Amine–Aldehyde Polycondensation Chemistry in Monolayer Films. An Approach Toward Achieving a Light Energy Transducing Molecular Organizate

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ABSTRACT: The grafting of *n*-octadecanal monomers contained in a monolayer at the air–water interface onto the linear polymer, poly-L-lysine, in the underlying aqueous subphase has been observed to give a viscous surface film. In addition to employing the classical techniques of surface chemistry, e.g., surface pressure–area and surface viscosity determinations, to study this reaction, the disappearance of *n*-octadecanal and poly-L-lysine were measured quantitatively by vapor phase chromatography and spectrometric assay techniques, respectively. Model systems reacting *n*-octadecanal with 1-aminopentane in the subphase and *n*-octadecylamine in the same surface film were also studied. A multicompartment monolayer trough was used to allow a series of chemical reactions to be performed upon a well-defined monolayer film. The ultimate goal of this work is the synthesis of a light energy transducing molecular organization based on an ultrathin polymer film.

The formation of pigmented molecular organizations such as micelles,[1a] vesicles,[1b] planar bilayer lipid membranes,[1c] and monomolecular layer arrays[1d] has allowed a variety of photophysical and photochemical studies to be performed as a function of two- and three-dimensional molecular geometry. The ultimate importance of such studies may lie in the understanding they provide for converting solar energy into electricity or storing this energy in chemical products.[2] In some instances, however, the interpretations placed on these observations have been limited by the mechanical fragility of the organization or the uncertainty of the location of individual components in the organizate. This report describes the chemistry and techniques of a specific approach whose ultimate goal is the synthesis of a well-defined and stable energy transducing molecular organization based on an ultrathin polymer film.

The realization of a system in which visible light energy is trapped and stored via an electron transfer reaction between an excited dye chromophore and a suitable electron acceptor (donor) requires the understanding and control of several processes on a molecular scale. The critical problem is to prevent dissipation of the trapped energy before it can be diverted to perform electrical work or stored as a stable, high-energy product. In general, the thermodynamically favored back reaction, which is followed by emission of light or degradation of the energy to heat, occurs too quickly to permit efficient energy conversion. To date, only the natural process of photosynthesis has been able to minimize the amount of energy lost in these ways. In attempting to overcome this problem, we[3] and others[1c,2,4,41] have proposed conceptual systems modeled, in part, on the structure and function of the photosynthetic apparatus.

In the approach described herein, the key processes of light-promoted charge separation, maintenance of this separation, and its utilization are to be determined largely by the unique structure of a synthetic organic film—a laminate of bimolecular thickness in which one side of the film is a light-sensitive electron donor (Dye) and the other is an electron acceptor (Q) with a thin separation barrier (≤25 Å) in between.

This asymmetric film separates two aqueous solutions containing soluble secondary oxidation–reduction couples (A,D). In operation, light is absorbed by the Dye producing an electronically excited state followed by an electron transfer between the Dye* and Q to create a trans membrane charge separation which is then maintained (in competition with a back electron transfer) by a rapid removal of these charges from each membrane surface by additional electron-transfer reactions. The membrane material and construction serves to orient and space the Dye,Q pair for maximum light absorption and charge separation, provide a potential barrier for transmembrane electron transfer which is expected to allow sufficient time for removal of charges from the surface, and act as a permeability barrier to prevent the diffusional recombination of water soluble A and D. The net effect derived from these processes is proposed to be the conversion of light energy into potential energy. The utilization of the separated oxidizing and reducing power may be to convert "energy poor" materials such as H_2O, N_2, or CO_2 into "energy rich" products such as H_2, NH_3, or CH_3OH. Alternatively, the charges can be transported to collector electrodes and made to do electrical work in an external circuit.

In order to achieve the molecular spatial organization required by this conceptual model, a variation of the membrane fabrication technique first proposed by Langmuir and Waugh,[5] revised by Takagi, Azuma, and Kishimoto,[6] and modified further by Montal and Mueller,[7] was adopted. The membrane formation procedure is shown in Figure 1. Two monolayers of differing composition are formed on a water surface divided by a hydrophobic partition containing a small aperture. In this adaptation, each monolayer is first polymerized into a cohesive two-dimensional sheet. Upon immersion of the partition, the two monolayer films adsorb onto the solid surface such that the hydrophobic interaction of the hydrocarbon tails with the surface determines the orientation. In the region of the aperture, the hydrocarbon surfaces of the monolayers coalesce to produce a single asymmetric film two molecules thick.

The strategy to synthesize a thin surface active polymer film notes that the deformations occurring during bilayer film formation due to the disparity in size between the film and the aperture support and induced by transient pressure differentials across the formed film require a flexible and elastic structure able to conform to angled surfaces, to absorb impact, and to return to its original shape without rupture. It has long been known that materials possessing such rubber elasticity have several important structural characteristics:[8] long polymer chains; sufficient internal mobility to allow the required rearrangements of chain configuration from random coil to one with chains aligned parallel to the axis of elongation during deformation; and occasional cross-linkages connecting these chains to provide a permanent network having the

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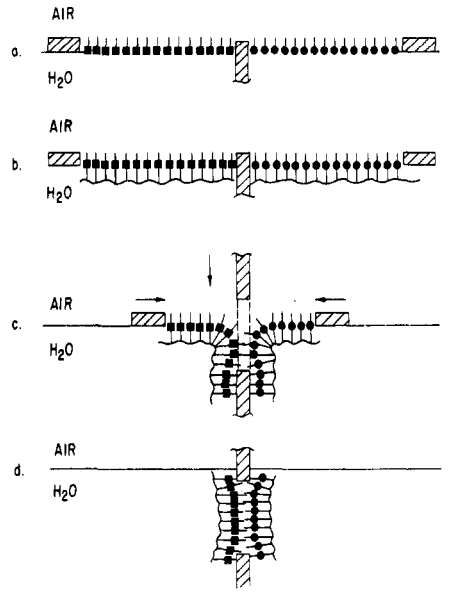
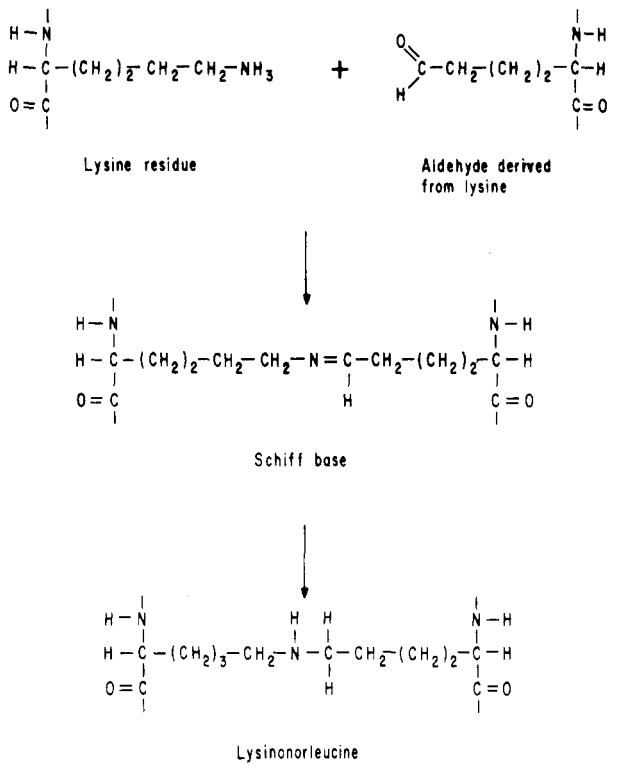


Figure 1. Formation of an asymmetric film by coalescence of two monomolelcular polymer films. (a) Two compositionally different monolayers are formed on a divided water surface. (b) Each monolayer is polymerized. (c) The hydrophobic partition is immersed through the surface films. (d) An asymmetric film is formed from the coalescence of the two monolayers in an aperture in the partition.

ability to recover its original dimensions. The synthetic path which hopes to incorporate these characteristics in a thin film grafts surfactant monomers formed in a monomolecular array onto linear, but randomly coiled, water-soluble polymer chains and then introduces cross-links between these chains to produce the surface active network desired.

There are a number of reports in the literature[9-14] in which a film of surfactant monomers has been reacted to produce what is suggested to be a polymeric sheet. In most instances, radical polymerizations of surfactant vinyl monomers, such as vinyl stearate, were utilized.[9-11] While much less studied,[12-14] condensation reactions offer the possibility of greater compositional control by directed chemical bond formation and are insensitive to oxygen inhibition.

Amine–aldehyde condensation chemistry was decided upon for a number of reasons: the condensation occurs rapidly at ambient temperature in an aqueous environment; the primary condensation product can be stabilized with respect to hydrolysis by further chemical reaction; surfactant monomers, water-soluble di- and polymeric amino and aldehyde compounds, are available in high purity and the chemistry of these reactions has been studied in detail (even if not completely understood). Further, this chemistry has been implicated in the formation of elastin, a rubber-like insoluble protein present in mammalian connective tissues which possesses unique elasticity and tensile strength.[15] Elastin is composed of linear polypeptide chains cross-linked via condensation reactions between lysyl and oxidized lysyl residues to give the reduced shiff base, lysinonorleucine, as well as more complex linkages.



Experimental Section

Materials. *N*-Octadecanal (StAld, Supelco, 99+%), poly-L-lysine (PL, HBr salt, methyl ester end caps, obtained in a variety of molelcular weight ranges 2000–183 000, as determined by viscosity, Sigma), *n*-octadecylamine ($StNH_2$, LaChat, 99%), *n*-octadecanol (StOH, Sigma, 98%), *n*-pentylamine (Aldrich, 99%), sodium borohydride ($NaBH_4$, Metal Hydrides Inc., Anal. Grade), and *o*-phthaladehyde (OPTA, Durrum) were used as obtained. Sodium cyanoborohydride ($NaBH_3CN$, Aldrich) was purified according to the method of Borch, Bernstein, and Durst.[16] All organic solvents (Burdick and Jackson) and inorganic reagents (analytical grade) were used without further purification. Triply distilled water from a quartz still was used in the preparation of the monolayer subphase solutions.

Procedures. Monolayers of StAld were spread from a dilute *n*-hexane solution (ca. 3×10^{-3} M) onto fresh, aqueous subphases. Surface pressure–area (Π–A) isotherms were measured on a thermostated trough (36 × 15 × 1 cm) equipped with a manual Langmuir film balance fabricated in this Laboratory. The construction details and operation of the multicompartment trough and Wilhelmy balance have been discussed previously.[17]

Relative surface viscosities, η_s^{rel}, were measured by a torsion pendulum system driven at a constant angular velocity. The viscometer was similar in design to Ellis et al.[18] The torsion pendulum had as a surface contacting element a paraffined Dural knife-edged disk (2.54 cm diameter). In measuring a surface film viscosity, one observes the steady state angular displacement, α, of the angle by which the surface element (pointer attached) lags behind the drive shaft (engineering circle attached, 2° divisions). The relative surface viscosity is obtained upon ratioing this angle to the angle observed with no film present (clean surface):[19]

relative surface viscosity

$$\eta_s^{rel} = \frac{\alpha_I - \alpha_0}{\alpha_{clean} - \alpha_0} \quad (\alpha_0 \equiv \text{rest position})$$

In the apparatus presently used, the lag on a clean water surface is 1°.

The attenuated total reflectance (ATR) infrared spectra (IR) of built-up monolayer films transferred off the water surface via the Langmuir–Blodgett technique to a hydrophobic (Me_2SiCl_2 treated) germanium plate (Herrick Scientific, 50 × 20 × 1 mm, θ = 45°, single pass parallel piped, 50 reflections) were recorded with a Perkin-Elmer Model 521 spectrometer.

Vapor phase chromatography (VPC) employed a Varian Model 1740 gas chromatograph equipped with a flame ionization detector and an OV-101 glass column (183 cm × 0.2 cm ID, 3% on 100/120 Var Aport 30) operated at 200 °C and 20 psi He. The retention times for StAld and StOH are 19.8 and 26.0 min, respectively, with the minimum amount detectable ≈ 1×10^{-11} mol.

Biuret Assay for Poly-L-lysine. Solutions of known PL-15K (molecular weight estimated to be 15 000 by viscosity) concentration were analyzed by well-established procedures[20] to provide

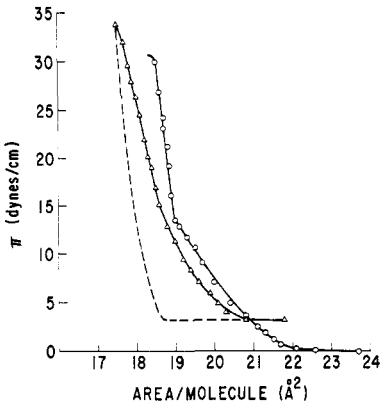


Figure 2. The Π–A isotherms (20 °C) of StAld (○) monolayer and a StAld–PL (15 K) (△) film after 90 min reaction time on pH 9.0, 5.0×10^3 M borate buffer. Compression (—) and expansion (- - -) isotherms were obtained at the same rate: 1 Å²/molecule/min.

a spectrometric calibration curve. Using the thin-film spectrometer built in this laboratory,[21] the minimum concentration detectable (S/N = 2) is ca. 2.0 μg/mL at the absorption maximum of the PL-15K/Cu^{2+} complex (525 nm) and the curve is linear up to at least 600 μg/mL. In a control experiment, 2750 ± 10 mL of a 1.0 μg/mL PL-15K solution in 5.0×10^{-3} M borate buffer, pH 9.0, was used to fill compartment 3. In order to obtain a higher PL-15K concentration for analysis, the subphase was removed from the trough in 500-mL portions and rotavaped to dryness before adding the biuret solution (10 mL). Control experiments demonstrate that 84–87% recovery of PL-15K from the trough can be achieved.

***o*-Phthalaldehyde Assay for Poly-L-lysine.** The exact procedure used has been published by Pierce.[22] Solutions of known PL-15K concentration were reacted with the OPTA β-mercaptoethanol stock solution and analyzed by the fluorescence section of the thin-film spectrometer[21] to provide a calibration curve. The maximum fluorescence intensity occurred at 445 nm when the sample was excited at 365 nm. The minimum concentration detectable is ca. 0.2 μg/mL PL-15K and the curve is linear from 0.2 to at least 60 μg/mL.

Results and Discussion

Surface Pressure–Molecular Area Isotherms. *N*-Octadecanal (StAld) and poly-L-lysine (PL) have been employed as the surfactant monomer and the linear, water-soluble polymer, respectively. PL, a basic polypeptide with methyl ester end caps, undergoes the random coil to helical transition at pH 10.5.[23]

The compression Π–A curve for StAld on pH 9.0 borate buffer (5.0×10^{-3} M, 20 °C) is shown in Figure 2. When a monolayer of StAld is spread on a subphase containing in addition 1.0 μg/mL PL having an average molecular weight of 15 000 (PL-15K), compressed to and held at Π = 10 dyn/cm, a gradual decrease in area/molecule is noted for the initial 45 min (total change ca. 2.5%), a leveling off is observed through 90 min, followed by a very slow increase until very nearly the original area is occupied at 8 h. The Π–A compression and expansion curve for this film at t = 90 min is also shown in Figure 2. It is to be noted that the film after having been compressed would slowly expand to occupy the area made available to it (insufficient trough surface area was available to allow full expansion in the experiment illustrated in Figure 2; hence, the minimum $\Pi \approx 3$ dyn/cm). Under these conditions, PL-15K itself has a small surface pressure, <1 dyn/cm.[24]

Relative Surface Viscosity Measurements. The measurement of intrinsic viscosity provides a convenient indication of bulk phase polymerizations. Indeed, most of the literature claiming to have prepared monomolecular polymer films has presented some surface viscosity

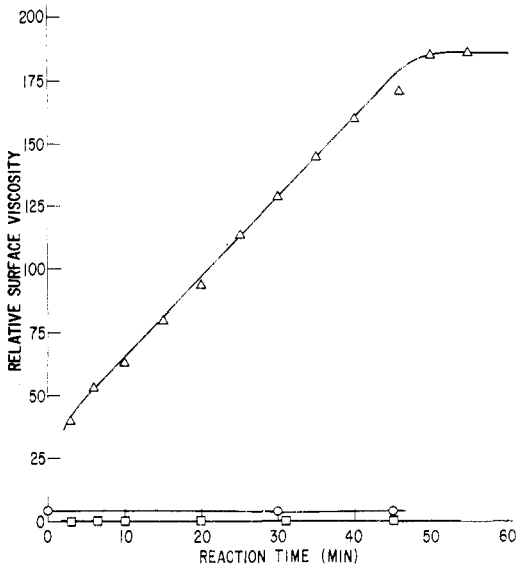


Figure 3. A comparison of relative surface viscosity vs. time for StAld alone (○), PL (15 K) alone (□) and StAld–PL (15 K) (△). All measurements were obtained on a subphase containing pH 7.0, 1.0×10^{-3} M phosphate buffer at 20 °C. Where used, the PL (15 K) concentration was 4.2 μg/mL. The measurements for StAld and StAld–PL (15 K) were done at a constant Π = 10 dyn/cm.

measurement in support.[9,11-14] When a StAld monolayer is formed and compressed to Π = 10 dyn/cm on a subphase containing 4.2 μg/mL PL-15K at pH 7.0 (phosphate), η_s^{rel} increases in a linear manner with time as illustrated in Figure 3. Note that η_s^{rel} has a low constant value for StAld without PL-15K in the subphase, is ca. zero for PL alone, and is not detectable for a monolayer of *n*-octadecanol with PL-15K in the subphase. At the maximum η_s^{rel}, the rotating surface element abruptly slips and begins to oscillate. Further measurements are marked by irreproducibility. The reason for this behavior is not clear although it would seem to indicate the loss of interaction between the surface element and the film and/or the rupture of the film itself.

A plot of η_s^{rel} vs Π for a StAld monolayer before and after 90 min reaction at Π = 10 dyn/cm with PL-15K emphasizes the changed nature of the surface film (Figure 4).

The viscosity changes during film reaction have been measured under a variety of conditions: PL concentration, PL molecular weight, pH, temperature, and StAld surface pressure. Increasing Π while holding other parameters constant resulted in a larger maximum η_s^{rel} obtained at a longer time. The viscosity range and time required to achieve a maximum were determined to be experimentally convenient when Π = 10 dyn/cm; hence, this value was chosen to be the reference surface pressure. Comparison of the 4.0 μg/mL labeled trace of Figure 5 with that presented in Figure 3 (4.2 μg/mL) shows a more rapid rise at pH 9.0 (borate) than at pH 7.0 (phosphate). Increasing the pH to 9.9 and 10.7 did not enhance the rate of η_s^{rel} increase. However, the higher pH was more difficult to maintain over long periods probably due to CO_2 diffusion into the N_2 blanketed trough atmosphere. It is clear from Figure 5 that decreasing the PL concentration slows the reaction. Variation of PL molecular weight showed no differences for 2, 15, and 30 K and a slowing of the initial rate for 85 and 183 K.

The measurement of surface viscosity has been used as a rapid means of surveying reaction kinetics assuming that a rise in the observed viscosity is related in some manner

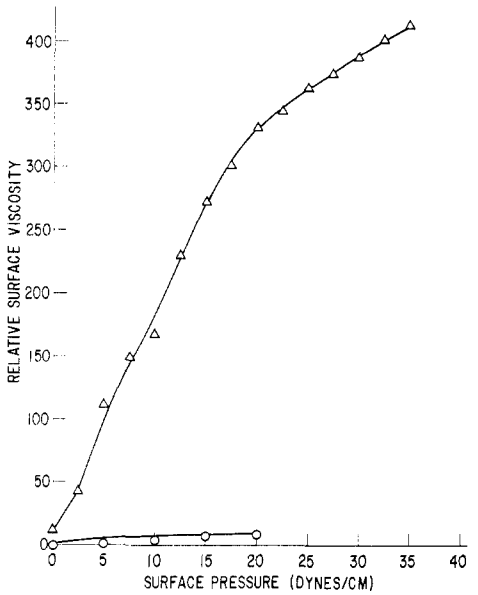


Figure 4. A comparison of relative surface viscosity vs. Π for StAld alone (○) and a StAld–PL (15 K) film (△) after 90 min reaction at Π = 10 dyn/cm (pH 7.0, 1.0×10^{-3} M phosphate buffer, 20 °C). Where used, the PL (15 K) concentration was 4.2 μg/mL.

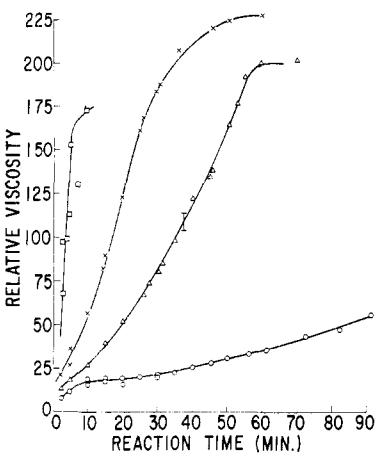


Figure 5. The influence of PL (15 K) concentration upon the rate of relative surface viscosity increase with reaction time: 4.0 μg/mL (□), 1.0 μg/mL (×), 0.4 μg/mL (△), and 0.1 μg/mL (○). Conditions: pH 9.0, 5.0×10^{-3} M borate buffer, 20 °C, Π = 10 dyn/cm.

to the degree of reaction. Some confidence in this assumption can be obtained from similar observations for the radical polymerizations of vinyl stearate monolayers,[13] polycondensation of glycine cetyl ester monolayers,[13] and saponification of stearate monolayers by Ca^{2+}.[25] Further, studies of the primary proton release in aqueous solution reactions between 1,5-pentanedial(glutaraldehyde) and PL showed that the pH optimum for the reaction rate was at pH 9–10,[26] the range in which η_s^{rel} also attains its maximum value. While kinetic viscosity measurements are useful to mark outer limits of reaction conditions within which it is prudent to conduct more detailed studies, the ambiquity over exactly what properties of the surface film–water interface system are being measured[27a] makes it reasonable to defer an analysis of these data until after a mass balance for the reaction has been achieved.

Multicompartment Monolayer Trough Reactions. Figures 3 and 5 would be more useful to the chemist if their ordinates were also labeled in percent reaction of starting material or percent formation of product. Two considerations must be taken into account when designing an experiment to determine the mass balance of a monolayer reaction as a function of time: the small amounts of material involved and the need to quench the reaction or otherwise obtain a chemically stable system such that later analysis will reflect the composition of the film while it was still at the air–H_2O interface. From Figure 2, the molecular area of StAld is 19.6 Å^2 (10 dyn/cm); hence, only ca. 0.1–0.2 mg is required to cover the surface area of a typical monolayer trough (~400–1000 cm^2). It is not too surprising that almost all surface reaction kinetics have been limited to physical measurements of the film on the trough surface,[27b] infrequent use of absorption spectroscopy of scraped films when chromophores are present,[27b,28] and rarer instances still of actual chemical analyses.[29] In order to solve these problems, a large area multicompartment trough was used to allow a series of chemical reactions to be performed upon a well-defined monolayer.[17]

The trough subphase is divided into a number of individual compartments by submerged hydrophilic glass barriers which extend the width of the trough and are covered by ca. 0.05 cm of H_2O. Hence, several compartments can be filled and emptied independently. Surfactant monolayers are constrained between the hydrophobic sides of the trough and two motorized hydrophobic surface barriers which can be driven independently to control the surface pressure (as measured by a Wilhelmy balance) or simultaneously to laterally transport the film from the surface of one compartment to another over the submerged barrier.

The reduction of StAld to *n*-octadecanol (StOH) by aqueous $NaBH_4$ illustrates the use of this technique. A monolayer of StAld (3.2 μmol, 0.86 mg) was formed on compartment 1 containing 5.0×10^{-3} M borate buffer (pH 9.0) and compressed to Π = 10 dyn/cm. The film was moved to compartment 2 (transfer time 1 min) which contained freshly prepared 1.0×10^{-2} M $NaBH_4$ in aqueous borate buffer (5.0×10^{-3} M, pH 9.0).[30] After 30 min, the film was moved back to compartment 1, compressed until collapsed into threads, "scraped" off the surface, and dissolved in 1.0 mL of $CHCl_3$. Vapor phase chromatographic (VPC) analysis indicated StOH corresponding to 84 mol % of the initially spread StAld with 1 mol % unreduced aldehyde. Control experiments have shown that 85 ± 2 mol % of either the original StAld spread and manipulated in the absence of $NaBH_4$ or StOH spread and manipulated in the presence of $NaBH_4$ can be recovered.

The manner in which the multicompartment trough was used for studying the reaction between StAld and PL-15K is shown in Figure 6. A monolayer of StAld (3.2 μmol, 0.86 mg) was formed and compressed to 10 dyn/cm on aqueous borate buffer (pH 9.0, 5.0×10^{-3} M) in compartment 1. The compressed film is moved laterally over borate buffer (same as in 1) in compartment 2 onto aqueous PL-15K (1.0 μg/ML, total ca. 2.8 mg) in compartment 3. After a period of time, the film was passed back over subphase 2 to wash out any unattached PL. The reaction is quenched by reduction with aqueous $NaBH_4$ (10^{-2} M, pH 9.0, borate buffer, 30 min) in compartment 1 and transferred with washing across compartment 2 onto the surface of 10^{-2} M NaOH in compartment 3. At this stage, or any intermediate one, the film may be transferred to a solid support (glass slide, CaF_2 and Ge IR plates) via the Langmuir–Blodgett technique[31] for spectral observation or collapsed, scraped from the surface, and dissolved in an appropriate solvent for chromatographic and spectral characterization.

Analysis of Unreacted StAld (StOH). Figure 7 shows the results of VPC analysis of reduced films scraped from the water surface and extracted with $CHCl_3$ to

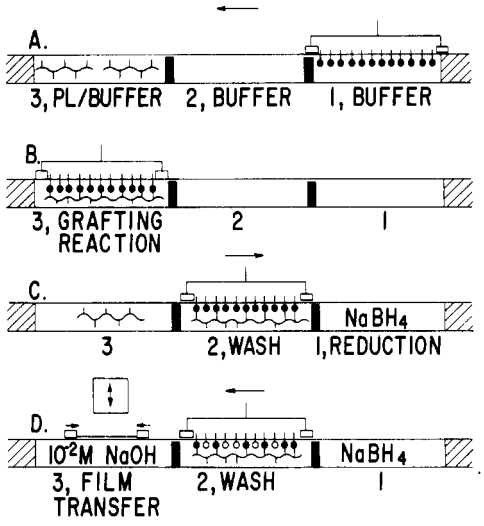


Figure 6. Schematic description of operations involved in grafting a monolayer of StAld onto PL and subsequent $NaBH_4$ reduction using the multicompartment trough. Operations: (A) form monolayer of StAld and compress to desired Π on surface of buffer in compartment 1; (B) shift film at constant area across buffer in second compartment onto PL containing subphase to start reaction; (C) shift film at constant area across wash buffer in compartment 2 onto alkaline $NaBH_4$ in compartment 1 to reduce surface film; (D) transfer film across wash compartment onto 10^{-2} M NaOH in compartment 1 for film removal from surface.

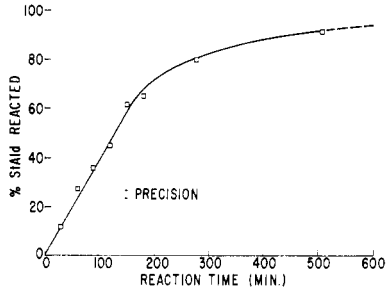


Figure 7. VPC analysis for unreacted StAld as StOH after $NaBH_4$ reduction as a function of StAld–PL reaction time. Conditions: 3.2 μmol of StAld, 1.0 μg/mL of PL (15 K), pH 9.0, 5.0×10^{-3} M borate buffer, 22 ± 2 °C, 10 dyn/cm.

dissolve the StOH (reduction product of StAld noted previously). As the reaction time lengthens, more H_2O and $CHCl_3$ insoluble white solid is obtained. The $CHCl_3$ soluble fraction shows only StOH and a small amount of unreduced StAld (≤2%). The disappearance of StAld occurs in a nearly linear fashion (0.42%/min or 1.3×10^{-2} μmol/min) until 180 min (ca. 66% consumption) when the reaction slows markedly leaving some 10% unreacted StAld after 8.5 h. The precision of the analysis is ±2% in the linear portion of the trace. If the grafted film is collapsed and scraped from the interface *before* $NaBH_4$ reduction, the precision of the VPC analysis for StAld drops to ±15–20% although the scatter of points shown in Figure 8 (data not obtained beyond 180 min) very nearly reproduces the more precise data of Figure 7. A number of films were extracted into CCl_4, the intensity of the carbonyl stretch frequency of StAld (1730 cm^{-1}) monitored in a 1.0 cm path length cell, and the StAld concentration obtained from an empirical calibration curve. The resultant data (unknown precision) for disappearance of StAld, also plotted in Figure 8, follow the VPC derived information.

Grafted, but unreduced, films were also transferred (Π = 25 dyn/cm, 10 monolayers per side) to a hydrophobic germanium ATR plate and their IR spectra obtained. Of major importance was the disappearance of the StAld

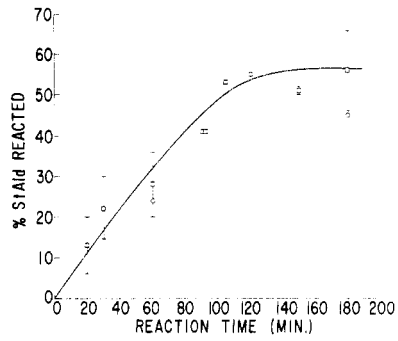


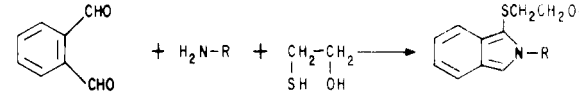
Figure 8. VPC (□) and IR (○) analysis of unreacted StAld after contact with PL (15 K) containing subphase. Conditions: 3.2 μmol of StAld, 1.0 μg/mL of PL(15K), pH 9.0, 5.0×10^{-3} M borate buffer, 22 ± 2 °C, 10 dyn/cm. The IR analysis monitored the 1730 cm^{-1} absorption in the recovered film in CCl_4, l = 1.0 cm.

carbonyl stretch frequency (1708 cm^{-1}) with reaction time and the simultaneous appearance of a broader band at 1645 cm^{-1} which can be assigned as the carbonyl amide I band of polypeptides.[32] No absorption was noted in the region of 1670 cm^{-1} in either of these spectra or those taken in CCl_4 solution indicating the absence of an imine ($R_2C{=}N{-}$) linkage. Major drawbacks of this technique were the inability to transfer the same amount of film to the ATR plate at each sampling[33] and the long time required (ca. 15 min) to complete these transfers. Hence, determination of the absolute percent disappearance of StAld and appearance of the carbonyl amide linkage were not possible.

VPC analysis has proven a most useful analytical tool in monitoring surface film reactions and has clearly shown that condensation chemistry continues long after the relative surface viscosity had increased to a value exceeding the range of the constant angular velocity torsion pendulum device used here. These results suggest that an alternative technique for determining the viscosity in this system would be to measure the damping constants of a torsion pendulum instantaneously set in oscillation to some angle significantly less than that observed to cause slippage.[9] While the sensitivity of this technique decreases with increasing viscosity, it would allow spanning a greater range of viscosities than is presently reported.

Analysis of Unreacted Poly-L-lysine. The disappearance of the other reactant, PL-15K, has been monitored by two different assay methods: biuret[20] and *o*-phthalaldehyde.[34] The biuret method is a classical polypeptide analysis which is simply performed by mixing a di- or higher polypeptide with an alkaline solution of cupric ions. The pink to purple color of the resulting peptide–Cu^{2+} complex forms the basis for quantitation by absorption spectrometry. Control experiments have demonstrated an 84–87% recovery of PL-15K from the trough can be achieved.

The *o*-phthalaldehyde (OPTA) method is based on the formation of a highly fluorescent product when reacted specifically with a primary amine in the presence of β-mercaptoethanol.[34] Simons and Johnson[35] have recently suggested that the fluorescent reaction products are 1-alkylthio-2-alkyl-substituted isoindoles:



While the OPTA method is more sensitive by tenfold and specific (to only primary amines) than the biuret test, it requires more careful attention to the timing of procedural operations due to the transient nature of the

Table I
Simultaneous Analysis of Unreacted StAld and PL-15K

initial				unreacted		grafted film		
StAld[a] × 10^{18}	lysyl[b] × 10^{18}	lysyl/ StAld	reaction time, min	StAld[a,e] × 10^{18}	lysyl[b] × 10^{18}	St[c] × 10^{18}	lysyl[d] × 10^{18}	lysyl/ St
1.9	7.6	4.0	275	0.39	5.1[f]	1.5	2.5	1.7
1.9	7.6	4.0	508	0.17	3.3[f]	1.7	4.3	2.5
1.9	5.0	2.6	275	0.44	2.1[g]	1.5	2.9	1.9

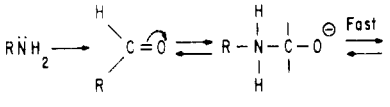
[a] Number of StAld molecules. [b] Number of lysylamino groups estimated from degree of polymerization, $X = 69$. [c] Number of n-$C_{18}H_{37}$ groups attached to PL = initial StAld − unreacted StAld. [d] Number of lysyl groups contained in surface film = initial lysyl − unreacted lysyl. [e] VPC analysis. [f] Biuret assay. [g] OPTA assay.

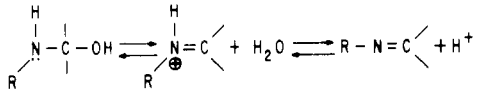
primary fluorescent product.

The preliminary results of several experiments providing simultaneous analysis of unreacted StAld by VPC and unreacted PL-15K by both the biuret and OPTA methods are shown in Table I and clearly indicate that PL-15K is being consumed.

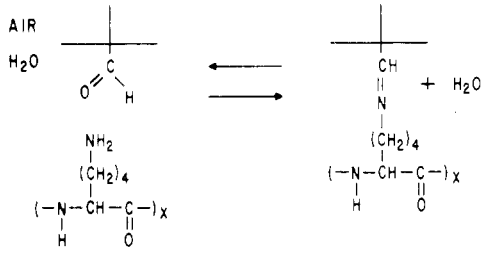
Amine–Aldehyde Condensation Chemistry. The disappearance of reactants, the formation of a water and chloroform insoluble surface film, and the increase in surface viscosity with increasing contact time between an insoluble StAld monolayer and a solution of PL-15K suggest the covalent attachment of the octadecyl hydrocarbon chain to the poly-L-lysine backbone. The entries of Table I indicate ca. two lysyl groups per attached octadecyl hydrocarbon chain or one free amino group for each lysylamino–StAld linkage.

The simplest means of covalent attachment is through the imino linkage formed in the condensation of an aldehyde and amine. For aliphatic amines in neutral or

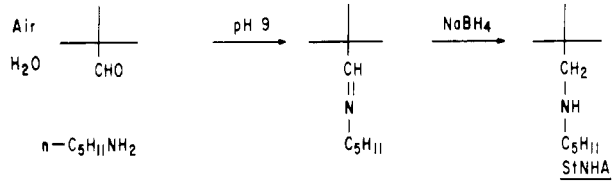




basic solution (pH >5) the attack (and loss) of free amine is fast while the loss (and attack) of water or hydroxide ion is rate determining.[36] The protonated imine is the reactive species, having a $pK \approx 7$. For aliphatic aldehydes, the equilibrium greatly favors hydrolysis in aqueous solution. Hence, the formation of the imine is kinetically rapid but is thermodynamically unfavorable. The poor reproducibility of StAld recovery from partially grafted but *unreduced* films provides some evidence for these complex equilibria and underlines the need to remove the imine from participation in the back reaction. Even then, however, there is *no* evidence for this functionality in the ATR infrared analysis of the grafted, viscous film; i.e., there is no absorption observed at 1670 cm^{-1} for the >C=N– stretch.



In order to utilize another analysis method, a model reaction was run between a StAld monolayer (Π = 10 dyn/cm) and n-pentylamine (5×10^{-3} M) dissolved in borate buffer subphase (5×10^{-3} M, pH 9.0). A large excess of amine to StAld was employed, 5×10^{3}:1. The resulting film was shifted onto a subphase containing $NaBH_4$ (6.6×10^{-3} M in same borate buffer system) to reduce any unreacted StAld to StOH as well as reducing the N-pentyloctadecylimine to N-pentyl-N-octadecylamine, StNHA. The film was finally moved onto buffer, collapsed, scraped off the surface, shaken with $CHCl_3$ to give a



slightly cloudy solution with little, if any, insoluble material, and analyzed by VPC. A standard sample of StNHA had been prepared from n-octadecyl bromide and n-pentylamine, purified and characterized. The VPC analysis showed: 63 mol % StOH, 6 mol % StAld, and <1 mol % StNHA with 31 mol % (relative to StOH) not accounted for. It should be noted that the amount of *unreacted* StAld (including StOH) from this reaction is nearly identical to that amount (~70%) *consumed* in the reaction with PL (Figure 7) at the same reaction duration (180 min). The reason for this discrepancy is not clear although it has been independently shown that the rate of initial proton release (ca. equal to formation of imine) in aqueous solution was much smaller for the reaction of a dialdehyde, 1,5-pentanedial, with a monofunctional amine, n-butylamine, than with PL at an equal concentration of amino groups.[26] This was the reason a 5000-fold excess of pentylamine was used in the monolayer reaction. Furthermore, a monofunctional aldehyde, acetaldehyde, reacted much more slowly with PL than did the dialdehyde. The authors reasoned from entropy considerations that the free aldehyde group of the dialdehyde should interact much more readily when the latter is already bound onto the macromolecule.

Since the imino bond can undergo facile aldol condensations with additional aldehydes and imines bearing α hydrogens to give dimers and polymeric material,[37,38] its lifetime in a close packed array of other reactive functional groups may be short. For that reason another model reaction was performed in which a reducing reagent was added to the same subphase as the n-pentylamine. Borch, Bernstein, and Durst[16] have shown that the cyanohydridoborate anion, BH_3CN^-, will react much more rapidly with protonated imines than with aldehydes at pH 7. Hence, reaction of an aldehyde with a primary amine in the presence of sodium cyanoborohydride leads to

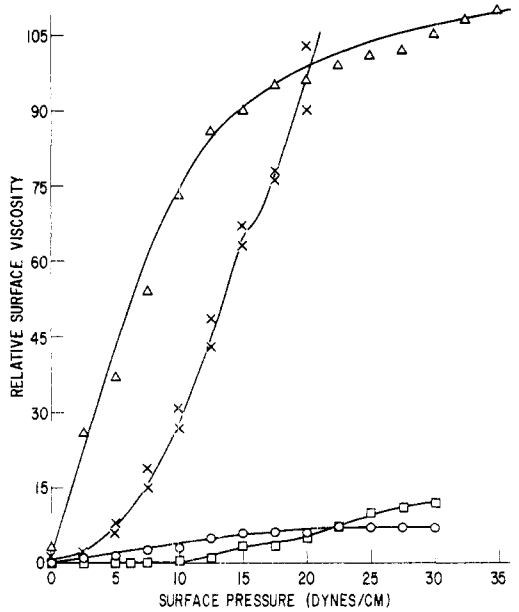
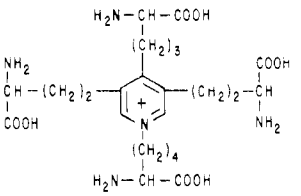


Figure 9. A comparison of relative surface viscosity vs. Π for StAld alone (○), $StNH_2$ alone (□), St_2NH alone (×) and a 1:1 StAld–$StNH_2$ mixed film (△) after 60 min reaction. Conditions: pH 7.0, 1.0×10^{-3} M phosphate buffer, 20 °C, 10 dyn/cm.

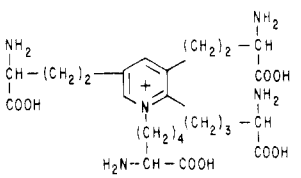
secondary amines via reductive amination of the carbonyl group. Reaction of a StAld monolayer (Π = 10 dyn/cm) with a subphase containing *n*-pentylamine (5×10^{-3} M), $NaBH_3CN$ (5×10^{-3} M), and phosphate buffer (5×10^{-3} M, pH 7.0) for 180 min and subsequent VPC analysis of the scraped film showed: 12 mol % StOH, 47 mol % StAld, and ~1 mol % ST NHA with 40 mol % (relative to StOH) not accounted for. It is clear that under these conditions BH_3CN^- is just not a competitive reagent for the imine if the latter is formed. A much higher BH_3CN^- concentration may be required. There is no evidence presently available concerning the composition or structure of the 31 and 40 mol % portion of the material which did not pass through the VPC under these conditions.

In a second set of model reactions, the amine as well as the aldehyde functionality was constrained to the interfacial region. A 1:1 mixed monolayer of *n*-octadecylamine ($StNH_2$) and StAld was spread from a freshly prepared (<1 min) hexane solution (1.0×10^{-3} M in each) which showed less than 2–4% IR C=O st intensity loss per minute. Higher concentrations and longer times before spreading led to the decrease of the carbonyl stretch absorption (1730 cm^{-1}) and concommitant rise of the imino stretch bond (1670 cm^{-1}) while still in the spreading solution. VPC analysis of scraped films resulting from mixed monolayers spread on pH 7.0 phosphate subphases (1×10^{-3} M) with and without $NaBH_3CN$ (5.5×10^{-2} M) for 60 min at Π = 10 dyn/cm showed less than 1% of the original $StNH_2$ and StAld. Under VPC/MS (mass spectrometry) conditions, where dioctadecyl amine could be analyzed and detected, the film spread on $NaBH_3CN$ containing subphase showed this compound (yield unknown) but that spread on only buffered subphase did not. Further, the relative viscosity of the surface film showed little change with time with $NaBH_3CN$ and a marked increase without. It should be noted that these results are suspect due to the fact that even highly purified $NaBH_3CN$[16] produces some unknown surface active material upon solution in water which continues to concentrate at the interface after repeated cleaning which may interfere with the viscosity determination and/or chemistry. Figure 9 shows the marked differences in η_s^{rel} vs. Π characteristics observed for this system. While the detailed description of this system is lacking, it is clear that constraining both amine and aldehyde to the same plane produces a more rapid and complete reaction than if the amine was dissolved in the subphase.

The ultimate fate of the imino linkage may be to enter into aldol condensation reactions with StAld giving dihydropyridines and pyridinium salts.[39] If this chemistry has occurred, then the second part of the overall synthetic strategy, cross-linking of linear chains to form a two-dimensional cohesive polymer film, has been achieved. It is to be noted that a minimum of eight of the cross-linkages in elastin (per 1000 amino acid residues) have been identified as the pyridinium compounds, desmosine and isodesmosine.[15,40]



Desmosine



Isodesmosine

This report has been concerned with developing the condensation chemistry, analytical techniques, and manipulative methodology which will produce a monomolecular polymeric surface film. Continued work will be required to assay the number of free lysyl amino groups present in the grafted film (degree of cross-linking) as well as to determine its overall structure and rheological properties.

In order to achieve the molecular organization to test the concept of energy conversion, two such polymer films must now be functionalized with light-absorbing electron-transfer components and formed into the laminated bilayer film. Preliminary studies have shown that pigments can be both mechanically entrapped in grafted films and covalently attached to films bearing amine and aldehyde functionalities while at the air–water interface. At the same time, an experimental subphase infusion/withdrawal technique has been developed to allow condensation and other chemical reactions to be performed simultaneously on two stationary surface films prior to coalescing their hydrophobic faces to form an ultrathin compositionally asymmetric polymer film.

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Water-Dependent Relaxation in Polymers. Study by the Thermally Stimulated Current Method

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ABSTRACT: The relaxation behavior of several polar polymers from 77 to 300 K was investigated as a function of moisture content by means of the thermally stimulated current method. The results were generally found in good agreement with published data obtained from dielectric and mechanical loss measurements. In all the polymers investigated, poly(methyl methacrylate), poly(ethyl methacrylate), polydiancarbonate, poly(ethylene terephthalate), polyamide 6–6, and aliphatic polycarbonates, the presence of water markedly affects the shape, amplitude, and maximum temperature of the relaxation peaks; as a general rule, water acts as a plasticizer for the motions involved but the variation in size of the corresponding peaks depends on the nature of the polymer and, in a given polymer, on the type of relaxation considered. New water-dependent relaxations also appear in certain polymers. These processes do not seem directly related to conformational motions but rather to breaking of hydrogen bonds of water–polymer complexes.

It is well established that water sorbed in hydrophilic polymers has a very pronounced effect on their relaxation behavior. Several dielectric and mechanical loss investigations, for example, have shown that the glass transition is always shifted to lower temperatures due to the plasticizing effect of water and that generally the secondary processes are markedly affected in size and position.[1-4] Additionally, a water-dependent new relaxation mechanism has been observed at low temperature in some polymers such as cellulose,[5] acetylated celluloses,[5] poly(methyl methacrylate),[6,7] polyimide,[8] polysulfone A,[4] and polyamides.[2] It seems that even in "dried" samples, residual water molecules could be mainly responsible for certain low-amplitude relaxations generally ascribed to local molecular motions of main chain or side groups. Such is probably the case, for example, for the β relaxation in poly(vinyl acetate), which is usually thought to arise from motions of the $OCOCH_3$ side group but strongly decreases in samples annealed under vacuum at temperatures higher than the glass transition temperature and can even become dielectrically undistinguishable.[9]

So far as we know, such water-dependent peaks have not been reported by the thermally stimulated current method (TSC). This method, based on the discharge current curves obtained during heating of dielectrics previously polarized at a high temperature with a dc field, is now currently used to study the relaxation properties in solid state.[10-12] Due to the very low equivalent frequency of this technique with regard to the dielectric loss method[10,13,14] the relaxation peaks are shifted to lower temperatures, which leads to a higher resolution of the spectrum. The

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