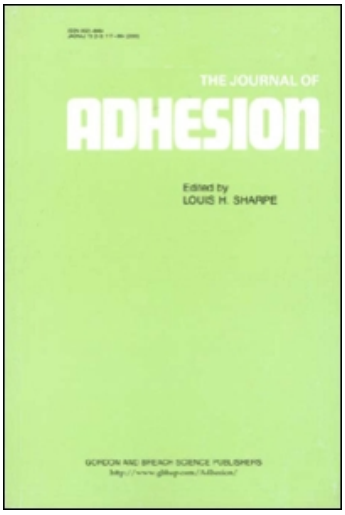

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Adhesion of Hydrophilic Particles to Polymer Substrates Immersed in Aqueous Media†

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Various factors affecting the extent of adhesion of hydrophilic particles to polymer surfaces have been evaluated. Specifically the kinetics of adhesion, and the influence of substrate surface tension, ionic strength, pH, and surface tension of the suspending liquid medium have been investigated. In addition the role of divalent cations has been assessed. The substrates examined exhibit a wide range of wettability and the hydrophilic particles used were both fresh and glutaraldehyde-fixed human erythrocytes.

For experiments in which the particles are suspended in solutions with an ionic strength of 0.15 or greater the kinetic studies reveal that the extent of adhesion increases rapidly initially and reaches a plateau value after approximately 30 mins. There is no evidence for a lag-time in the onset of particle adhesion, suggesting that electrostatic double layer forces are negligible under these experimental conditions. For a bulk particle concentration 1×10^6 cells/ml the plateau value of adhesion corresponds to a surface coverage of no more than 10% for the most densely covered substrate. For a given set of experimental conditions the level of saturation adhesion is determined by the wettability of the substrate material. The extent of particle adhesion to any given substrate material is influenced considerably by the respective surface tension of the adhering particles (γ_{PV}), the substrate material (γ_{SV}) and the suspending liquid media (γ_{LV}). For conditions where $\gamma_{LV} > \gamma_{PV}$ the extent of

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particle adhesion decreases with increasing substrate surface tension, γ_{SV}. For conditions where $\gamma_{LV} < \gamma_{PV}$ the opposite pattern of behaviour is found.

The extent of particle adhesion to various polymer surfaces is also a function of solution pH and ionic strength. At a constant pH of 6 virtually no erythrocyte adhesion occurs at ionic strengths less than 0.05. Adhesion increases with increasing ionic strength and reaches a limiting plateau value at an ionic strength of approximately 0.1. The actual level of the plateau value is quite different for each polymer. Particle adhesion is also pH dependent. For conditions of constant ionic strength and variable pH the extent of erythrocyte adhesion at and below pH 6 is constant for each polymer substrate and extends over a substantial domain of high ionic strength and low pH. As the pH is increased erythrocyte adhesion decreases and reaches a second plateau at pH 8 and above.

The pH and ionic strength studies suggest that at low ionic strength double layer repulsion plays a critical role in preventing particle adhesion. With increasing ionic strength the double layer becomes more compressed allowing a closer approach of the particles to the substrate and hence an increased van der Waals attraction giving rise to increased erythrocyte adhesion.

KEY WORDS Adhesion kinetics; cell-surface interactions; particle adhesion; polymer surfaces; erythrocyte adhesion; effect of pH, ionic strength and surface tension on adhesion.

1 INTRODUCTION

There is widespread interest in particle adhesion to various substrates for theoretical[1,2] as well as practical reasons.[3,8] Understanding of the process of particle adhesion has direct implications for a large number of fields including artificial organs; electrophotography; separation of liquid–solid mixtures in filtering, screening and flocculation; sintering, pelleting and briquetting; fluidizing and pneumatic conveyance of powders; reactor fouling and various cleaning processes. In the present article we wish to review our work on hydrophilic particle adhesion to polymer substrates immersed in aqueous solutions. The presence of the liquid medium alters considerably the interaction energies involved and needs to be considered as will be discussed later in this article.

2 EXPERIMENTAL ASPECTS

(a) Hydrophilic Particles

The hydrophilic particles examined in this work are glutaraldehyde-fixed human erythrocytes (red blood cells). Our reasons for

selecting these objects as our model particles include the following:

(1) Erythrocytes are readily available in large quantities and exhibit remarkably homogeneous batch properties including size, shape and chemical composition;

(2) Glutaraldehyde-fixation results in the preparation of particles which are stable over long periods of time and in virtually all environmental conditions;

(3) Fixation prevents the loss of proteins and glycoproteins from the cell surface which might otherwise preadsorb onto carefully prepared substrates rendering interpretation of the experimental data more difficult;

(4) Fixation increases cell rigidity, thereby stabilizing the area of contact between the cell and the substrate material.

(5) Fixation produces only a small change in the net surface charge of the cell surface[9] or in the local distribution of the charge-bearing glycoproteins in the membrane lipid bilayer.[10]

(6) The hydrophilicity of fixed erythrocytes has been determined by a number of independent methods.[11] The surface tension of fixed human erythrocytes is approximately 65 ergs/cm^2.

Thus these cells represent a well-characterized, readily-available source of hydrophilic particles ideally suited for studies of the type described here.

(b) Polymer Substrates

The adhesion experiments were performed using the substrate materials listed in Table I. Preparation of the substrates was performed as described previously.[12] Smooth films of these polymers (with the exception of polystyrene and sulphonated polystyrene which are commercially available as thin films) were made in a hydraulic heat press (Wabash, Ind.) by pressing strips of the polymers between chromic-acid-cleaned glass slides. All surfaces with the exception of sulphonated polystyrene have no ionizable groups or semi-permanent charges. They are also inert in the sense of not containing chemical reactive groups such as hydroxyl, amine or isocyanate. These polymers thus represent a class of materials

TABLE I
Solid substrates used in the erythrocyte adhesion experiments

Material	Source	Preparation	Contact angle θ_{H_2O} with water	Surface tension γ_{SV} (ergs/cm^2)
Fluorinated ethylene-propylene copolymer (FEP)	Commercial Plastics, Toronto, Canada	Heat Press	110 ± 3	16.4
Dimethyl-dichlorosilane (SIL)	Eastman-Kodak, Rochester, N.Y.	Vapour deposition	108 ± 2	17.6
Polystyrene (PS)	Central Research Lab., Dow Chemical Co.	Film	95 ± 2	25.6
Low density polyethylene (LDPE)	Commercial Plastics, Toronto, Canada	Heat press	84 ± 4	32.5
Acetal resin (Ac)	Commercial Plastics, Toronto, Canada	Heat press	64 ± 1	44.6
Sulfonated polystyrene (SPS)	Central Research Lab., Dow Chemical Co.	Film	24 ± 3	66.7

whose principal property, insofar as it affects particle adhesion, is their surface tension, γ_{SV}. The surface tension of the polymers (*cf.* Table I) was calculated from contact angle data using the equation of state approach.[13]

(c) Suspending Liquids

For the purpose of the experiments to be described here the fixed-erythrocytes were suspended at a concentration of 1×10^6 cells/ml in aqueous solutions of various ionic strengths, pH and liquid surface tensions. The specific experimental conditions are described at the appropriate position in the text.

(d) Static Adhesion Test

Particle-substrate adhesion has been investigated in a number of different experimental systems including the inclined plane,[14–16] centrifuge methods,[17–19] aerodynamic or hydrodynamic methods,[20] gravimetric methods[1,6,7,12] the rotating disc method[21–24] and powder-bed methods.[25,26] The present results were obtained using a static adhesion test which involves the deposition of particles onto a flat horizontal surface from a stagnant suspension. This system has the advantage that it avoids the complexities such as turbulence, shear forces, *etc.*, which might arise as the result of fluid flow. The method has been described in detail previously[6,7,12] and hence will be discussed only briefly here. One millilitre of the particle suspension, at a concentration of 1×10^6 cells/ml, was placed on the surfaces and was retained in wells formed in Teflon® molds separated from the polymers by Silastic® gaskets. The height of the cell suspension is approximately 1 cm. The system is then incubated for the desired time and at the selected temperature. Thereafter the surfaces are placed, without exposure to air, in a large volume of the suspending fluid and rinsed under standardized conditions in order to remove any non-adherent particles. For this purpose we employ a rinsing device described previously.[27] Thereafter the substrates are air-dried. Then the number of particles adhering to the various surfaces is determined, after brief immersion in deionized distilled water to dissolve any salt crystals, using an automated image analysis system (Omnicon 3000, Bausch and Lomb, Roches-

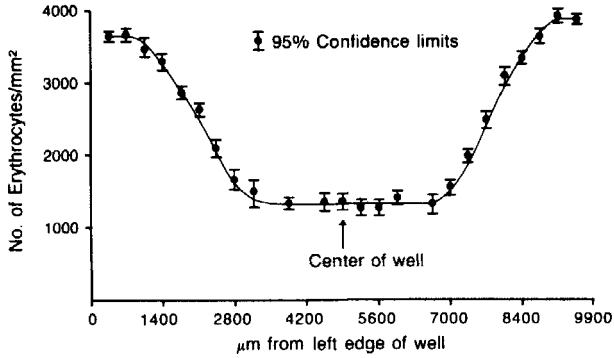

FIGURE 1 The influence of "wall-effects" in determining the extent of particle (glutaraldehyde-fixed erythrocytes) adhesion to a FEP surface. The erythrocytes, at a concentration of 1×10^6 cells/ml in Hanks Balanced Salt Solution (pH 7.2, ionic strength of 0.15) were retained in a Teflon® mold. Contact time was 30 minutes.

ter, NY). For this purpose a Wild–Leitz Metalloplan microscope connected to a Bosch camera containing a Chalnicon tube was used. A 32X, long-working-distance objective lens was employed. Computer controlled automation of the microscope stage permitted rapid evaluation of 126 separate fields of view of known area for each well. In view of "wall-effects" in these adhesion tests, illustrated in Figure 1, only the central portions of each well are assessed. The data from each of these individual fields measurements were then averaged and expressed as the number of particles (erythrocytes) adherent per unit surface area of substrate material. For each experiment at least six wells per type of polymer surface were examined. Each experiment was repeated at least three times.

3. THEORETICAL ASPECTS

It is generally accepted that the main forces responsible for the adhesion of small particles, suspended in a liquid, to a solid surface are long-range interactions which result from a combination of van der Waals forces and electrostatic forces. Both of these primary forces may under certain circumstances be either attractive or repulsive. The actual strength of the subsequent adhesive bond may

also depend on factors such as substrate roughness and deformability, particle size and shape, the nature of the particle, surface contamination. At the present time it is widely accepted that particle adhesion is best described in terms of the DLVO theory.[28,29]

(a) DLVO Predictions of Particle Adhesion

In recent years a theoretical description of particle sedimentation and adhesion to flat horizontal substrates out of stagnant suspensions, such as described in the present work, has become available.[30,31] In the essence this approach takes into account the rate of sedimentation of the particles in the suspending medium and the rate of escape over the potential barrier as given by the DLVO theory. The authors give an expression for the number of particles adhering to a solid surface as a function of time. Denoting t^* as the time needed for the furthest cell to reach the surface by diffusion, then for times $t < t^*$ the number of particles adhering to the surface is given by:

$$n = C_0 V\left(t + \frac{e^{-Pt} - 1}{P}\right) \tag{1}$$

where n is the number of particles adhering per unit area; C_0 is the initial bulk concentration of the particles in suspension; V is the sedimentation velocity and P is the probability per unit time that a particle will adhere to the solid surface. For time t larger than $3/P$, Eq. (1) can be approximated by:

$$n = C_0 V\left(t - \frac{1}{P}\right) \tag{2}$$

The rate at which particles adhere to the solid surface is then proportional to the sedimentation velocity V. For time t greater than t^*, the authors[31] obtained an expression which predicts that all available particles will adhere to the solid surface.

Overall, the theoretical predictions are as sketched in Figure 2. For a different solid surface, P may vary and hence the extrapolated intersection with the time axis may be different. However, as may be seen from Eq. (2), at times $t < t^*$ but $t > 3/P$, the rate of particle adhesion would be independent of the solid surface. Thus this

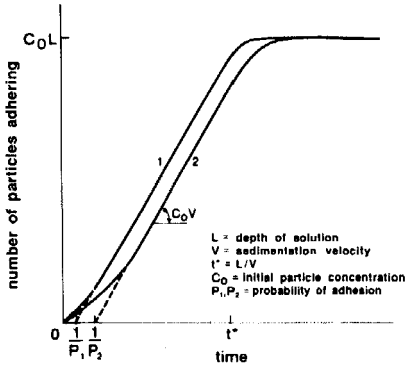

FIGURE 2 Theoretical plot of particle adhesion as a function of time—from Ruckenstein, *et al.*[30,31] Schematic curves shown for two different probabilities of adhesion. L = depth of solution; V = sedimentation velocity; $t + = L/V$; C_0 = initial particle concentration; P_1, P_2 = probability of adhesion.

DLVO model predicts that the process of particle adhesion would be kinetically controlled and should lead to complete coverage of the substrate material.

4. EXPERIMENTAL VERIFICATION

In an attempt to test this theoretical model we have examined the kinetics of particle adhesion to a number of different substrates.[7,32,33] Shown in Figures 3 and 4 are the results of experiments with both fresh and glutaraldehyde-fixed erythrocytes, suspended in Hanks Balanced Salt Solution, pH 7.2 and an ionic strength of 0.15. For details of the experimental conditions see legends to the figure. It should be noted that the general features of the curves for the two types of hydrophilic particles are identical.

Comparing the curves of Figures 3 and 4 with the theoretical predictions[30,31] as given in Figure 2 and described by Eqs (1) and (2), we note that these relations do not describe our results adequately. According to this theory, for times greater than 3/P, but less than t^*, the rate of particle adhesion to various solid surfaces would have to be identical. In other words, after lag times possibly different for the various substrates the curves would all

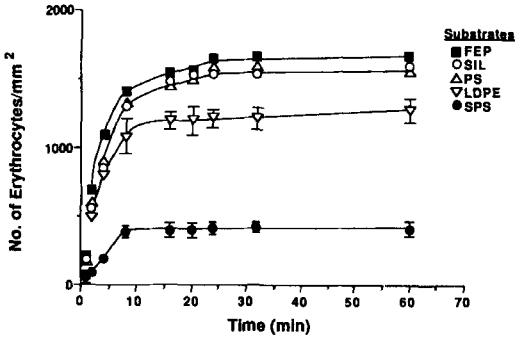

FIGURE 3 Kinetics of glutaraldehyde-fixed human erythrocyte adhesion to various surfaces. Cell concentration is 1×10^6 cells/ml in Hanks Balanced Salt Solution (pH 7.2; ionic strength = 0.15). Substrates as indicated. The associated error limits are 95% confidence limits. (For graphical reasons errors are shown only for selected cases; errors are similar in all cases.)

have to be parallel to each other. This is clearly not the case. There is no evidence in the experimental curves for the existence of lag times, and the slopes of the curves are a function of the surface tension of the substrate, as discussed previously.[6,7,12,32,34] Furthermore, the derived theoretical expressions imply that the rate of adhesion will remain constant until it reaches t^*. For a depth of the

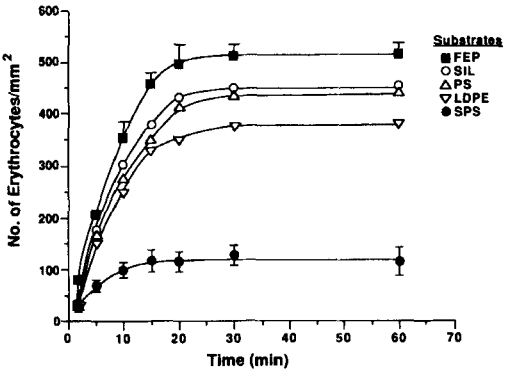

FIGURE 4 Kinetics of fresh human erythrocyte adhesion to various surfaces in Hanks Balanced Salt Solution (pH 7.2; ionic strength = 0.15). Cell concentration is 1×10^6 cells/ml. Substrates as indicated. The associated error limits are 95% confidence limits. (For graphical reasons error are shown only for selected cases; errors are similar in all cases.)

solution in the well of approximately 1 cm as in these experiments, t^* is of the order of 10 hrs, in contrast to the 20–30 minutes after which no more particles adhered to the surface. The extent of adhesion is most pronounced on the hydrophobic FEP surface. At the saturation level of adhesion on the FEP surface a maximum surface coverage of only approximately 10–15% is observed. Thus the traditional DLVO type of description of particle adhesion fails on two accounts to describe the experimental observations of the present work: (1) Within the limits of these experiments no lag time in the adhesion process is noted; and (2) Complete surface coverage of the substrate is not observed. Thus we conclude that the process of particle adhesion out of a solution with an ionic strength of 0.15 or greater is not transport controlled but is determined by properties of the solid substrate, *cf.* Figures 3 and 4.

Both the rate of adhesion and the saturation level of particle adhesion to each of the surfaces is substrate dependent. Plots of the number of particles adhering per unit surface area for the various substrates as a function of substrate surface tension for the various contact times result in a generally linear decrease in the extent of particle adhesion with increasing substrate surface tension.

5. THERMODYNAMIC MODEL FOR PARTICLE ADHESION

These observations are consistent with a theoretical description of particle adhesion based on surface thermodynamic considerations. Such an approach indicates that a properly identified thermodynamic potential, the grand canonical potential, which we simply call the free energy,[7] will be minimized at equilibrium. This implies that the process under consideration, *e.g.* particle adhesion, will be favoured if the process itself causes the thermodynamic function to decrease. The process will not be favoured if it would cause the free energy function to increase.

In order to make quantitative predictions about the likelihood of a particular process, it is necessary to "model" the process: Consider a particle (P) initially suspended in a liquid (L) attaching to a solid (S) which is also immersed in the same liquid as illustrated schematically in Figure 5. In the absence of specific interactions the

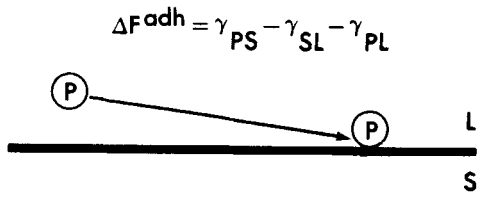

FIGURE 5 Schematic representation of the process of particle adhesion. *P*, particle; *L*, suspending liquid; *S*, substrate materials.

change in free energy (ΔF^{adh}) due to the process of adhesion is

$$\Delta F^{adh} = \gamma_{PS} - \gamma_{PL} - \gamma_{SL} \tag{3}$$

where γ_{PS}, γ_{PL} and γ_{SL} are the particle–solid, particle–liquid and solid–liquid interfacial tensions, respectively. The validity and restrictions of this model have been described in detail elsewhere.[11,35,36] It should be emphasized that this model considers only van der Waals interactions. The relationship between interfacial tensions and van der Waals forces has been described in detail elsewhere.[37,38]

It is clear from Eq. (3) that the free energy of adhesion of a particle to a surface depends not only on the surface tension of the adhering particles and the polymer surface but is also dependent on the surface tension of the suspending liquid. As an illustration shown in Figure 6 is the theoretical free energy of adhesion (ΔF^{adh}) for fixed-erythrocytes to substrates of various surface tensions, for two conditions of the liquid surface tension. The input data required for the development of such a plot are the respective surface tensions of the adhering particles (γ_{PV}), the polymer substrates (γ_{SV}) and the suspending liquid medium (γ_{LV}). This information is given in the legend to this figure. Consideration of such theoretical calculations as illustrated in Figure 6 lead to a distinction between two situations: For

$$\gamma_{LV} > \gamma_{PV} \tag{4}$$

ΔF^{adh} becomes more positive with increasing γ_{SV} predicting a decrease of particle adhesion with increasing substrate surface tension, γ_{SV}, over a comparatively wide range of γ_{SV} values. On the

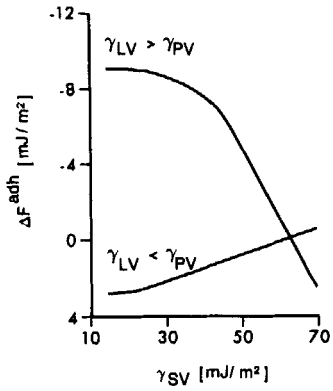

FIGURE 6 The free energy of adhesion (ΔF^{adh}) for fixed human erythrocytes as a function of substrate surface tension, γ_{SV}. (A) $\gamma_{LV} > \gamma_{PV}$: $\gamma_{LV} = 72.8$ ergs/cm^2 and $\gamma_{PV} = 64.6$ ergs/cm^2. (B) $\gamma_{LV} < \gamma_{PV}$: $\gamma_{LV} = 59.8$ ergs/cm^2 and $\gamma_{PV} = 64.6$ ergs/cm^2.

other hand, when

$$\gamma_{LV} < \gamma_{PV} \tag{5}$$

the opposite pattern of behaviour is predicted.[6,34] For the limiting case of the equality

$$\gamma_{LV} = \gamma_{PV} \tag{6}$$

ΔF^{adh} becomes equal to zero independently of the value of γ_{SV} implying that under these conditions the extent of particle adhesion does not depend on substrate surface properties and in principle should be zero if no other effects, such as electrostatic interactions, come into play.

6. EXPERIMENTAL VERIFICATION

As an illustration of the influence of the substrate surface tension on the extent of erythrocyte adhesion shown in Figure 7 are photomicrographs of the substrate after rinsing to remove non-adherent cells. In Figures 7a and 7b the suspending liquid surface tension was 72.8 ergs/cm^2 and the two substrates, fluorinated ethylene-propylene copolymer (FEP) and sulphonated polystyrene (SPS), have surface tensions of 16.4 and 66.7 ergs/cm^2, respectively. In

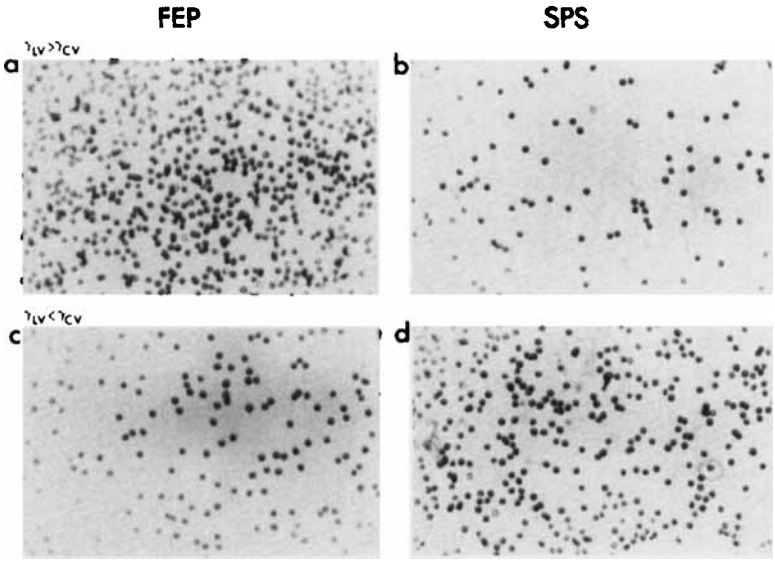

FIGURE 7 Photomicrographs of erythrocyte adhesion under varying experimental conditions. (a) Fluorinated ethylene-propylene (FEP, $\gamma_{SV} = 16.4$ ergs/cm^2); $\gamma_{LV} = 72.8$ ergs/cm^2. (b) Sulphonated polystyrene (SPS, $\gamma_{SV} = 66.7$ ergs/cm^2); $\gamma_{LV} = 72.8$ ergs/cm^2. (c) Fluorinated ethylene-propylene (FEP, $\gamma_{SV} = 16.4$ ergs/cm^2), $\gamma_{LV} = 59.8$ ergs/cm^2 (d) Sulphonated polystyrene (SPS, $\gamma_{SV} = 66.7$ ergs/cm^2); $\gamma_{LV} = 59.8$ ergs/cm^2.

Figure 7c and 7d we show photomicrographs of the extent of adhering erythrocytes to the same two surfaces when the liquid surface tension had been substantially lowered to a value of $\gamma_{LV} = 59.8$ ergs/cm^2. A comparison of Figure 7a and 7b with Figures 7c and 7d reveals quite clearly that as the liquid surface tension is varied so the pattern of adhesion is changed. As the surface tension of the suspending liquid medium is lowered from 72.8 to 59.8 ergs/cm^2, the number of adhering particles per unit surface area decreases in the case of FEP but increases for SPS under otherwise identical conditions. The effect of varying γ_{LV} on the extent of particle adhesion is perhaps even more clearly illustrated by considering one and the same substrate and two different γ_{LV} values, *e.g.* by comparing Figure 7a with Figure 7c or Figure 7b with Figure 7d.

The quantitative results of experiments in which the particles, at a constant bulk concentration, are suspended in various aqueous mixtures having different surface tensions are summarized in Figure 8. For the purpose of these experiments the fixed erythrocytes were suspended, at a bulk concentration of 1×10^6 erythrocytes/ml, in buffered Hanks Balanced Salt Solutions containing varying amounts of dimethyl sulfoxide (DMSO), a surface-tension-lowering additive. The pH and temperature of these solutions was held constant at pH 7.2 and 25°C respectively. The surface tension, measured by means of the Wilhelmy Plate Method,[39] of these solutions are given in Table II. For complete experimental details see legend to Figure 8. The theoretical predictions inherent in Figure 6 and their implications are substantiated experimentally as shown in Figure 8. At the lowest DMSO concentration, corresponding to the highest surface tension, γ_{LV}, of the suspending medium, particle adhesion *decreases* with increasing substrate surface tenson γ_{SV}. As the DMSO concentration is increased and the surface tension γ_{LV} correspondingly lowered, the change in the extent of particle adhesion with increasing γ_{SV} become less pronounced. At a certain intermediate γ_{LV}, particle adhesion becomes independent of γ_{SV}

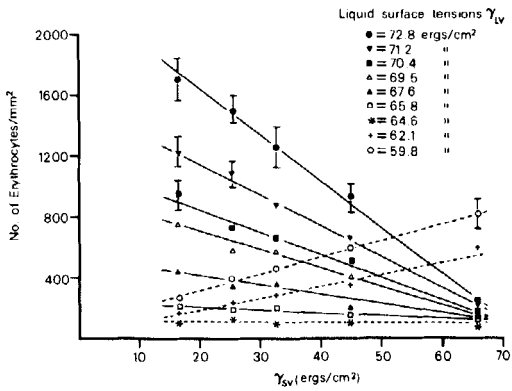

FIGURE 8 Erythrocyte adhesion as a function of substrate surface tension γ_{SV} for various liquid surface tensions. The cells at a concentration of 1×10^6 cells/ml were suspended in Hanks Balanced Salt Solution containing varying amounts of a surface tension lowering additive, dimethylsulfoxide (DMS). The pH of the solution was 7.2. Indicated error limits are 95% confidence limits. (For graphical reasons error limits are given only for selected cases; the errors are similar in all cases.)

TABLE II
Suspending media for the erythrocyte adhesion experiments

Medium	Concentration	Surface tension γ_{LV} (ergs/cm^2)
HBSS[a]	—	72.8
HBSS–DMSO 1[b]	1% (vol/vol) DMSO	71.2
HBSS–DMSO 3	3% (v/v)	70.4
HBSS–DMSO 5	5% (v/v)	69.5
HBSS–DMSO 10	10% (v/v)	67.6
HBSS–DMSO 12	12% (v/v)	65.8
HBSS–DMSO 12.5	12.5 (v/v)	64.6
HBSS–DMSO 15	15% (v/v)	62.1
HBSS–DMSO 18	18% (v/v)	59.8
H_2O–DMSO 12.5	12.5% (v/v)	64.4

[a] Hanks Balanced Salt Solution, comprising in mg/L: anhydr. $CaCl_2$: 140.0; KCl: 400.0; KH_2PO_4: 60.0; $MgCl_2 \cdot 6H_2O$: 100.0; $MgSO_4 \cdot 7H_2O$: 100.0; NaCl: 8000.0; $NaHCO_3$: 350.0; $NaHPO_4 \cdot 2H_2O$: 60.0; glucose: 1000.0; ionic strength, $\mu = 0.15$; pH 7.26.
[b] Dimethyl sulfoxide.

and finally, at yet lower values of the surface tension γ_{LV}, particle adhesion *increases* with increasing γ_{SV}. Aside from the practical interest of these data there are two further points to be made. First the thermodynamic model underlying Eq. (3) describes the qualitative features of erythrocyte adhesion remarkably well. The agreement between theoretical predictions and experimental observations suggests for the present experimental conditions (ph 7.2, ionic strength $\simeq 0.15$) that the extent of particle adhesion to polymer surfaces is primarily governed by van der Waals forces. Secondly, the thermodynamic model predicts that in the case of $\gamma_{LV} = \gamma_{PV}$ (Eq. (6)), ΔF^{adh} should be independent of γ_{SV}, implying that the extent of adhesion should be independent of γ_{SV}, a situation that is indeed contained in the data of Figure 8. To investigate this concept further, the slopes of the straight lines in Figure 8 were plotted *versus* γ_{LV} in Figure 9, by means of a second order polynomial curve fit. It is inferred that the slope becomes equal to zero at a value of γ_{LV} which is characteristic for the adhering particles. This value, according to the predictions of the thermodynamic mode, is equal to the surface tension, γ_{PV}, of the particles themselves. Figure 9 reveals that for the fixed human erythrocytes the adhesion

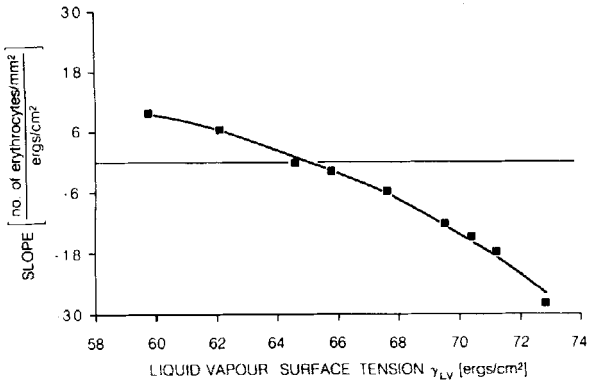

FIGURE 9 Slopes of the straight lines of Figure 8 *versus* liquid surface tension. The slope is equal to zero for $\gamma_{LV} = \gamma_{PV}$.

slope becomes equal to zero when $\gamma_{LV} = 64.6$ ergs/cm^2, implying that the surface tension of these particles is equal to 64.6 ergs/cm^2 at 25°C. This is in good agreement with the surface tension values obtained for these particles by means of other independent techniques.[11,32,40] This issue is discussed in more detail later in this article.

Thus the thermodynamic model for particle adhesion proposed in Eq. (3) and illustrated in Figure 6 can be used to describe qualitatively erythrocyte adhesion to a range of polymer surfaces under conditions of varying γ_{LV}. The model, however, does not describe entirely all the features of the pattern of particle adhesion. When $\gamma_{LV} = \gamma_{PV}$ there is an absence of van der Waals interactions and hence the extent of erythrocyte adhesion is expected to be *independent* of the surface properties of the substrate materials, and

$$\Delta F^{\text{adh}} = 0 \tag{7}$$

Under these conditions ($\gamma_{LV} = \gamma_{PV}$), however, a small level of adhesion to all the polymer surfaces was observed. In this case, particle adhesion cannot be ascribed to van der Waals attraction; in a polar liquid such as water, electrostatic interactions may be implicated. To investigate this possibility further, the adhesion of erythrocytes was measured at the liquid surface tension close to the cell surface tension of 64.6 ergs/cm^2. This was done at 12.5% (v/v) DMSO concentrations at an ionic strength $\mu = 0.15$ (in HBSS), *i.e.*

just as in Fig. 8, and similarly at $\mu = 0.075$ (half-strength HBSS) and at $\mu = 0$. The results are given in Figure 10. At $\mu = 0.15$, and in the absence of van der Waals interactions, an average of 104 adhering particles per square millimeter is observed, while at $\mu = 0.075$ considerably fewer ($\simeq$68) particles per unit surface are found. At $\mu = 0$ only 6 particles per square millimeter were observed. Thus, lowering the ionic strength of the suspending medium significantly decreases the residual level of particle adhesion under these experimental conditions, *i.e.*, when the van der Waals forces are zero. This reduction in the number of adhering cells may be due to electrokinetic phenomena such as a decrease in ionic strength being accompanied by an increase in ζ-potential as discussed below.

Plurivalent cations, *e.g.* Ca^{2+} ions, are often implicated in particle adhesion studies. Since these ions were present in the buffer system (HBSS) used in these studies, a divalent chelating agents, Na_2 EDTA, was admixed into the buffer in order to bind and effectively remove these cations. As shown in Fig. 10, this indeed resulted in a significant decrease in the extent of particle adhesion to the polymer surfaces. These results together with the data presented in Figure 8 suggest that, in the absence of any specific interactions, two major types of forces play a role in determining the overall extent of particle adhesion to polymer surfaces: van der Waals attractive

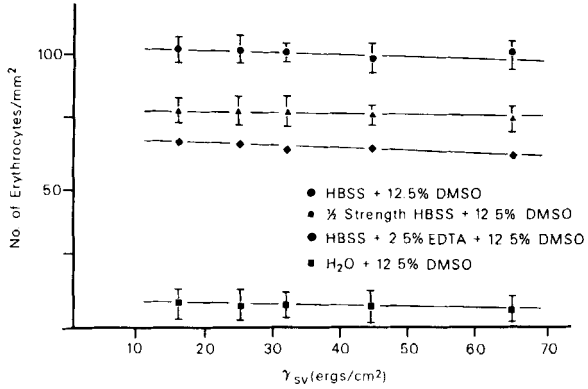

FIGURE 10 Erythrocyte adhesion as a function of substrate surface tension γ_{SV} in high and low ionic strength media and in the presence of a chelating agent. Indicated error limits are 95% confidence limits.

forces (between the adhering particles and the polymer substrate) and plurivalent cationic bridging.

The data presented thus far would suggest that the most important of these forces are, by a large margin, the van der Waals interactions; the level of particle adhesion out of HBSS onto a Teflon® surface is $\simeq$1700 cells/mm^2. When the van der Waals attraction is reduced to zero, and under otherwise identical conditions, the level of particle adhesion is reduced to approximately 100 cells/mm^2 ($\simeq$6%). Under these conditions both plurivalent cationic bridging and ionic strength effects contribute to the residual level of particle adhesion. These conclusions are based on experiments in which the particles are suspended in buffered solutions having an ionic strength of 0.15 and pH 7.2.

7. ROLE OF SOLUTION pH AND IONIC STRENGTH

The effect of ionic strength has been briefly referred to above. In order to define its role in particle adhesion more precisely a systematic study was undertaken. The data contained in Figure 8 and in 10 are surprising since there is an apparent absence of electrical double layer effects on particle adhesion. In order to address this question a study on the role of solution pH and ionic strength in determining the extent of particle adhesion to polymer surfaces was undertaken. Experimental details have been reported elsewhere.[34]

(a) Solution pH

Results of experiments to determine the effect of pH on particle adhesion are shown in Fig. 11. These experiments were performed at a constant ionic strength of 0.15, *i.e.* the particles were suspended in 150 mM NaCl solutions buffered to the described pH with 3×10^{-4} M sodium bicarbonate. The pH range examined extended from pH 5.0 to pH 9.0 at 0.5 pH intervals. This range was selected since it has been established[41,42] that the particles' surface charge density did not vary over this pH range as assessed by electrophoretic mobility measurements in 0.15 M NaCl at the various pH values. Thus, changes in the extent of particle adhesion cannot be

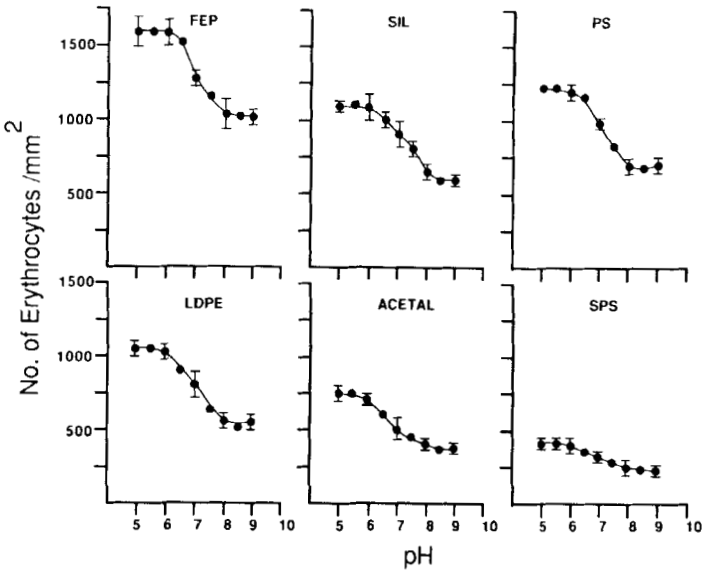

FIGURE 11 The effect of pH on erythrocyte adhesion to polymers of different surface tension. The cells, at a concentration of 1×10^6 cells/ml, were suspended in various buffered pH solutions at a constant NACl concentration of 150 mM. Errors indicated are 95% confidence limits.

ascribed to changes in particle zeta-potential and must be due to other factors.

There are several points to be made about the data contained in Fig. 11. First we note that a decrease in pH below 6.0 does not result in any further change in the level of particle adhesion for any of the polymer substrates examined (Plateau No. 1). As the pH of the suspending solution is increased above pH 6.0 there is a marked decrease in the level of particle adhesion until a pH value of approximately 8.0 is reached. Thereafter, the level of particle adhesion does not change anymore, *i.e.* a second plateau is reached for each substrate (Plateau No. 2). The values of these plateau levels of particle adhesion are determined by substrate surface properties in agreement with earlier observations with respect to Figures 3, 4 and 8. This is illustrated in Figure 12 in which the two plateau levels of particle adhesion are plotted as a function of substrate surface tension. We note that the two plateau levels of

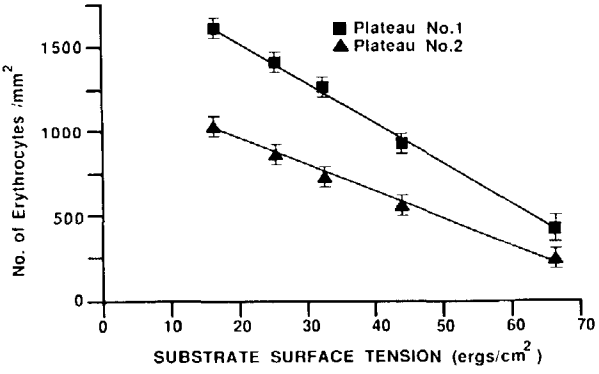

FIGURE 12 Plateau values of erythrocyte adhesion plotted as a function of substrate surface tension. Errors indicated are 95% confidence limits.

adhesion each give rise to a straight-line plot of the extent of particle adhesion with increasing substrate surface tension. Particularly noteworthy, however, is the observation that the slopes of these two lines are different. These differences are significant and suggest that the particle-polymer interaction is altered as a result of the pH changes. As discussed above these changes cannot be ascribed to differences in the effective zeta-potentials and an alternative explanation must be sought.

Interpreting the two straight lines naively from the point of view of the thermodynamic model described earlier and the associated stipulation that only van der Waals forces are operative, one would conclude for the present case of constant liquid surface tension that the Plateau No. 2 curve belongs to particles having a surface tension which is different from that of the particles described by Plateau No. 1. To be specific, the thermodynamic model would suggest that the particles of Plateau No. 2 have a higher surface tension (*i.e.* are more hydrophilic) than those of Plateau No. 1. The implication of these conclusions is that changes in pH conditions have, in some as yet undetermined manner, altered the surface tension of the particles whilst the net surface charge of the particles remains unchanged.

(b) Ionic Strength

The results of experiments performed in order to assess the effect of solution ionic strength on hydrophilic particle adhesion are summarized in Figure 13. For the purpose of these experiments the pH of the solution was maintained at a constant value of 6.1. This pH value was selected since it falls within the pH range giving rise to one of the two plateaux in Fig. 12. Thus, the points corresponding to an ionic strength of 0.15 (*i.e.* 150 mM NaCl concentration) in Figure 13 are identical to those of pH 6.1 in Figure 12. We note from Figure 13 that the level of particle adhesion is near zero for the lowest ionic strengths and increases with increasing ionic strength. Finally, at ionic strengths near 0.1 (*i.e.* sodium chloride concentrations of 100 mM) the extent of erythrocyte adhesion reaches a limiting plateau value. In general, this plateau level of particle adhesion decreases with increasing substrate surface tension. A plot of this level of particle adhesion as a function of

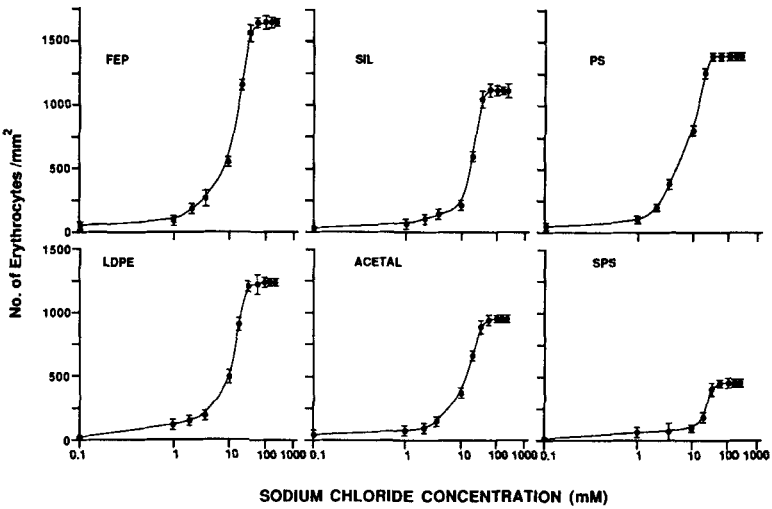

FIGURE 13 The effect of ionic strength on erythrocyte adhesion to polymers of different surface tension. The cells at a concentration of 1×10^6 cells/ml were suspended in various ionic strength solutions at a constant pH of 6.1. Errors indicated are 95% confidence limits.

substrate surface tension has already been given in Figure 12 (Plateau No. 1).

These findings suggest that at low ionic strength double layer repulsion prevents particle adhesion more or less completely. With increasing ionic strength the double layer thickness decreases so that the particles can approach the solid substrates more closely so that the van der Waals forces will become appreciable, causing particle adhesion. For ionic strengths of 0.1 (corresponding to a NaCl concentration of 100 mM) and above, the van der Waals forces apparently overpower all electrostatic effects.

8. IRREVERSIBLE PARTICLE ADHESION

Adhesion of fixed erythrocytes to polymer surfaces out of solution of high ionic strength (0.15) is irreversible in the sense that subsequent immersion of the substrate in deionized-distilled water does not result in particle detachment. This observation is valid for all pH conditions examined. This is illustrated in Table III in which it is shown that there is no significant difference in the level of particle adhesion to any of the polymer surfaces, for each of the pH conditions examined, when the surfaces are rinsed either in water or in high-ionic-strength (150 mM NaCl) media. The implication of this observation is that under experimental conditions of a suspending solution ionic strength of 0.15 and a solution pH in the range of 5–9, particle adhesion is irreversible, a pattern of behaviour which is characteristic of a process that has occurred at the primary energy minimum.[29] It has yet to be established whether solutions of low ionic strength also give rise to irreversible adhesion or whether under such experimental conditions particle adhesion is reversible reflecting a process occurring at the secondary energy minimum.

9. DISCUSSION

Earlier in this article we discussed the apparent failure of a typical DLVO approach to describe the experimental results of erythrocyte adhesion to a wide range of substrate surface properties. It appears that a pH of approximately 7.0 and an ionic strength of 0.1 or

TABLE III

Effect of ionic strength on erythrocyte detachment. For the initial cell-polymer exposure the erythrocytes were suspended in the various pH solutions as indicated with an ionic strength of 0.15. Errors are 95% confidence limits assuming a Student *t*-distribution

Polymer surface	150 mM NaCl rinse					Deionized distilled water rinse				
	pH					pH				
	5.0	6.0	7.0	8.0	9.0	5.0	6.0	7.0	8.0	9.0
FEP	1610 ± 100	1602 ± 90	1524 ± 60	1021 ± 120	1000 ± 76	1645 ± 80	1590 ± 110	1545 ± 78	1008 ± 75	1000 ± 69
SIL	1098 ± 56	1103 ± 68	897 ± 104	644 ± 80	597 ± 42	1051 ± 70	1146 ± 85	909 ± 60	665 ± 75	578 ± 56
PS	1418 ± 26	1382 ± 55	1180 ± 30	882 ± 40	870 ± 68	1447 ± 56	1359 ± 42	1162 ± 38	877 ± 52	853 ± 36
LDPE	1243 ± 58	1217 ± 54	998 ± 86	756 ± 45	747 ± 58	1236 ± 61	1238 ± 76	1049 ± 58	743 ± 38	719 ± 31
Acetal	926 ± 68	898 ± 47	709 ± 64	580 ± 42	559 ± 30	949 ± 25	861 ± 89	748 ± 60	570 ± 45	518 ± 24
SPS	393 ± 38	386 ± 48	316 ± 15	237 ± 40	226 ± 31	406 ± 42	365 ± 31	297 ± 24	260 ± 52	216 ± 45

higher, erythrocyte adhesion is primarily governed by van der Waals interactions between the particles and the various substrate materials. The experimental data can be predicted in large part by means of a thermodynamic model which implicitly considers that van der Waals forces only are operative. Under the stated experimental conditions particle adhesion is irreversible, a pattern of behaviour which is consistent with a process occurring at the primary energy minimum.

Critical to the success of the thermodyanamic model for particle adhesion is the ability to determine the surface tension of the adhering particles. Over the past decade several strategies have been developed to determine the surface tension of small particles. These strategies include contact angle measurements,[43] the adhesion strategy,[6,12,33] droplet sedimentation[44,45] and sedimentation volume[46–49] studies, and the freezing front method.[37,38,50–55] These techniques have recently been reviewed in detail elsewhere.[11,40] A comparison of the results obtained with these independent techniques for glutaraldehyde-fixed human erythrocytes is given in Table IV. As illustrated it is clear that the results obtained with the different techniques are in good agreement. As yet we have not been able to perform contact angle measurements on fixed-erythrocytes. We believe this is because of the rigid nature of these cells.

As noted earlier, the extent of particle adhesion decreases with increasing substrate surface tension. There is, however, one minor, although significant, exception to this general trend. Adhesion to a siliconized glass surface is considerably less than that noted on a

TABLE IV
Surface tension of glutaraldehyde-fixed human erythrocytes obtained *via* different techniques. Corrected to 22°C assuming $\frac{d\gamma}{dT} = 0.1$ ergs/cm^2°C

Method	Particle surface tension (ergs/cm^2)
Adhesion	64.6
Freezing Front	64.2
Droplet Sedimentation	64.3
Sedimentation Volume	64.5

hydrophobic FEP surface even though these surfaces both have the same surface tension of approximately 17 ergs/cm^2. Generally, the level of erythrocyte adhesion on a siliconized surface is similar to that observed on a polystyrene surface which has a surface tension of approximately 26 ergs/cm^2. This observation is illustrated in Figures 3 and 4, respectively. Thus, siliconized glass behaves as one would expect of a more hydrophilic surface. There are two possible, not mutually excluding, explanations for this pattern of behaviour: 1. The hydrocarbon (silane) layer may not be complete and would thus allow contact between the hydrophilic glass surface and the erythrocytes. This possibility, however, is not likly since contact angle measurements performed on various sections of the siliconized surface suggest a homogenous surface. 2. The second possibility, which may be operative even if the silane coating is perfect, is a phenomenon known as "screening".[2,56,57] This is due to the fact that the depth of the van der Waals interaction between two phases is of the same order as the separation distance between the two phases. Thus, since the silane layer is probably only a few Angstroms thick, and since the van der Waals interaction are appreciable over hundreds of Angstroms, the approaching cells will interact not only with the silane layer, but also with the underlying glass substrate. This type of apparent anomaly in the behaviour of siliconized surfaces has been observed not only with respect to particle adhesion[32] but also with macromolecule (protein) adsorption.[58]

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