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Title: The Influence of Surface Functional Groups on β-Lactoglobulin Adsorption Equilibrium

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Interactions between proteins and contact surfaces can have important implications in the food industry. Such interactions contribute to the course of fouling of membrane surfaces and they appear to mediate bacterial and spore adhesion to some degree as well. In addition to protein and solution properties, interfacial behavior is strongly influenced by contact surface properties. Among these, hydrophobicity and the potential to take part in acid-base interaction have received considerable attention, but in a quantitative sense we know very little about their respective influences on protein adsorption. It was the purpose of this research to quantify the equilibrium adsorptive behavior of the milk protein β-lactoglobulin as it is influenced by the presence of different contact surface functional groups.

Monocrystalline and polished silicon surfaces were modified to be hydrophilic by oxidation and hydrophobic by silanization with

dichlorodiethylsilane (DDES), dichlorodimethylsilane (DDMS), and dichlorodiphenylsilane (DDPS), each used at concentrations of 0.82, 3.3, and 82 mM. Surface hydrophobicities were evaluated with contact angle methods. Adsorption isotherms were constructed after allowing each modified silicon surface to independently contact β-lactoglobulin (0.01 M phosphate buffer, pH 7.0) at concentrations ranging between 200 and 2000 mg/L for eight h at room temperature. Surfaces were then rinsed and dried. Optical properties of the bare- and film-covered surfaces, necessary for calculation of adsorbed mass, were obtained by ellipsometry.

Plots of adsorbed mass as a function of protein concentration exhibited attainment of plateau values beyond a protein concentration of about 200 mg/L. At high silane concentration, the plateau values associated with surfaces exhibiting ethyl groups were observed to be greatest followed by those exhibiting phenyl, methyl, then hydrophilic (OH) groups. At the low DDMS and DDES concentrations (0.82 and 3.3 mM), adsorbed mass did not increase beyond that value recorded for the hydrophilic surface. This is likely due to some critical spacing of methyl and ethyl groups being required to produce a favorable hydrophobic effect on adsorption. For surfaces treated with dichlorodiphenylsilane, adsorbed mass increased with silane concentration. Apparently, a favorable acid-base interaction effected by the hydrophilic surface is inhibited by the presence of small amounts of methyl and ethyl groups, but somewhat less inhibited by the presence of phenyl groups because the latter have the ability to undergo acid-base interaction.

The Influence of Surface Functional Groups on β-Lactoglobulin Adsorption Equilibrium

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INTRODUCTION

Protein deposition onto solid surfaces is important to the food processing industry. It has been found that protein adsorption to surfaces can result in several problems. For example, it can lead to fouling due to subsequent adsorption of other organic materials. With respect to heat exchange equipment, deposition leads to a reduction in heat transfer efficiency which results in either insufficient heat treatment of the processed food or to increased energy costs. Fouling also results in increased cleaning and labor costs as well as lost production during periods of downtime. Protein adsorption has also been observed to mediate bacterial biofilm development on food contact surfaces (Tosteson and Corpe, 1975; Bryers 1987). The danger associated with consuming foods contaminated by some microbial species is well documented, and dairy foods have received wide attention as sources for such contamination in the food supply. Control of these kinds of problems seems best attained by focusing attention on the early events that occur at the fluid food-contact surface interface.

Protein adsorption involves noncovalent attachment of segments of the molecule to the solid surface. In order for the molecule to adsorb, solvent molecules must be released from both the solid and the protein surface. This process is accompanied by a gain in entropy;

protein adsorption is often said to be entropically driven (Lee and Ruckenstein, 1988).

Protein adsorption equilibrium is sometimes found to follow a Langmuir-type isotherm (Al-Malah et al., 1992; Luey et al., 1991; Lee and Kim 1974). A steep initial slope over very low concentrations followed by leveling off at higher concentrations is observed in this type of isotherm.

Protein adsorption is a process described as irreversible or at least only partly reversible. The amount adsorbed to a surface remains on that surface even after contacting it with protein-free solution (Jonsson et al., 1982; Daeschel et al., 1992).

Much effort has been devoted to investigating numerous adsorption- influencing factors. Excellent reviews are available in the literature concerning that work (Andrade, 1985; Norde,1986; Horbett and Brash, 1987).

Protein adsorption exhibits a diversity in behavior from one surface to another. This diversity is a result of the complexity of the protein itself as well as other factors. Protein properties which contribute to this diversity in behavior include isoelectric point, net charge and charge distribution, three-dimensional structure in solution, location and nature of hydrophobic domains and conformational stability. The medium pH, temperature, ionic strength, protein equilibrium concentration and hydrodynamics are contributing factors to the protein adsorption process. Solid surface energetics and energetic heterogeneity, charge density, composition, and chemistry play key roles in protein adsorption as well. In general, contact surface

properties are often described as being either hydrophobic or hydrophilic in character. However, hydrophobicity can be due to the presence of any functional group able to exhibit a favorable hydrophobic attraction with similar groups or domains found in the protein molecule. Food contact surfaces can therefore exhibit similar hydrophobicities, but differ in performance, and although it is often used as an independent variable in describing various interfacial processes, hydrophobicity might explain surface behavior in only very well controlled circumstances. The extent to which a given functional group can produce a hydrophobic or other type of effect on food protein adsorption has not been investigated extensively.

Objective and Method of Approach

It was the purpose of this research to study the equilibrium adsorptive behavior of β-lactoglobulin (β-lg; a milk protein) as a function of contact surface chemistry. Hyperpure silicon surfaces were modified to be hydrophilic by oxidation and hydrophobic by derivatization with dichlorodiethylsilane, dichlorodimethylsilane, or dichlorodiphenylsilane, each used at concentrations of 0.82, 3.3, and 82 mM. Surface properties were evaluated with contact angle methods. Adsorption isotherms were constructed after allowing each modified silicon surface to independently contact β-lg (0.01 M phosphate buffer, pH 7.0) at concentrations ranging between 200 and 2000 mg/L for eight hours at room temperature. Surfaces were then mildly rinsed to remove loosely bound protein, then dried by nitrogen

flow and kept in a dust-free desiccator. Optical properties of the bare- and film-covered surfaces, necessary for calculation of adsorbed mass, were obtained by ellipsometry.

LITERATURE REVIEW

Protein Adsorption

Protein adsorption takes place when protein molecules are added to a solution into which a solid material is immersed (Lundstrom 1987). Much work has been conducted to determine the parameters affecting protein adsorption and to develop a model that can accurately describe it.

Some factors that affect protein adsorption and have been studied to some extent include pH, ionic strength, hydrophobic interactions, conformation of the protein in solution, and the surface characteristics of the material onto which adsorption takes place. A brief summary of the literature concerning these factors is provided in the following subsections.

pH Effects

The pH of the buffer solution is an important factor that affects protein adsorption. Much work has been conducted to describe this effect (Lee and Ruckenstein 1988; Luey et al., 1991; Zsom 1986; Norde and Lyklema 1989). Generally, it has been shown that maximum adsorption takes place at or near the isoelectric point. That is, at the pH where the net charge on the protein molecule is zero, the molecule is in its most compact form and requires less contact surface area to adsorb (Lee and Ruckenstein 1988; Luey et al., 1991; Elgersma

et al., 1990; Hegg et al., 1985).

The effect of pH on protein adsorption onto hydrophilic and hydrophobic surfaces has been investigated (Luey et al., 1991). Hydrophilic surfaces are negatively charged, and a protein molecule in buffer at a pH below its isoelectric point would be positively charged. A favorable electrostatic interaction arises due to the oppositely charged surfaces. However, some soft (nonrigid) proteins (e.g. serum albumin) may adsorb to surfaces that have the same charge as the protein itself. This has been attributed to an additional driving force arising from structural alterations that may overcome unfavorable contributions from hydrophilic dehydration and electrostatic repulsion (Norde and Lyklema 1989). However, adsorption to hydrophilic surfaces at a pH higher than the isoelectric point of the protein is smaller than that compared with lower pH values. The significant increase in the negative surface charge of the protein as a result of its existing at a value of pH greater than its isoelectric point is the reason behind this decrease in the adsorbed mass of a protein. Moreover, this depends on the protein, e.g., β-lg exists as an expanded monomer at pH value higher than its isoelectric point (pH 7.5-8.90) and therefore would require more contact surface area per molecule for adsorption.

Additional driving forces for adsorption, such as hydrophobic interaction, are not considered to be major factors in adsorption onto hydrophilic surfaces, due to their strong affinity for water molecules (Luey et al., 1991). On the other hand, electrostatic interaction on hydrophobic surfaces is considered to be a minor force relative to the hydrophobic interaction. On hydrophobic surfaces, at pH values higher

than the isoelectric point of β-lg, the adsorbed mass was greater than that at lower pH. This was related to the protein being more unstable at high pH, leading to more hydrophobic regions of the molecule being exposed to water molecules and a greater amount adsorbed (Luey et al., 1991).

Ionic Strength Effects

The degree to which ionic strength affects protein adsorption depends on the role that electrostatics play in the adsorption driving force. Increasing the ionic strength causes an increase in the shielding of the charge of the protein molecule. This reduces the electrostatic effects and causes the protein molecule to become more globular. Luey et al. (1991) observed that as NaCl concentration was increased at constant pH (8.90), the amount of β-Lg adsorption to hydrophilic silicon increased. β-Lg at pH 8.90 is negatively charged, causing a repulsion between molecule and surface. Increasing the NaCl concentration in solution shielded the protein molecule by increasing the amount of positively charged ions surrounding it. In that case shielding reduced the electrostatic repulsion and was probably a factor in the observed increase in amount adsorbed, along with attainment of a more globular configuration of the protein (Lee and Ruckenstein 1988). On the other hand, it could be inferred that if the electrostatic interaction between protein and surface is attractive, increasing ionic strength would reduce the amount adsorbed.

At hydrophobic surfaces, however, increasing ionic strength

caused a decrease in the plateau amount adsorbed. Here, the observed behavior was opposite that at hydrophilic surfaces which were influenced more strongly by electrostatics. It is possible however, that incorporation of sodium ions into the protein structure yielded a more stable molecule, and therefore lower amount adsorbed (Luey et al., 1991).

Hydrophobic Interactions

Hydrophobic interactions contribute to the entropy driving force for adsorption of proteins from aqueous solution. Generally it is believed that protein adsorption is entropically driven. Lee and Ruckenstein (1988) suggested that there are two positive entropic contributions: the first one being due to the entropy gain caused by dehydration of the protein molecule and surface and the second due to an entropy gain by the structural changes that occur in the less rigid, partially dehydrated, protein molecule. Since hydrophobic surfaces have a low affinity for water molecules, the protein will change conformation to remove contact of these regions with the aqueous environment by either burying the hydrophobic regions internally or associating them with similar regions on the solid surface (Luey et al., 1991).

Molecular Effects

The chemistry and physics of the protein molecule have their

own effect on adsorption. Not all proteins adsorb to the same extent on the same surface. The relative binding characteristics to solid surfaces for different proteins can be ascribed to what is known about their molecular structures and the stability of those structures (Brock and Enser 1987). Some relatively small globular proteins with a polar surface and a hydrophobic interior inaccessible to a hydrophobic solid surface in non-denaturating solvent, have been observed not to bind to the surface under specific conditions e.g. high salt concentration (0.5 M), high temperature (20 °C) and long exposure to these conditions (24 h) (Brock and Enser 1987). However, under the same conditions a larger globular protein will preferentially adsorb to the surface. For example, actin is a globular protein that fails to adsorb to a hydrophobic surface under the above mentioned conditions, while bovine serum albumin (BSA) is a globular protein that does bind. BSA is larger than actin and has three distinct structural domains. The tertiary structure of each domain is stabilized by an extensive network of disulphide linkages (Brock and Enser 1987). The connection between the domains exists only through the polypeptide backbone and no participation of the disulphide linkages takes place between the domains. Consequently, relative to actin, the tendency of BSA to adsorb is less likely a result of the denaturation of its tertiary structure, and possibly due to previously buried surface being exposed by partial separation of its domains. Also, surface properties of the protein in its native conformation could render it less polar than actin (Brock and Enser 1987).

Non-globular proteins (elongated molecules) have more surface

area available for interaction with solid surfaces than do globular proteins. However, proteins exhibit a varying degree of conformational adaptability, which allows them to change their structures as conditions (e.g. pH) in the surrounding environment change (Luey et al., 1991). At low pH, for example, BSA partially denatures and the globular shape is changed to a threadlike conformation; however, caseins, even under non-denaturating conditions, exhibit a considerable conformational adaptability, and can take part in hydrophobic interaction. This was evident from qualitative similarities in binding properties between caseins molecules and hydrophobic surfaces (Brock and Enser 1987).

In other cases, Norde and Lyklema (1978) have observed that plateau amounts of protein adsorbed to negatively charged polystyrene surfaces are virtually independent of changes in pH for bovine pancreas ribonuclease (RNase), while those for human plasma albumin (HPA) vary by as much as a factor of 2. The occurrence of structural changes in HPA was inferred from changes observed in the amount adsorbed as a function of pH. The amount of RNase adsorbed to the surfaces were observed to be independent of changing solution conditions.

Surface Effects

Lee and Kim (1974) studied the adsorption of albumin, γ-globulin, and fibrinogen from solution to different hydrophobic polymers using internal reflection infrared spectroscopy. The polymers chosen were

poly(dimethyl siloxane) (SR) (silastic rubber), fluorinated ethylene/propylene copolymer (FEP), and a segmented copolyether-urethane-urea (PEUU) based on polypropylene glycol. The adsorption process was carried out under flow and static conditions at room temperature. A net adsorption value was given by taking the difference between the ratios of the amide I (C=O stretching) band at 1640 cm^{-1} to a standard band for each polymer before and after adsorption. This adsorption value was related to the real surface concentration by calibration. The standard bands were CH_3 for SR, CF_2 for FEP, and CH_2 for PEUU at 1400, 980, and 1450 cm^{-1} respectively. The PEUU surface adsorbed more than the SR and FEP surfaces. The researchers related that mainly to hydrogen bond formation with protein. FEP does not have the ability to form this type of bond with protein so it adsorbed less (Lee and Kim, 1974).

In his investigation of the dependence of preferential adsorption of BSA oligomers on the surface properties of monodisperse polystyrene latices, Zsom (1986) used polystyrene latices with different charge densities as his substrates.

The latices were purified by centrifugation and concentrated to about 10% solids. The latices exhibited considerable differences in their hydrophilicities based on the percentage of particle surface covered with $-O-SO_3^-$ groups. BSA samples contained different fractions of monomeric, dimeric, and polymeric species. Zsom found that the total amount of adsorbed protein increased with increasing concentration of dimeric and or polymeric species, with the exception of dimer-enriched BSA adsorption on a low surface charged latex. For

the monomeric and dimeric BSA, the total amount adsorbed decreased with increasing surface charge density up to a value of -15 $\mu C\ cm^{-2}$ but further increase in charge density yielded greater adsorbed amounts. Preferential adsorption of high molecular weight species of BSA increased with increasing surface charge density of PS latices. The plateau adsorption level was reached within 1 h on latices with low surface charge densities, but the adsorbed amount increased over a longer period of time on latices with high surface charge densities (Zsom, 1986). Zsom pointed out that since the number of $-O-SO_3^-$ groups available for interaction with one molecule of BSA monomer was so different for the latices, the diversity in adsorption data found, especially for the adsorbed fractions of monomer, dimer, and polymer was hardly surprising. Employing ion-exchange and hydrophobic interaction liquid chromatography, Zsom observed only slight differences in behavior of monomeric and dimeric BSA. Thus gross differences in ionic and hydrophobic character of monomer and dimer BSA were not evident (Zsom, 1986). Apparently, in addition to electrostatic interactions, the preferential adsorption phenomena were controlled by the molecular size (Zsom,1986).

Lee and Ruckenstein (1988) studied the adsorption equilibrium and kinetics of the protein BSA onto polymeric surfaces of different hydrophilicities using a radiolabeling technique. The solid surfaces employed were: (a) a hydrophobic glass; (b) a less hydrophobic, siliconized glass; (c) PMMA of intermediate hydrophobicity; (d) a hydrophilic hydrogel; (e) a less hydrophilic hydrogel; and (f) a high surface free energy glass. The adsorption process was allowed to occur

for 20 h at room temperature. Results of this study indicated that the hydrophobic surfaces adsorbed more than the hydrophilic ones, but the most hydrophobic surface adsorbed less than the least hydrophobic or the surface of intermediate hydrophobicity. Glass (f) adsorbed BSA in an amount comparable to the hydrophobic solids (b) and (c). Adsorption to these surface was interpreted in terms of Hamaker constant.

The Hamaker constant provides a quantitative indication of the strength of interaction between a protein molecule and a solid surface located in water. Solids used in this study were divided into two classes. Solids (a), (b) and (c) had large Hamaker constants. Solids (d) and (e) had Hamaker constants which were one order of magnitude smaller (Lee and Ruckenstein, 1988). Solids characterized by high values of Hamaker constant were expected to adsorb greater amounts of protein than solids of smaller Hamaker constants. However, this was true for solids (b) through (e) but was not with solid (a). This was in part attributed to the approximations involved in the evaluation of components of the Hamaker constant for solid (a) (Lee and Ruckenstein, 1988).

Al-Malah et al., (1992) evaluated surface energetic and temperature effects on the apparent equilibrium adsorption behavior of β-lg using ellipsometry. Seven different silicon surfaces chemically modified by dichlorodimethylsilane to exhibit varying hydrophobicities were used. Completely silanized and unsilanized (hydrophilic) silicon surfaces were used as substrates at 2, 27, and 52 °C as well. Isotherms were also constructed for acrylic, polycarbonate, polyester, glass, and

#304 stainless steel surfaces at 37 and 55 ° C. They observed that the plateau values of adsorbed mass increased as the degree of silicon surface silanization increased. However, β-lg did not sense a surface as having any hydrophobic character until the silane concentration applied to it exceeded 0.005%. This was related to some critical spacing of methyl groups being required to produce a hydrophobic effect. This was also related to contact angle data used to characterize the surfaces being only a macroscopic indication of surface hydrophobicity (Al-Malah et al., 1992).

Adsorbed mass was greater at hydrophobic surfaces at each temperature, although adsorption was depressed at 2 °C, and no significant differences were observed in the adsorbed mass at 27 and 52 °C. The lack of a difference in adsorption behavior at 27 and 52 °C was related to the thermal stability of β-lg (Al-Malah et al., 1992).

Adsorption of β-lg on the engineering materials was somewhat different. The most hydrophobic solid among the polymers (polycarbonate) adsorbed less than the most hydrophilic solid (acrylic) and that was related to the molecular structure of each polymer. While acrylic is a soft, extensible material resulting from high molecular flexibility in addition to an amorphous, coiled structure, polycarbonate is a rigid, inextensible material and to some degree is crystallizable (Al-Malah et al., 1992). The researchers found that glass (a high-energy surface) adsorbed the greatest mass of β-lg while stainless steel adsorbed the lowest mass. This was explained partially in terms of surface energetics. Adsorption of β-lg to the engineering materials at 37 and 55 °C was observed to be somewhat similar with a slightly

greater adsorbed amount observed at the higher temperature for each surface, except for polycarbonate.

In any event, the investigators pointed out that adsorption of β-lg to the engineering surfaces could not be attributed to surface hydrophobicity alone. Rather it can be to some extent explained with reference to flexibility of the polymer structure. A flexible structure, like an amorphous structure, provides greater surface area for adsorption than a rigid structure. Moreover, an amorphous structure is more likely to undergo surface restructuring to accommodate an adsorbate than is a crystallizable structure (Al-Malah et al., 1992).

Protein adsorption from solution has been studied on a variety of organic materials. The adsorption of proteins to agarose gels, for example, containing hydrophobic alkyl residues has been studied extensively (Er-el et al., 1972, Jennissen and Heilmeyer, 1975). Adsorption to such gels depends on the alkyl residue chain length and their density on the agarose particles. Er-el et al., (1972) synthesized a homologous series of hydrocarbon-coated Sepharoses varying in the length of their alkyl side chains (Seph-C1 to Seph-C6). These hydrocarbon-coated Sepharoses were used to purify proteins. Mixtures of D-glyceraldehyde 3-phosphate dehydrogenase (GAPD) and phosphorylase b were passed through short columns of these modified Sepharoses. The researchers found that GAPD was not adsorbed or retained by any of these columns. However, while Seph-C1 did not retain phosphorylase b, Seph-C2 retarded the enzyme, and higher alkyl Sepharoses (Seph-C4 to Seph-C6) adsorbed it. They found that out of several other proteins tested (lysozyme, BSA, and bovine

γ-globulin) only phosphorylase b was retained by Seph-C4. The investigators noted that the length of the hydrocarbon on the Sepharose beads has a marked effect on the capacity of the column to bind phosphorylase b, passing from no retention, through retardation, to reversible binding up to a very tight binding, as the hydrocarbon chain is gradually lengthened. The retention of phosphorylase b on Seph-C4 was attributed to the interactions between the hydrocarbon side chains and hydrophobic pockets in the enzyme.

Jennissen and Heilmeyer (1975) investigated adsorption of some skeletal muscle enzymes to alkyl-amine-substituted agarose gels. They found that the capacity of the gels for phosphorylase kinase increased exponentially as a function of the degree of substitution, then reached plateau values with methyl-, ethyl-, and butylamine-derivatized gels. The binding capacity of the gels was greater when butylamine was used as compared to ethyl-, or methylamine substituted agaroses. However, adsorption of some proteins to these gels did not occur unless a "critical gel hydrophobicity" had been reached. This was observed to depend on the hydrophobicity of the protein molecule as well. This critical hydrophobicity can be obtained either by increasing the degree of substitution of an alkyl residue or by elongation of the alkyl chain.

MATERIALS AND METHODS

Materials

Monocrystalline and polished silicon wafers (1-0-0 orientation, resistivity = 0.8-2 ohm cm) were obtained from Wacker-Chemitronic GMBH (Germany). Ammonium hydroxide, hydrogen peroxide (30%), and hydrochloric acid as well as acetone, ethanol, trichloroethylene, and sodium phosphate (mono and dibasic), were of analytical grade. β-lg from bovine milk, which contained the genetic variants A and B (3 x crystallized and lyophilized; lot No 98F8080) was from Sigma Chemical Co. (St. Louis, MO). Dichlorodiethylsilane (DDES) (97% purity; lot No. 1719-35-5), dichlorodimethylsilane (DDMS) (99% purity; lot No. 11905cx), and dichlorodiphenylsilane (DDPS) (99% purity; lot No. 04027TV) as well as a controlled-atmosphere chamber (AtmosBag™) were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI).

Methods

Surface Modification

Silicon wafers were cut into plates of approximately 1 x 2 cm with a tungsten knife. Ammonium hydroxide, hydrogen peroxide, and deionized, distilled water were combined (1:1: 5) (v : v) and used to wash the plates at 80 °C for 10 minutes. This step was followed by

thorough rinsing with deionized, distilled water. Then the plates were washed again in hydrochloric acid, hydrogen peroxide, and deionized, distilled water (1: 1: 5) (v : v) at 80 °C for 10 minutes and again thoroughly rinsed in deionized, distilled water. This treatment renders the silicon plates hydrophilic. The plates were then dried with nitrogen to remove water from the surface. Hydrophilic surfaces were rendered hydrophobic or partially hydrophobic by using one of three different concentrations of each silane. The desired concentration (0.82, 3.3, or 82 mM) was obtained by transferring an appropriate amount of silane to 50 ml trichloroethylene. The mixture was stirred with a glass rod then poured into a glass petri dish. Individual silicon plates were quickly immersed in the mixture using clean, stainless steel forceps. Surfaces were allowed to react with the silane for one hour. The plates were then transferred to a petri dish containing 50 ml trichloroethylene and rinsed with trichloroethylene, acetone, and finally ethanol. The rinsing step was followed by drying with nitrogen. Surfaces were stored in a dust-free desiccator until use. This procedure was followed for surfaces prepared by both DDES and DDMS. Surfaces to be contacted with DDPS were prepared in an inert nitrogen atmosphere (Atmosbag™). The remaining steps were identical to those described for DDES and DDMS treatments.

Surface Characterization

A contact angle method was used to measure the nondispersive component of the work required to remove selected diagnostic liquids

from each sample surface. The contact angle formed by a sessile drop on a given surface was measured with a contact angle goniometer equipped with an environmental chamber (NRL Model No. 100-00115; Rame-Hart, Inc., Mountain Lakes, NJ).

The goniometer and contact angle technique are described elsewhere (McGuire and Kirtley 1988). The technique can be briefly summarized as follows. Young's equation (a force balance) for a drop of liquid at equilibrium on a plane surface is written in its simplest form as

$$\gamma_S = \gamma_{SL} + \gamma_L \cos\theta \quad (1)$$

in which γ_S is the solid surface free energy (mJ/m^2); γ_{SL} is the solid-liquid interfacial free energy (mJ/m^2); γ_L is the liquid surface free energy (mJ/m^2 or surface tension, mN/m) and θ is the contact angle in degrees (°).

The energy required to part a unit area of liquid from a solid is referred to as the work of adhesion, W_a (mJ/m^2). The Dupre' equation (an energy balance) defines this work of adhesion as

$$W_a = \gamma_S + \gamma_L - \gamma_{SL} \quad (2)$$

Because γ_{SL} cannot be measured experimentally, combining equations (1) and (2) yields:

$$W_a = \gamma_L (1 + \cos\theta) \quad (3)$$

For the purpose of developing a more quantitative treatment of contact angle data, the London dispersion force contribution to the surface free energy of a solid, γ_S^d, and a liquid, γ_L^d, were introduced (Fowkes 1964). Liquid surface free energy may be written as

$$\gamma_L = \gamma_L^{ab} + \gamma_L^d \quad (4)$$

and solid surface free energy as

$$\gamma_S = \gamma_S^{ab} + \gamma_S^{d} \quad (5)$$

where superscripts ab and d refer to the nondispersive (polar, or "acid-base") and dispersive force contributions to surface energy.

Using test liquids with known γ_L, and methodology (McGuire and Kirtley 1988) for the evaluation of γ_L^{d}, γ_L^{ab} and γ_S^{d}, the nondispersive component of the work of adhesion, W_a^{ab}, can be determined as

$$W_a^{ab} = \gamma_L (1 + \cos\theta) - 2 (\gamma_L^{d} \gamma_S^{d})^{1/2} \quad (6)$$

The diagnostic liquids used included a series of ethanol/water solutions (10, 20, 30, and 40% v/v), diiodomethane and 1-bromonaphthalene. The liquid surface tension of each diagnostic liquid was reported elsewhere (McGuire and Kirtley 1988).

W_a^{ab} was calculated for each test liquid contacted with a given solid surface using equation (6). A plot of W_a^{ab} vs. γ_L^{ab} could then be constructed for each surface. The relationship between W_a^{ab} and γ_L^{ab} was observed to be linear for each modified silicon surface; that is, $W_a^{ab} = k \gamma_L^{ab} + b$. The value of the ordinate intercept (b) has been interpreted as a measure of the reduction in surface energy of the solid resulting from adsorption of vapor from the diagnostic liquid; i. e., the spreading pressure, Πs (McGuire and Kirtley, 1988). The slope (k) has been suggested (McGuire and Kirtley, 1988) to be related only to the nondispersive component of solid surface energy, and not dependent on the diagnostic liquids used.

The nondispersive component of the work of adhesion between each solid surface and water ($W_a^{ab}{}_{water}$) was calculated according to

$$W_a^{ab}{}_{water} = k (\gamma_L^{ab}{}_{water}) + b \quad (7)$$

$W_a^{ab}{}_{water}$ provides an index of surface hydrophobicity (McGuire, 1990). The parameters k, b, and $W_a^{ab}{}_{water}$ were recorded for each surface.

Adsorption Equilibrium

β-lactoglobulin was weighed and dissolved in an appropriate volume of phosphate buffer (pH 7.0 and 0.01 M) to prepare a stock solution. The solution was stirred with a magnetic stirrer in a 500 ml beaker until all the protein was dissolved. This step was followed by filtration through a Millipore filter(pore size 0.22 μm Lot NO. HOMM91223 A, Millipore Corporation, Bedford, MA). Desired volumes were transferred to 50-ml beakers containing appropriate buffer volumes to yield some desired final concentration which ranged from 200-2000 mg/L, then stirred for 5 minutes. Approximately 15 ml of the final concentration was transferred to individual 30 ml beakers. Silicon plates were carefully immersed in each beaker; each beaker was then sealed with parafilm and left for 8 hours under static conditions at room temperature. After adsorption, loosely bound protein was removed by rinsing as follows. Each surface was carefully removed from its solution, and deionized, distilled water was gently poured on it for several seconds. Then the surface was "dip-rinsed" three times using three 500 ml beakers each containing 400 ml deionized, distilled water. Rinsing was performed for about 15 seconds in each beaker with light hand stirring. This was followed by drying with a nitrogen flow (Elwing et al 1988). The surfaces were kept in dust-free desiccators until the optical constants Ψ and Δ for

each film were measured by ellipsometry. Three replicates were performed simultaneously.

Measurement of Adsorbed Mass

The adsorbed mass of β-Lg on each surface was evaluated with an automated ellipsometer (Model L 104 SA, Gaertner Scientific Corp., Chicago, IL).

Ellipsometry is an optical technique used for the measurement of the optical constants of reflecting surfaces, and to determine the thickness and refractive index of very thin films deposited onto these surfaces. In principle, a laser beam of known physical properties is transmitted to a film-covered surface and reflected. The physical properties of the beam undergo changes upon reflection, and these changes are measured. For a transparent film covering a reflecting substrate, these changes are totally dependent on film thickness, and film and substrate refractive index. If the electrical field oscillation is resolved into two orthogonal components, reflected light can be characterized by the angle Δ, defined as the change in phase, and the angle Ψ, the arctangent of the factor by which the amplitude ratio changes. These are evaluated simultaneously with the aid of a microcomputer interfaced to the ellipsometer. Resolution of ellipsometrically measured Ψ and Δ into a film refractive index (n_f), and thickness, d, is obtained using a computer program (Krisdhasima et al., 1992) based on methods detailed by McCrackin et al. (1963).

Knowing the refractive index and thickness of a film, the

adsorbed mass can be evaluated according to the following Lorentz-Lorenz relationship, as experimentally validated by Cuypers et al. (1983) :

$$\Gamma = 0.3\ d\ f(n)\ (n_f - n_b)/\ [(A_p/M_p) - V_{20}\ (n_b^2 - 1)/(n_b^2 + 2)] \quad (8)$$

where

$$f(n) = (n_f + n_b)/[(n_f^2 + 2)\ (n_b^2 + 2)] \quad (9)$$

Γ ($\mu g/cm^2$) is the adsorbed mass; d (nm) is the film thickness; A_p (cm^3/mol) is the molar refractivity of protein; M_p ($g\ mol^{-1}$) is the protein molecular weight; and V_{20} ($cm^3\ g^{-1}$) is the partial specific volume of protein at 20 °C. The refractive indices n_f and n_b refer to that of the entire "mixed" film and of pure buffer, respectively. When the protein film is rinsed and dried, the remaining layer is considered to be a mixture of protein and air in which case $n_b = n_{air} = 1.000$, and

$$\Gamma = 0.1\ d\ (M_p/A_p)\ (n_f^2 - 1)/(n_f^2 + 2) \quad (10)$$

In this study the angle of incidence used was 70 °, and the light source was a 1-mW helium-neon laser with a beam wavelength of 6328 Å. The bare substrate optical constants, Ψ_s and Δ_s, are necessary for evaluation of film thickness and refractive index and were measured for each modified silicon surface before contact with protein solution. For film covered surfaces, Ψ and Δ were recorded at 10 to 25 different locations on each surface.

For all experiments, Ψ_s and Δ_s were averaged, and used with individual point measurements of the film-covered surface to calculate n_f and d, then Γ, at each point. These individual point measurements of adsorbed mass were then averaged for each surface. The molecular weight : molar refractivity ratio used was 3.796 g/cm^3 (Luey et al.,

1991).

Isotherm Construction

The equilibrium relationship between adsorbed mass of β-Lg and its equilibrium concentration was described by a Langmuir-type model of the following form;

$$\Gamma = \Gamma_{max} C_{eq}/(b + C_{eq}) \quad (11)$$

Where Γ = adsorbed mass ($\mu g/cm^2$), C_{eq} = equilibrium concentration (mg/L), and Γ_{max}/ b is the initial slope of a plot of Γ versus C_{eq} . Γ_{max} is the plateau value of adsorbed mass and b (mg/L) is a function constant.

Statistical Analysis

Analysis of variance (ANOVA) of completely randomized design (CRD) with three replications was performed using statistical analysis system (SAS) statement PROC GLM (SAS, 1988). The analysis was performed for equation (11) parameters Γ_{max} and b. The estimated adsorbed mass, Γ, at each level of protein concentration was also evaluated.

RESULTS AND DISCUSSION

Surface Characterization

The degree of silanization was varied by reacting hydrophilic silicon surfaces with DDMS, DDES, or DDPS at molar concentrations of 0.82, 3.3 and 82 mM. Increasing the number of functional groups on a given silicon surface should cause that surface to become more hydrophobic. A plot of the nondispersive component of the work of adhesion, W_a^{ab} (mJ/m^2), vs. the nondispersive component of test liquid surface free energy, γ_L^{ab} (mJ/m^2), is shown in Figure 1 for each type of surface. As shown, the resulting relationship between W_a^{ab} and γ_L^{ab} is linear with respect to all silanized and hydrophilic silicon surfaces. Surfaces with high slope are considered to be hydrophilic. Figure 1a shows that surfaces treated with 0.82 mM DDMS are still relatively hydrophilic, while applying 3.3 mM DDMS caused a sharp decrease in hydrophilicity (or increase in hydrophobicity) to a degree similar to that caused by applying 82 mM DDMS. The same is observed with surfaces treated with DDES (Figure 1b). Surfaces treated with DDPS however, are uniquely different. The decrease in hydrophilicity was gradual. It can be seen that surfaces treated with 3.3 mM DDPS still exhibit some hydrophilicity (Figure 1c) as compared to DDMS or DDES-treated surfaces at the same molar concentration.

$W_a^{ab}{}_{water}$, γ_S^d, k, and b for each surface are presented in Table 1. The values of slope (k) and intercept (b) defining the linear relationship between the nondispersive component of the work of

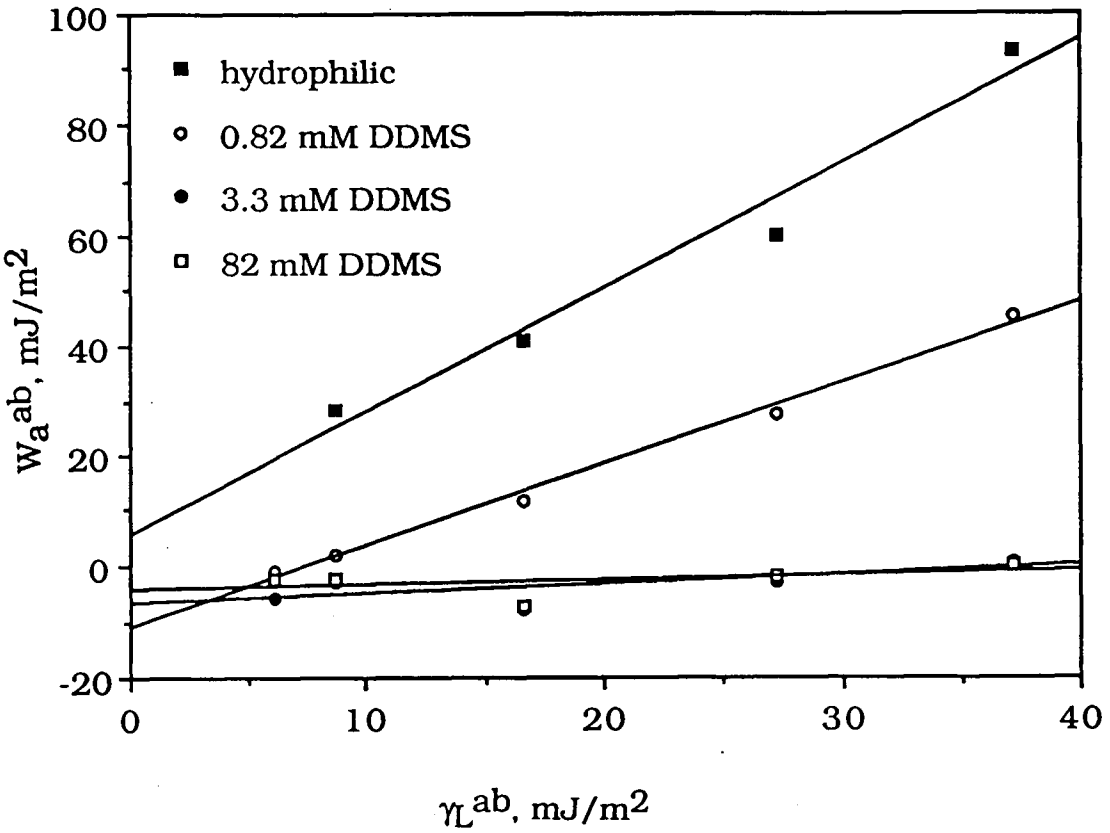

Figure 1a. The relationship between the nondispersive component of the work of adhesion and liquid surface tension for DDMS-treated silicon surfaces and hydrophilic silicon surfaces.

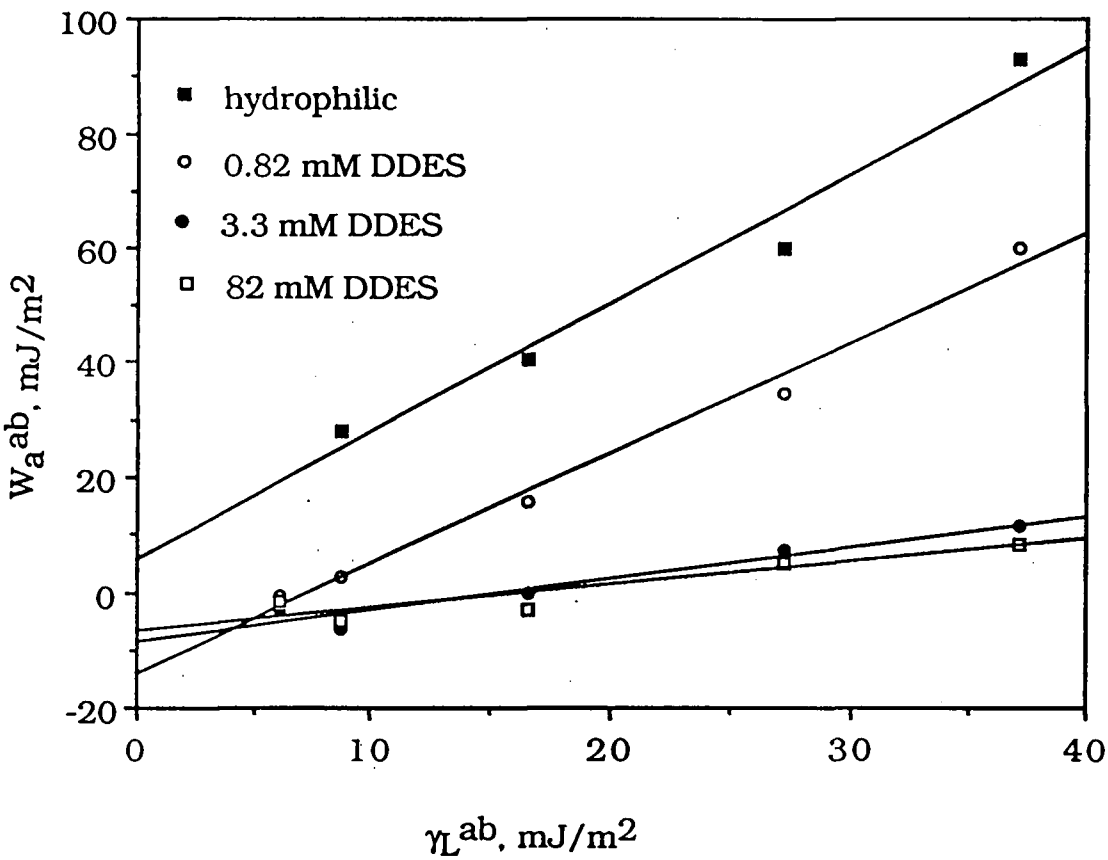

Figure 1b. The relationship between the nondispersive component of the work of adhesion and liquid surface tension for DDES-treated silicon surfaces and hydrophilic silicon surfaces.

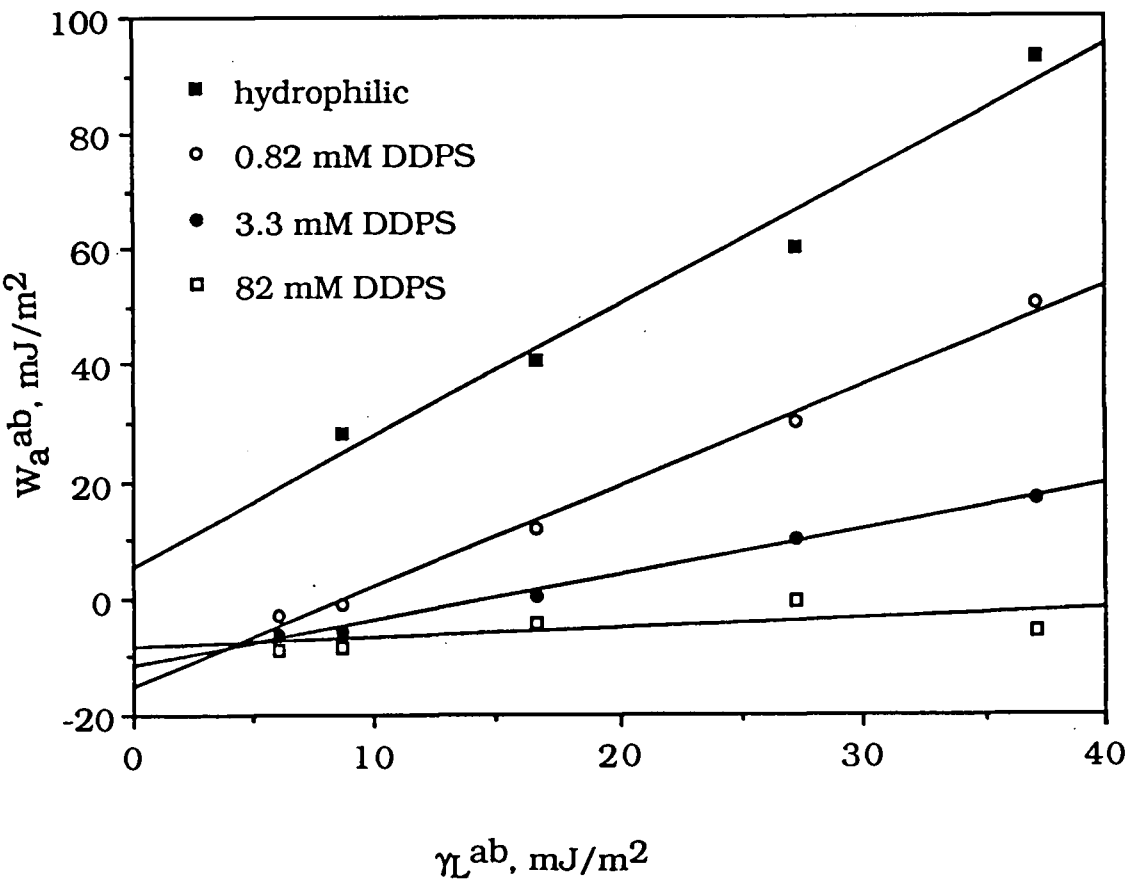

Figure 1c. The relationship between the nondispersive component of the work of adhesion and liquid surface tension for DDPS-treated silicon surfaces and hydrophilic silicon surfaces.

Table 1. Solid surface properties related to their hydrophobic-hydrophilic balance. The associated standard errors are reported in parentheses. (k is dimensionless; $W_a^{ab}{}_{water}$, γ_s^d, and b are reported in mJ/m2)

Surface type	$W_a^{ab}{}_{water}$	γ_s^d	k	b
Hydrophilic	88.37	17.0*	2.24	5.18
			(0.28)	(7.02)
0.82 mM silane				
DDES-treated	57.02	41.34	1.92	-14.29
			(0.12)	(2.67)
DDMS-treated	43.50	35.80	1.47	-11.10
			(0.07)	(1.50)
DDPS-treated	48.80	42.79	1.73	-15.45
			(0.07)	(1.67)
3.3 mM silane				
DDES-treated	11.46	31.96	0.54	- 8.60
			(0.08)	(1.88)
DDMS-treated	0.0	24.85	0.17	- 6.98
			(0.10)	(2.25)
DDPS-treated	17.23	42.50	0.78	-11.74
			(0.04)	(0.87)
82 mM silane				
DDES-treated	7.96	30.50	0.40	- 6.90
			(0.10)	(2.23)
DDMS-treated	0.0	20.59	0.08	- 4.31
			(0.11)	(2.43)
DDPS-treated	0.0	44.22	0.17	- 8.64
			(0.12)	(2.68)

* from Yang's Thesis, 1990

adhesion and the nondispersive component of the test liquid surface tension were used to calculate $W_a^{ab}{}_{water}$ according to equation (7) for each surface. The slope of the plots increases with increasing surface hydrophilicity. A low value of $W_a^{ab}{}_{water}$ is thus associated with a hydrophobic surface and a high value with a hydrophilic surface. Regarding the chain length of the functional group on a surface, a longer chain should exhibit a higher hydrophobic character (Er-el et al., 1972, Jennissen and Heilmeyer, 1975). It can be inferred that since a phenyl group is larger than an ethyl or methyl group, surfaces with phenyl groups attached to them should be most hydrophobic. However, this is not evident in Table 1. "Molecular hydrophobicity" is a microscopic property; Table 1, however, lists only macroscopic properties of the surfaces and a clear correlation between surface hydrophobicity and chain length is not expected. This is because the contact angle cannot be expected to distinguish among chain length. As shown, surfaces with methyl groups exhibited the most hydrophobic character according to both $W_a^{ab}{}_{water}$ and k. This, however, could simply be related to the amount of a given functional group on the surface. Methyl groups are small compared to ethyl and phenyl groups. Thus, more of them could attach to the surface at a given silane concentration as there would be less steric hindrance during silanization. This would lead to a higher number of methyl groups on the surface and increased hydrophobicity as measured by this contact angle method. The differences in $W_a^{ab}{}_{water}$ among surfaces treated with the same silane at different concentrations is large. This is expected since the increase in the number of the functional groups

on the surface would contribute further to the increased hydrophobicity of the surface.

Representative plots of W_a^{ab} versus silane concentration, generated from data recorded with 20% ethanol, are shown for each type of silicon surface in Figure 2. Figure 2 indicates that while the potential for hydrophobic attraction increases with increasing silanization, it is accompanied by a potential for acid-base interaction that is decreasing. However, the drop in this acid-base potential is slower on DDPS-treated surfaces than that on DDES- and DDMS-treated surfaces.

Schematic representations of silicon surfaces with methyl, ethyl, phenyl, and hydroxyl groups attached to them are shown in Figure 3. It is important to note that we have no evidence that the functional groups are spread uniformly across these surfaces, and it is possible that relatively large, hydrophobic "islands" are present on surfaces prepared at higher silane concentrations. The density of the functional groups on the surface, the effect of steric hindrance, and thus the resultant hydrophobicity of the surface could be inferred from the data obtained by the contact angle method and shown in Table 1

Adsorption Isotherms

Effects of Silane Concentration

The averaged value of adsorbed mass plotted against apparent equilibrium protein concentration for each treatment is shown in

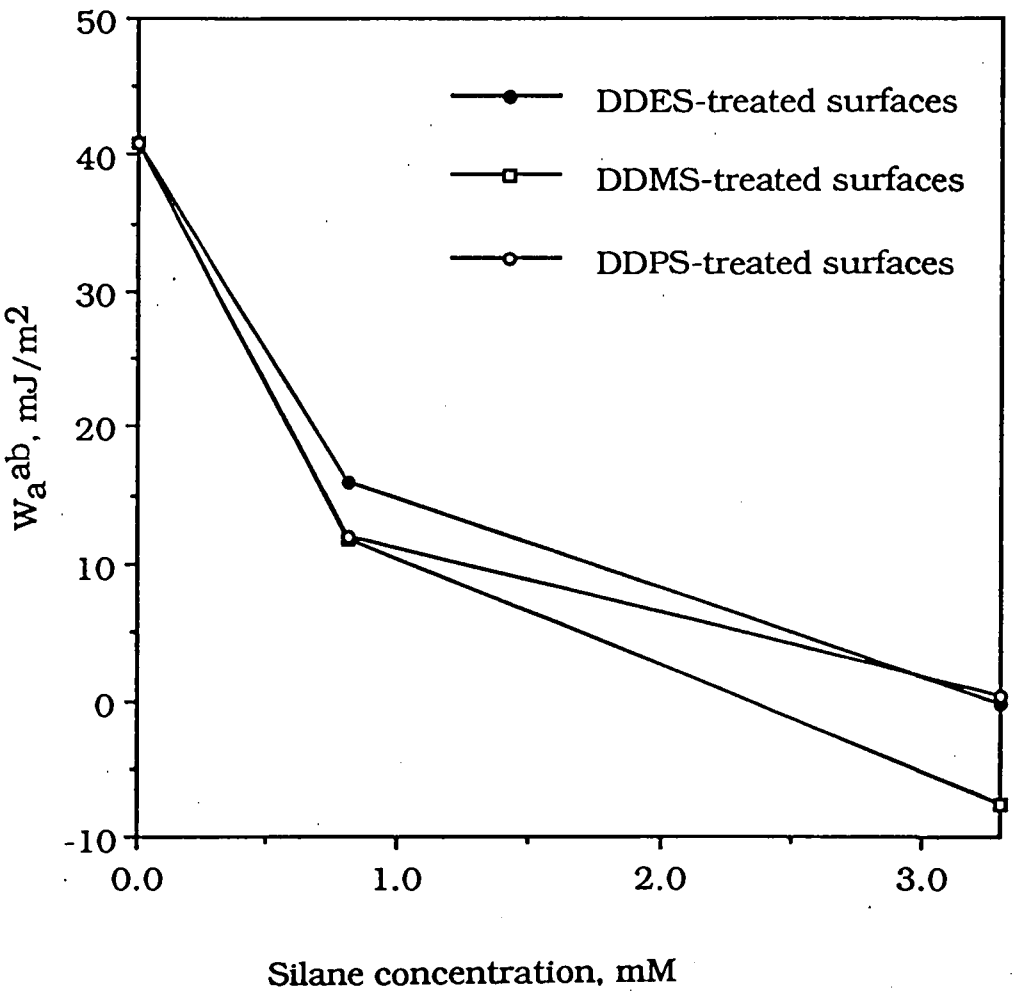

Figure 2. Acid-base contribution to the work of adhesion exhibited by DDMS, DDES, and DDPS-treated surfaces using 20% ethanol as the test liquid.

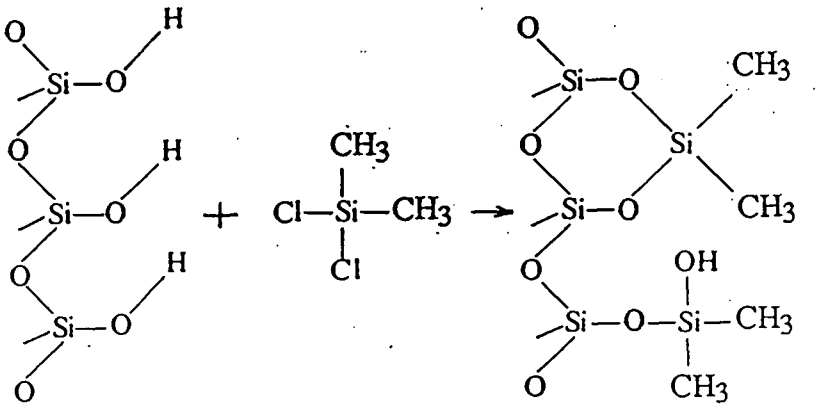

Figure 3. Schematic representation of the modified silicon surfaces treated with DDMS. Methyl groups can be substituted by ethyl or phenyl groups by using DDES or DDPS respectively.

Figure 4. Figure 4 indicates that equation (11) describes the pattern of the experimental data reasonably well. However, data obtained from surfaces treated with DDPS at 3.3 mM (Figure 4c) show a wider scatter, probably because some experimental errors occurred during silanization. DDPS is a moisture sensitive silane so silanization wasperformed under a controlled atmosphere. However, it is possible that an improperly sealed chamber resulted in more heterogeneous surfaces.

Silicon surfaces treated with DDMS have methyl groups covalently attached to Si groups on the surface. The number of these methyl groups should vary somewhat according to the amount of DDMS applied. Figure 4a shows that at high DDMS concentration, the adsorbed mass is significantly greater than that at lower DDMS concentrations (Table 2). This is probably because increasing methyl group density on the surfaces causes an increase in surface hydrophobicity. This is shown in Table 1 where surfaces treated with a high concentration of DDMS have a smaller $W_a^{ab}{}_{water}$ value than surfaces treated with low DDMS concentration.

The difference in adsorbed mass among surfaces prepared at lower DDMS concentrations is not significantly different at the 5% level (Table 2). Apparently, at lower DDMS concentrations, the surface does not have a number of hydrophobic interacting sites (methyl groups) sufficient to allow a strong interaction with hydrophobic regions on the protein molecule. As the amount of these interacting sites increases, the chance of favorable hydrophobic interaction is enhanced; moreover, at lower DDMS concentration methyl groups may

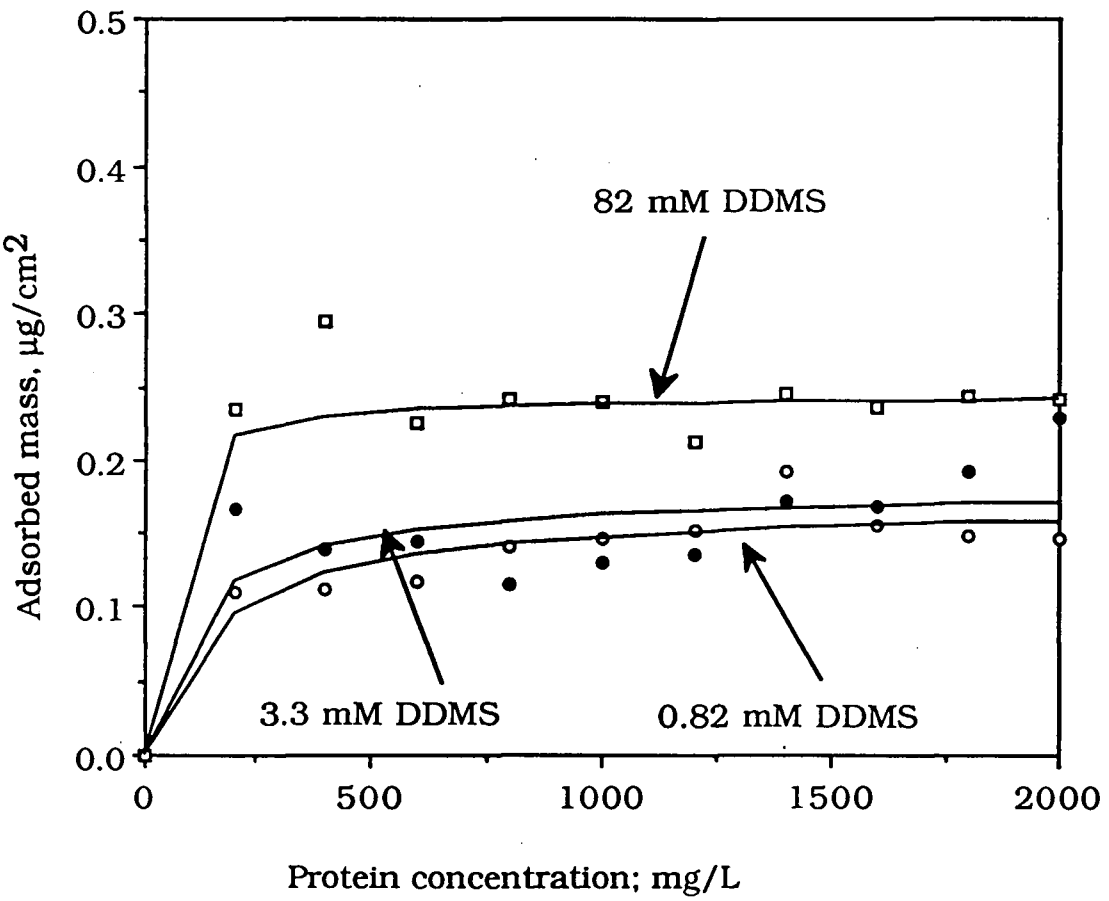

Figure 4a. β-lactoglobulin adsorption isotherms constructed for silicon surfaces treated with 0.82, 3.3, and 82 mM dichlorodimethylsilane.

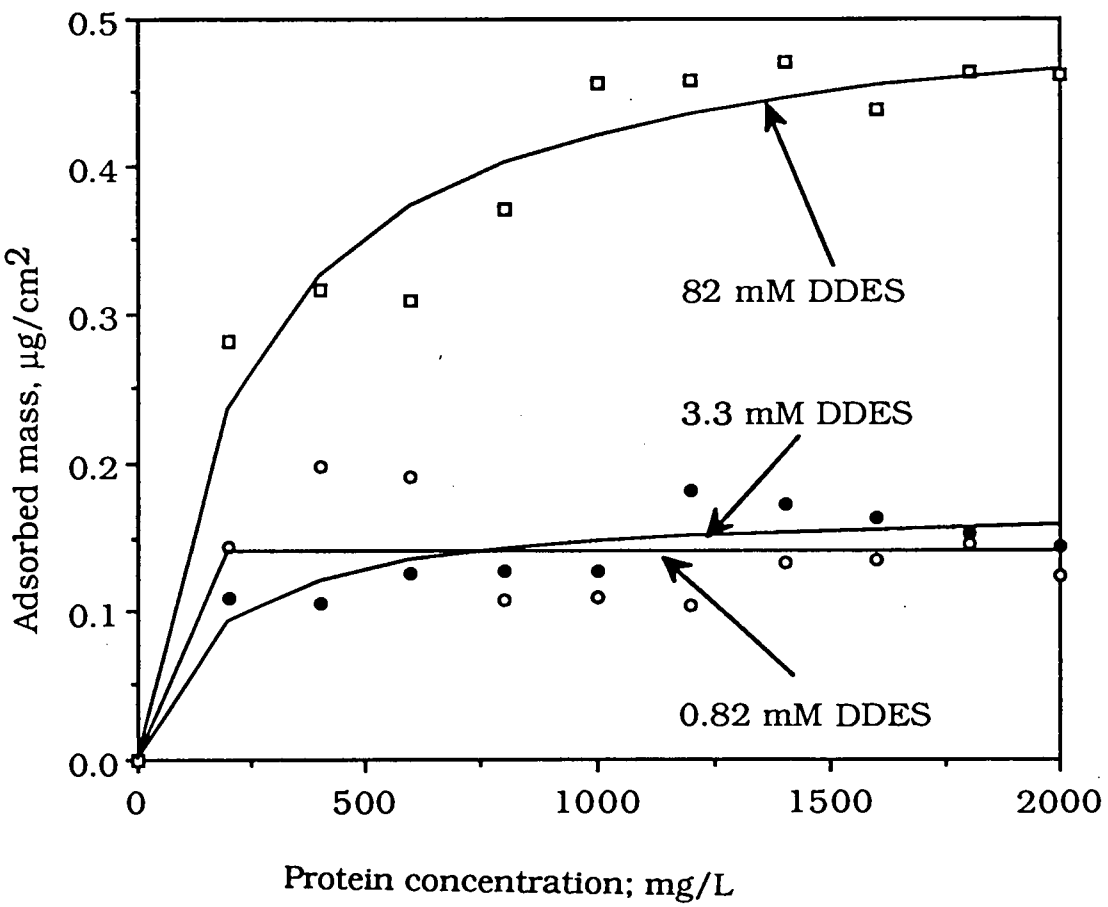

Figure 4b. β-lactoglobulin adsorption isotherms constructed for silicon surfaces treated with 0.82, 3.3, and 82 mM dichlorodiethylsilane.

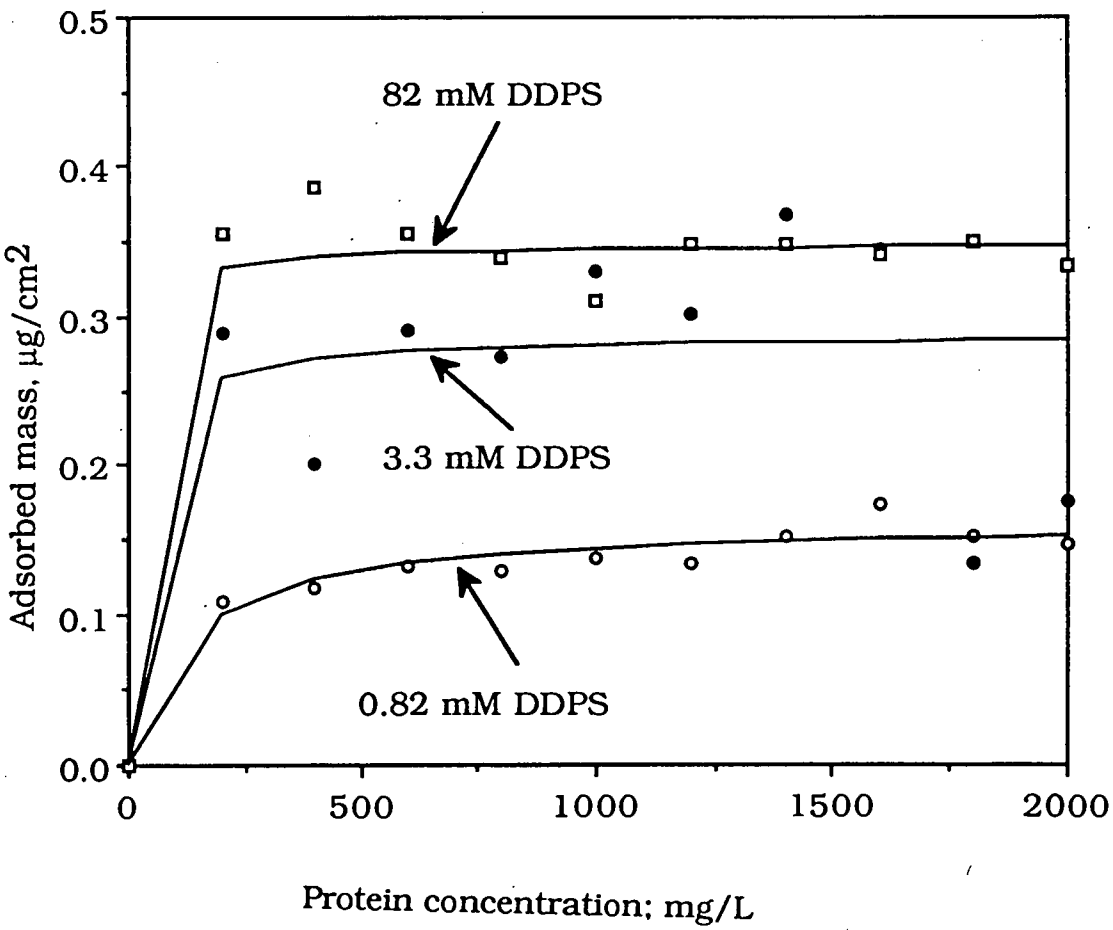

Figure 4c. β-lactoglobulin adsorption isotherms constructed for silicon surfaces treated with 0.82, 3.3, and 82 mM dichlorodiphenylsilane.

interfere with any favorable acid-base interaction present between hydrophilic sites on the surface, and protein. This is evident from the rapid drop in W_a^{ab} as the amount of DDMS is increased (see Figure 2).

Figure 4b shows β-Lg adsorption behavior on silicon surfaces treated with DDES. The same pattern of increased adsorbed mass at high silane concentration is noticed on comparison with Figure 4a. The adsorption behavior at surfaces treated with DDES at lower silane concentrations should be explainable by the same reasoning used to describe similar behavior at the DDMS-treated surfaces, i. e., the number of interacting sites on the surface (ethyl groups) is not great enough to produce a strong, favorable hydrophobic interaction with the adsorbing protein molecule. Also, ethyl groups at this level may have interfered with any favorable acid-base interaction accompanying protein adsorption on hydrophilic surface sites, consistent with the rapid decrease in W_a^{ab} observed as the concentration of DDES is increased (Figure 2).

The increase in hydrophobicity on going from 0.82 to 3.3 mM (as measured by contact angle) seems to be uncorrelated with adsorbed mass on both DDMS and DDES-treated surfaces. The 3.3 mM concentration does not provide sufficient functional groups for generating favorable hydrophobic interaction as far as protein adsorption is concerned. But this hydrophobicity is enough to cause a repulsive interaction between a drop of water and the surface. The interaction between a drop of water and a solid surface is a macroscopic characteristic of the interface, however, while protein interaction with a surface is a microscopic phenomenon. At low DDMS

and DDES concentrations, protein molecules apparently do not "recognize" the surface as hydrophobic, leading to lower than expected values of adsorbed mass. With reference to Figures 4a and 4b, at high silane concentration, the alkyl groups are more closely packed, possibly leaving little or no space to accommodate hydrophilic interaction. At low silane concentration, however, there are probably fewer alkyl groups on the surface and greater space between them (see Figure 3). This could inhibit the protein molecule from unfolding, and cause adsorption to take place by relatively few noncovalent contacts.

Figure 4c shows that the adsorbed amount of β-Lg increased rather steadily with DDPS concentration. The interaction between protein molecules and the DDPS-treated surface is due in part to the ability of phenyl residues to interact with different reactive groups in the protein molecule. Due to its π-electron basicity, a phenyl group can interact with carbonyl oxygen, sulfur groups, amino groups and aromatic moieties of the protein molecule (Burley and Petsko 1988). An example of attractive, oxygen-aromatic interactions is seen with study of drugs and peptides bound to deoxyhemoglobin A (Perutz et al., 1986). One such attraction is due to interaction between the carbonyl oxygen atoms of aspartate-94 and cysteine-93 of the β chain of deoxyhemoglobin A with the phenyl ring of ethacrynic acid (Perutz et al., 1986). Each of these types of interaction can be referred to as "acid-base" attractions. Figures 1 and 2 as well as Table 1 indicate that the surface capacity for acid-base interaction decreases with increasing silane concentration. At the intermediate silane concentration, however, DDPS-treated surfaces exhibit the greatest

capacity for acid-base interaction. At this DDPS concentration, adsorption may be due to cooperative forces of attraction that include both hydrophobic and acid-base attractive forces.

The means of the regressed parameters gained by fitting equation (11) to the data of Figure 4, with their associated standard errors, are shown in Table 2. Results shown in Table 2 indicate that the values of b show a wider variation than that of the curve-fitted plateau values, Γ_{max}, though the means of b for most of the treatments are not significantly different. The parameter b of equation (11) is a function constant. Γ_{max}/b is the initial slope of the isotherm i.e the slope at zero equilibrium concentration. Therefore b has some physical meaning only at zero protein equilibrium concentration where Γ_{max} has a physical significance at higher protein equilibrium concentration. Since we do not have a zero equilibrium concentration in this experiment, the curve-fitted plateau values, Γ_{max}, should be considered as a more reliable characteristic of the isotherms than the value of the initial slope. The plateau value in adsorbed mass generally increased as silane concentration increased for a given silane. However, an apparent decrease in adsorbed mass is observed with increasing hydrophobicity at silane concentrations of 0.82 and 3.3 mM relative to hydrophilic surfaces, with the exception of the DDPS-treated surfaces at 3.3 mM.

Effects of Silane Type

To enable comparison of adsorbed mass among surfaces prepared at identical levels of silane concentration, the fitted lines of Figure 4

Table 2. The means and the associated standard errors (in parentheses) of the regressed parameters of equation (11) describing β-lg adsorption to modified silicon surfaces.

Material	Γ_{max}, $\mu g/cm^2$		b, mg/l	
Hydrophilic surfaces	0.268^{c*}	(0.0464)	466.795^{a}	(197.61)**
Silanized silicon surfaces (0.82 mM)				
DDES-treated	0.139^{d}	(0.0016)	0.000^{c}	(0.0003)
DDMS-treated	0.171^{d}	(0.008)	156.271^{bc}	(27.973)
DDPS-treated	0.160^{d}	(0.00065)	120.284^{bc}	(21.374)
Silanized silicon surfaces (3.3 mM)				
DDES-treated	0.172^{d}	(0.0101)	169.626^{bc}	(21.673)
DDMS-treated	0.180^{d}	(0.016)	106.563^{bc}	(92.221)
DDPS-treated	0.286^{c}	(0.0156)	21.727^{c}	(21.726)
Silanized silicon surfaces (82 mM)				
DDES-treated	0.522^{a}	(0.0077)	241.074^{b}	(18.311)
DDMS-treated	0.244^{c}	(0.0039)	25.617^{c}	(25.616)
DDPS-treated	0.348^{b}	(0.0045)	9.699^{c}	(9.689)

* Means with the same letters are not significantly different at the 5% level of significance

** The pooled standard error for the means of Γ_{max} = (0.0172) and for b = (71.289)

are replotted in Figure 5.

Adsorption of β-Lg to surfaces treated with DDES is significantly greater than that to surfaces treated with DDMS at high silane concentration (82 mM). This is illustrated in Figure 5a and Table 2. This can possibly be explained with reference to the relative amounts of the alkyl groups on the silicon surfaces and the length of these alkyl residues. Jennissen (1976; 1978) studied the adsorption and desorption of skeletal muscle enzymes on hydrophobic alkyl-agaroses. He found that if the chain length of the alkyl residue is increased from methyl to ethyl to butyl, a decrease in the adsorption exponents (1/n, a term in the power function of the Freundlich equation written as $\log a = \log \alpha + 1/n \log c$; where a is the amount of adsorbed protein, c is the alkyl residue density, and α is the "adsorption constant") is observed at constant a for phosphorylase kinase adsorption on going from methyl-(1/n = 5.8) to ethyl-(1/n = 3.6) to butyl-Sepharose (1/n = 3.5). The concentration of the butyl residue was varied to study its effects on the adsorbed amount of phosphorylase b. The results showed that as the butyl-residue density increased, the adsorption increased and reached a plateau, while the adsorption exponent decreased. He concluded that the number of binding sites necessary for adsorption of a protein molecule decreased with increasing alkyl-residue chain length and density.

β-Lg has surface sites that can react favorably with alkyl groups. The favorable hydrophobic intereaction may start when a critical number of alkyl residues (yielding some critical hydrophobicity) comes into contact with corresponding sites on the surface of the protein.

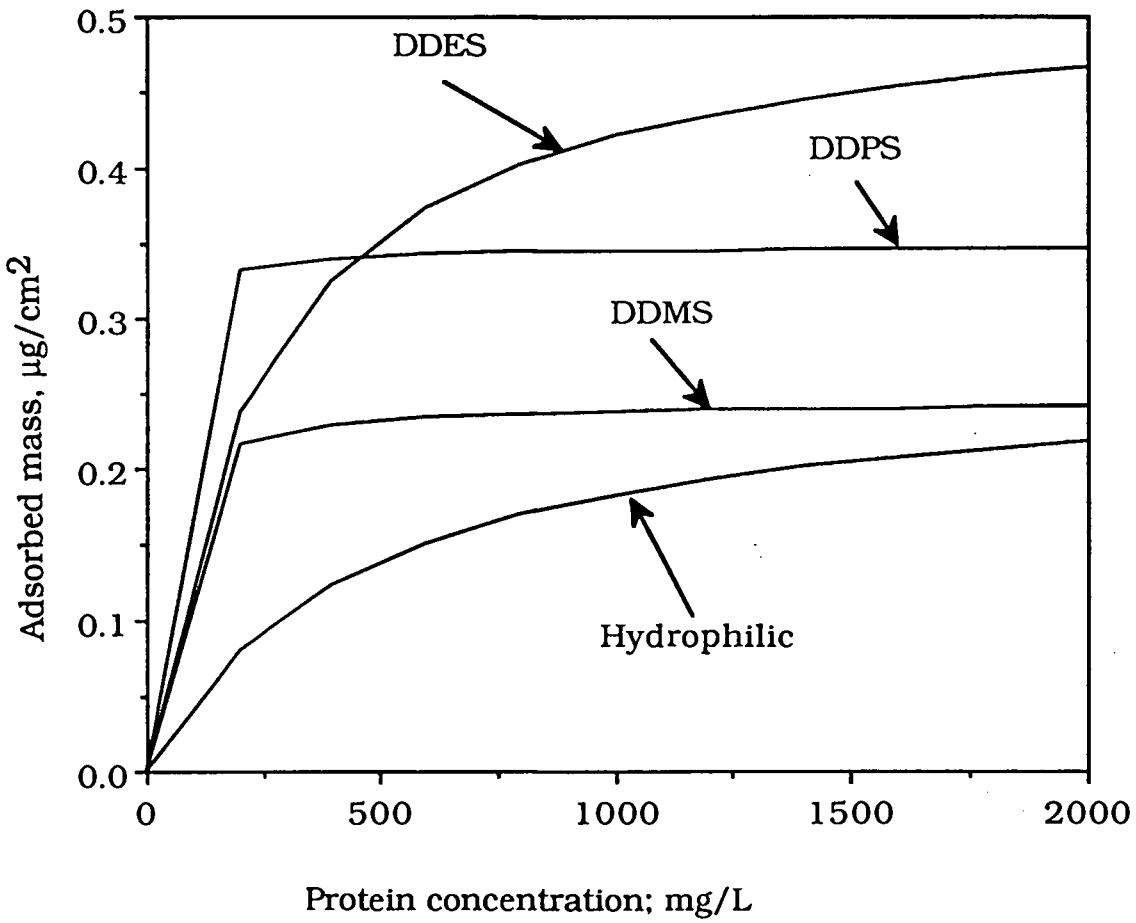

Figure 5a. Comparison of the adsorption behavior of β-lactoglobulin to surfaces treated with 82 mM silane and hydrophilic silicon surfaces.

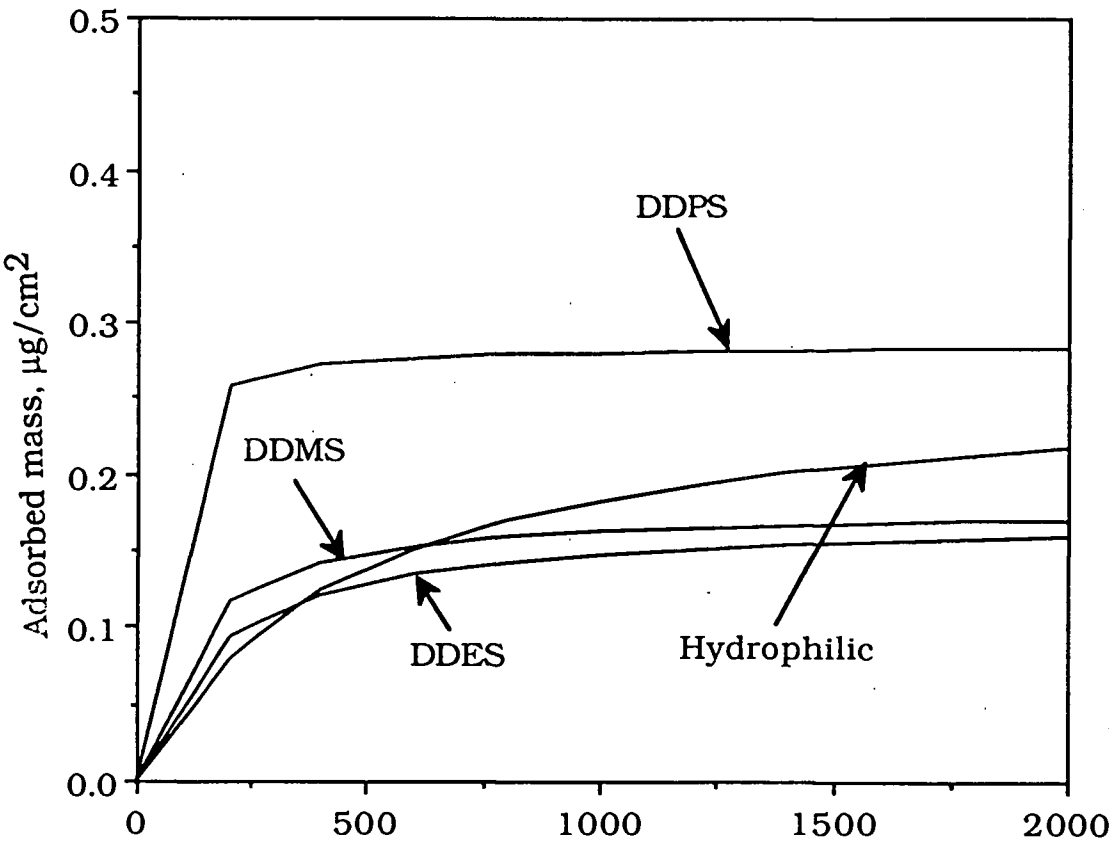

Figure 5b. Comparison of the adsorption behavior of β-lactoglobulin to surfaces treated with 3.3 mM silane and hydrophilic silicon surfaces.

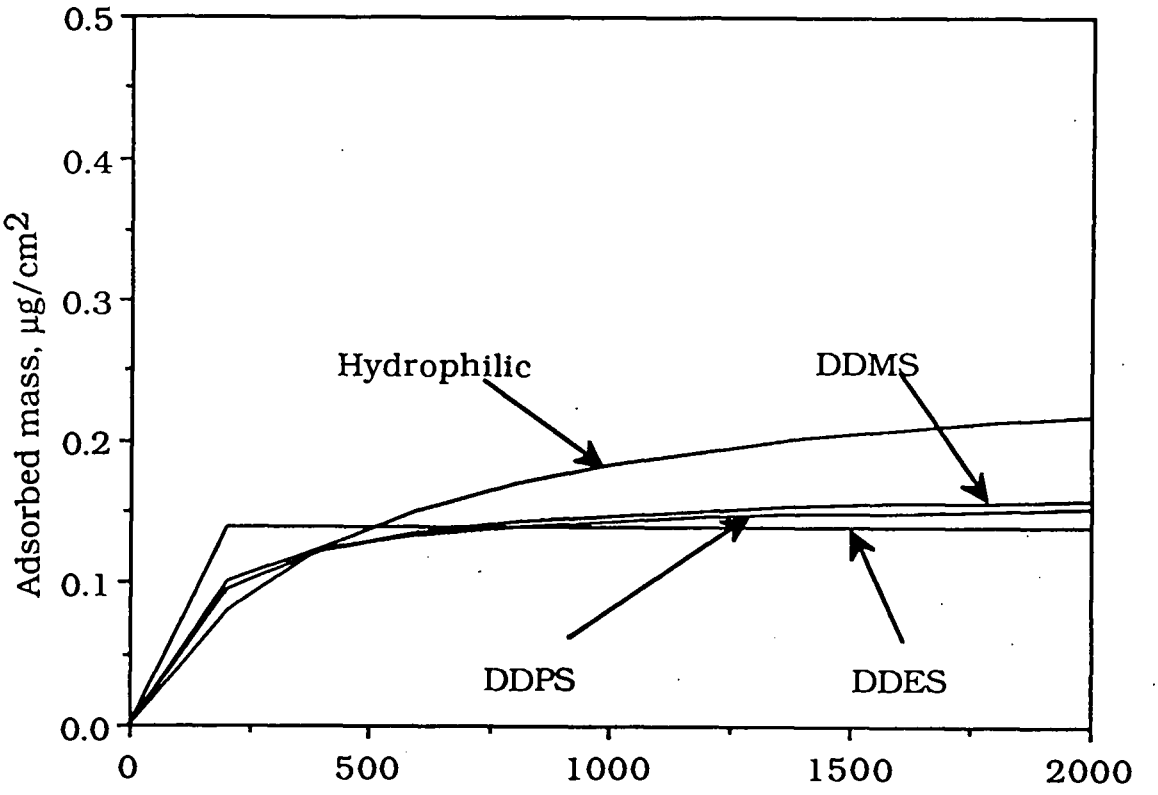

Figure 5c. Comparison of the adsorption behavior of β-lactoglobulin to surfaces treated with 0.82 mM silane and hydrophilic silicon surfaces.

The more alkyl residues that make contact with the protein, the greater the adsorbed amount. Most of the alkyl residues are available to the first arriving molecules adsorbed; thereafter each incoming protein molecule meets a smaller number of available alkyl residues for multivalent binding until the critical value of alkyl residue is reached and adsorption ceases to increase (Jennissen, 1976).

In this work, the number of alkyl residues covalently bound to the silicon surface was not determined. But results obtained here show that at low molar concentrations, the adsorbed amount on DDES-treated surfaces was not significantly different from that on DDMS-treated surfaces (see Figure 5b, 5c and Table 2). This could be related to a smaller number of ethyl groups attached to the surface than the number of methyl groups for the same silane concentration. Both chain length and number of residues have an effect. Since methyl groups are smaller than ethyl groups their density on the surface could be greater due to a higher mass diffusivity of the silane. Protein molecules would require fewer, and find fewer multivalent reaction sites on DDES-treated surfaces.

At higher silane concentrations, the relative rates of mass transfer would be less important if the reaction were kinetically controlled. At high silane concentration, the pattern of the isotherms for the DDES- and DDMS-treated surfaces is consistent with chain length. With respect to adsorption onto surfaces treated with DDPS at the high silane concentration the adsorption might be expected to be greater than that to surfaces treated with DDES since phenyl groups are bigger than ethyl groups and therefore more hydrophobic. The results

are not consistent with that thinking, however, possibly due to steric hindrance experienced by the phenyl groups during silanization, leading to fewer of them being anchored to the surface. Protein adsorption to these surfaces at 3.3 mM, however, was significantly greater than that onto DDMS- and DDES-treated surfaces. The adsorption to DDPS-treated surfaces at this stage could be related to the contribution of both hydrophobic and acid-base attractive forces.

CONCLUSIONS

β-Lg equilibrium adsorption to solid silicon surfaces is influenced by the type and amount of functional groups attached to the surface. Though low molar concentrations of ethyl, methyl and phenyl functional groups increased the hydrophobicity of the surface, the experimental data indicated that β-Lg adsorption is not perfectly correlated to this hydrophobicity increase. Apparently, a decrease in the potential for acid-base interaction initially afforded by hydrophilic (unsilanized) surfaces occurred on surfaces treated with low concentrations of DDMS and DDES. This acid-base interaction potential however, was apparently enhanced by phenyl groups attached to the surface when 3.3 mM concentration of DDPS was used. A measurable, attractive hydrophobic effect was observable only for surfaces silanized at the highest concentration selected. Since adsorption has been generally observed to increase as surface hydrophobicity increases, these data lead us to conclude that contact angle measurements can not alone be used to characterize the hydrophobicity of the surface in an effort to predict relative amounts of protein adsorption. The contact angle is influenced by macroscopic properties of a surface while protein adsorption is influenced by microscopic properties.

RECOMMENDATION

Results of this study indicate that the functional groups of the substrate have an effect on the adsorbed mass of β-Lg. This effect varies with the type of functional group. Some functional groups affect the adsorbed mass of β-Lg through an acid-base interaction. Groups capable of such an interaction are the hydroxyl and phenyl groups. Phenyl groups may have enhanced acid-base attraction at the intermediate silane concentration. To verify the acid-base effects contributed by phenyl groups, it would be interesting to study the effect of salt concentration on the adsorbed mass and observe how increasing or decreasing the salt concentration will affect the acid-base contribution to the interaction between the protein and the DDPS-treated surfaces. For example, adding NaCl with varying concentrations at different pH's should affect the adsorbed amounts. When a pH higher than the isoelectric point of the protein is used, the protein molecule would be negatively charged and the adsorption to DDPS-treated surfaces should decrease due to the unfavorable electrostatic interactions. Adding NaCl at this pH would cause shielding of the surface and would result in a more protein adsorbed. If, however, the pH employed was below the isoelectric point of the protein, the protein molecule would be positively charged and an electrostatic repulsion would occur. Adding NaCl in this case would contribute to the increased counterions which would shield the surface and allow favorable interaction leading to an increased adsorbed amount on the surface.

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APPENDICES

APPENDIX A

The results obtained from the analysis of variance (ANOVA) of equation (11) parameters are presented in Table 1. Table 1 indicates that there is a highly significant effect of the silane type on the adsorbed amount of β-lg. However, a highly significant interaction between silane type and silane concentration is observed (Figure 1 a, b, and c). Such results would indicate that the adsorbed amount of protein is also silane concentration dependent. In other words, there are differential contributions from the main effects (treatment or silane type and silane concentration) to the magnitude of the parameters of equation (11). Moreover, Table 1 indicates that the coefficient of variation (C. V) were below 16% except for the parameter b where the C. V is 66.26% is observed. This high value might be attributed to the lack of accuracy in estimating the parameter b.

Table 1. Observed mean squares and coefficient of variation of equation (11) parameters (Γ_{max}, b) describing ß-lactoglobulin adsorption to modified silicon surfaces and the estimated adsorbed mass (Γ)at each level of protein concentration.

Source of Variation	Df[1]	Γ_{max}	b	Γ_{200}	Γ_{400}	Γ_{600}	Γ_{800}
Treatment (T)	2	0.01649**	16786.4615*	0.018464**	0.01413**	0.01312**	0.01282**
Concentration (C)	2	0.11163**	153.2768^{ns}	0.05394**	0.06897**	0.07786**	0.08371**
T x C	4	0.024784**	34967.1947**	0.00861**	0.00816**	0.01028**	0.01239**
Error	18	0.00027	3923.7846	0.00046	0.00016	0.0001	0.0001
Total	26						
CV(%)		6.64	66.26	11.96	6.27	4.75	4.44

Table 1 continued. Observed mean squares and coefficient of variation of the estimated adsorbed mass (Γ) at each level of protein concentration.

Source of Variation	Df[1]	Γ_{1000}	Γ_{1200}	Γ_{1400}	Γ_{1600}	Γ_{1800}	Γ_{2000}
Treatment (T)	2	0.012905**	0.013084**	0.01329**	0.01348**	0.01367**	0.01384**
Concentration (C)	2	0.08784**	0.09091**	0.09328**	0.09516**	0.0967**	0.098**
T x C	4	0.01416**	0.01561**	0.0168**	0.01779**	0.01862**	0.01934**
Error	18	0.0001	0.00011	0.00012	0.00013	0.00013	0.00015
Total	26						
CV(%)		4.47	4.59	4.75	4.89	5.02	5.14

** Highly significant at 1% level; * significant at 5% level; ns not significant
[1] Degrees of freedom

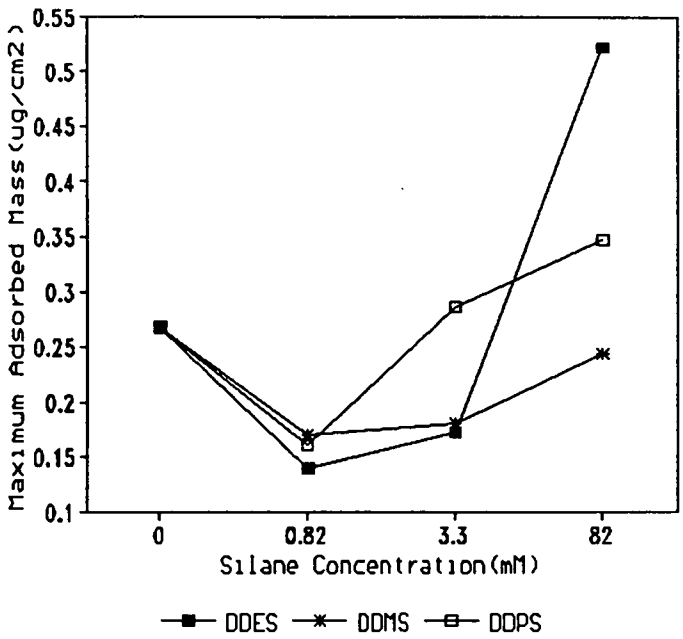


Figure 1a. Plot of the means of the maximum adsorbed mass versus silane concentration

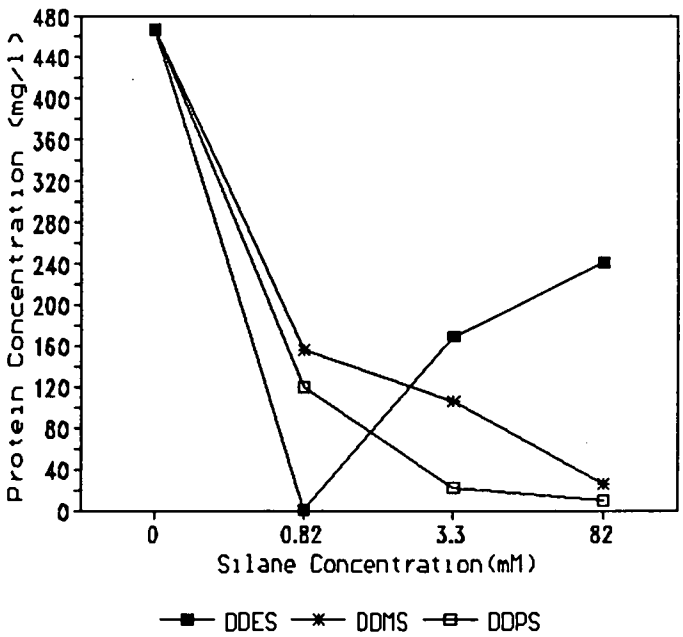
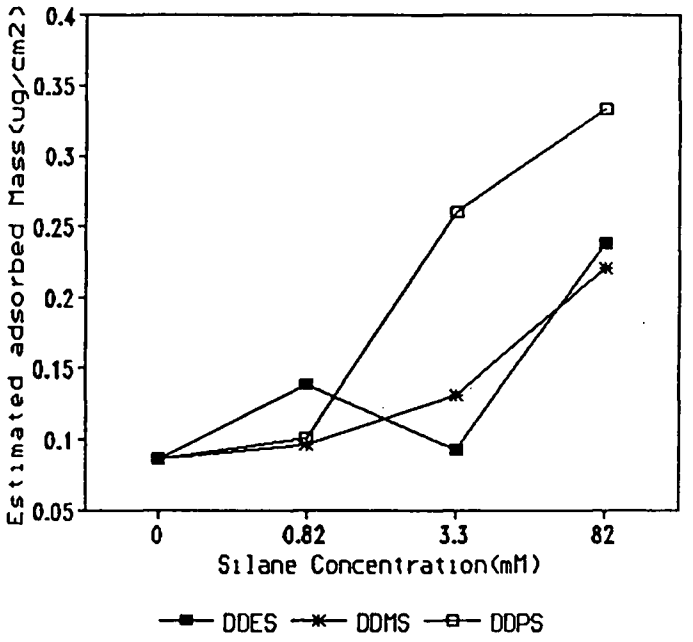
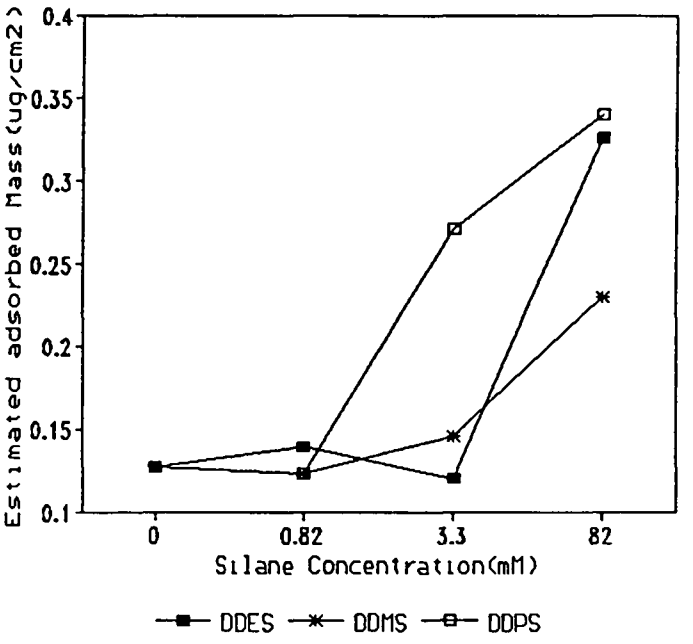


Figure 1b. Plot of the means of the parameter (b) of equation (11) versus silane concentration

Figure 1c. Plots of the means of the estimated adsorbed mass at each level of protein concentration versus silane concentration.

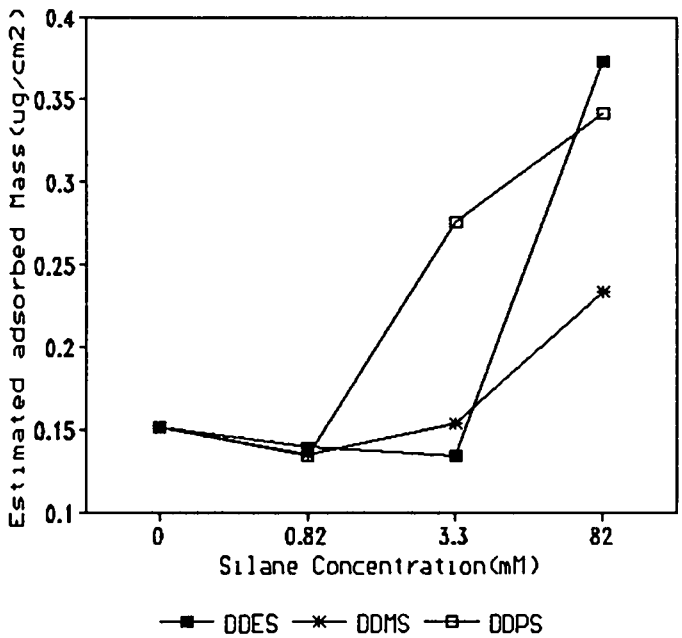


At 200 (mg/l)

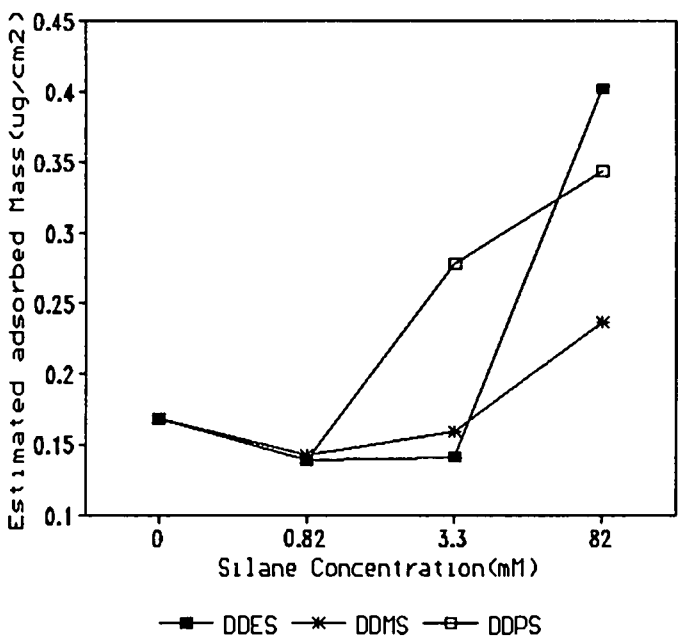


At 400 (mg/l)

Figure 1c. (Continued)

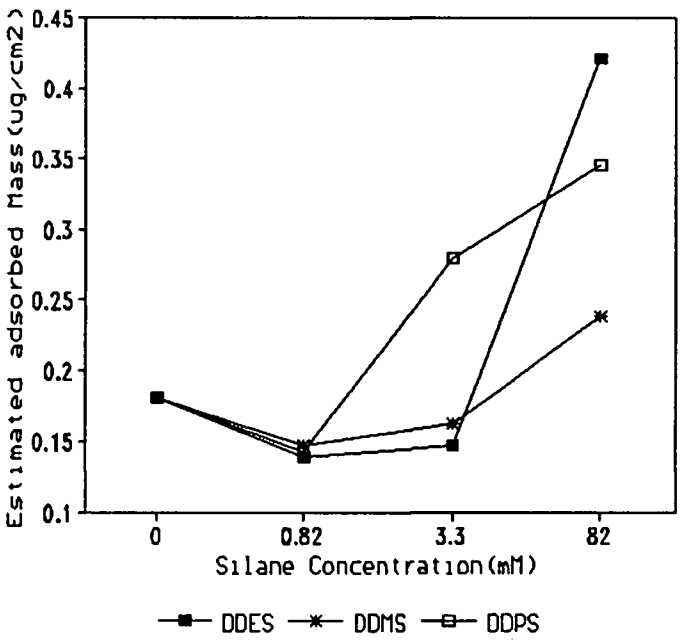


At 600 (mg/l)

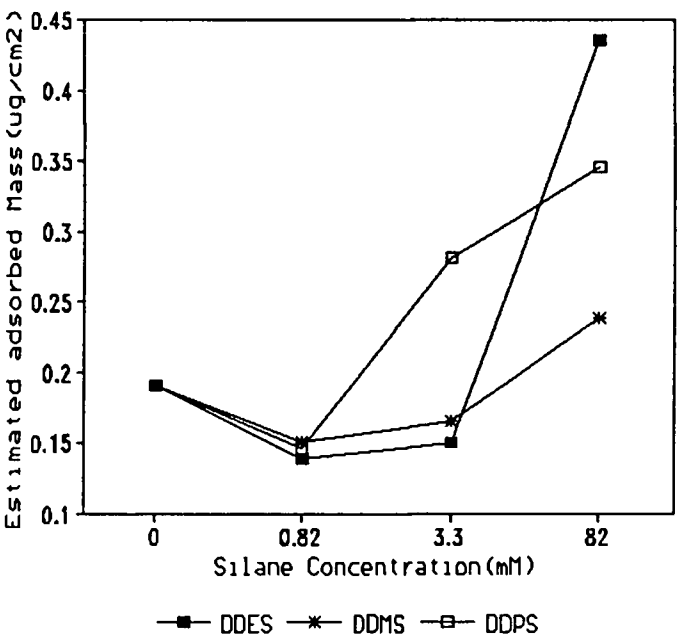


At 800 (mg/l)

Figure 1c. (Continued)

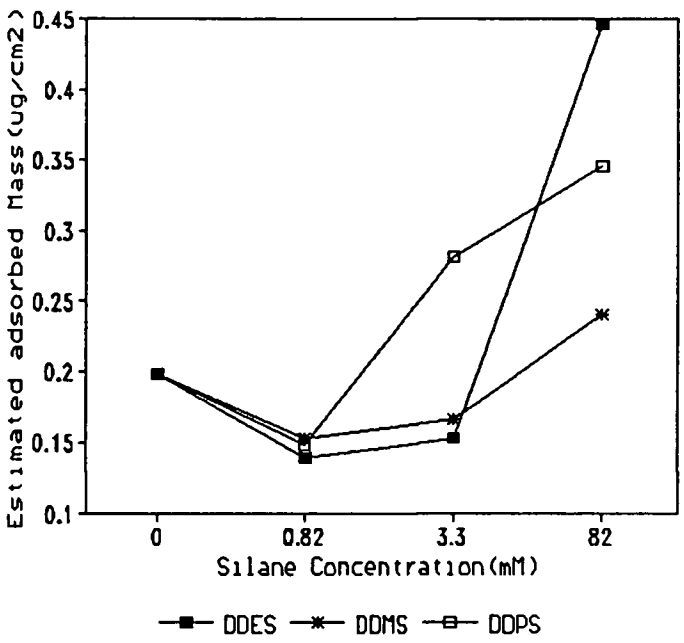


At 1000 (mg/l)

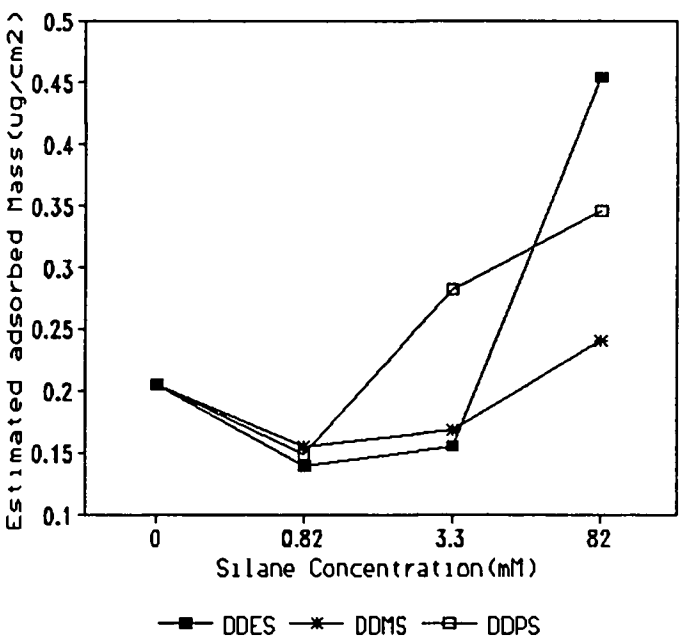


At 1200 (mg/l)

Figure 1c. (Continued)

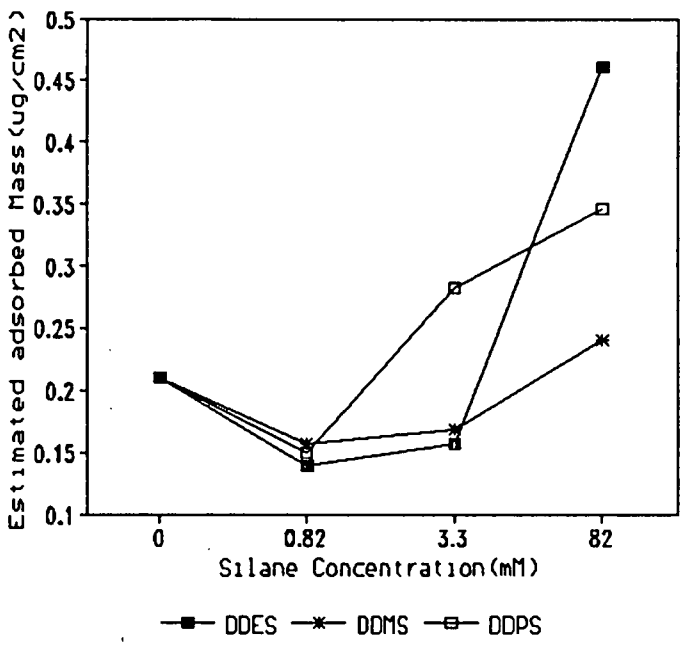


At 1400 (mg/l)

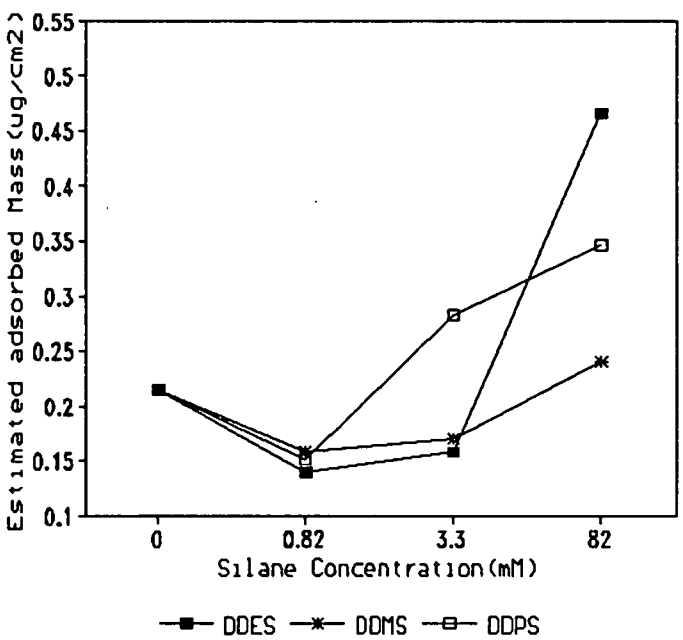


At 1600 (mg/l)

Figure 1c. (Continued)



At 1800 (mg/l)



At 2000 (mg/l)

APPENDIX B

All raw data are available with Dr. Joseph McGuire at Bioresource Engineering Department.