the free energy of aggregation is negative, it will occur; and, one would expect sufficiently large positive free energies of attraction to result in a spontaneous repulsion of the particles, side chains or cells which are involved [9,10]. The large number of types and instances of non-covalent attractive and repulsive interactions in cellular systems makes it clearly worthwhile to attempt to develop methods which provide direct quantitative measurements of their energies.

This paper describes the relationship between (a) the equilibrium constant for particles or cells separated by partition in a biphasic liquid system and (b) the difference in the surface energy of the particles in each phase. Phase-partition and surface-energy determinations provide two independent methods of obtaining a measure of the interfacial free energy at the surface of living cells.

Biphasic aqueous mixtures of poly(ethylene glycol) and Dextran allow determination of cell surface energy differences by both the phase-partition and contact-angle methods. These biphasic mixtures are advantageous, since they maintain cell viability while allowing measurement of both the hydrophobic (dispersive) and hydrophilic (polar) contributions to cell surface energy. Biphasic mixtures of water-soluble polymers have been used as a practical means of separating cell types, subcellular fragments and enzymes [11,12], but have only recently been applied to the measurement of interfacial free energies at cell surfaces [13,14].

Contact angles provide a measurement of cell surface free energy, and have been used to describe the uptake of hydrocarbons by hydrocarbon-utilizing bacteria [15,16], phagocytosis [17] and the adhesion of cells to solid substrates [15,18]. The most reliable method used by surface physicists to obtain the surface energy of a solid is to obtain a series of contact angles ($\theta$) on the solid with similar liquids having different surface tensions ($\gamma_{12}$). A plot of $\cos\theta$ against $\gamma_{12}$ or $\gamma_{12}^{-1/2}$ usually yields a straight line. The value of $\gamma_{12}$ corresponding to $\cos\theta = 1$ is the value of the surface energy, or the critical interfacial tension for spreading, $\gamma_c$, of the solid. This subject has been reviewed extensively [15, 19,20]. Empirical relationships have been used to obtain essentially the same results with fewer measurements, but they are limited in the range of surface tensions and types of surface to which they apply [21]. Application of these empirical relationships to the problem of phase partition is discussed in the following papers of this series.

The value of knowing the surface energy of a cell lies in its utility in the calculation of the net free energy change which occurs on contact with other interfaces (liquid/solid, liquid/liquid or cell/liquid). Contact angles in biphasic liquid systems of hydrocarbon and water have enabled us to correctly describe the interaction of kerosene with the surface of *Corynebacterium lepus* and of hexadecane with the surface of a hydrocarbon-utilizing bacterium, *Acinetobacter calcoaceticus* [15,16]. The contact angles of water drops on cells under hydrocarbon give a measure of the dispersive van der Waals' interactions, which, fortunately are the only van der Waals' interactions applicable to interactions involving hydrocarbons. Certain plastics have been characterized using similar techniques [22,23].

However, in order to study the fusion of protoplasts in aqueous media it was

necessary to find immiscible liquid/liquid pairs which would provide measurements of both the polar and dispersive interactions. The aqueous biphasic mixtures of high molecular weight polymers commonly used for cell and particle separation [11] provided an ideal solution to this problem. Poly(ethylene glycol) and Dextran form immiscible phases when combined in aqueous solution at concentrations above the critical point, and a dilution series of such a mixture provides a series of similar liquid pairs which have progressively decreasing interfacial tensions, $\gamma_{12}$. Using droplets of the dense phase on cells immersed in the light phase of such a biphasic system, it was possible to measure the interfacial free energy at the surface of protoplasts from *Zea mays* and *Aureobasidium pullulans* and from these to predict the relative frequencies of self- and inter-specific protoplast fusions [13]. A second advantage of using biphasic mixtures of aqueous polymers is that the interfacial tension between the phases is very low (approx. $1.0 \cdot 10^{-4}$ erg/cm$^2$) compared to aqueous/hydrocarbon or fluorocarbon interfaces (approx. 50 erg/cm$^2$). This allows 3—4-fold greater sensitivity in the measurement of interfacial free energies at the cell surface than is possible at present by any other means. The interfacial tension between these phases is generally quite similar to that of the cell/medium interface. In addition, such mixtures are commonly used in biological preparations and have no known physiological disadvantages. These mixtures have also recently been used to determine cell surface energies for a variety of blood cell types [14].

The partition of cells between the phases of aqueous mixtures of incompatible polymers has been used extensively for the separation of cells, cellular particulates and proteins [1—8,11,12,24,25]. This process depends fundamentally on the difference between the interfacial free energy of the particle in one phase compared to that in the other phase [11,13,15], but as yet this technique has not been used for the determination of cell-medium interfacial free energies. Recent work [1—8] has clearly demonstrated the utility of partition coefficients in biphasic aqueous polymer mixtures in the characterization of the hydrophobicity of the surface of cells. The work of Zaslavsky and coworkers [1—5] has compared the partition of surfactants to that of cells, and in essence, provides a measure of cell surface hydrophobicity akin to the HLB (hydrophilic-lipophilic balance) scale used to characterize surfactants, emulsifiers and detergents [26—29]. The HLB scale is a linear function of the logarithm of the partition coefficient of the surface-active agent between an aqueous medium and a hydrocarbon such as octane, and thus, it can be used to distinguish between large differences in hydrophobicity. Partition in aqueous biphasic polymer mixtures is exquisitely sensitive to small differences in the hydrophobicity of relatively hydrophilic molecules and particles.

Since both the determination of contact angles with liquids of known surface or interfacial tension on solids, and the distribution of solid particles between two liquid phases measure the difference between the interfacial free energies at the solid surface in the two liquids, it should be possible to combine or relate these measurements. However, at this time, only contact-angle measurements allow calculation of the actual values of the interfacial free energies involved. The following section describes the relevant theory.

## Methods

*The expected relationship between partition coefficients, surface free energies and contact angles*

Fig. 1A depicts the possible results of partitioning a single type of cell or particle between two immiscible liquids. Either there is a complete separation of the particles into the two bulk phases, or there is also an accumulation of particles at the interface. Firstly, we will consider the free energy changes accompanying complete separation (Fig. 2, A—C).

At equilibrium, the partitioning of the cells depends on their chemical potential in each phase. The net chemical potential is the sum of several free energy terms, including the standard chemical potential, a concentration term, a surface free energy (hydrophobicity) term and an electrostatic term. Gravitational contributions will be neglected. Electrostatic effects (the Nernst equation) are important for experimental situations in which the charge on the cells or the potential between the phases varies, usually as a result of changes in electrolyte composition. Interactions between the components of the system may cause a concomitant hydrophobicity change (e.g., phosphate and poly(ethylene glycol)). If the electrolyte composition is constant, the electrostatic contribution to the chemical potential will be constant, and the partition coefficient will be determined by differences in surface energies. Such measurements form the basis for the theory of the hydrophobic effect [30,31].

The net chemical potentials, $\mu$, of the cells in each phase are given by Eqns.

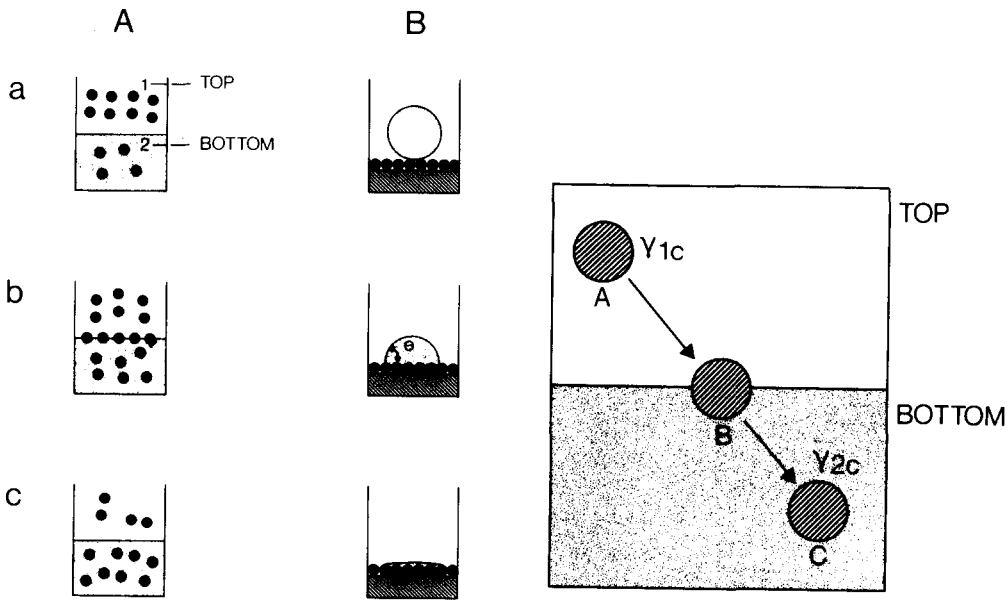


**Fig. 1. The relationship between partition coefficients (A) and contact angles (B) of two liquids (1 and 2) and cells or particles of a solid phase (•). High (a) or low (c) contact angles correspond to low or high partition coefficients, respectively. Intermediate situations are described by category b.**

**Fig. 2. Surface free energy ($\gamma$) changes accompanying the transfer of a cell or particle from one liquid phase (A) to the interface (B) and to the other liquid phase (C).**

2a and b.

$$\mu_{1c} = \mu^{\circ}_{1c} + \boldsymbol{k}T\ln f_{1c}[\text{cells}]_1 + A\gamma_{1c} + ze\Psi_{1c} \quad (2a)$$

$$\mu_{2c} = \mu^{\circ}_{2c} + \boldsymbol{k}T\ln f_{2c}[\text{cells}]_2 + A\gamma_{2c} + ze\Psi_{2c} \quad (2b)$$

where $\mu^{\circ}$ = standard chemical potential, $\boldsymbol{k}$ = Boltzmann's constant, $T$ = absolute temperature, $f$ = activity coefficient, $A$ = surface area of the cell or particle, $z$ = total charge on the cell or particle, $e$ = electronic charge, $\Psi$ = electrical potential.

The standard chemical potential, $\mu^{\circ}$, refers to a standard state for the cells. Here, this is chosen so that in the limit of ideal dilution, the activity coefficients, $f$, approach unity [32]. Choice of the units of concentration (e.g., fraction of the total or cells/ml) will alter the meaning of the standard state [32, 33]. Thus, the activity coefficients include all non-idealities arising from finite cell concentrations (cell-cell interactions). All contributions to the interfacial free energy at the cell/solution interface are included in the term $A\gamma$.

At equilibrium, the net chemical potentials of the cells in each phase are equal. Equating Eqns. 2a and b above results in Eqn. 3, which describes the partition coefficient as a function of the interfacial, electrostatic and concentration-related free energy changes which occur as a cell moves from phase 1 to phase 2 (Fig. 2).

$$-\boldsymbol{k}T \ln K_{eq} = A\ \Delta\gamma + ze\ \Delta\Psi + (\mu^{\circ}_{2c} - \mu^{\circ}_{1c}) + \boldsymbol{k}T \ln\left(\frac{f_{2c}}{f_{1c}}\right) \quad (3)$$

where $\Delta\gamma = \gamma_{2c} - \gamma_{1c}$ and $\Delta\Psi = \Psi_{2c} - \Psi_{1c}$.

The relative importance of the terms in Eqn. 3 depends on the experimental situation. By definition, the difference between the standard chemical potentials will be constant. Depending on how the experiment is performed, the partition coefficient can depend either on the electrical potential difference or on the solid/liquid interfacial free energy difference between the phases. Linear relationships have been observed in both cases (Refs. 34 and 35 and below), and thus we must assume that the activity coefficient ratio term is either nearly constant or relatively small.

Another consideration is the large size and heterogeneity of the cells. Partition of a large object between two phases is different from partition of an ideal dimensionless point in that all parts of the surface are bound together and must move cooperatively. This is akin to the cooperativity accompanying a physical phase change such as melting or freezing. The effect of this is the introduction of an exponent to each concentration term, or a factor, $\omega$, in the expression for the free energy of partition:

$$\omega\boldsymbol{k}T\ln K_{eq}$$

In practice, it is probably impossible to measure accurately and independently the effective surface area of a cells, $A$, because of the heterogeneous, fibrous and colloidal nature of the glycocalyx [36]. Nevertheless, use of measured values of $A$ may normalize results obtained with different cell types. Estimation and interpretation of $\omega$ are also difficult. These considerations lead to an empirical version of Eqn. 3 in which the constants have been combined into

experimentally determined parameters (Eqn. 4):

$$-\log K_{eq} = \alpha\Delta\gamma + \delta\Delta\Psi + \beta \quad (4)$$

Eqn. 4 predicts a linear relationship between the log of the partition coefficient and either the difference in interfacial free energy or the potential difference between the phases, or a linear combination of the two. A similar result was obtained by Bronstead [11]. Thus, for a series of biphasic systems with constant potential but differing in values of $\Delta\gamma$, one would expect a linear relationship between $\log K_{eq}$ and $\Delta\gamma$ (e.g., constant salt composition but variable polymer concentrations). Alternatively, for a series of biphasic systems with constant $\Delta\gamma$ but differing $\Delta\Psi$, one would expect a linear relationship between $\log K_{eq}$ and $\Delta\Psi$. These possibilities are expressed in Eqns. 4a and b, using the parameter, $\beta$, as a generalized constant:

$$-\log K_{eq} = \alpha\Delta\gamma + \beta \quad (4a)$$

$$-\log K_{eq} = \delta\Delta\Psi + \beta \quad (4b)$$

Eqn. 4 could be useful in both the experimental characterization of the liquid-liquid separation of cells and particles and in determining the optimal conditions for industrial processes involving liquid-liquid separations. In carefully studied systems, this type of relationship should allow calculation of either solid/liquid interfacial free energies or liquid/liquid interfacial tensions from the partition or adhesion of solid particles. The partition coefficient could apply to both physical or biological phases (e.g., phagocytosis). Eqn. 4a applies specifically to alterations in the surface energy at the cell surface, such as undoubtedly occur with changes in membrane lipid composition.

*Relationship to contact angles*

The interfacial energies at the surfaces of solids are notoriously difficult to determine experimentally [15,19,37]. However, the difference between the interfacial energies of two liquids and one solid can be directly determined by the measurement of the contact angle, $\theta$, between the solid and a drop of one liquid immersed in the second liquid (Fig. 1B, b). The relationship between these quantities was developed in 1805 by Young [38] and is given in Eqn. 5.

$$-\gamma_{12}\cos\theta = \gamma_{2c} - \gamma_{1c} = \Delta\gamma \quad (5)$$

Assuming equilibration, and disregarding gravitational effects in both the partitioning experiments and the measurement of contact angles [11,39], it is possible to combine Eqns. 4a and 5 to obtain the following expression, which it has not been possible to find in the literature to date (Eqn. 6).

$$\log K_{eq} = \alpha\gamma_{12}\cos\theta + \beta \quad (6)$$

An extension of this relationship which has particular applicability to problems involving the adhesion of particles to solids or tissues can also be developed. Eqn. 6 describes the relationship between partition coefficients and contact angles for particles which have completely separated into either phase 1 or phase 2 of the two-phase system (Fig. 1A, a and c). Frequently, a considerable fraction of the cells or particles accumulate at the interface between the two

phases, and partition coefficients are often given comparing the concentraion of cells in one phase to that of those in the interface or of the interface plus those in the other phase [1—5,11]. The relationships between the contact angle and these two partition coefficients are given below (Eqns. 7 and 8), and both require knowledge of the concentrations of particles $[x]_i$ at the arbitrarily thin interface (i). Unfortunately, this quantity is difficult to measure experimentally in liquid-liquid systems and thus, great care will be required to obtain data allowing these two relationships to be tested adequately. However, if the interface is a solid surface, it should be possible to obtain more reliable data [40].

$$\log\left(\frac{[x]_i}{[x]_1}\right) = \alpha\gamma_{12}(\cos\theta + 1)^2 + \beta \tag{7}$$

$$\log\left(\frac{[x]_i[x]_2}{[x]_1}\right) = \alpha\gamma_{12}(\cos\theta + 1)(\cos\theta + Q) + \beta \tag{8}$$

where

$$Q = \frac{[x]_2}{[x]_i + [x]_2}$$

Fig. 1 depicts the relationships between the partition coefficient (A) and the expected contact angle (B). The range of interfacial tensions, $\gamma_{12}$, which results in measurable contact angles depends on the relative magnitudes of the interfacial energies at the two solid/liquid interfaces. The selection of an appropriate pair of immiscible liquid phases is therefore quite difficult. Situations which result in complete spreading ($\theta = 0$) of a drop of one fluid on the cells immersed in the other, or in a contact angle of 180°, should yield almost complete partition of the cells into one phase provided the interfacial tension is low enough. In general, a given two-phase system in conjunction with a solid gives measurable contact angles and partition coefficients over a range of about two orders of magnitude for the difference, $\gamma_{2c} - \gamma_{1c}$. Complete spreading of the drop occurs at the critical interfacial tension for spreading, $\gamma_c$, which is itself an extremely valuable measure of cell surface energy (see below). In aqueous systems, the observed differences range from about 50 erg/cm$^2$ for very hydrophobic surfaces, to as low as $10^{-4}$ erg/cm$^2$ for very hydrophilic surfaces.

*Experimental methods*

Partition coefficients of cells between the phases of aqueous polymer mixtures were measured as follows. Poly(ethylene glycol) ($M_r$ 6000, Sigma, St. Louis, MO) and Dextran T500 ($M_r$ 500 000, Pharmacia, Uppsala) solutions were prepared at the concentrations given in Table I in Dulbecco's phosphate-buffered saline (Gibco, Paisley, 320-1885). Separation of the two phases which formed spontaneously was accomplished by centrifugation at 500 rev./min. Cells were washed once with the less dense (light) phase (poly(ethylene glycol)-rich), and were resuspended in it. Following thorough mixing, an equal volume of the dense phase (Dextran-rich) was added and the cells were thoroughly mixed with a vortex mixer. The emulsion which formed was first allowed to settle by gravity for 4 h, and then centrifuged at 200 rev./min for 15 min. This speed is insufficient to pellet the cells in these polymer solutions. Samples were

TABLE I

COMBINATIONS OF POLY(ETHYLENE GLYCOL) AND DEXTRAN USED TO FORM BIPHASIC MIXTURES USED FOR THE DETERMINATION OF BOTH PARTITION COEFFICIENTS AND CONTACT ANGLES

Interfacial tensions are given for the two phases which form spontaneously at room temperature [42]. These combinations result in approximately equal volumes of the two phases.

| | Polymer mixture | | Interfacial tension (mdyne/cm) |
|---|---|---|---|
| | Poly(ethylene glycol) ($M_r$ 6000 (% w/w)) | Dextran ($M_r$ 500 000 (% w/w)) | |
| A | 6.0 | 8.0 | 66 |
| B | 4.4 | 7.0 | 20 |
| C | 4.0 | 6.0 | 7.4 |
| D | 4.0 | 5.0 | 3.1 |

taken from both the light and dense phases and cell concentrations were determined in a Coulter counter, model ZF. The results presented are the averages of triplicates of four experiments.

Contact angles were measured by the horizontal-projection technique [41] utilizing an optical bench with a collimated light beam (Spindler and Hoyer, Model 30123) and the zoom lens assembly of a Wild M405 stereomicroscope equipped with a modified lens column having a single centrally located lens providing parfocal adjustment throughout the magnification range. A Wild 10× or 15× goniometer eyepiece was used for the direct measurement of contact angles. Droplets 0.1—0.5 mm in diameter were projected on a ground-glass screen to a diameter of approx. 20—100 mm for measurement. The results presented are the averages of approx. 100 measurements for each experimental situation; the standard error of the mean was relatively constant at about 0.5% of the mean. It was found that the most consistent results were obtained by using the following procedure. Cells were prepared as for the partitioning experiment, but collected on a 25 mm diameter, 0.22 or 0.45 $\mu$m pore diameter Millipore filter (using a type XX10025000 filter holder) at a density of $2—5 \cdot 10^3$ cells/mm$^2$. To prevent drying, the filter was left on the porous glass support until the moment before transfer to a 25 ml bath containing the light phase of the polymer mixture. It was then removed from the filter support, cut in half and both halves were placed in the bath. Drops of the heavy phase were then introduced just below the liquid surface from a pasteur pipet drawn out to a tip diameter of approx. 0.1 mm. The drops slowly settled to the surface of the layer of cells and it took 15—20 min for equilibrium to be established. Contact angles of the drops on the exposed edges of the Millipore filter provided a test of the consistency of the interfacial tension between the phases. Interfacial tensions between the phases of the polymer mixtures were essentially those reported by Ryden and Albertsson [42], however, they were checked by the hanging-drop technique [43] using capillaries approx. 10—50 $\mu$m in diameter and differential interference optics [14].

Cells used in this study were lymphocytic cell lines donated by B. Weimann, and were of Balb/c origin: K (A-MuLV transformed) [44], BM18-4(A-MuLV transformed in vitro) [45] and ABLS-1 (A-MuLV transformed in vivo [46]. The

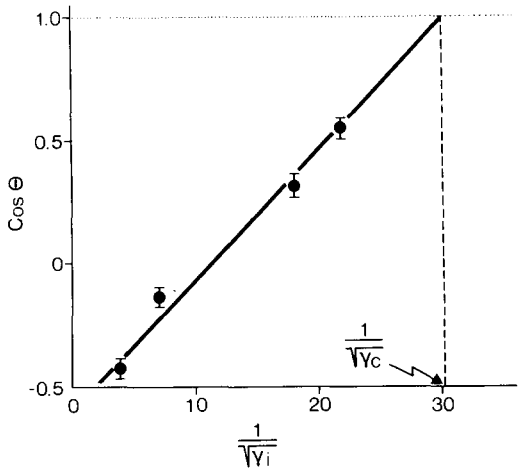


Fig. 3. Linear relationship observed between $\cos\theta$ and $\gamma_i^{-1/2}$ for cell line K. The value of $\gamma_i$ corresponding to $\cos\theta = 1.0$, is $\gamma_c$, the critical interfacial tension for spreading.

cell lines were maintained in Iscove's modified Dulbecco's medium (Gibco, Grand Island, NY, 785220) containing 10% fetal calf serum and were incubated in 5% $CO_2$/95% air at 37°C and 100% humidity. Cells were harvested in mid-exponential phase. The relationship between $\cos\theta$ and $\gamma_i^{-1/2}$ was linear, as expected (Fig. 3).

Cell volumes were determined with $^3H_2O$, using [$^{14}$C]inulin to correct for extracellular space [47]. Cells were incubated for 30 min then centrifuged through a layer of Versilube F-50 silicone fluid (General Electric, Waterford, NY) into a 0.7% solution of Triton X-100 in 10% NaCl, in a Beckman Microfuge (10 000 × $g$, 1 min). The plastic tubes were then placed in solid $CO_2$ and the frozen tips were cut off through the silicone layer and placed in scintillation vials with Instagel (Packard) scintillation fluid. Intracellular water volume measured in this way was corrected for a cell water content of 85% to calculate cell surface areas. The results are given in Table II.

TABLE II

PARAMETERS OF EQN. 6 IN THE TEXT RELATING THE CONTACT ANGLE, $\theta$, TO THE PARTITION COEFFICIENT, $K_{eq}$, FOR CELLS IN BIPHASIC AQUEOUS MIXTURES OF POLY(ETHYLENE GLYCOL) AND DEXTRAN

The parameter $r^2$ is the coefficient of correlation for the least-squares fit to the lines in Fig. 3. Cell surface areas and critical interfacial tensions, $\gamma_c$, are also given.

| Cell type | Cell surface area ($\mu m^2$) | $\gamma_c$ ($\mu N \cdot m^{-1}$) | Characteristics of the relationship $\log K_{eq} = \alpha\gamma_{12}\cos\theta + \beta$ | | |
|---|---|---|---|---|---|
| | | | $\alpha$ | $\beta$ | $r^2$ |
| K | 450 | 1.1 | 21.89 | —0.547 | 0.99 |
| BM18-4 | 320 | 3.3 | 17.06 | —0.865 | 0.97 |
| ABLS-1 | 320 | 3.6 | 20.19 | —1.017 | 0.98 |

## Results and Discussion

A linear relationship was found between the logarithm of the partition coefficient and the difference in interfacial free energies of the cells in the two phases as predicted by Eqn. 6. These results are given in Fig. 4. Table II gives the slopes ($\alpha$), intercepts ($\beta$) and correlation coefficients ($r^2$) for the linear least-squares fit to the data points as well as the values of $\gamma_c$. Excellent linearity is observed for all three cell types, indicating that partition between the phases of aqueous polymer mixtures is directly related to measurements of the interfacial free energies of cell surfaces. Interpretation of the parameters $\alpha$ and $\beta$ is, however, difficult. The value of $\alpha$ is much smaller than that expected from $A/kT$, which may indicate that $\omega$ is of the order of $10^6$—$10^7$.

The finding of Albertsson and Baird [48] that the partition coefficient of cells increases as the polymer concentration is reduced under conditions of constant salt concentration is confirmed by these data, as demonstrated by the positive values of $\alpha$ for all three cases.

The values of $\alpha$ and $\beta$ thus serve to characterize the hydrophobicity of the cell surface as measured by the two techniques. The line is a function of the critical surface tension for spreading, $\gamma_c$ [14], but cannot yet be used as an independent measure of it. These parameters may also be affected by specific van der Waals' interactions at the cell surface, as has been suggested by the work of Walter et al. [49] and Ericksson et al. [50].

The influence of the charge between the phases on the equilibrium partitioning of cells in aqueous biphasic mixtures has been discussed at some length [5,49], but those discussions have not dealt with the van der Waals' forces acting at the cell surface (cell surface energies). Walter et al. [49] realized the possibility of these effects and investigated the influence of cell membrane lipids on phase partition in Dextran/poly(ethylene glycol) mixtures. Correlations were found between the partition coefficient and the degree of polyunsaturation of membrane fatty acids and the content of phosphatidylcholine. A

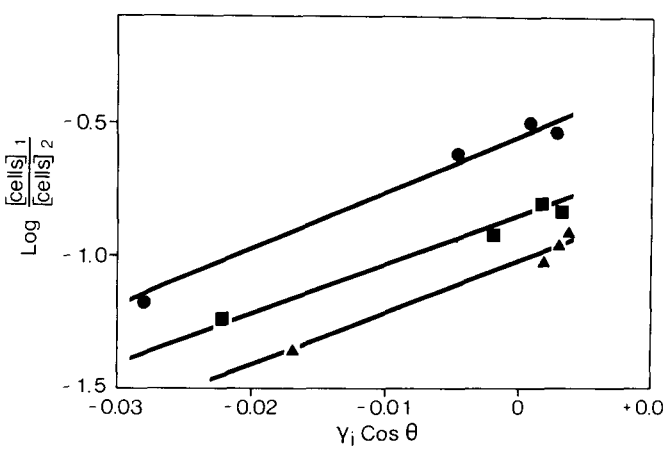


Fig. 4. Linear relationships between $\gamma_i \cos\theta$ and $\log K_{eq}$ for transformed lymphocytic cell lines in biphasic mixtues of poly(ethylene glycol) and Dextran. The cell lines are: K (●), ABLS-1 (▲) and BM18-4 (■).

negative correlation was found with sphingomyelin content. Increased lipid content does not necessarily lead to increased cell surface hydrophobicity, since all of these lipids are amphipathic and the nature of their interactions with extracellular hydrophobic molecules is difficult to predict without knowledge of the HLB of the lipids. Certainly, it would be interesting to measure contact angles on both sides of monolayers of these lipids, or of cell surface proteins with the relevant biphasic systems. A set of measurements of this type has recently been completed (Schürch, S., McIver, D.J.L. and Gerson, D.F., unpublished data), and may have relevance to the vertical modulation of membrane-bound surface receptors by changes in membrane lipid composition [51].

In conclusion, a relationship between the partition coefficient of cells in biphasic systems and the difference between the interfacial energies at the cell surface in each phase has been derived and successfully tested experimentally. The results obtained demonstrate the importance of surface and interfacial energy differences in the phase-partition process, and distinguish these from electrostatic effects. The relationship provides two independent methods for determining relative interfacial energies at the cell surface (contact angles and partition coefficients). This methodology increases the accessibility of knowledge of cell surface energies to those interested in such physical events at the cell surface as engulfment, aggregation, adhesion or spontaneous self-sorting in embryonic development [52].

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## References

1 Zaslavsky, B.Y., Miheeva, L.M., Mestechkina, N.M., Pogorelov, V.M. and Rogozhin, S.V. (1978) FEBS Lett. 94, 77—80
2 Zaslavsky, B.Y., Miheeva, L.M. and Rogozhin, S.V. (1978) Biochim. Biophys. Acta 510, 160—167
3 Miheeva, L.M., Zaslavsky, B.Y. and Rogozhin, S.V. (1978) Biochim. Biophys. Acta 542, 101—106
4 Zaslavsky, B.Y., Mestechkina, N.M. and Rogozhin, S.V. (1979) Biochim. Biophys. Acta 579, 463—465
5 Zaslavsky, B.Y., Miheeva, L.M. and Rogozhin, S.V. (1979) Biochim. Biophys. Acta 588, 89—101
6 Walter, H., Webber, T.J., Michalshi, J.P., McCoombs, C.C., Moncla, B.J., Krob, E.J. and Graham, L.L. (1979) J. Immunol. 123, 1687—1695
7 Malmström, P., Nelson, P., Jönsson, A., Sjögren, H.O., Walter, H. and Albertsson, P.A. (1978) Cell. Immunol. 37, 409—421
8 Magnusson, K.E., Kihlstrom, E., Norlander, L., Norqvist, A., Davies, J. and Normark, S. (1979) Infect. Immun. 26, 397—401
9 Van Oss, C.J., Absolom, D.R., Grossberg, A.L. and Neumann, A.W. (1979) Immunol. Commun. 8, 11—29
10 Van Oss, C.J., Absolom, D.R. and Neumann, A.W. (1979) Sep. Sci. Technol. 14, 305—317
11 Albertsson, P.A. (1971) Partition of Cell Particles and Macromolecules, 2nd edn., Wiley, New York
12 Walter, H. (1978) in Methods of Cell Separation (Castimpoolas, N., ed.), Vol. 1, 307—354, Plenum Press, New York
13 Gerson, D.F., Meadows, M.G., Finkelman, M. and Walden, D.B. (1980) in Advances in Protoplast Research (Ferenczy, L. and Farkas, G.L., eds.), pp. 447—456, Akademiai Kiado, Budapest
14 Schürch, S., Gerson, D.F. and McIver, D.J.L. (1980) Biochim. Biophys. Acta, in the press
15 Gerson, D.F. and Zajic, J.E. (1979) in Immobilized Microbial Cells (Venkatsubramanian, K., ed.), ACS Symp. Ser. Vol. 106, pp. 29—57, Am. Chem. Soc., Washington, DC
16 Neufeld, J.R., Zajic, J.E. and Gerson, D.F. (1980) Appl. Environ. Microbiol. 39, 511—517

17 Van Oss, C.J., Gillman, C.F. and Neumann, A.W. (1975) Phagocytic Engulfment and Cell Adhesive ness, Dekker, Inc., New York
18 Neumann, A.W., Absolom, D.R., van Oss, C.J. and Zingg, W. (1979) Cell Biophys. 1, 79—92
19 Good, R.J. (1979) Surf. Colloid Sci. 11, 1—30
20 Good, R.J. (1977) J. Colloid Interface Sci. 59, 398—419
21 Neumann, A.W., Good, R.J., Hope, C.J. and Sejpal, M. (1974) J. Colloid Interface Sci. 49, 291—304
22 Andrade, J.D., King, R.N., Gregonis, D.E. and Coleman, D.L. (1979) J. Polymer Sci. 66, 313—336
23 Andrade, J.D., Ma, S.M., King, R.N. and Gregonis, D.E. (1979) J. Colloid Interface Sci. 72, 488—494
24 Albertsson, P.A. (1977) Endeavour 1, 69—73
25 Kroner, K.H. and Kula, M.R. (1978) Process Biochem. 13, 7—9
26 Davies, J.T. and Rideal, E.K. (1968) Interfacial Phenomena, Academic Press, New York
27 Lin, I.J. and Marszall, L. (1976) J. Colloid Interface Sci. 57, 85—93
28 Schott, H. (1971) J. Pharm. Sci. 60, 648—649
29 Allured, S.E. (1973) McCutcheon's Detergents and Emulsifiers, Allured Publ. Co., Ridgewood, NJ
30 Tanford, C. (1973) The Hydrophobic Effect, Wiley, New York
31 Wolfenden, R.V., Cullis, P.M. and Southgate, C.C.F. (1979) Science 200, 576—577
32 Moore, W.J. (1972) Physical Chemistry, 4th ed., Longman, London
33 Tanford, C. (1979) J. Phys. Chem. 83, 1802—1803
34 Walter, H., Selby, F.W. and Garza, R. (1967) Biochim. Biophys. Acta 136, 148—150
35 Gerson, D.F. and Akit, J. (1980) Biochim. Biophys. Acta 602, 281—284
36 Costerton, J.W., Brown, M.R.W. and Sturgess, J.M. (1979) Pseudomonas Aeruginosa (Doggett, R.G. ed.), Academic Press, New York
37 Neumann, A.W. and Good, R.J. (1979) Surf. Colloid Sci. 11, 31—92
38 Young, T. (1805) Philos. Trans. R. Soc. London 95, 65—87
39 Good, R.J. and Koo, M.N. (1979) J. Colloid Interface Sci. 71, 283—292
40 Gerson, D.F. and Scheer, D. (1980) Biochim. Biophys. Acta 602, 506—510
41 Fox, H.W. and Zisman, W.A. (1950) J. Colloid Sci. 5, 520—525
42 Ryden, J. and Albertsson, P.A. (1971) J. Colloid Interface Sci. 37, 219—222
43 Ambwani, D.S. and Fort, T. (1979) Surf. Colloid Sci. 11, 93—120
44 Weimann, B.F. (1978) Cold Spring Harbor Symp. Quant. Biol. 41, 103—124
45 Rosenberg, N. and Baltimore, D. (1976) J. Exp. Med. 143, 1453—1456
46 Premkumar, E., Potter, M., Singer, P.A. and Sklar, M.D. (1975) Cell 6, 149—159
47 Kiefer, H., Blume, A.J. and Kaback, H.R. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 2200—2204
48 Albertsson, P.A. and Baird, G.D. (1962) Exp. Cell Res. 28, 296—322
49 Walter, H., Krob, E.J. and Brooks, D.E. (1976) Biochemistry 15, 2959—2964
50 Ericksson, E., Albertsson, P.A. and Johansson, G. (1976) Mol. Cell. Biochem. 10, 123—128
51 Borochov, H. and Shinitzky, M. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 4526—4530
52 Steinberg, M.S. (1975) J. Theor. Biol. 55, 431—443