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<p>Two substrates that are amenable to analogous silane chemistry provided the basis for characterizing alkylsilane layers formed by octadecyltrichlorosilane (OTS) and dimethyloctadecylchlorosilane (DMOCS) reactions. Electrochemical analysis of the capacitance was used to measure an apparent thickness, $d(A)$, and the degree of surface coverage, θ, for deposited silane layers on Si/SiO₂ electrodes. Elemental analysis was performed to determine the carbon load resulting from comparable silane reactions on glass bead substrates. Glass bead substrates were also examined by FTIR spectroscopy to evaluate the hydrocarbon incorporation and hydration of surface silanol groups. A semiquantitative measure of hydrocarbon deposition was derived from FTIR spectra and shown to correlate well with the electrochemical and analytical data over a defined range. The results indicate that under the anhydrous conditions used and large reagent excess, silane reaction with OTS produces complete monolayers</p>			
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28 A thick whereas only partial monolayers could be achieved (80% surface coverage) with DMOCS. An assay for surface activation of the enzyme phosphodiesterase provided further evidence that the OTS was distributed as a monolayer uniformly covering the substrate surface. A description of the silane layer and techniques for their evaluation are important when such alkylsilane layers are utilized in commercial processes or as elements in complex structures such as artificial biomembranes.

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**Surface-Bound Alkyl Monolayers:
Electrochemical and Structural Characterization**

by

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Submitted to

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INTRODUCTION

Organic monolayers have been widely studied and utilized as models for biological membranes and as building blocks for artificial membranes. The most common approach (Langmuir-Blodgett) employs compressed monolayers from an air-water interface that can be deposited on a polar, planar substrate. This technique is, however, not readily adaptable to the formation of large-scale structures or biomembranes for the commercialization of biosensors. As an alternative, it has been noted (1) that hydrophobic monolayers with properties very similar to Langmuir-Blodgett monolayers can be formed by reactions of silane compounds with solid substrates. These monolayers can provide the basis for formation of a variety of complex, ordered structures.

We propose that covalently immobilized monolayers of alkyl chains formed by use of silane chemistry can be used to initiate the self-assembly of surface-bound biomembranes that could couple membrane receptors to an optical or electrical substrate for biosensor development and other applications. Such a generic technique would be applicable for most receptors amenable to reconstitution by detergent dialysis (2). Here we report the physical characterization of surface-bound alkyl layers formed on two substrate types, glass microspheres and Si/SiO₂ electrodes. A variety of techniques, including electrochemical capacitance analysis, FTIR spectroscopy, elemental analysis, and enzymatic activation are used to probe the structure and composition of the surface layer.

Our results support the conclusion that under anhydrous conditions, predominantly monolayer type structures are formed with octadecyltrichlorosilane (OTS). For the monofunctional reagent, dimethyloctadecylchlorosilane (DMOCS), complete monolayer coverage was not achieved even with higher

reagent concentrations and longer reaction times. FTIR measurements were correlated with carbon analysis and surface capacitance to evaluate the extent and integrity of the deposited alkyl layers. FTIR provides a simple, semiquantitative technique for monitoring surface hydration and carbon loading before and after silanization. The FTIR parameters characterizing silanol and hydrocarbon groups may be useful for evaluating or understanding the behavior of other silane chemistry-based systems, for example, bonded phases on silica chromatography media or interfacial bonding layers in reinforced plastics and composites (3).

EXPERIMENTAL

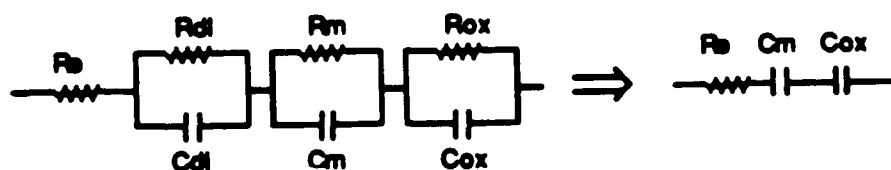
Substrates. To prepare Si/SiO₂ electrodes, 5 mm square chips were cut from n-type and p-type silicon wafers (EG&G Reticon, Inc., Sunnyvale, CA) that were coated on one side with a layer of SiO₂ 960 Å thick. Doping density of the underlying silicon was on the order of 10¹⁴ per cc. Ohmic contact was provided on the reverse side by a gold layer to which leads were attached with silver epoxy (Epoxy Technology, Inc., Billerica, MA). The electrode assembly was sealed in glass tubing with the SiO₂ surface exposed. Glass microspheres 37 μm in diameter were purchased from Ferro, Inc. (Jackson, MS) and chromatography medium (pellicular ODS, 37-53 μm) from Whatman (Hillsboro, OR). The glass microspheres were prepared for alkylsilanization by washing in concentrated HCl, boiling in 1% Liquinox, and soaking in chromate/sulfuric acid cleaning solution. Finally, they were treated with 3 N NaOH, then 0.1 N HCl and rinsed thoroughly with H₂O. Beads were dried for at least 2 hours at 110 °C before storage.

Alkylsilanation of Substrates. OTS or DMOCS solutions (v/v %) were freshly prepared each time by diluting silane reagent in anhydrous solvent I, 80:12:8 n-hexadecane/carbon tetrachloride/chloroform, under argon in a plastic glove bag (Atmosbag from Aldrich Chemical, Milwaukee, WI). Si/SiO₂ electrodes or glass beads were dried for 2 hr at 60 °C immediately before alkylation. Substrates were reacted by immersion in the silane solution at room temperature under dried argon with occasional agitation. Reactions were terminated by rinses with solvent I followed by chloroform washes. Electrodes were then cured at 100 °C under vacuum for 10-12 hr. Glass beads were used either with or without the cure step.

Compositional Analysis. Carbon loading of treated glass bead substrates was assessed by elemental analysis for carbon performed on a 440 CHN/OS Analyzer (Control Equipment/Leeman Labs, Lowell,

MA). Samples of 30-60 mg were run in triplicate or quadruplicate and corrected for the instrument/capsule blank. FTIR spectra were recorded on a Cygnus 100 Spectrometer (Mattson Instruments, Inc., Madison, WI). For transmission spectra, approximately 10-30 mg of beads were pressed between NaCl windows and then infused with CCl_4 to match the bead refractive index and reduce scattering. Bead spectra were analyzed after subtraction of the CCl_4 spectrum to minimize solvent peaks at 1540, 1220, and 980 cm^{-1} . In order to compare hydrocarbon loading for equivalent samples sizes, $\log R_{3000}$ (integrated intensity between 3000 and 2750 cm^{-1} ratioed to ΔA_{900} , the absorbance at 900 cm^{-1} after subtraction of the background at 2300 cm^{-1}) was plotted vs. ΔA_{900} and fitted with a linear regression program. Values for R_{3000} reported in tables correspond to a $\Delta A_{900} = 0.5$ unless otherwise noted. The parameter R_{3700} was computed and could be analyzed analogously to R_{3000} in order to evaluate surface hydration. The integrated intensity from 3700 to 2500 cm^{-1} was employed with correction for the superimposed hydrocarbon peak if necessary. Diffuse reflectance (DRIFT) spectra were recorded with a diffuse reflectance accessory (DRA-200 from Harrick Scientific Corp., Ossining, NY) and ratioed to a KBr powder background.

Electrochemical Analysis. Surface layers on the Si/SiO₂ electrodes were analyzed by AC impedance techniques (4) with the instrumentation shown in Figure 1. A small amplitude (≈ 10 mv peak to peak) sinusoidal AC function was superimposed on the DC potential applied to the control or coated electrode and a lock-in amplifier used to measure the phase difference and ratio of peak values between the input voltage and output current waveforms. With the treated electrodes studied here, the impedance was almost purely capacitive and was evaluated from the quadrature component of the total impedance. The capacitance analysis is based on the following equivalent circuit:



where R_e is the electrolyte resistance; C_{dl} , the double layer capacitance; R_{dl} , the double layer polarization resistance; C_m is the membrane (or silane layer) capacitance ; R_m , the membrane (silane layer) resistance; C_{ox} , the oxide layer capacitance; and R_{ox} , the oxide resistance. R_{dl} , R_m , and R_{ox} are assumed to be sufficiently large under the conditions employed here that they make a negligible contribution to the measured impedance. Since the double layer capacitance ($20-40 \mu\text{F}/\text{cm}^2$) is at least an order of magnitude greater than that expected for the membrane or oxide, the resulting capacitive circuit can be reduced to the series capacitors as shown above.

Phosphodiesterase Activity. Activation of the rod outer segment phosphodiesterase (PDE) results in the hydrolysis of cGMP and liberation of a proton. The assay for activity is based upon measurement of the resulting pH change in a weakly buffered assay solution as described by Yee & Liebman (5). Rhodopsin-containing rod outer segment membranes were isolated from bovine retinas (J.A. Lawson, Co., Lincoln, NE). Peripheral enzymes (which include PDE and the G-protein activator, transducin) from the rod outer segment membranes were extracted into 5 mM Tris buffer, pH 7.4, containing 0.1mM EDTA, 1 mM MgCl_2 , 1 mM dithiotreitol, 50 μM phenylmethylsulfonyl fluoride, 10 μM leupeptin, and 10 μM pepstatin. The extract was concentrated 10-fold in an Amicon Centricon-10 device (Danvers, MA). The assay for activation in the absence of light was conducted by suspending glass beads (≈ 300 mg) in 0.5 ml of 120 mM NaCl, 2 mM MgCl_2 , 1 mM dithiothreitol, 10 mM octylglucoside, and 10 MM HEPES at pH 7.9. The low concentration of detergent was required to prevent exclusion of hydrophobic coated beads and did not interfere with PDE activity. A micro electrode set-up was used to monitor pH after the addition of GTP and cGMP to final concentrations of 250 μM and 1 mM, respectively.

RESULTS AND DISCUSSION

The objective of the alkylsilanization step is to covalently attach long-chain alkyl groups to the substrate surface in order to effectively substitute for one half of a membrane bilayer leaflet during the formation of membrane-like structures incorporating receptors. Reactions were performed with two different octadecylsilane reagents that differed in their reactivity toward the substrate surface. DMOCS is limited to monolayer formation while OTS, with three hydrolyzable groups, has the potential for forming thick polymeric films (1 and references cited therein). In order to evaluate the extent of surface coverage by C_{18} -silane, reactions were performed in parallel on Si/SiO₂ electrodes and glass beads. The latter were subjected to elemental analysis and examined by FTIR spectroscopy to characterize the substrate surface and the incorporation of alkylsilane. Capacitance measurements on the electrodes provided complementary information about the thickness and integrity of the alkylsilane layer (see below).

Analysis by FTIR Spectroscopy

Figure 2 shows the transmission FTIR spectra for control (cleaned, untreated) beads (trace A) and for glass beads after anhydrous reaction with DMOCS and OTS (traces B and C). Also shown for comparison (trace D) is the spectrum of a commercial bonded phase chromatographic support (pellicular medium) prepared by reaction with C_{18} -silane and specified to have a carbon load in the 1-2% range. Bound C_{18} hydrocarbon is reflected in the triplet at 3000-2800 cm^{-1} that is superimposed on the broad silanol band. The inset (Figure 2) expands this region from traces B and C. The characteristic complex band with major peaks at 2960, 2925, and 2850 cm^{-1} was observed for all silane-reacted samples. It was clear from the spectra that pellicular medium carried a much greater carbon load than did beads reacted

with OTS or DMOCS under anhydrous conditions in our experiments. In addition to the absorption bands from C₁₈-chains, the pellicular medium exhibited a prominent band with a peak at 1123 cm⁻¹. Since the bonded phases of chromatography media generally contain heterogeneous alkyl polysiloxane structures (6), absorption from Si-O-Si bonds in this frequency range is expected. A peak with similar frequency (1100 cm⁻¹) was observed in the difference spectrum of aged vs fresh OTS and is probably indicative of polymerization (unpublished observations). By contrast, there was no detectable absorbance in this region for any of the silanized beads in our experiments. Thus FTIR observations provide evidence that, under our reaction conditions, OTS is bound predominantly as a monolayer in spite of its potential for forming more complex structures through side to side polymerization.

The hydrocarbon peaks are superimposed on a very broad absorption band that is seen in all bead spectra from about 3700 to 2600 cm⁻¹. It reflects both adsorption of water on the substrate surface (7) and surface silanol groups in various states of hydrogen bonding. As observed by others (3) curing at 80-100 °C reduced the amplitude of this absorption and shifted the frequency spectrum toward higher values reflecting the loss of adsorbed H₂O (3400 cm⁻¹) and the relative increase of hydrogen-bonded surface silanols (3500-3680 cm⁻¹). However, at temperatures below 200 °C the primary layer of strongly hydrogen-bonded water remains since drying *in vacuo* above 500 °C (7) is required to convert most of these to free silanol groups (3750 cm⁻¹).

The glass substrate is highly absorbing below 1500 cm⁻¹ and the plateau value of this absorbance (A₉₀₀) was used as a measure of the amount of sample (silica medium) in the IR beam. Normalization of the integrated intensities for both the surface silanols and the bound alkyl chains to this value provided parameters, R₃₇₀₀ and R₃₀₀₀, that could be used to compare the surface-bound C₁₈ and substrate surface silanols for different samples (see below). The transmission spectra recorded with beads suspended in a

high refractive index solvent show most of the same features as diffuse reflectance (DRIFT) spectra of silanized glass beads (Figure 3) as well as DRIFT spectra reported for silane-treated glass fibers (3). (The large peak at 1290 cm^{-1} seen in each sample in Figure 3 is attributed either to a silica band or an impurity adsorbed onto the KCl). With diffuse reflectance techniques, quantitative or semi-quantitative applications can be achieved if care is taken to minimize or correct for differences in surface area and effective pathlength (8). The transmission technique was used here because it gave comparable results with small sample sizes and required no special sampling apparatus.

Elemental Analysis of Carbon Loading

To provide a quantitative measure of the C_{18} coverage that could be related to the FTIR parameters, alkylsilanated bead samples were subjected to elemental analysis. Results of the carbon analysis are given in Table I along with the R_{3000} values from the FTIR characterization of glass beads reacted with OTS and DMOCS at reagent concentrations varying from 0.1 to 4%. Control beads always carried a finite amount of hydrocarbon, as was noted by MacDonald (7) on the basis of IR spectra. This probably represents strongly adsorbed surface impurities which may or may not be displaced during the silanization reaction.

In initial experiments using 0.1 to 1% silane for times under an hour, DMOCS-treated beads consistently exhibited 50% or less carbon loading relative to OTS. An example from such an experiment is included in Table I. For comparison, the values of R_{3000} calculated from the DRIFT spectra (Figure 3) for similar samples are 0.37, 1.22, and 2.45 for control, DMOCS- and OTS-reacted beads, respectively.

In an attempt to achieve monolayer coverage with DMOCS and to optimize the extent of reaction in general, reagent concentration and reaction times were increased. In these experiments, the DMOCS reaction appeared to reach a plateau with on average only 60% of the surface-bound carbon found for OTS-

reacted samples (see Table I). This fraction corresponds to the overall ratio of R_{3000} observed for 4% DMOCS reaction relative to that with 2% OTS. Values of R_{3000} reported in Table I for these optimized conditions are the means from 3 different experiments comparing carbon loading for the two reagents on the same batch of cleaned, dried substrate. The ratios of R_{3000} values for DMOCS vs. OTS reaction in the individual experiments were 0.33, 0.77 and 0.83.

Included in Table I for reference are the data for Whatman pellicular chromatography medium. The carbon analysis is in accord with the carbon loading specified by the manufacturer (1-2%). The value for R_{3000} , although an order of magnitude greater than that seen for our OTS-reacted beads, is not proportional to the carbon content determined by elemental analysis. Overall, the data in Table I show that there is a good correlation between carbon load and R_{3000} values in the range up to 250 μg carbon/g beads. Thus the IR parameter is a useful, semi-quantitative measure of surface coverage for evaluating silanization reactions on silica substrates.

Alkylsilanization reactions are very sensitive both to solvent dryness and to the substrate surface (6). Hydration of the surface was difficult to control by use of the moderate drying conditions that could be applied to both the glass beads and the electrodes used in other experiments. Variation in C_{18} -chain incorporation for the same reagent concentration was observed to vary as much as 2-fold in our experiments, apparently on account of differences the variables noted above. A measure of the surface hydration was provided by R_{3700} values. For cleaned and alkylsilanted beads these varied in the range from 30 to 150, with the lowest value observed for cured, OTS-reacted beads and the highest for DMOCS-treated beads equilibrated at room humidity. When desiccated samples were exposed to humidity, R_{3700} values increased more rapidly for the DMOCS-reacted beads than for the OTS-reacted beads, indicating that the surface of the former is more easily hydrated. This parameter could be reversibly modulated by

cycles of drying and hydration.

The amount of carbon incorporated by silanization can be compared with the value expected for hypothetical full monolayer coverage. Quantitative reaction would incorporate 116 $\mu\text{g/g}$ carbon for a 37 μm diameter bead with ideal spherical surface based on an estimate of 8 $\mu\text{mol/m}^2$ (9) for surface silanols on silica. In their studies, the actual surface area was determined by use of nitrogen adsorption isotherms. Given the distribution of sizes and the irregular surface structure of the microspheres used in our experiments, the actual surface area will be several-fold larger than that of the ideal sphere. However, it is also known that the surface density for the reaction is limited by the Van der Waals size of the alkyl group so that only about 60% of silanols can be reacted for a trimethylsiloxy layer (9).

Thus, the limiting value of carbon incorporation, 244 $\mu\text{g/g}$, observed in our study is reasonable for monolayer coverage of the glass microsphere surface within the uncertainty limits noted above. Given that the FTIR spectra exclude significant amounts of branched polymeric structures for the OTS-treated beads, the results of the carbon analysis suggest that OTS-reacted surfaces approach complete monolayer coverage. Capacitance results (see below) provide support for this picture. By contrast, DMOCS reaction does not generally lead to full coverage of the surface and allows the surface to become more readily hydrated under atmospheric conditions. Both the extent and the size of "gaps" in the C_{18} monolayer have been explored by other techniques.

Capacitance Measurements on Silanized Electrodes

Electrochemical techniques provide a means for acquiring detailed information about the molecular structure at an electrode surface. To take advantage of this technique for characterizing surface-bound structures produced by silanization, semiconductor/oxide electrodes (Si/SiO_2) were used as substrates to

approximate the surface chemistry of glass microspheres. Electrodes were subjected to the same silanization reactions as were glass beads. Capacitance values of the deposited layers were determined from the quadrature component of the impedance at a bias potential chosen such that the silicon substrate is in the accumulation condition and, therefore, conductive. For this condition, the space charge capacitance of the semiconductor is eliminated and the measured capacitance reflects the oxide layer in series with the silane layer in the presence of electrolyte.

Capacitance values measured in this way for the bare, oxidized electrode surface and the silane-treated surface are reported in Table II. It also summarizes dimensions of silane (ODS=octadecylsilane) layer derived from capacitance data for electrodes reacted with OTS and DMOCS under various conditions. Capacitance was calculated from the relationship for series capacitors in the proposed equivalent circuit:

$$\frac{1}{C_T} = \frac{1}{C_{SiO_2}} + \frac{1}{C_{layer}}$$

where C_T is the total measured capacitance for the silane-coated electrode, C_{SiO_2} is the capacitance measured for the bare (oxidized) electrode, and C_{layer} is the capacitance of the structure incorporated at the surface. The thickness of a layer was estimated from the relationship $C_{layer} = \epsilon \cdot \epsilon_0 / d$ by assuming a value of 3 for the dielectric constant. A similar capacitive analysis on silicon wafers has been reported to detect antigen binding to substrate-bound antibodies (10).

The dimensions tabulated in Table II assume full coverage of the surface with an insulating hydrocarbon layer. The length of an extended C_{18} silane molecule is calculated to be 25.6 Å. Values of the thickness determined for the surface layer with two different sets of OTS-reacted electrodes are in good agreement with theory as well as with measurements by Polymeropoulos & Sagiv (11) of the dielectric

thickness for monolayers of C₁₈ fatty acid (25.7 Å) and OTS (28.0 Å) at metal-insulator-metal junctions. The apparent dimensions of the surface layer calculated from the capacitance measurements on DMOCS-reacted electrodes are smaller. However, since both OTS and DMOCS have the same alkyl chain length, a value for the fractional coverage, $\Theta = d/d_0$, is reported where d is the apparent dimension and d_0 (25.6 Å) is the theoretical thickness of one monolayer. On this basis, the capacitance measurements are interpreted to suggest that the DMOCS-treated electrodes carry incomplete monolayers covering from 30 to 80% of the surface, which is consistent with the elemental analysis and FTIR data in Table I.

A second way to examine the extent of reaction at the electrode surface is to measure the shift in the the flat band potential (ψ) as a function of pH. The pH sensitivity of the surface potential reflects both the surface charge density and the reactivity of dissociable groups at the electrode surface (12). For the Si/SiO₂ electrodes used here, these are silanol groups and the electrode behavior was close to Nernstian with a slope of 45mv/pH unit. By measuring the ψ /pH curves for silane-treated electrodes, it was possible to calculate the fraction of silanol groups still chemically active at the surface after silanization and from this to infer the degree of reaction.

On the basis of such measurements, 52% of the surface silanol groups on Si/SiO₂ electrodes were blocked after OTS (1%) reaction for 3 hr, conditions that appeared by other analyses to yield full monolayer coverage with C₁₈ chains. For 0.5 hr reactions with either OTS or DMOCS at 1%, only 22-24% of the surface silanols were reacted, and C₁₈ coverage for glass beads, as estimated by FTIR, was corresponding less than for the longer reaction time (Table I). The parallel increase of chemically blocked silanols and C₁₈ coverage to their respective theoretical limits is consistent with the picture that both silane reagents are reacting as monomers to form monolayers in which packing of the C₁₈-chains limits coverage.

Functional Assay for Surface Coverage

In the course of experiments investigating the reconstitution of photoreceptor membrane function on the surface of silanized glass beads, it was observed that the bare glass beads strongly activate an enzyme, cGMP phosphodiesterase (PDE), even in the absence of light which is the physiologic activator. PDE bound at the membrane surface in native membranes is activated via a complex cascade (13) triggered by a light-activated membrane receptor. In our experiments, an extract containing PDE and the required cofactors for activation was exposed to clean and modified glass beads with various surface modifications in order to establish dark (control) levels of enzymatic activity.

The activation of the enzyme by increasing amounts of bare glass beads is demonstrated in Figure 4. Enzymatic activation by a constant amount of beads was also seen to decrease as a function of reaction time with OTS and DMOCS. These phenomena are likely related to the observations that PDE can be activated by polyanions such as heparin or silicate (14). The surface activation of PDE in the dark was then utilized as a probe of surface accessibility for different silane-reacted samples, as shown in Table III.

Reaction with OTS and DMOCS resulted in significant reduction in the enzymatic activation produced by the glass beads. Beads that had been cured directly after reaction showed a further reduction, with virtually no PDE activation observed for the cured, OTS-reacted beads. The results in Table III indicate that PDE activation can be used to discriminate degrees of coverage as well as molecular details of the silane layer.

The non-physiologic activation of PDE is thought to require contact of at least one inhibitory subunit with the activating surface (15). For the 10 kilodalton molecular weight subunit that is involved, the molecular diameter given a globular structure would be between 25 and 30 Å. For an elongated structure, the molecular diameter would be less. Thus, activation of PDE probably requires defects in the surface-

bound matrix with dimensions between 5 and 30 Å. The enzyme, PDE, can be envisioned as a probe with "lower resolution" than the ionic species that mediate surface accessibility detected by the shift in flatband potential measurements.

As shown in Table III, low surface accessibility for OTS samples ($\approx 10\%$ of controls) could be reduced to virtually zero by post-reaction curing. Curing eliminates bound water and may enhance lateral bond formation between OTS molecules covalently reacted at the substrate surface. Taking the stoichiometry of OTS reaction that is consistent with monolayer coverage (Table I) together with the observation that curing prevents surface activation of PDE altogether, provides strong evidence that OTS-reacted beads bear a uniform monolayer of C_{18} chains. Under these same conditions, electrochemical flat band potential shifts indicate that 50% of silanols are still accessible to small ions. For DMOCS-reacted beads, some PDE activation was always observed and remained after curing. The extent to which DMOCS reaction allows PDE activation is consistent with the incomplete surface coverage measured by other techniques, and suggests that bare patches, of a size to accommodate protein molecules, exist on the DMOCS-reacted bead surface.

SUMMARY AND CONCLUSIONS

Two substrates that are amenable to analogous silane chemistry provided the basis for characterizing the properties of alkylsilane layers formed from OTS and DMOCS reaction. Electrochemical analysis of the system provides a structural picture of the surface-bound matrix that is complementary to the chemical analysis. FTIR spectroscopy proved to be a convenient technique for monitoring the bead surface silanols

and for providing semi-quantitative estimates of the C_{18} incorporation under different conditions.

The dimensions of the surface layer determined from capacitance measurements with the OTS-reacted electrode ($\approx 28 \text{ \AA}$) are consistent with an intact monolayer. That full monolayer coverage by C_{18} chains appears to correspond to about 50% reaction of available silanol groups is expected on the basis of chemisorption experiments with a less bulky silane (9). All measurements on DMOCS-reacted substrates indicated that the silane layer covered at most 80% of the surface.

In addition to the hydrocarbon fingerprint, FTIR spectra reflected the hydration state of surface silanol groups on the alkylsilanated beads. For OTS-treated beads the kinetic accessibility of the substrate surface to H_2O vapor appears to be restricted, as reflected in the low values (≤ 100) of R_{3700} . However, this surface retains the potential for hydration, as reflected by the reactivity of the surface silanols at equilibrium with the electrolyte in the measurement of flat band potential. For DMOCS-treated beads that have only 40-80% surface coverage by C_{18} monolayer, flatband potential measurements indicated correspondingly more free silanol groups. R_{3700} values for DMOCS-treated beads ranged from 110-150, presumably because the surface is more readily available for adsorption of water vapor.

Finally, the differential in the surface activation of the enzyme PDE reflects an important difference between the silane layers formed by OTS and DMOCS. For the latter, the accessibility of the substrate to the surface of a protein subunit suggests that these structures could also support insertion of the hydrophilic terminus of a transmembrane receptor protein. Conversely, OTS-reacted substrates may be more effective in binding hydrophobic entities. The two types of surface-bound monolayers provide a basis for exploring the formation of surface-bound biomembranes that are an alternative to Langmuir-Blodgett films and can potentially incorporate a wide variety of lipophilic molecules and protein receptors in membrane-like matrices covalently attached to a transducing substrate.

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FIGURE LEGENDS

Figure 1. Block diagram of experimental set-up.

Figure 2. FTIR transmission spectra of glass bead samples. A, cleaned beads; B, DMOCS-reacted (4%, 6hr); C, OTS-reacted (2%, 3 hr);D, Whatman Pellicular ODS. Inset shows an expanded region of traces B and C.

Figure 3. DRIFT spectra of glass bead samples. A, cleaned beads; B, DMOCS-reacted (0.1%, 0.5 hr); C, OTS-reacted (0.1%, 0.5 hr).

Figure 4. Activation of PDE (dark activity) in the presence of non-silanized glass beads.

TABLE I

<u>Bead Coating</u>	<u>Elemental Analysis</u> $\mu\text{g carbon/g beads}$	<u>FTIR</u> R_{3000}^a
control, clean	13 ± 6^b	0.36
DMOCS ($\leq 1\%$)	53 ± 7^b	0.74
OTS ($\leq 1\%$)	143 ± 26^b	2.06
DMOCS (4%, 6 hr)	147 ± 40^c	$1.39 \pm .45^c$ (n = 3)
OTS (2%, 3hr.)	244 ± 131^c	$2.31 \pm .64^c$ (n = 3)
Whatman beads	15084 ± 185^b	22.7

^a Ratio of integrated intensity ($3000\text{-}2750\text{ cm}^{-1}$) to $A_{900}\text{-}A_{2300}$

^b mean \pm s.d. for quadruplicate determinations on a sample

^c mean \pm s.d. for 3 sets of reacted beads

TABLE II

CAPACITANCE CHANGES AND DERIVED DIMENSIONS FOR SILANE (ODS) LAYERS

	C (nF cm ⁻²) ^a		d (Å) ^b	
	Si/SiO ₂	Si/SiO ₂ /ODS	ODS Layer	θ
DMOCS/1%	53.1	52.3	3754	7.3 ± 2.5 (n=3) 0.29
OTS/1%	39.7	38.1	1033	27.5 ± 7.2 (n=4) 1.07
DMOCS/4%/6hr	40.2	38.9	1331	20.7 ± 5.0 (n=6) 0.81
OTS/2%/3hr	42.2	40.3	1052	30.2 ± 16 (n=6) 1.18

^a mean value from n electrodes

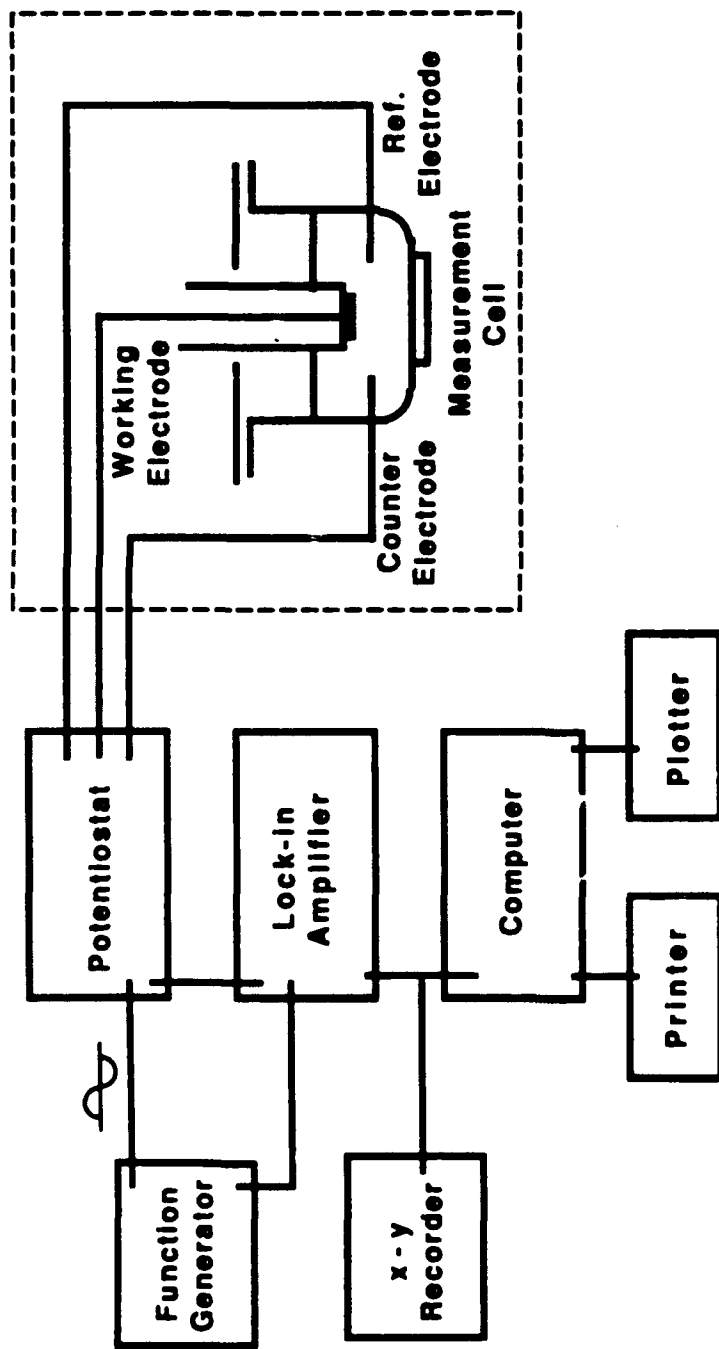
^b mean value and S.D. of d(Å) calculated for each of n electrodes

TABLE III**ACTIVATION OF PDE BY GLASS BEAD SURFACES**

	PDE activity (dark) in nmol H⁺/min		
	Control (untreated)	Reacted	Reacted, cured
Silane-type			
DMOCS, ≤ 2%	146 (100-150)^a	35 (15-75)	8
OTS, ≤ 2%	118 (100-150)	12 (10-30)	0 (0-5)

^a Parentheses indicate range of values observed in similar experiments (n = 2-4).

Figure 1



Block Diagram of Experimental Set-Up

Figure 2

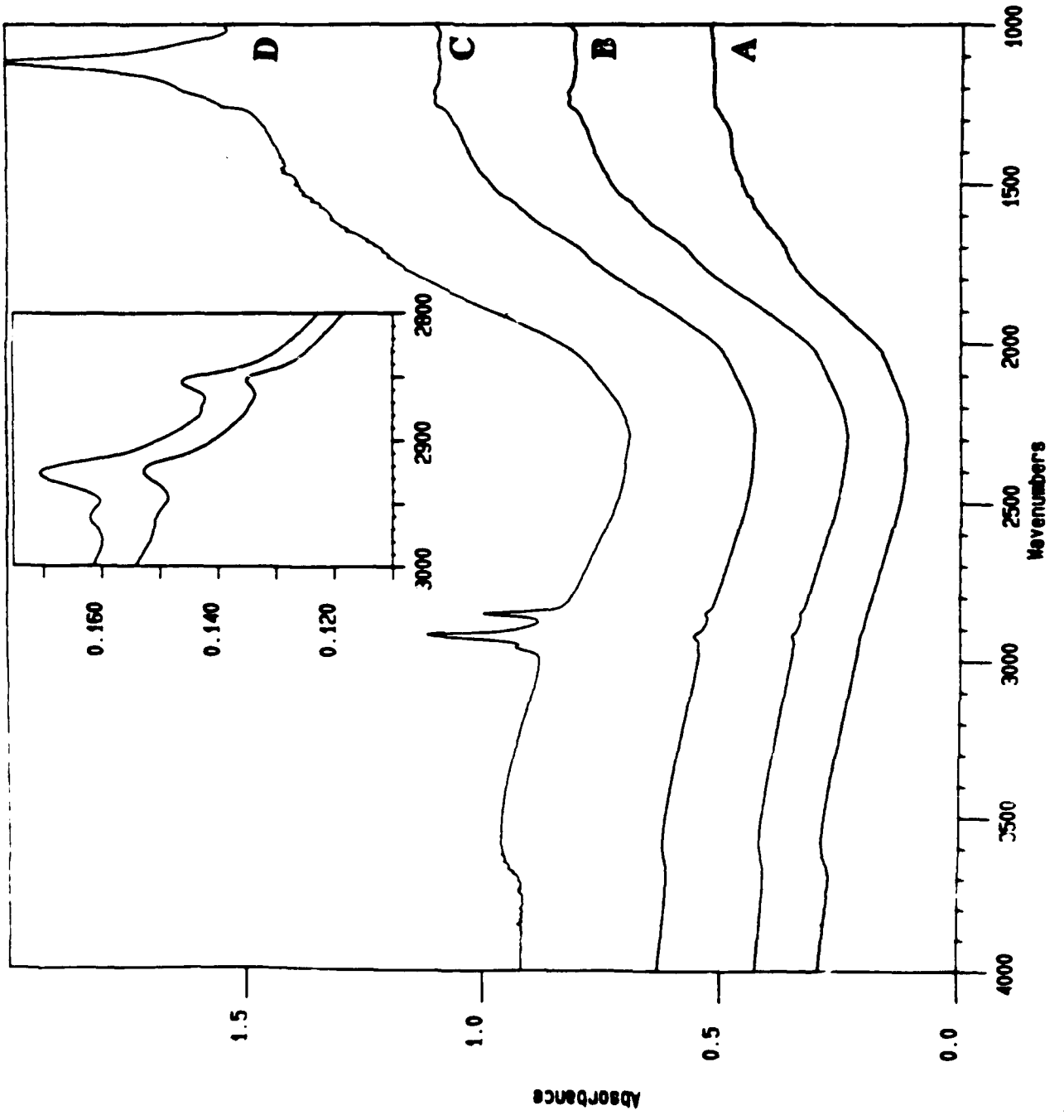
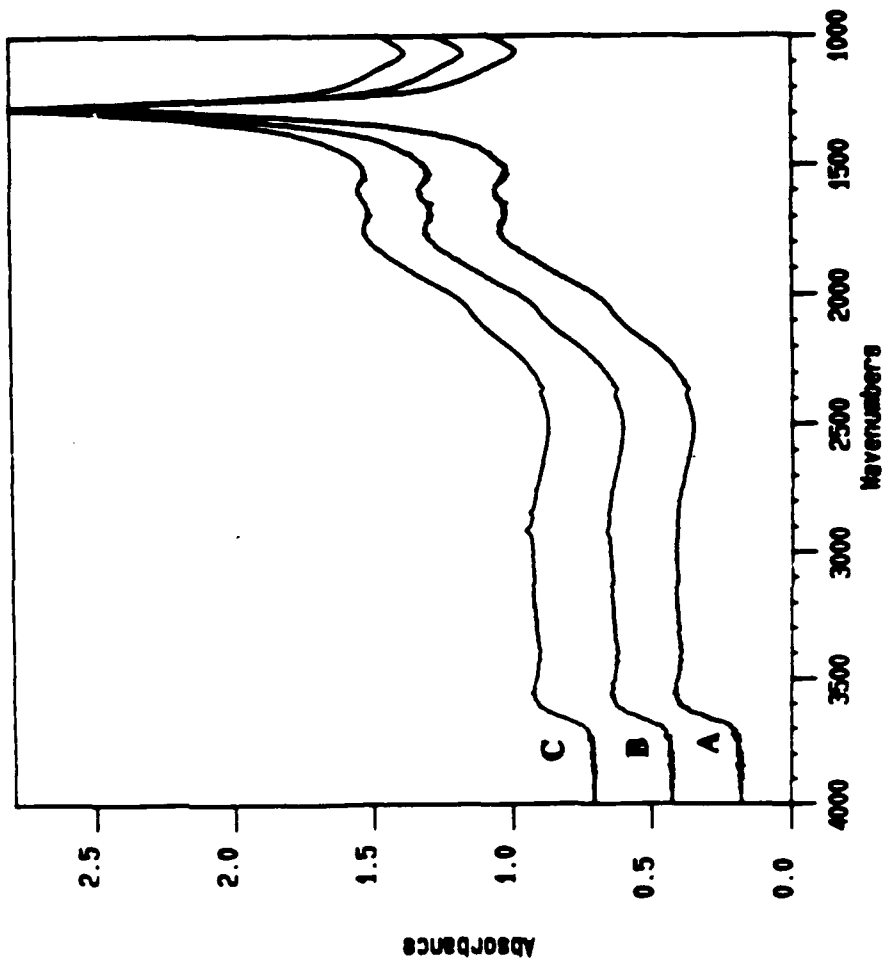
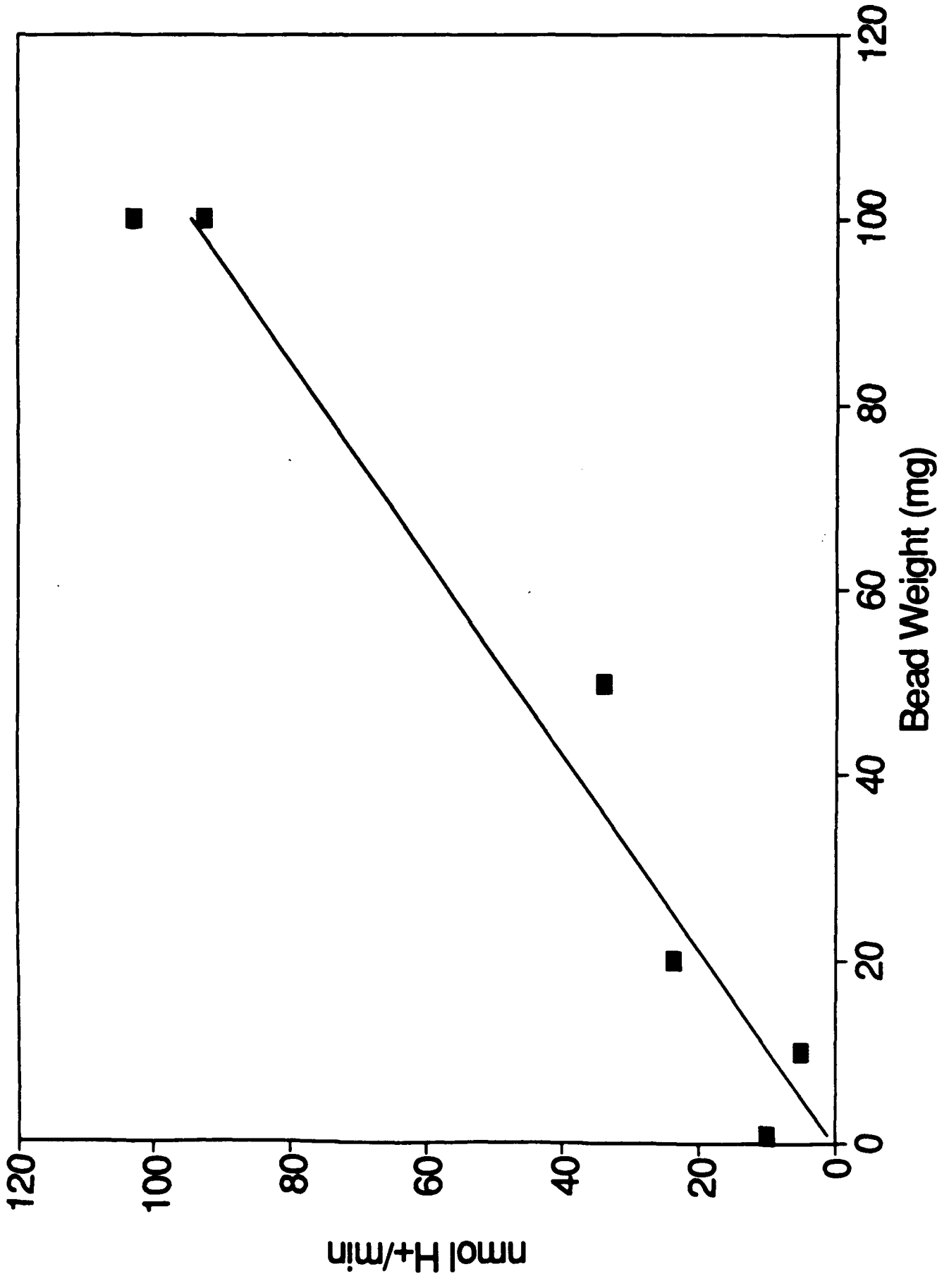


Figure 3





▲ Figure 4

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