

NRL Report 6318

Surface Chemistry of Proteins and Polypeptides

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November 30, 1965



U.S. NAVAL RESEARCH LABORATORY
Washington, D.C.

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ABSTRACT

This report is a review of recent literature in the field of polypeptide and protein surface chemistry, concentrating on the last decade. Theoretical approaches to polymers at liquid interfaces are considered, and surface measurement techniques are briefly outlined.

The various classes of polypeptides are considered, followed by a discussion of proteins in general and specific proteins in particular. Enzymatic activity, structure, and adsorption at various surfaces are considered also.

PROBLEM STATUS

This is an interim report; work on the problem is continuing.

AUTHORIZATION

NRL Problem C02-18A
RUDC-4B000/652-1/F101-99-01

Manuscript submitted June 30, 1965.

SURFACE CHEMISTRY OF PROTEINS AND POLYPEPTIDES

INTRODUCTION

This report is a review of certain aspects of the interfacial properties of proteins and synthetic polypeptides. These polymeric materials are of importance from a variety of viewpoints. To the biologist, they represent a class of natural products intimately associated with the vital processes of all viable organisms whose cells are of microscopic and submicroscopic dimensions. As each cell and organelle has its share of membranes, it is evident that the surface properties of natural products are important to the functioning of the organism. To the physical and polymer chemist, the fact that proteins and synthetic polypeptides may exist in well-defined conformations presents the opportunity to study the effects of the highly asymmetrical environment at an interface upon such structures. Since the amino acid residues found in proteins include highly nonpolar side chains combined with a polar backbone and other highly polar chains, it is to be expected, and indeed is found, that these polymers are highly surface active, making them important in technology for applications requiring stable foams and strong adsorption at interfaces. Conversely, their strong surface activity may hamper certain operations in which proteins are required but where adsorption and foaming are undesirable.

Previous reviews of this field have been published by Bull (1947), Rothen (1947, 1956), Cumper and Alexander (1951), Cheesman and Davies (1954), Fraser (1957), and in the very recent book of Davies and Rideal (1963) which contains some material dealing with protein surface chemistry. A description of some techniques is given by Sobotka and Trurnit (1961).

SURFACE PRESSURE, EQUATIONS OF STATE, AND MOLECULAR WEIGHTS AT INTERFACES

A fundamental parameter characterizing the interaction of a surface-active substance with the interface between two bulk phases is the change in surface tension produced at the interface by the presence of the surface-active compound. This difference in surface tension is measured in dynes/cm and may also be considered the surface pressure, because historically the similarity to a two-dimensional pressure is accentuated by the use of the Langmuir trough in its measurement. As surface tension is numerically equal to the surface free energy in ergs/cm², the surface pressure π is equal to the change in the surface free energy brought about by the presence of the surface-active agent.*

The surface pressure-area relation of compounds which form surface films may be analyzed by a method analogous to osmometry; an element of the apparatus confines a component of interest in a restricted region of the surface, while permitting other components free access to another region of the surface which is considered to be in a reference state. To expand the surface region on which the compound of interest is confined, we must place more diluent into that region, at the expense of the surface region which is free of the compound. The two regions may be separated, as in the case of the Langmuir trough, or the entire free surface may be covered, as is possible with the Wilhelmy balance, where the equilibrium is established with the bulk of liquid below the surface, whose state is considered to be in equilibrium with bulk liquid under a clean

*Surface pressure is often also denoted by F .

surface. Following Bull (1950) we may let the activity of the bulk solvent be A_0 and a be the activity on a clean surface in equilibrium with it. Then, for transfer of 1 mole of solvent from clean surface to film-covered area,

$$\begin{aligned} \pi A_1 &= A_1(\gamma_0 - \gamma_1) \\ &= RT \ln \frac{a}{a_0} \approx -RT \ln \frac{N_1}{N_0} \quad (\text{if we may take } a_i \approx N_i) \\ &= -RT \ln (1 - N_2) \\ &= -RT \ln \left(1 - \frac{n_2}{n_1 + n_2} \right) \end{aligned} \quad (1)$$

where the subscripts 1 and 2 refer to solvent and film molecules respectively, n_i and N_i denote the number of moles and the mole fraction of component i in a surface of area A , A_1 is the molar area of solvent, N_0 (the mole fraction of solvent in the clean surface) is equal to unity, and γ is the surface tension. If we now let the film be very dilute, we may expand the logarithm, and assuming that the total area $A = A_1 n_1 + n_2 \delta_2$, where δ_2 is the area of film occupied by a mole of film-forming substance,

$$RT \ln n_2 = \pi A_1 (n_1 + n_2) \approx \pi A_1 n_1 = \pi (A - n_2 \delta_2),$$

and Eq. (1) becomes

$$\pi A = RT \ln n_2 + n_2 \delta_2 \pi. \quad (2)$$

If we plot πA versus π , the intercept on the πA axis determines n_2 and the slope determines δ_2 . Note that δ_2 is the apparent area based on the assumption that the area of a mole of water is invariant with surface concentration. This is not necessarily correct. Also, the activity may be proportional to concentration only in the case of molecules of circular section (Davies and Llopis (1955)).

This treatment holds in the region of infinite dilution and is used to justify extrapolation of the curve of πA versus π to $\pi = 0$ to find molecular weight. However, we have no thermodynamically rigorous theory at those higher pressures where measurements are possible. Therefore, theories which must be tested by experiment have been developed and applied to polymer and protein systems with the purpose of enabling extrapolations to be made with confidence, and also explaining the general behavior of the force-area curve.

Purely Entropic Theories

Singer's Theory - The theories applicable to protein systems which have been recently in use are those of Singer (1948) and of Frisch and Simha (1956). Singer's treatment is based upon the Flory-Huggins development of polymer theory. It assumes that there exists at the surface or interface of the polymer solution a quasi-lattice of sites, each of which may be occupied or vacant. The entropy of the polymeric system occupying these sites in a given manner may be calculated by statistical methods, and the free energy may be derived by assuming that there is no intermolecular attraction or repulsion (Davies (1954b)). The resulting equation is

$$\frac{\pi A_0}{kT} = \left[\ln \left(\frac{A}{A - A_0} \right) + \frac{(X-1)}{X} \frac{Z}{2} \ln \left(1 - \frac{2A_0}{A} \right) \right]. \quad (3)$$

In Eq. (3), A_0 is the limiting molecular area per residue, A is the available area per residue (or monomer unit) in the chain. The parameter Z is the lattice coordination number of the chain segments, i.e., the number of possible sites on which the neighboring residues may be situated. For a rigid chain $Z = 2$, and the chain configuration is fixed. Flexibility may be defined as $100 (Z - 2)/Z = \omega$. This assumes that the upper limit of Z is 4, i.e., a "square lattice." Here X is the degree of polymerization or DP , k is Boltzmann's constant, and T is temperature.

If $X = 1$ or $Z = 2$, Eq. (3) becomes

$$\pi = \frac{kT}{A_0} \ln \left(\frac{A}{A - A_0} \right). \quad (4)$$

If $Z = (2 + \epsilon)$ where ϵ is a small number,

$$\pi A = kT + 1/2 \pi A_0. \quad (5)$$

This is Bull's equation, except for the factor $1/2$.

Plots of πA versus π for polymers and proteins are often linear at the air/water interface where the cohesion is sufficient for low flexibility. If Z is not close to 2, Eq. (3) predicts that the force-area curve will be convex upward. In the oil/water interface $Z = 2$, so we expect curvature which prevents good extrapolation.

The value of Z depends on charge and ionic strength through their effect upon the net cohesive energy; thus, flexibility may be reduced by changes in pH or salt concentration. When cohesion is appreciable, the system is described by an equation relating electrostatic effects to surface pressure, presented by Davies and Llopis (1955); the equation has been derived on a different basis by Isemura, Hamaguchi, and Ikeda (1957). A discussion is presented by Davies and Rideal (1963) and by Payens (1960).

Frisch and Simha's Theory - It is possible that the polymer may not be completely in the interface but may loop into one or both phases forming the interface, especially when compressed. This consideration leads to the relation

$$\pi = \frac{X kT}{A_0} \left[\ln \left(\frac{A}{A - p A_0} \right) - \left(\frac{p^2 A_0}{A - p A_0} \right) \right] - J p^2 \frac{kT A_0^2}{A^2} \quad (6)$$

where A_0 = close-packed area, and J is a coefficient allowing for lateral entropic and enthalpic interaction between those residues fixed in the interface. In this equation,

$$p = 2\alpha / (\pi f_i X)^{1/2} \quad (7)$$

where f_i is a function of internal flexibility of the molecule, X is the DP , and α is called the intramolecular segment condensation coefficient, which expresses the interactions within the polymer molecule. At low π Eq. (6) becomes (since $A \gg A_0$)

$$\pi A = X p kT. \quad (8)$$

This implies that molecules with large DP can form loops more easily and return to the interface to have units act as "independent" kinetic units, lowering their apparent molecular weights. This is not verified experimentally (Davies and Rideal (1963)), since for several proteins and copolypeptides the proper molecular weights are obtained at air/water and oil/water interfaces when the Singer equation is used at very low π (<50 millidynes). This does not preclude its applicability to other experimental situations involving other polymers and interfaces or higher pressures. In practice, distinguishing

between applicability of the Singer or Frisch-Simha theories on the basis of πA experiments is difficult (Jaffe and Loutz (1964), Dieu (1956 a, b, c)). Jaffe and Loutz have shown that both equations may be cast into the form of the virial equation

$$\pi A = kT (1 + B_1 \pi + B_2 \pi^2 + \dots) \quad (9)$$

and that using either Eq. (3) or (6) one can fit the force-area curves reasonably well by adjusting the parameters available. In both the case of the Singer equation and that of Frisch and Simha, however, Jaffe and Loutz point out that the choice of parameters may be ambiguous and that there is no guarantee that any of the parameters chosen bears a simple relation to the property of the polymer it purports to describe. Davies and Rideal (1963) have also indicated that Z should be regarded as an empirical factor because of the many oversimplifications of the theory. Jaffe and Loutz propose to use the function of the first two "virial coefficients" (B_2/B_1^2) to describe the polymer, empirically establishing an assignment of flexibility to values of the ratio. This parameter is independent of a model; insight into details of molecular structure is lacking as yet.

It has also been emphasized (Dieu (1956 a, b, c)) that both Singer's and Frisch and Simha's treatments have neglected intermolecular enthalpy effects. A term in A_0^2/A^2 may be introduced in an attempt to account for enthalpy of mixing in Singer's treatment (Davies (1954 b), Davies and Llopis (1955), Ter Minassian-Saraga and Prigogine (1954)), and the enthalpy of interaction of adjacent residues in the "surface plane" is allowed by Frisch and Simha. There has been at least one case where the Frisch and Simha theory seems to fit the data better than Singer's equation, the case of polyvinyl alcohol at a benzene/water interface (Dieu 1956 a). Dieu points out, however, that even in cases where the Frisch and Simha theory fits better than Singer's theory, values of Z are obtained which do not seem to fit expected chain properties. This may, at the present state of development, be attributed to the lack of recognition of enthalpy effects in the treatment.

In general, one may say that for molecules of low flexibility at the air/water interface one may obtain a good determination of molecular weight (Davis and Rideal (1963), p. 241, Dieu (1956 a, b, c), Davies (1954 b), Davies and Llopis (1955), Bull (1947)). High flexibility (induced by the presence of an oil/water interface or high charge on the macromolecule) or extensive intermolecular association (as in polyleucine) renders extrapolations of the force-area curve uncertain. Jaffe and Loutz (1964) have shown that with flexible polymers, even in the extremely low pressure range below 0.04 dyne/cm, one cannot get a good extrapolation for determining molecular weights. The fact that πA versus π plots for many proteins are indeed linear at the air/water interface lends credence to the notion that not much intermolecular interaction occurs in these systems, although this is not conclusive. Surface rheology studies (Cheesman and Davies (1954), Imahori (1952 a-d), Davies (1954 a, b)) to be described later will also have a bearing on this point. A summary of results of molecular weight determination is given by Cheesman and Davies (1954) together with a discussion of elasticity determinations based on Singer's equation. Subsequent data are listed in papers by Dieu (1956 a-c), Jaffe and de Coene (1957), Harrap (1955 a, b), and Ter Minassian-Saraga (1956).

An illustration of the effect of these ambiguities is the study by Davies and Llopis (1955) of the copolymer 1:1:2 lysine-glutamic acid-leucine. They found that at neutral pH at the air/water interface they could easily get a linear πA versus π plot which gave the correct molecular weight. At extremes of pH, they were forced to very low pressures to overcome the curvature resulting from flexibility induced by charging the molecule. At the oil/water interface, they observed effects very much like the dissociations reported for proteins, and only when the measurements were carried to extremely low π was it clear that the correct molecular weight would be found at $\pi \rightarrow 0$. They point out that, because of the many approximations in the theory, no absolute significance should be attached to the values of Z .

The importance of taking enthalpy into consideration is strikingly illustrated by the fact that the temperature dependence of π is much greater than the proportionality to absolute temperature indicated by both theories (Dieu (1956 a-c), Llopis and Rebollo (1956), Llopis and Albert (1959 a, b)).

Davies and Llopis (1955) have indicated that an energy of interaction may be included in the value of the flexibility ω by writing $\omega = \omega_0 e^{-E/RT}$, which shows that flexibility may change with temperature. This is equivalent to an energy of interaction because it biases the coordination number, $Z = \omega + 2$, in favor either of a greater number of polymer-solvent contacts or of polymer-polymer contacts, depending on the value of E .

Another indication that the Singer and Frisch-Simha theories are not adequate is the observation by Bull (1950) and Dieu (1956 a-c) that the πA versus π curves for proteins are often neither linear nor monotone curves, but show two linear portions separated by a kink. Each linear portion may be extrapolated to yield the same molecular weight. This is taken as evidence of the existence of at least two surface states (Bull (1950), Dieu (1956 a-c)). Experiments on synthetic polypeptides (Isemura and Hamaguchi (1952, 1953)) also suggest this possibility, which will be discussed further when considerations of surface configuration are undertaken (see "Nonpolar Polypeptides"). However, Cheesman and Davies (1954) interpret the kink as evidence of an interaction, saying it shows "stickiness" of the molecules.

Theories Involving Energy of Interaction

Silberberg's Theory - Silberberg (1962) has developed a theory for adsorption of an isolated, flexible molecule at an infinite planar surface. It is assumed that for each segment placed at the surface the internal energy of the system is reduced by an adsorption energy wkT . It is then shown that the polymer molecule will adjust itself so that alternate runs of P_S segments are all in the surface and loops of P_B segments whose size is not a function of molecular weight are all out of the interface. For a molecule composed completely of adsorbable units, on a surface which is everywhere adsorbing, the loops are small, and even at small w the molecules stay close to the surface. Otherwise, the loops are much larger. Restrictions limiting the re-entry of an adsorption loop into the surface have a similar effect. The result is a thin bulk phase of high polymer concentration near the surface. The molecules of polymer are rather flattened, with about half of the segments in the surface phase in some cases. The inability to wash the polymer from the interface (i.e., apparent irreversibility), the similarity of its isotherm to that of Langmuir, and a slow approach to equilibrium are predicted. The surface equation of state derived covers the limiting cases in the literature. Although the treatment conforms with the Gibbs isotherm, the apparent irreversibility of polymer adsorption is explained by the prediction that the adsorption will be very strong even at extremely low solution concentration. The surface equation of state in the limit of low θ is

$$A_0 \pi = kT \left(\frac{\theta/p}{X} + \frac{\sigma^2}{2} \beta_s \right) \quad (10)$$

where X is the number of segments per polymer molecule, θ is fraction of surface sites occupied by polymer segments, β_s is a complicated function, and p is a function of polymer segments at the surface.

It is a consequence of the model that the ends of the molecule stay in the surface.

Motomura and Matuura's Theory - Motomura and Matuura (1963 a,b) have also recently derived a theory which takes the energy of interaction into account and which reduces to the Singer theory when the energy is negligible. This equation also predicts phase changes in the films if the temperature is low enough and energy large enough.

The force-area curve is almost independent of the degree of polymerization χ if χ is sufficiently large (i.e., 7300). Their equation is

$$\frac{\pi}{\pi_0} = \frac{Z}{2} \ln \left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \frac{A_0}{A} \right] - \ln \left[1 - \frac{A_0}{A} \right] - \frac{Z}{2} \ln \frac{\left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \frac{A_0}{A} \right] (\beta_M + 1) - 2 \left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \right] \frac{A_0}{A}}{\left(1 - \frac{A_0}{A} \right) (\beta_M + 1)} \quad (11)$$

where $\pi_0 = kT/A_0$, A_0 is the area occupied by a monomer unit, Z is the coordination number of a site in a two-dimensional surface lattice, χ is the degree of polymerization, and β_M is defined by the complex function

$$\beta_M^2 = \left\{ \frac{\left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \right] \left(1 - \frac{A_0}{A} \right) \frac{A_0}{A}}{\left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \frac{A_0}{A} \right]^2} (e^{2w/ZkT} - 1) + 1 \right\} \quad (12)$$

in which $-2w/Z$ is the energy of contact between neighboring monomer units.

These authors point out that the success of Singer's equation may be due to the fact that Z in Singer's theory behaves similarly to the energy term. They do not account for the possibility that some chains may loop into the bulk phases.

The quantity π/π_0 may exhibit a maximum and a negative minimum when plotted against A/A_0 . This is characteristic of a two-phase film and occurs when $w/kT > 0.864$.

Motomura and Matuura examined poly(vinyl acetate), poly(methyl acrylate), and poly(methyl methacrylate), i.e., PVAc, PMA, and PMMA. In the case of PVAc a value of w was obtained which allowed a good fit to the theory, assuming $Z = 4$, below 6 dynes/cm. Above this pressure, deviations occurred which were attributed to squeezing out of chain segments from the surface.

Molecules of PMA are less expanded as w/kT is higher, and the theory fits better. This is expected, because the lattice approximation should not be as valid for expanded films.

In both the PVAc and PMA cases, $w/kT < 0.864$, so that phase changes were not expected. For PMMA, $w/kT > 0.864$ and the film is of condensed type; it shows vanishingly small π at large areas, with π becoming measurable at ca. $30A^2/\text{monomer}$. The predicted curve becomes negative at $23.5A^2/\text{residue}$; below this the theory predicts a phase transitional region, which is supported experimentally by a fluctuation of the surface potential.

Studies of these films showed that the higher the value of w/kT , the more viscoelastic the film, as would be expected. A similar parallelism between viscoelasticity and cohesive energy was found in studies on bovine serum albumin (BSA) and gamma globulin. The ΔH of spreading and area of onset of non-Newtonian viscosity increased with pH in the case of BSA, while both parameters were independent of pH in the case of gamma globulin (Llopis and Albert (1959 a, b)).

While these results are very promising, especially since phase transitions may be handled, it is this reviewer's feeling that more data must be forthcoming before the newer theories may be fully evaluated.

Electrostatic Effects on the Force-Area Curve

The effect of charge upon the force-area curve of polymers may be separated into intermolecular and intramolecular effects. If the polymer molecules are charged, then repulsion of like charges will decrease the net cohesion of the polymer molecule.

If one writes the flexibility ω as

$$\omega = \omega_0 e^{-E/kT} \quad (13)$$

where ω_0 is the flexibility in the absence of cohesive effects (i.e., at the oil/water interface and at zero charge), electrostatic effects will appear in the parameter E , as will cohesive energy. At very high area where intermolecular effects may be neglected safely, the oil/water interface in the isoelectric region allows the establishment of a base curve for which $E = 0$; electrostatic (and cohesive) contributions to the force-area curve at the air/water interface may be obtained by subtraction. One thus obtains the "excess electrostatic surface pressure" (π_{el}) experimentally (Davies and Llopis (1955), Payens (1960), Davies and Rideal (1963), Chapter 5).

The interfacial potential Ψ may be calculated according to the Gouy theory, and one may write

$$\pi_{el} = \int_0^{\Psi} \sigma d\Psi' \quad (14)$$

where π_{el} is the electrostatic contribution to the surface pressure, and σ is the charge density of the monolayer. This leads to

$$\pi_{el} = 6.1 \sqrt{C} \left\{ \cosh \left[\operatorname{arc} \sinh \left(\frac{134}{\sqrt{C}} \right) \right] - 1 \right\} \quad (15)$$

(Payens (1960), Davies (1951)). Here, C is the concentration of 1:1 electrolyte. Intermolecular contributions, which may be evaluated at higher pressures, are evident since the apparent potentials decrease with area faster than indicated for isolated charges of the valency of the polymer. The contributions of the intermolecular and intramolecular effects may thus be considered as contributing to the effective potential. An effective charge may be derived by fitting such data.

The value of Z may be related to the electrostatic effect through the relation

$$Z - 2 = \omega = \omega_0 \exp \left(\frac{-E_{\text{cohesion}} + \pi_{el} A^*}{kT} \right) \quad (16)$$

and the value of A^* derived from the slope of a plot of π_{el} versus $\log \omega$. The value of A^* so obtained is considered to be the area of the minimum kinetic unit (Joly (1948)) in the polymer in the course of its molecular motion.

The effective charge perturbing the πA curve in this study was found to be 20 percent of the true value of the macromolecular charge and corresponds to the estimate of the charge expected to be at the periphery of the molecule, with the rest of the charge

screened by counter-ions. The size of the kinetic unit appears to be that of five residues, a small fraction of the size of the molecule.

Thermodynamics of the Force-Area Curve

One may attempt to characterize the degree of disruption of molecular structure when a molecule is spread by the value of the free energy change on spreading. This is not a clear-cut analysis as yet, because it is not known how to resolve the observed change on spreading into its components corresponding to changes in solvation, polarization of the interfaces, changes in solution structure, and so on. Neglecting these matters, attempts have been made to compare the entropies of unfolding for various substances. Davies (1953 c) [Cheesman and Davies (1954)] used the following approach.

If one accepts the model of Singer (1948), one can calculate the entropy S_s of a spread polymer with respect to a reference state in which the polymer backbone chain is rigid from the high area portion of the force-area curve. This quantity was given by Davies (1953) as

$$S_s = \left(I - \frac{2}{X} \right) R \ln (Z - 1) \quad (17)$$

using the same notation as in the section titled "Singer's Theory."

The entropy change on compression of the film between any two areas can be calculated if one assumes that the free energy change has only an entropy term and no enthalpy term. Subtracting from this the entropy change which would be found for a completely rigid molecule undergoing expansion or compression between the same areas gives

$$\Delta S_s = \frac{1}{XT} \int_{A_1}^{A_2} \pi dA - \frac{1}{XT} \int_{A_1}^{A_2} \pi_r dA. \quad (18)$$

The entropy of the molecule at any area can be calculated relative to a reference state of the rigid main chain by evaluating π_r , the predicted value for a rigid chain (i.e., $Z = 2$). The Singer theory allows this to be done if the effect of side chains is neglected. Accepting this limitation, we may determine the entropy per residue at the limiting area relative to the rigid reference state.

It turns out for proteins (Davis (1953 c), Cheesman and Davies (1954)) that the experimental unitary entropy of expansion from the limiting area to high area is equal to the calculated unitary entropy of the molecule at high area relative to the rigid molecule reference state.

But this is not true in the cases of polyalanine and polyleucine, for which the unitary expansion entropy is less than that calculated from the Singer theory. (This implies that the limiting area in the case of these peptides does not correspond to a rigid molecule, while the rigid molecule at limiting area is consistent for the proteins.)

The value of S_s calculated in this way may be considered the entropy of surface denaturation if the Singer model of copolypeptide monolayers is accepted and if the entropy of the reference state chosen is indeed the same as that of the native molecules. For example, the experimental data indicate that, for methemoglobin at the oil/water interface, $Z = 2.12$, corresponding to $S_s = +0.22$ eu, while for polyalanine and polyleucine one has $S_s = +1.6$ eu through use of Eq. (18).

Davies states that the values of s_s are similar for most proteins and makes a comparison with the thermal denaturation entropy of trypsin, which is +0.76 eu. The inference is that a number of linkages modified by thermal denaturation are not destroyed by surface denaturation. Other evidence leading to the conclusion that surface denaturation is not complete (found in sections titled "Lysozyme," "Ovalbumin," and "Structural Alteration of Spread Films") makes the argument more convincing, since the validity of this type of argument rests upon Singer's treatment, now known to be inadequate. This approach may be refined using the more satisfactory treatments of the theory of the force-area curve. At this stage of development, the treatment measured a quantity which may be more closely related to the free energy than to the entropy of the transition. We may cite some of the evidence which has been obtained by heating solutions and monolayers. Kaplan and Fraser (1953) found that fibers formed from protein monolayers were quite different when formed from native spread proteins instead of denatured ones, and that if proteins were heated prior to spreading the fibers formed were of the denatured type. Trapeznikov (1948) found a change in surface viscosity η_s at the temperature of bulk denaturation. These observations indicate that the properties of the film are dependent upon elements of structure which are affected by denaturation.

A second approach to the thermodynamic parameters of the polymeric monolayer is the study of the systems at different temperatures. Llopis and Albert (1959 a, b) have done this for bovine serum albumin and globulin. In both cases the enthalpy of spreading on acid at low temperature is negative, i.e., favors spreading. At higher temperatures the enthalpy term is positive. On alkaline substrates, cohesion always predominates. More work on the thermodynamics of the process is required for full understanding, but it is already clear that Singer's equation will have to be improved upon, as will the theory of Frisch and Simha, to take enthalpy effects into account. The theories of Motomura and Matuura (1963 a, b) and of Silberberg (1962 a, b) have not had sufficient application to be adequately judged.

These approaches are not adequate to evaluate the surface denaturation process, because they pertain to the already spread material. Comparisons based on Eq. (18) depend upon the assumption that the molecules at the point of minimum compressibility, or the collapse pressure, are in a state whose entropy corresponds to the solution or solid state. Although there are indications that this may be true (Benhamou (1954), MacRitchie (1963)) (as discussed later in the "Collapse" section), the issue is not settled. It would appear to be more suitable to measure the equilibrium involved in the adsorption from solution to the surface if such an equilibrium could be established. Guastalla and Benhamou (1961) attempted to evaluate the equilibrium parameters in such a situation and found that hemoglobin has too high a molecular weight to be suitable for the experiment; insulin, which is much smaller, appeared to be on the borderline of feasibility.* The data on the entropy and enthalpy of expansion of the already spread monolayer, in addition to the data from the adsorption and spreading steps, are required for a complete description of the surface denaturation process.

SURFACE PARAMETERS AND TECHNIQUES AT FINITE PRESSURES

Molecular weights are studied in the low-pressure region of the force-area curve, where properties of the molecules at the surface may be studied in a highly dilute state. In the highly expanded state, the theoretical attempts to describe molecular behavior have led to estimates of the intrinsic flexibility and size of the solute molecules in what is hoped to be a situation essentially free of intermolecular interaction. When one

*A study of this process with a series of polypeptides as a function of molecular weight would be useful.

compresses a film so that the molecules interact, a different type of information is forthcoming. Experimental parameters, other than the surface pressure will now be considered.

Interfacial Viscosity and Viscoelasticity

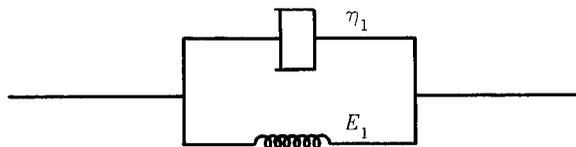
A review of interfacial viscosity and viscoelasticity was presented by Joly (1964). Polymer monolayers may be classified, rather empirically, by interfacial viscosity properties. One finds that condensed films and expanded films have quite different viscosity-area curves. In the case of the condensed film, one finds a high viscosity at areas larger than the limiting area found from a πA experiment. For expanded films, we find that the interface viscosity η_s drops outside the range of high surface pressures (Isemura and Hamaguchi (1954)).

A high surface viscosity would seem to imply intermolecular interaction. This may come about mechanically through chain entanglement (Davies (1953 c)) or through chemical interaction (Isemura and Hamaguchi (1954)), e.g., electrostatic salt linkage, hydrogen bonds. High surface viscosity at high area signifies that a network of some sort is formed by condensed films between molecules before compression sufficient to pack them tightly is reached. In contrast, expanded films show a large increase in interfacial viscosity only when the area is reduced to the limiting area of the force-area curve (Isemura and Hamaguchi (1954)). This implies that the repulsion effects which keep the film expanded also serve to inhibit chemical or mechanical intermolecular interactions at areas larger than the close-packed area.

Isemura and Hamaguchi (1954) emphasize the role of electrostatic interactions in producing surface viscosity, while Davies (1953 c) interpreted the increase of film viscosity of polypeptides at an oil/water interface as evidence that a loss of intramolecular cohesion allows sufficient interaction to occur at higher area, as chains are compressed. Increase of η_s at high charge may also be the result of expansion (see also Joly (1964)). A theory of viscosity based upon the general theory of rate processes has been formulated by Eyring and coworkers (Glasstone, Laidler, and Eyring (1941)). Conclusions reached from a study of activation areas are cited in the "Proline and Hydroxyproline" section. Ikeda and Isemura (1959) found that a strongly cohesive condensed polymer exhibits an area of activation which extends over the whole molecular area, while for expanded films with weaker cohesion, the area corresponds to only a small section of the molecule. Earlier, Joly (1948) had observed, also on the basis of the Eyring theory, that since all proteins seemed to have approximately the same viscosity coefficient at the onset of non-Newtonian viscosity, the kinetic subunits of the protein monolayer must be of similar area. Calculation of the activation energy of flow led to a value of $90A^2$ for this unit. Joly's treatment relies upon (a) an estimation of the contributions of the various components of the molecule to the interaction energy and (b) the deduction that the spacing of the kinetic units at the onset of non-Newtonian viscosity is such as to let the width of an assumed hexagonal pseudolattice be twice the diameter of the kinetic unit (assumed spherical). Ikeda and Isemura do not give the details of their calculation. The strongly cohesive polymers of poly(phenylalanine), polyleucine, etc., have a much higher density of nonpolar amino acids than the proteins.

An alternative method of analysis of viscoelasticity in films is shown in the series of works treating the rheology of materials as represented by a mechanical model. The mechanical analogy is a system of springs and dashpots; the springs represent elastic elements, and the dashpots, viscous elements. These elements may be combined in various ways, such as:

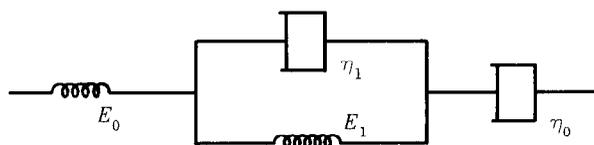
1. Voight Element:



2. Maxwell Element:



3. Four-Parameter Model:



In an appropriate mathematical analysis of the result of a stress-strain measurement, where strain is followed as a function of time, and the stress is permitted to decay, the parameters of such models, i.e., the E_i and η_i , may be evaluated. Such a treatment is given by Motomura and Matuura (1963 b), as well as by others. The relation between the type of model, the values of the constants, and what goes on in the actual film is still obscure. One may say that the existence of elastic elements implies the presence of a network of some kind in the film, and Tachibana and Inokuchi (1953) have found a relationship between kinks or plateaus in the force-area curve and the onset of instantaneous elasticity (i.e., nonzero E_0). They have also postulated that Hookean elasticity depends upon the strength and exchange rate of network bonds.*

The last paragraph of the paper by Motomura and Matuura indicates the type of conclusion which may be drawn. After showing that the viscoelastic parameters were larger for PMMA than for PMA, and were smallest for PVAc, they wrote: "In the preceding paper, the value of w/kT , which was related to the cohesive force between monomers, was estimated for the three polymers, and it was found that the order was PMMA > PMA > PVAc. The present result might show a parallelism between the values of viscoelasticity and w/kT . It seems proper from this point that linear polymer monolayers are made up of a two-dimensional network structure produced by extended polymer molecules and their rheological behavior is determined mainly by the cohesive force between segments"

Polymer films may, to some extent, be grouped according to the mechanical model describing them (Tachibana, Inokuchi, and Inokuchi (1959)); group A has an elastic element only. It is suggested that films of this type form a two-dimensional network and

*However, they have since shown that they can eliminate instantaneous elasticity without changing the force-area curve by use of an organic spreading solution (Tachibana, Inokuchi, and Inokuchi (1957)).

become elastic before the spread molecules are closely packed. Group A does not show viscoelasticity until the gel point is reached, while group B (the four-parameter model) gradually becomes viscous and begins to show viscoelasticity only when a multilayer is formed by compression.

It is important that large stresses not be imposed on the films during the measurement, since some interactions are very easily broken (Cheesman and Sten-Knudsen (1959), Tachibana and Inokuchi (1953)).

The molecular weight of flexible chain polymers, which cannot be derived from π - A relations, may be derived from the η_s - A curve on an empirical basis in the cellulose acetate, PVAc, and poly(vinyl alcohol) (PVA) series, where the relation

$$(\mu) = AM^\alpha \quad (19)$$

has been tested by Jaffe and Loutz (1958). They find, for PVA, $A = 10^{-7}$ and $\alpha = 1.2$; for PVAc, $A = 10^{-4.5}$ and $\alpha = 0.63$; for cellulose acetate, $A = 1.26 \times 10^{-7}$ and $\alpha = 0.97$. Here, (μ) is the limit at zero concentration (i.e., $\pi \rightarrow 0$) of the reduced surface viscosity coefficient

$$\mu = \left(\frac{\Delta_f}{\Delta_w} \right) \left(\frac{1}{C} \right). \quad (20)$$

In Eq. (20), Δ_f and Δ_w are the slopes of the plots of the log of the amplitude of an oscillating bob in the surface against the number of swings for film-covered and clean water surfaces, respectively. This is analogous to the Staudinger relation for bulk intrinsic viscosity. When a periodic disturbance is used, the motion of the sensor may be analyzed in terms of a complex modulus, whose real and imaginary parts correspond to the elastic modulus and viscosity (Tschoegl and Alexander (1960a, b)).

It was shown by Boyd and Vaslow (1958) that surface viscosity may be a sensitive detector of interaction in surface films. If one makes a mixed film which forms a two-dimensional solution, then

$$\log \Phi = X_1 \log \Phi_1 + X_2 \log \Phi_2 + X_1 X_2 \quad (21)$$

where $\Phi = 1/\eta_s =$ fluidity and X is an interaction parameter. This equation assumes approximate equality of molecular areas. In the case of protein interaction, Joly (1948, 1949) concluded that proteins when spread may be considered as aggregates of kinetic subunits, and the subunits were about $90A^2$ for all the cases considered. In such a case, the value of η_s may be a better indication of film interaction than the perturbation of force-area curves (Joly (1964)).

The measurement of surface viscosity is not a simple problem. The various instruments used do not in general yield results which are in agreement, and the instrumental setups to be used depend upon the nature of the film. A review of this topic was given by Jarvis (1962), and discussions are also presented by Davies and Rideal (1963, Chap. 5) and by Joly (1964).

The possible relationships of hysteresis of the force-area curve on rapid cycling, viscoelasticity, and the degree of spreading are discussed further in the "Ovalbumin" section.

Interfacial Potential

The measurement of interfacial potential is carried out with either a vibrating electrode or a radioactive electrode. The vibrating electrode functions as one plate of a capacitor, and the other plate is the interface in question. The capacity is determined by rapidly varying the distance between the electrode and the interface and measuring the current flow, or ac signal, across this element of the circuit. This procedure was pioneered by Yamins and Zisman (1933). Another method, which has become popular in recent years because of the increased availability of sources of ionizing radiation, employs the "radioactive electrode." In this case, air between a metal electrode and the interface is rendered conducting by ionizing radiation from a radioactive source in the metal electrode; the potential is measured with respect to a reference electrode immersed in the hypophase. In either case, the quantity of interest is the change in surface potential resulting when an interfacial layer is placed at the previously clean interface.

Interfacial potentials are measurable and stable in the instance where the concentration and mobility of charge on at least one side of the interface are sufficiently low that the interfacial potential is not screened from the sensing electrode by interposition of a net space charge between the electrode and the interface. Such a situation exists when the solubility of ionized material is too small to screen the interfacial charge, as at a gas/condensed phase interface or when a very nonpolar material such as paraffin oil is one bulk phase. In the case of more polar oils, one may not get a stable potential indication if the concentration of ionic species is large enough to reduce the thickness of the ionic double layer to a dimension of the order of the distance of the sensing electrode from the interface (Davies and Rideal (1963, Chap. 2)).

When a monolayer, or adsorbed layer, situates itself at an interface of the type described, the change in potential observed with respect to the potential of the clean interface may be considered to result from a number of components. Davies and Rideal consider convenient the following resolution of the potential into components. The observed potential change ΔV is related to the interfacial dipole moment by the Helmholtz relation for an uncharged monolayer:

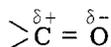
$$\Delta V = 4\pi n \mu_D \quad (22)$$

where n is the number of molecules of the film-forming substance per unit area of interface and μ_D is the vertical component of the dipole moment per molecule of such substance.

One may resolve μ_D into three components: μ_1 , corresponding to reorientation of solvent molecules caused by the presence of the material placed on the clean surface; μ_2 , corresponding to alignment of the dipoles of the polar end of the material; and μ_3 , corresponding to any unbalanced dipoles of the nonpolar or hydrophobic part of the molecule. The component μ_3 is included because even a nonpolar group, when lined up at an interface, may exhibit a permanent dipole resulting from the asymmetric condition existing at the end of the nonpolar portion of a molecule, e.g., as does the CH_3 group at the end of a paraffin chain. In addition, an ionized head group may contribute a component termed χ_0 caused by counter ions near the surface. The result is then

$$\Delta V = 4\pi n [(\mu_1 + \mu_2 + \mu_3) + \chi_0] = 4\pi n (\mu_D + \chi_0) \quad (23)$$

Study of the surface potential is helpful in determining the orientation of polar groups in the monolayer. The amide group contains the



dipole, which is the major contributor to μ_D of a nonpolar amino acid residue. If the oxygen atom points downward into the aqueous phase, we should therefore expect a positive value of μ_D .

Two papers have recently appeared which must be taken into consideration when performing or interpreting measurements of surface potential. Chambers (1963) has observed that a radioactive electrode will cause changes in the film adjacent to it; therefore, the electrode should not be used for any length of time at a single location. He has presented data showing the magnitude of the effect. Secondly, L. Ter Minassian-Saraga (1956) has pointed out in a review article that the presence of an electrode, even if not radioactive, will have an effect upon the film directly under it. She, therefore, recommends that when measuring ΔV in a transition region one should work quickly, as far as possible from the surface, and with an electrode as large as the film area, although this will eliminate detection of islands.

Microelectrophoresis

The microelectrophoretic technique (reviewed by Fraser (1957)) is of value in studying protein adsorption on solid particles or on droplets of emulsion. Studies of oil emulsions stabilized with lipids allowed evaluation of the charge on emulsion particles, which in turn allowed correlation of adsorption and clarification with charge as explained below (see "Emulsions"). Recently, Bull and co-workers, and Benhamou, de Mende, and Guastalla have examined several systems. One of the uncertainties in this field appears to be that adsorbed proteins have mobilities similar to those of dissolved proteins. Bull (1958) has shown, by means of the ionic strength dependence of the mobility of BSA and ovalbumin adsorbed on glass, that the effective radii of the particles correspond to very large particles and not to particles of the dimensions of the protein molecule. The apparent charge was found to be smaller than that calculated upon the basis of a proton titration, however. Bull reconciled results by postulating adsorption of the protein in a manner such that much of its charge points inward toward the highly polar glass surface, reducing the charge density on the outer surface.

Further studies of proteins adsorbed on various adsorbents have been made; however, microelectrophoresis does not appear to be on sufficiently solid footing as yet, either theoretically or experimentally, to make general statements concerning interpretation of the data within the scope of this review. The reader is referred to the papers of Barnett and Bull (1959), Chatteraj and Bull (1959), and Benhamou, de Mende, and Guastalla (1961).

Transferred and Adsorbed Monolayers on Solids

The Langmuir-Blodgett technique of transferring spread films from liquid surfaces to solid surfaces has proved to be useful and has led to a series of analyses which have helped to elucidate the structure and reactivity of monolayers. Descriptions of the technique have been given in recent reviews (Sobotka and Trurnit (1961), Rothen (1956)). Essentially, a layer from the liquid surface may be transferred to a metal or lipid-coated metal slide with an area which in some cases is very close to the area of the original monolayer; in other cases it is not, depending upon the surface pressure to which the film is subjected during transfer. The transfer of a monolayer may occur when a plate of glass, metal, mica, or a substance coated with paraffin or a fatty acid or other lipid film, is passed through the monolayer while the required surface pressure is maintained.

In the case of protein films, which are highly compressible and elastic, the thickness of a transferred film depends on the pressure to which the film is subjected during transfer. If the pressure is in the range of 2 to 6 dynes, the thickness of the transferred protein film is close to the thickness before transfer. For incompressible films, the transfer ratio is close to unity; for elastic films, such as proteins, the transfer ratio is variable with film pressure. Proteins may also be adsorbed directly from solution onto a plate immersed within the solution.

In either case, the plates may be coated prior to transfer or adsorption of the protein film with a layer of a dielectric material, most often barium stearate, which serves as an aid in determining the thickness of film and also may serve to modify the nature of the surface of the plate.

Films adsorbed from solution are usually thicker than transferred monolayers. Both the adsorbed and transferred films may be analyzed for enzymatic activity or susceptibility, and the optical thickness and refractive index may be measured. The case of bovine serum albumin has been analyzed in detail, and the results (summarized by Bateman and Adams (1957) and Bateman (1959)) indicate that the differences in the structures of adsorbed and of transferred films are reflected in the thickness and refractive index of the films:

| Type of Film | Refractive Index | Thickness (A) | Mass (mg/m ²) |
|--------------|------------------|---------------|---------------------------|
| Transferred | 1.5-1.6 | 9.1-9.6 | 1.1-1.2 |
| Adsorbed | 1.2-1.4 | 15-20 | 0.8-2 (pH 3-4) |

The contrasting optical properties of the two types of film are attributed to differences in structure. The transferred monolayers are thought to consist of a layer of nearly close-packed polypeptide chains lying in the plane of the film, with side chains vertical. The films formed by direct adsorption may consist of globular protein; there would be interstices, which might be filled (to raise the observed refractive index to 1.5 to 1.6) by adsorption at higher pH or salt concentration so that the amount of protein adsorbed rises to about 3 mg/m², with no change in thickness.

The protein layers so attached to slides may be studied in several ways. Film thickness and refractive index may be measured optically with an ellipsometer (Rothen (1956)) or by spectral analysis of reflected light (Bateman (1959, 1963)). A few studies of transferred films have raised the question as to whether monolayers may in some way recoil on transfer, so that small regions which would not affect the determination of average thickness may form which contain a thicker structure; the evidence is presented in "Structural Alteration of Spread Films."

These techniques, individually or in combination, are used in attempting to define the configuration of polypeptides and proteins at interfaces.

SYNTHETIC POLYPEPTIDES

General Considerations

A few studies of polypeptides were considered in a review by Cheesman and Davies (1954). Subsequently, much work has been done on a greater variety of polymers, especially in Japan by Isemura and co-workers.

The earlier studies of proteins showed that the area of close packing was, in most cases where good spreading was established, approximately 15A²/residue. This corresponds to the β type of structure, with side chain R groups alternating up and down. Since it was to be expected that hydrophobic groups would project upward into the nonpolar phase and hydrophilic groups downward into the aqueous phase, it seemed reasonable that the amino acid sequences of proteins would prove to be alternating hydrophobic and hydrophilic residues (Bull (1947)). The postulate soon foundered upon the facts of life as two important types of study were performed: first, the experimental elucidation of amino acid sequences, and second, studies on synthetic polypeptides. The sequence

studies showed that such sequences were not found in proteins, and the poly(amino acid) studies showed that such sequences were not required to lead to the observed results (Cheesman and Davies (1954)).

As a result of these studies, three general rules have emerged concerning the orientation of side chains (Davies (1953 b), Cheesman and Davies (1954)):

1. Polymers consisting only of nonpolar residues are folded at the air/water interface with the side chains alternately up into the air and down into the water. At the oil/water interface, the chains are all free to enter the oil phase.
2. Hydrocarbon side chains with ω -polar groups tend to lie flat on the air/water surface unless surrounded by hydrocarbon residues, in which case they are lifted up into the air. Isolated hydrocarbon side chains show the same effect at high areas. At the oil/water surface, the ω -polar groups tend to rise up into the oil when the film is compressed.
3. If part of a molecule is ionized, it can never completely leave the aqueous phase.

The question of secondary structure of polyamides and proteins is one to which much work has been addressed. In monolayers, the situation is as yet not completely understood.

The fact that the observed limiting areas of most polypeptides correspond to $\sim 15\text{A}^2$ /residue seems strongly indicative of the β type, or pleated sheet, structures, but there exist some data which seem to complicate the issue, as discussed below.

Nonpolar Polypeptides

Table 1 abstracts the data available to Cheesman and Davies at the time of their review in 1954, concerning the highly nonpolar amino acids, poly(DL-leucine), poly(DL-alanine), and poly(DL-phenylalanine). Using this data, they arrived at the following idea of the structure of nonpolar amino acids.

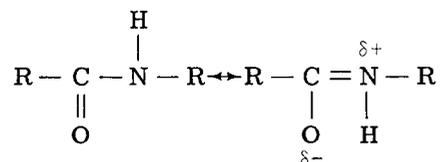
Since the molecular model studies show that the side chains have cross sections of about 30A^2 , the 17A^2 value of A_π indicates alternate up and down orientation (Davies (1953 a, b)). A similar observation had been made by Cumper and Alexander (1950) for poly(DL-phenylalanine). Since ΔV is higher at the acid air/water than at the oil/water interface, they deduce that the $>C=O$ dipoles are more nearly vertical at the air/water interface in acid. At pH 7 or 13, lower potentials are produced at the air/water interface. They postulate that this is caused by hydrogen bonds causing the $>C=O$ dipoles to become more horizontal. At the oil/water interface, cohesion is lost and flexibility increases. Cohesion now is not enough to maintain a hydrogen-bonded structure, and ΔV becomes almost independent of pH. All chains tend to be in the oil, so that ΔV becomes lower as the chains move into the oil and, as determined from studies of models, reorient the $>C=O$ groups from the vertical. The alternating side chain configuration no longer present, we cannot compress the poly(phenylalanine) monolayer beyond 25A^2 /residue without getting strong intermolecular interaction. This shows up in both the force-area curve and interfacial viscosity curve, while at the air/water interface one could compress poly(phenylalanine) to 14A^2 /residue. This configuration is called the "oil form." The postulated configuration of the polypeptides was deduced from studies of dipole moment and their change with pH. This change was attributed to a decreasing tendency to form hydrogen bonds between peptide groups as the subphase becomes more acid. Crisp's study (1946) of nylon, in which it was found that the usually extremely compact film became expanded at low pH, was apparently largely responsible for this idea. It was later found that other polyamide and polypeptide films behaved similarly (Davies (1953 b)). In studies of detergent binding to gelatin, Pankhurst (1949) and Yamashita and Isemura (1962)

Table 1
Surface Properties of Some Nonpolar Polypeptides

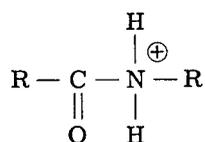
| Polymer | Inter- face | A_{η}^* (A ² /residue) | | | A_{η}^* (A ² /res) | μ (mD) | | | Flexibility (%) | | Theoretical Area per Side Chain Cross Section (A ²) |
|--------------------|----------------|--|------------------|-------|--|--|--|--|-----------------|--------|---|
| | | pH 2 | pH 6-8 | pH 13 | | pH 2 | pH 6-8 | pH 13 | pH 2 | pH 6-8 | |
| Leucine | A/W | 19 | 17 | 17 | | 188 (at 20 A ² /res) | 137 (at 20 A ² /res) | 113 (at 20 A ² /res) | | | 30 |
| | O/W | | | | | 97 (at 20 A ² /res) | 85 (at 20 A ² /res) | 85 (at 20 A ² /res) | 65 | | |
| Phenyl- alanine | A/W | | 14 | | 14 <small>(small η_s for $A > 25A^2/res$)</small> | 105 (at 100 A ² /res) | 127 (at 100 A ² /res) | 113 (at 100 A ² /res) | | | ~23 |
| | O/W | | | | 25 | | ~0 | | 65 | 25 | |
| Alanine | A/W | | expanded | | 14.7 | | | | | | |
| | O/W | | 23 (expanded) | | | | | | | | 15 |

* A_{η} and A_{η} are limiting areas measured by surface pressure and surface viscosity respectively.

raised the possibility of peptide groups becoming protonated at low pH (about pH = 2). Pankhurst cites evidence from titration curves that anomalous proton uptake occurs at about pH 2. He recalled that Pauling suggested that protonation of the amide group could occur to destroy the resonance responsible for the double bond character and thus the stiffness of the peptide group. The resonance



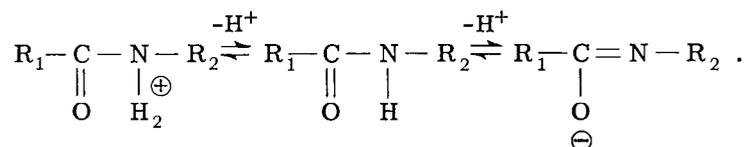
could be prevented by protonation, and



would be the stable form.

This would have three effects. First, the partial double bond character of the peptide bond would be reduced, so that the stiffness of the main chain would also be reduced. Second, the positive charge introduced would increase the already positive charge at this pH, which would increase the electrostatic repulsion effects, thus increasing π at constant area, or area at constant π , or increasing the flexibility. Third, a change of electric moment as the pH is lowered would also result. Pauling (1953) has stated that the degree of amide protonation is expected to be "very small and without significance." Klotz and co-workers (Hanlon and Klotz (1965), Hanlon, Russo, and Klotz (1963)) have obtained evidence for the protonation of the amide group by the IR spectral change of amides in acid; however, this has been done in organic acid solvents, and the pK in aqueous solution is expected to be much lower. At any rate, it appears that this phenomenon is only significant in the very acid range. The range of the change in properties with pH of nonpolar peptides (Davies (1953b), Yamshita and Isemura (1962)) seems too great to be explained solely on the basis of amide protonation, although there may be differences between pK at the interface and in the bulk solution.

At high pH, Schauenstein and Perko (1953) have shown that under certain circumstances the peptide group may become ionized by losing a proton, thus becoming negatively charged. The high pH effect is not completely elucidated; such factors as the amino acid content of the polymer and molecular weight seem to have a prominent effect upon the ionization. The peptide group is thus amphoteric, and the effect of pH upon the proteins and polypeptides must be considered from this point of view. According to these ideas, the range of possible ionization for the peptide group appears to be



The effect of ionization of this type upon the dipole moment of the monolayer has not yet been determined, to the reviewer's knowledge.

A large number of interesting polypeptide structures have been investigated by Isemura and Hamaguchi and co-workers. In 1952 and 1953 they published a study of force-area and surface viscosity-area curves on polypeptides as a function of the length of the side chains (see Figs. 1 and 2). It was found that the α -aminocaprylic acid polymer marked the transition between long and short side chains. This polypeptide has a side chain six carbon atoms long. The longer chains (8, 10, and 12 carbon atoms) produced plateaus in the force-area curves on compression, in which the pressure was independent of area over a range of area from 15 to 20 or 25A²/residue, depending on the chain length. For each of these polymers, the value of π in the plateau region was a function of temperature, while the areas bounding the plateau region remained constant. The shorter chain of caprylic acid (C₆) was not sufficient to maintain a constant-pressure plateau, but its graph showed an inflection at 30°C and an increasing tendency toward a plateau as the temperature was decreased. The smaller four-carbon side chains of nor-leucine, which is α -aminocaproic acid, showed behavior similar to that of protein monolayers, in that no inflection or plateau was formed. The interpretation of the plateau was that a conformational change was taking place, and a modified form of the Clausius-Clapeyron equation was used to estimate the internal energy and enthalpy changes occurring in the transition. These were of the order of 300 to 600 cal/mole, as the chain length increased from six to ten carbon atoms. The compressibility is very low at the low-area end of the transition, and the low-area form has the higher entropy.*

The idea of a transition is supported by measurement of the thickness of the film, when picked up by the Blodgett technique. For example, with poly(aminocaprylic acid), the film thickness was 9A at low π , and 15 or 16A at high π . These numbers were said to

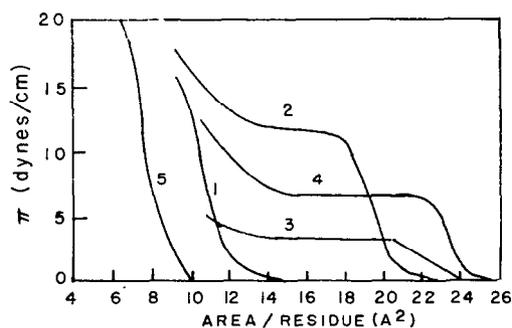


Fig. 1 - The surface pressure-area curves of poly(DL- α -aminocaproic acid) (1), poly(DL- α -aminocaprylic acid) (2), poly(DL- α -aminocapric acid) (3), poly(DL- α -aminolauric acid) (4), and poly(γ -methyl-L-glutamate) (5)

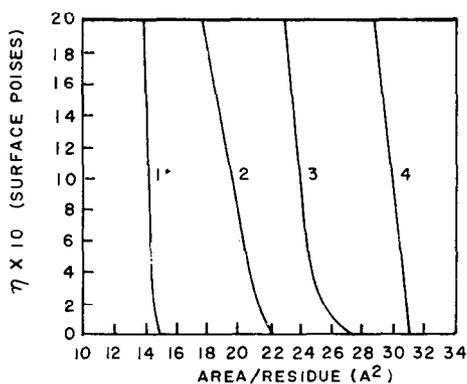


Fig. 2 - The surface viscosity-area curves of polypeptide monolayers on distilled water

*It is also of interest that while protein films do not show plateaus if compressed slowly, Dervichian (1952) reports that rapidly compressed proteins do. He explains this phenomenon by postulating that the plateau occurs if molecular rearrangements cannot occur as rapidly as the compression of the film. Longer chains will take a longer time for rearrangement. The rearrangement in poly(aminocaproic acid) seems to become more rapid at higher temperature, and the incipient "plateau" seems to vanish at 46°C, although it is pronounced at low temperature.

correspond to models for the α - β transition in polypeptides (see footnote, page 21). Experiments investigating the surface viscosity of these polymers yielded curves which rose suddenly from very low values and were extrapolated almost linearly to zero pressure at areas of $15\text{A}^2/\text{residue}$ for chain lengths less than 6. With longer chain lengths, they showed significant η_s even at lower π , demonstrating interactions of the longer chains at higher area.

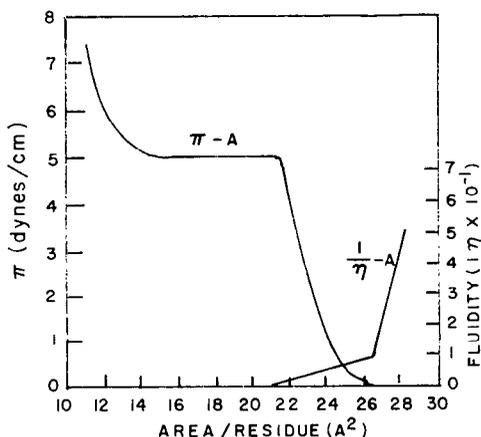


Fig. 3 - The force-area and fluidity-area curves of poly(DL- α -amino-capric acid) at 23°C

spreading solvents used with nonpolar polypeptides, as is also suggested by Davies and Rideal (1963, Chap. 5); but DCA may affect the conformation of the polypeptide, since it is recognized to be a helix-breaking or protonating solvent at high concentrations (Urnes and Doty (1961), Hanlon and Klotz (1965)).

Poly(amino acids) with less than four carbon atoms in hydrocarbon side chains behaved similarly to the esterified glutamate polymer on neutral subphases but did not exhibit a change in limiting area on acid. When the viscosity was plotted, however, a rise from essentially zero was noted at $15\text{A}^2/\text{residue}$, with a jump at 10A^2 , where π starts to increase. This may be interpreted as another transition, but one whose free energy change is small enough that only an imperceptible increment in surface pressure is needed to induce it. Poly(γ -benzyl-L- and DL-glutamate) give results similar to those of the longer chain polymers but with areas corresponding to packing of the bulky side chains. Of the two, the L-polymer is more compact.

The case of amilan, which is an ϵ -amino acid polymer, shows increases in viscosity at 80 to $90\text{A}^2/\text{residue}$, and an A_0 of $32\text{A}^2/\text{residue}$, with a kink in the force-area curve at 80A^2 . This clearly shows the much more expanded configuration of this polymer, which is not very similar to the proteins and α -amino acid polymers.

This interpretation of the data has been criticized by Bamford, Elliott, and Hanby (1956). They point out that:

1. Poly(DL-leucine) has A_0 of 18A^2 ; the α -helix model occupies $19\text{A}^2/\text{residue}$.

When the fluidity (the reciprocal of η_s) of the long-side-chain polymer films is plotted against area (Fig. 3), two linear sections are obtained: a high-slope region at very high area where π is almost zero, and a lower slope at higher concentration where the pressure starts to rise, the slope falling off to approximately zero where the plateau region of pressure starts. The methyl glutamate chain (esterified) behaves differently; the force-area curve showed $A_0 = 9.8\text{A}^2/\text{residue}$ at neutral pH and on acid, $14.7\text{A}^2/\text{residue}$, as discussed above. Corresponding to this difference in force-area curves, the surface viscosity-area relations show a similar difference in limiting areas.

It should be noted that spreading of very coherent polymers raises problems (Crisp (1958), Davies and Rideal (1963, Chap. 5)). Isemura and co-workers in their studies (quoted in "Synthetic Polypeptides") were often able to attain good spreading with the inclusion of dichloroacetic acid (DCA) in the

2. Poly(α -methyl-L-glutamate) has $A_0 = 9.8A^2$ on H_2O , but $14.7A^2$ on formic acid (i.e., they get a transition to what looks something like the β structure at $14.7A^2$ with formic acid, in agreement with solution studies).*

3. Poly(γ -benzyl-DL-glutamate) has A_0 of $24A^2$ /residue as compared with a calculated area of $22.4A^2$ for an α -helix.

4. Poly(γ -benzyl-L-glutamate) gives $21A^2$, as compared with a calculated area of $21.8A^2$ for α -helix.

5. For several of the following, steep parts of the force-area curve at $\sim 24A^2$ (which may signify α -helix) were noted (Fig. 1):

$$\text{for poly(DL-amino)} \left\{ \begin{array}{l} \text{caproic acid (C-6)} \quad 14.6A^2 = A_0 \\ \text{caprylic acid (C-8)} \quad 14.7 = A_0 \\ \text{capric acid (C-10)} \quad 14.8 = A_0 \\ \text{lauric acid (C-12)} \quad 14.7 = A_0. \end{array} \right.$$

These data show the possibility of an α -helical configuration reverting to β on greater compression. Bamford, Elliott, and Hanby (1956) also point out what may be an inconsistency in Cheesman and Davies' treatment (1954) of the interfacial potential data, namely, that Cheesman and Davies postulate that at high pH, at the air/water interface, the $>C=O$ groups are horizontal and so have a very small moment. But for poly-leucine, ΔV at the oil/water interface is even smaller, although the presence of the oil draws the nonpolar groups up and so must make the $>C=O$ groups point down. The inconsistency might be accounted for if the α -helix is considered, especially since A_0 is found to be $18A^2$, which is very close to the calculated value of $19A^2$.

Subsequently, Malcolm (1962) investigated the conformation of several synthetic polypeptides at the air/water interface. He found that poly(α -D-amino-n-butyric acid), poly(DL-leucine), poly(γ -methyl-L-glutamate), poly(γ -ethyl-L-glutamate), and poly(γ -benzyl-L-glutamate) at pH 2 all showed very little deuterium-hydrogen exchange, as at that time was expected for the α -helix; and the swept-off films showed the α type of IR spectrum when dried. We must keep in mind, however, that the spectra were taken of dried films, and changes of configuration may occur during the sweeping and drying.

Perhaps equally significant is the observation that these polymers show A_0 about equal to that calculated from models for the helix to within 10 percent (20 to $25A^2$ /residue). He also found that high pH and temperature led to increased exchange rates with eventual slow change to the β type of spectrum. Malcolm also found electrical dipole moments, which implies orientation of water structure, since only a small dipole moment is forthcoming from a helix lying on the surface with nonpolar side chains. He suggests that at the oil/water interface α -helices will also be found, since he expects an oil phase would be more conducive to helix formation. In addition to the fact that the monolayers studied spectrally by Malcolm were dried, one may also criticize the interpretation of the exchange studies. It has been shown (review by Schellman and Schellman (1964)) that deuterium-hydrogen exchange experiments may depend not only upon the relative helicity

*This was called an α - β transition. Low (1953) criticized this interpretation, because she feels that the α -helix should show an area $\geq 20A^2$ /residue. This has been answered by Isemura and Hamaguchi (1954) who state that the areas of the α and β forms should be proportional to their length and assume that the interchain packing is similar in the two configurations, but their opinion seems to be out of the mainstream of current thought. (Bamford, Elliott, and Hanby (1956).)

of the polypeptide main chain but also upon denial of access of solvent to hydrophobically bonded regions and that exchange is faster at higher pH. In the case of monolayer structures it remains unclear, as yet, what effect the presence of the surface and the orientation and polarization of the surface phase have upon the rates of exchange. Therefore, while Malcolm's work is strong evidence for the existence of the helical configuration, it is not yet conclusive.

As another alternative to these structures, we should note the position of Crisp (1958), who feels that polymers may collapse into a three-dimensional, essentially liquid-like state. He lists six arguments criticizing the packing of molecules into the β -like configuration at high pressures:

1. Since the $20\text{\AA}^2/\text{residue}$ configuration requires close packing of chains both above and below the plane of the polar amide groups (in the case of nonpolar side chains), the polar groups are expected to be shielded from the water. He does not feel such a configuration would lead to stable monolayers.

2. To put the side chains into their alternating up and down configuration requires submerging half of them. The plateau region which is supposed to be the transition between the large-area configuration (chains not oriented up and down) and the β -type configuration is characterized by a given surface pressure for a given chain length and temperature. It turns out that the required surface pressure is less for a long chain than for a short one, despite the fact that more energy is required to submerge a long chain.

3. Similar transitions occur in the poly(methylsiloxanes), for example, where there are no side chains. This fact suggests that other kinds of configurational changes than that in the β - α transitions may manifest themselves as plateaus in the force-area curve.

4. The surface potential is the product of the surface moment and surface concentration of such moments. If molecules are being aligned so that their surface moments become normal to the interface and their surface concentration is increased by compression, it is expected that the surface potential ΔV will increase. In fact, for poly(methacrylate) polymers which have similar force-area relations, the surface moment declines to zero over the plateau region, which argues against alignment and is what is expected for a substance entering into a three-dimensional condensed state. The argument may be carried over to the polypeptide case, although potential data for these polypeptides are, to this reviewer's knowledge, not available. Bull (1947) has mentioned a similar effect as protein monolayers are compressed.

5. Crisp further notes that the entropy of spreading, i.e., $d\pi/dT$, is negative at high π , which implies that compressing a compact film is creating a disordered structure. (The entropy of spreading at high area is positive, as would be expected for a loose film.)

Crisp also points out that an increase of π occurs halfway along the plateau; he ascribes this to the formation of a double layer, which has increased stability. This is also found in stearic acid at $10\text{\AA}^2/\text{molecule}$. Up to this point the collapse may be reversed, although with hysteresis. Schäfer (1949) also found this phenomenon and attributed it to breaking up of the film and immersing flakes.

6. Also observed was hysteresis on relaxation with plateau-forming polymers. Crisp attributes this to the difficult formation of a three-dimensional phase and a lag in removal from that phase. Langmuir and Waugh (1938), on the other hand, and Dervichian (1952) blamed immersion of hydrophilic parts of protein chains, as did Ellis and Pankhurst (1954a). In the case of stearic acid, striae appear after the "double layer" stage of compression has been reached. In some materials one sees an increase in pressure on collapse beyond this point. Crisp feels that the very high pressure phase is a collapsed overfilm which may be seen with a dark-field microscope.

Several of Crisp's arguments may be countered, at least qualitatively, without attempting to elucidate the structure of the highly compressed and collapsed films. His second point (involving this comparative free energy of immersion of a hydrocarbon chain) may be countered by noting that the cohesive energy in aligning the upper portion of the layer will be an increasing function of chain length. Further, the closely packed chains in the aqueous phase will present their CH_3 ends to the aqueous phase, which will not then be in contact with the entire chain, but with a surface whose extent is independent of chain length. In addition, the cohesive energy of these chains will also increase with chain length much as for those in the upper part of the layer.

His third point regarding transitions in other types of molecules, implies that some transition is occurring. It does not concern itself with what kind. His fourth point may be resolved into a discussion of the nonpolar chains and the polar parts of the molecule. It is not clear what effect the orientation of the nonpolar chains will have upon the molecular dipole moment; one must consider solvation especially. As for the polar groups of the molecule, two opposing views of the α - β transition are available. If the views presented by Cheesman and Davies (1954), Bamford, Elliott, and Hanby (1956), and Low (1953) are believed, then compression must correspond to conversion of the α form to the β form. In this case, compression results in alignment of dipoles and should increase any dipole moment. Conversely, Isemura and Hamaguchi (1954) feel that the opposite is true, that the α form has a smaller area. The helical form may very well have only a small dipole moment. A further complication is the fact that the different components of dipole moment observed cannot be resolved at the present state of knowledge.

Crisp's fifth point, regarding the signs of entropy of spreading, may perhaps be interpreted in terms of solvation; the highly compressed form may have increased entropy if water of hydration is released.

The sixth point concerns itself with the difficulty of making and breaking a compact structure and may have a large number of possible interpretations.

This leaves point 1 and the polar part of 4 — namely, that in the proposed β configuration the polar parts of the molecule may not come in contact with the aqueous phase, and also that if the β configuration is correct, there is no reason for the resultant dipole to vanish, although this latter point is questionable without further measurements.

The effect of ionic strength on uncharged polymers is expected to be small, and this is found to be true in comparison with the electrolytic polypeptides.

The effect of urea on hydrogen-bonding materials has been investigated very extensively. It has long been recognized that urea, with two amine groups and a carbonyl group, may function as a competitive reactant. Urea has been especially popular among protein chemists because of its close resemblance to a peptide structure. In recent years, the hydrogen-bond-breaking function of urea has been questioned; the action of this reagent is possibly related to weakening of what are called hydrophobic interactions, which are visualized as association of hydrocarbon, or other hydrophobic groupings, to avoid dispersal in an aqueous phase. Some experiments in which urea has had an effect upon the critical micelle concentration of detergents have been interpreted from this point of view, as has a study of the solubility of protein analogs in urea solution by Tanford (1964). A number of works are cited by Scheraga (1964). In the case of monolayers of the straight-chain poly(α -amino acids), and poly(α -benzyl-L-glutamate), Isemura, Hamaguchi, and Kawasato (1955) found that the limiting area increased with urea concentration (Figs. 4a, 4b). This is reflected in the slopes of the πA versus π curves, indicating a larger co-area. The shapes of the force-area curves were not significantly affected, although they were shifted to higher area. The shift may be due to an increase in limiting area because urea may bind to whatever structures exist in the monolayer (Bamford, Elliott, and Hanby (1956)). Binding of urea to peptide groups is also considered to take place by Steinhardt and Beychok (1964).

NRL Report 6318

Surface Chemistry of Proteins and Polypeptides

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November 30, 1965



U.S. NAVAL RESEARCH LABORATORY
Washington, D.C.

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ABSTRACT

This report is a review of recent literature in the field of polypeptide and protein surface chemistry, concentrating on the last decade. Theoretical approaches to polymers at liquid interfaces are considered, and surface measurement techniques are briefly outlined.

The various classes of polypeptides are considered, followed by a discussion of proteins in general and specific proteins in particular. Enzymatic activity, structure, and adsorption at various surfaces are considered also.

PROBLEM STATUS

This is an interim report; work on the problem is continuing.

AUTHORIZATION

NRL Problem C02-18A
RUDC-4B000/652-1/F101-99-01

Manuscript submitted June 30, 1965.

SURFACE CHEMISTRY OF PROTEINS AND POLYPEPTIDES

INTRODUCTION

This report is a review of certain aspects of the interfacial properties of proteins and synthetic polypeptides. These polymeric materials are of importance from a variety of viewpoints. To the biologist, they represent a class of natural products intimately associated with the vital processes of all viable organisms whose cells are of microscopic and submicroscopic dimensions. As each cell and organelle has its share of membranes, it is evident that the surface properties of natural products are important to the functioning of the organism. To the physical and polymer chemist, the fact that proteins and synthetic polypeptides may exist in well-defined conformations presents the opportunity to study the effects of the highly asymmetrical environment at an interface upon such structures. Since the amino acid residues found in proteins include highly nonpolar side chains combined with a polar backbone and other highly polar chains, it is to be expected, and indeed is found, that these polymers are highly surface active, making them important in technology for applications requiring stable foams and strong adsorption at interfaces. Conversely, their strong surface activity may hamper certain operations in which proteins are required but where adsorption and foaming are undesirable.

Previous reviews of this field have been published by Bull (1947), Rothen (1947, 1956), Cumper and Alexander (1951), Cheesman and Davies (1954), Fraser (1957), and in the very recent book of Davies and Rideal (1963) which contains some material dealing with protein surface chemistry. A description of some techniques is given by Sobotka and Trurnit (1961).

SURFACE PRESSURE, EQUATIONS OF STATE, AND MOLECULAR WEIGHTS AT INTERFACES

A fundamental parameter characterizing the interaction of a surface-active substance with the interface between two bulk phases is the change in surface tension produced at the interface by the presence of the surface-active compound. This difference in surface tension is measured in dynes/cm and may also be considered the surface pressure, because historically the similarity to a two-dimensional pressure is accentuated by the use of the Langmuir trough in its measurement. As surface tension is numerically equal to the surface free energy in ergs/cm², the surface pressure π is equal to the change in the surface free energy brought about by the presence of the surface-active agent.*

The surface pressure-area relation of compounds which form surface films may be analyzed by a method analogous to osmometry; an element of the apparatus confines a component of interest in a restricted region of the surface, while permitting other components free access to another region of the surface which is considered to be in a reference state. To expand the surface region on which the compound of interest is confined, we must place more diluent into that region, at the expense of the surface region which is free of the compound. The two regions may be separated, as in the case of the Langmuir trough, or the entire free surface may be covered, as is possible with the Wilhelmy balance, where the equilibrium is established with the bulk of liquid below the surface, whose state is considered to be in equilibrium with bulk liquid under a clean

*Surface pressure is often also denoted by F .

surface. Following Bull (1950) we may let the activity of the bulk solvent be A_0 and a be the activity on a clean surface in equilibrium with it. Then, for transfer of 1 mole of solvent from clean surface to film-covered area,

$$\begin{aligned} \pi A_1 &= A_1(\gamma_0 - \gamma_1) \\ &= RT \ln \frac{a}{a_0} \approx -RT \ln \frac{N_1}{N_0} \quad (\text{if we may take } a_i \approx N_i) \\ &= -RT \ln (1 - N_2) \\ &= -RT \ln \left(1 - \frac{n_2}{n_1 + n_2} \right) \end{aligned} \quad (1)$$

where the subscripts 1 and 2 refer to solvent and film molecules respectively, n_i and N_i denote the number of moles and the mole fraction of component i in a surface of area A , A_1 is the molar area of solvent, N_0 (the mole fraction of solvent in the clean surface) is equal to unity, and γ is the surface tension. If we now let the film be very dilute, we may expand the logarithm, and assuming that the total area $A = A_1 n_1 + n_2 \delta_2$, where δ_2 is the area of film occupied by a mole of film-forming substance,

$$RT \ln n_2 = \pi A_1 (n_1 + n_2) \approx \pi A_1 n_1 = \pi (A - n_2 \delta_2),$$

and Eq. (1) becomes

$$\pi A = RT \ln n_2 + n_2 \delta_2 \pi. \quad (2)$$

If we plot πA versus π , the intercept on the πA axis determines n_2 and the slope determines δ_2 . Note that δ_2 is the apparent area based on the assumption that the area of a mole of water is invariant with surface concentration. This is not necessarily correct. Also, the activity may be proportional to concentration only in the case of molecules of circular section (Davies and Llopis (1955)).

This treatment holds in the region of infinite dilution and is used to justify extrapolation of the curve of πA versus π to $\pi = 0$ to find molecular weight. However, we have no thermodynamically rigorous theory at those higher pressures where measurements are possible. Therefore, theories which must be tested by experiment have been developed and applied to polymer and protein systems with the purpose of enabling extrapolations to be made with confidence, and also explaining the general behavior of the force-area curve.

Purely Entropic Theories

Singer's Theory - The theories applicable to protein systems which have been recently in use are those of Singer (1948) and of Frisch and Simha (1956). Singer's treatment is based upon the Flory-Huggins development of polymer theory. It assumes that there exists at the surface or interface of the polymer solution a quasi-lattice of sites, each of which may be occupied or vacant. The entropy of the polymeric system occupying these sites in a given manner may be calculated by statistical methods, and the free energy may be derived by assuming that there is no intermolecular attraction or repulsion (Davies (1954b)). The resulting equation is

$$\frac{\pi A_0}{kT} = \left[\ln \left(\frac{A}{A - A_0} \right) + \frac{(X-1)}{X} \frac{Z}{2} \ln \left(1 - \frac{2A_0}{A} \right) \right]. \quad (3)$$

In Eq. (3), A_0 is the limiting molecular area per residue, A is the available area per residue (or monomer unit) in the chain. The parameter Z is the lattice coordination number of the chain segments, i.e., the number of possible sites on which the neighboring residues may be situated. For a rigid chain $Z = 2$, and the chain configuration is fixed. Flexibility may be defined as $100 (Z - 2)/Z = \omega$. This assumes that the upper limit of Z is 4, i.e., a "square lattice." Here X is the degree of polymerization or DP , k is Boltzmann's constant, and T is temperature.

If $X = 1$ or $Z = 2$, Eq. (3) becomes

$$\pi = \frac{kT}{A_0} \ln \left(\frac{A}{A - A_0} \right). \quad (4)$$

If $Z = (2 + \epsilon)$ where ϵ is a small number,

$$\pi A = kT + 1/2 \pi A_0. \quad (5)$$

This is Bull's equation, except for the factor $1/2$.

Plots of πA versus π for polymers and proteins are often linear at the air/water interface where the cohesion is sufficient for low flexibility. If Z is not close to 2, Eq. (3) predicts that the force-area curve will be convex upward. In the oil/water interface $Z = 2$, so we expect curvature which prevents good extrapolation.

The value of Z depends on charge and ionic strength through their effect upon the net cohesive energy; thus, flexibility may be reduced by changes in pH or salt concentration. When cohesion is appreciable, the system is described by an equation relating electrostatic effects to surface pressure, presented by Davies and Llopis (1955); the equation has been derived on a different basis by Isemura, Hamaguchi, and Ikeda (1957). A discussion is presented by Davies and Rideal (1963) and by Payens (1960).

Frisch and Simha's Theory - It is possible that the polymer may not be completely in the interface but may loop into one or both phases forming the interface, especially when compressed. This consideration leads to the relation

$$\pi = \frac{X kT}{A_0} \left[\ln \left(\frac{A}{A - p A_0} \right) - \left(\frac{p^2 A_0}{A - p A_0} \right) \right] - J p^2 \frac{kT A_0^2}{A^2} \quad (6)$$

where A_0 = close-packed area, and J is a coefficient allowing for lateral entropic and enthalpic interaction between those residues fixed in the interface. In this equation,

$$p = 2\alpha / (\pi f_i X)^{1/2} \quad (7)$$

where f_i is a function of internal flexibility of the molecule, X is the DP , and α is called the intramolecular segment condensation coefficient, which expresses the interactions within the polymer molecule. At low π Eq. (6) becomes (since $A \gg A_0$)

$$\pi A = X p kT. \quad (8)$$

This implies that molecules with large DP can form loops more easily and return to the interface to have units act as "independent" kinetic units, lowering their apparent molecular weights. This is not verified experimentally (Davies and Rideal (1963)), since for several proteins and copolypeptides the proper molecular weights are obtained at air/water and oil/water interfaces when the Singer equation is used at very low π (<50 millidynes). This does not preclude its applicability to other experimental situations involving other polymers and interfaces or higher pressures. In practice, distinguishing

between applicability of the Singer or Frisch-Simha theories on the basis of πA experiments is difficult (Jaffe and Loutz (1964), Dieu (1956 a, b, c)). Jaffe and Loutz have shown that both equations may be cast into the form of the virial equation

$$\pi A = kT (1 + B_1 \pi + B_2 \pi^2 + \dots) \quad (9)$$

and that using either Eq. (3) or (6) one can fit the force-area curves reasonably well by adjusting the parameters available. In both the case of the Singer equation and that of Frisch and Simha, however, Jaffe and Loutz point out that the choice of parameters may be ambiguous and that there is no guarantee that any of the parameters chosen bears a simple relation to the property of the polymer it purports to describe. Davies and Rideal (1963) have also indicated that Z should be regarded as an empirical factor because of the many oversimplifications of the theory. Jaffe and Loutz propose to use the function of the first two "virial coefficients" (B_2/B_1^2) to describe the polymer, empirically establishing an assignment of flexibility to values of the ratio. This parameter is independent of a model; insight into details of molecular structure is lacking as yet.

It has also been emphasized (Dieu (1956 a, b, c)) that both Singer's and Frisch and Simha's treatments have neglected intermolecular enthalpy effects. A term in A_0^2/A^2 may be introduced in an attempt to account for enthalpy of mixing in Singer's treatment (Davies (1954 b), Davies and Llopis (1955), Ter Minassian-Saraga and Prigogine (1954)), and the enthalpy of interaction of adjacent residues in the "surface plane" is allowed by Frisch and Simha. There has been at least one case where the Frisch and Simha theory seems to fit the data better than Singer's equation, the case of polyvinyl alcohol at a benzene/water interface (Dieu 1956 a). Dieu points out, however, that even in cases where the Frisch and Simha theory fits better than Singer's theory, values of Z are obtained which do not seem to fit expected chain properties. This may, at the present state of development, be attributed to the lack of recognition of enthalpy effects in the treatment.

In general, one may say that for molecules of low flexibility at the air/water interface one may obtain a good determination of molecular weight (Davis and Rideal (1963), p. 241, Dieu (1956 a, b, c), Davies (1954 b), Davies and Llopis (1955), Bull (1947)). High flexibility (induced by the presence of an oil/water interface or high charge on the macromolecule) or extensive intermolecular association (as in polyleucine) renders extrapolations of the force-area curve uncertain. Jaffe and Loutz (1964) have shown that with flexible polymers, even in the extremely low pressure range below 0.04 dyne/cm, one cannot get a good extrapolation for determining molecular weights. The fact that πA versus π plots for many proteins are indeed linear at the air/water interface lends credence to the notion that not much intermolecular interaction occurs in these systems, although this is not conclusive. Surface rheology studies (Cheesman and Davies (1954), Imahori (1952 a-d), Davies (1954 a, b)) to be described later will also have a bearing on this point. A summary of results of molecular weight determination is given by Cheesman and Davies (1954) together with a discussion of elasticity determinations based on Singer's equation. Subsequent data are listed in papers by Dieu (1956 a-c), Jaffe and de Coene (1957), Harrap (1955 a, b), and Ter Minassian-Saraga (1956).

An illustration of the effect of these ambiguities is the study by Davies and Llopis (1955) of the copolymer 1:1:2 lysine-glutamic acid-leucine. They found that at neutral pH at the air/water interface they could easily get a linear πA versus π plot which gave the correct molecular weight. At extremes of pH, they were forced to very low pressures to overcome the curvature resulting from flexibility induced by charging the molecule. At the oil/water interface, they observed effects very much like the dissociations reported for proteins, and only when the measurements were carried to extremely low π was it clear that the correct molecular weight would be found at $\pi \rightarrow 0$. They point out that, because of the many approximations in the theory, no absolute significance should be attached to the values of Z .

The importance of taking enthalpy into consideration is strikingly illustrated by the fact that the temperature dependence of π is much greater than the proportionality to absolute temperature indicated by both theories (Dieu (1956 a-c), Llopis and Rebollo (1956), Llopis and Albert (1959 a, b)).

Davies and Llopis (1955) have indicated that an energy of interaction may be included in the value of the flexibility ω by writing $\omega = \omega_0 e^{-E/RT}$, which shows that flexibility may change with temperature. This is equivalent to an energy of interaction because it biases the coordination number, $Z = \omega + 2$, in favor either of a greater number of polymer-solvent contacts or of polymer-polymer contacts, depending on the value of E .

Another indication that the Singer and Frisch-Simha theories are not adequate is the observation by Bull (1950) and Dieu (1956 a-c) that the πA versus π curves for proteins are often neither linear nor monotone curves, but show two linear portions separated by a kink. Each linear portion may be extrapolated to yield the same molecular weight. This is taken as evidence of the existence of at least two surface states (Bull (1950), Dieu (1956 a-c)). Experiments on synthetic polypeptides (Isemura and Hamaguchi (1952, 1953)) also suggest this possibility, which will be discussed further when considerations of surface configuration are undertaken (see "Nonpolar Polypeptides"). However, Cheesman and Davies (1954) interpret the kink as evidence of an interaction, saying it shows "stickiness" of the molecules.

Theories Involving Energy of Interaction

Silberberg's Theory - Silberberg (1962) has developed a theory for adsorption of an isolated, flexible molecule at an infinite planar surface. It is assumed that for each segment placed at the surface the internal energy of the system is reduced by an adsorption energy wkT . It is then shown that the polymer molecule will adjust itself so that alternate runs of P_S segments are all in the surface and loops of P_B segments whose size is not a function of molecular weight are all out of the interface. For a molecule composed completely of adsorbable units, on a surface which is everywhere adsorbing, the loops are small, and even at small w the molecules stay close to the surface. Otherwise, the loops are much larger. Restrictions limiting the re-entry of an adsorption loop into the surface have a similar effect. The result is a thin bulk phase of high polymer concentration near the surface. The molecules of polymer are rather flattened, with about half of the segments in the surface phase in some cases. The inability to wash the polymer from the interface (i.e., apparent irreversibility), the similarity of its isotherm to that of Langmuir, and a slow approach to equilibrium are predicted. The surface equation of state derived covers the limiting cases in the literature. Although the treatment conforms with the Gibbs isotherm, the apparent irreversibility of polymer adsorption is explained by the prediction that the adsorption will be very strong even at extremely low solution concentration. The surface equation of state in the limit of low θ is

$$A_0 \pi = kT \left(\frac{\theta/p}{X} + \frac{\sigma^2}{2} \beta_s \right) \quad (10)$$

where X is the number of segments per polymer molecule, θ is fraction of surface sites occupied by polymer segments, β_s is a complicated function, and p is a function of polymer segments at the surface.

It is a consequence of the model that the ends of the molecule stay in the surface.

Motomura and Matuura's Theory - Motomura and Matuura (1963 a,b) have also recently derived a theory which takes the energy of interaction into account and which reduces to the Singer theory when the energy is negligible. This equation also predicts phase changes in the films if the temperature is low enough and energy large enough.

The force-area curve is almost independent of the degree of polymerization χ if χ is sufficiently large (i.e., 7300). Their equation is

$$\frac{\pi}{\pi_0} = \frac{Z}{2} \ln \left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \frac{A_0}{A} \right] - \ln \left[1 - \frac{A_0}{A} \right] - \frac{Z}{2} \ln \frac{\left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \frac{A_0}{A} \right] (\beta_M + 1) - 2 \left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \right] \frac{A_0}{A}}{\left(1 - \frac{A_0}{A} \right) (\beta_M + 1)} \quad (11)$$

where $\pi_0 = kT/A_0$, A_0 is the area occupied by a monomer unit, Z is the coordination number of a site in a two-dimensional surface lattice, χ is the degree of polymerization, and β_M is defined by the complex function

$$\beta_M^2 = \left\{ \frac{\left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \right] \left(1 - \frac{A_0}{A} \right) \frac{A_0}{A}}{\left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \frac{A_0}{A} \right]^2} (e^{2w/ZkT} - 1) + 1 \right\} \quad (12)$$

in which $-2w/Z$ is the energy of contact between neighboring monomer units.

These authors point out that the success of Singer's equation may be due to the fact that Z in Singer's theory behaves similarly to the energy term. They do not account for the possibility that some chains may loop into the bulk phases.

The quantity π/π_0 may exhibit a maximum and a negative minimum when plotted against A/A_0 . This is characteristic of a two-phase film and occurs when $w/kT > 0.864$.

Motomura and Matuura examined poly(vinyl acetate), poly(methyl acrylate), and poly(methyl methacrylate), i.e., PVAc, PMA, and PMMA. In the case of PVAc a value of w was obtained which allowed a good fit to the theory, assuming $Z = 4$, below 6 dynes/cm. Above this pressure, deviations occurred which were attributed to squeezing out of chain segments from the surface.

Molecules of PMA are less expanded as w/kT is higher, and the theory fits better. This is expected, because the lattice approximation should not be as valid for expanded films.

In both the PVAc and PMA cases, $w/kT < 0.864$, so that phase changes were not expected. For PMMA, $w/kT > 0.864$ and the film is of condensed type; it shows vanishingly small π at large areas, with π becoming measurable at ca. $30A^2/\text{monomer}$. The predicted curve becomes negative at $23.5A^2/\text{residue}$; below this the theory predicts a phase transitional region, which is supported experimentally by a fluctuation of the surface potential.

Studies of these films showed that the higher the value of w/kT , the more viscoelastic the film, as would be expected. A similar parallelism between viscoelasticity and cohesive energy was found in studies on bovine serum albumin (BSA) and gamma globulin. The ΔH of spreading and area of onset of non-Newtonian viscosity increased with pH in the case of BSA, while both parameters were independent of pH in the case of gamma globulin (Llopis and Albert (1959 a, b)).

While these results are very promising, especially since phase transitions may be handled, it is this reviewer's feeling that more data must be forthcoming before the newer theories may be fully evaluated.

Electrostatic Effects on the Force-Area Curve

The effect of charge upon the force-area curve of polymers may be separated into intermolecular and intramolecular effects. If the polymer molecules are charged, then repulsion of like charges will decrease the net cohesion of the polymer molecule.

If one writes the flexibility ω as

$$\omega = \omega_0 e^{-E/kT} \quad (13)$$

where ω_0 is the flexibility in the absence of cohesive effects (i.e., at the oil/water interface and at zero charge), electrostatic effects will appear in the parameter E , as will cohesive energy. At very high area where intermolecular effects may be neglected safely, the oil/water interface in the isoelectric region allows the establishment of a base curve for which $E = 0$; electrostatic (and cohesive) contributions to the force-area curve at the air/water interface may be obtained by subtraction. One thus obtains the "excess electrostatic surface pressure" (π_{el}) experimentally (Davies and Llopis (1955), Payens (1960), Davies and Rideal (1963), Chapter 5).

The interfacial potential Ψ may be calculated according to the Gouy theory, and one may write

$$\pi_{el} = \int_0^{\Psi} \sigma d\Psi' \quad (14)$$

where π_{el} is the electrostatic contribution to the surface pressure, and σ is the charge density of the monolayer. This leads to

$$\pi_{el} = 6.1 \sqrt{C} \left\{ \cosh \left[\operatorname{arc} \sinh \left(\frac{134}{\sqrt{C}} \right) \right] - 1 \right\} \quad (15)$$

(Payens (1960), Davies (1951)). Here, C is the concentration of 1:1 electrolyte. Intermolecular contributions, which may be evaluated at higher pressures, are evident since the apparent potentials decrease with area faster than indicated for isolated charges of the valency of the polymer. The contributions of the intermolecular and intramolecular effects may thus be considered as contributing to the effective potential. An effective charge may be derived by fitting such data.

The value of Z may be related to the electrostatic effect through the relation

$$Z - 2 = \omega = \omega_0 \exp \left(\frac{-E_{\text{cohesion}} + \pi_{el} A^*}{kT} \right) \quad (16)$$

and the value of A^* derived from the slope of a plot of π_{el} versus $\log \omega$. The value of A^* so obtained is considered to be the area of the minimum kinetic unit (Joly (1948)) in the polymer in the course of its molecular motion.

The effective charge perturbing the πA curve in this study was found to be 20 percent of the true value of the macromolecular charge and corresponds to the estimate of the charge expected to be at the periphery of the molecule, with the rest of the charge

screened by counter-ions. The size of the kinetic unit appears to be that of five residues, a small fraction of the size of the molecule.

Thermodynamics of the Force-Area Curve

One may attempt to characterize the degree of disruption of molecular structure when a molecule is spread by the value of the free energy change on spreading. This is not a clear-cut analysis as yet, because it is not known how to resolve the observed change on spreading into its components corresponding to changes in solvation, polarization of the interfaces, changes in solution structure, and so on. Neglecting these matters, attempts have been made to compare the entropies of unfolding for various substances. Davies (1953 c) [Cheesman and Davies (1954)] used the following approach.

If one accepts the model of Singer (1948), one can calculate the entropy S_s of a spread polymer with respect to a reference state in which the polymer backbone chain is rigid from the high area portion of the force-area curve. This quantity was given by Davies (1953) as

$$S_s = \left(I - \frac{2}{X} \right) R \ln (Z - 1) \quad (17)$$

using the same notation as in the section titled "Singer's Theory."

The entropy change on compression of the film between any two areas can be calculated if one assumes that the free energy change has only an entropy term and no enthalpy term. Subtracting from this the entropy change which would be found for a completely rigid molecule undergoing expansion or compression between the same areas gives

$$\Delta S_s = \frac{1}{XT} \int_{A_1}^{A_2} \pi dA - \frac{1}{XT} \int_{A_1}^{A_2} \pi_r dA. \quad (18)$$

The entropy of the molecule at any area can be calculated relative to a reference state of the rigid main chain by evaluating π_r , the predicted value for a rigid chain (i.e., $Z = 2$). The Singer theory allows this to be done if the effect of side chains is neglected. Accepting this limitation, we may determine the entropy per residue at the limiting area relative to the rigid reference state.

It turns out for proteins (Davis (1953 c), Cheesman and Davies (1954)) that the experimental unitary entropy of expansion from the limiting area to high area is equal to the calculated unitary entropy of the molecule at high area relative to the rigid molecule reference state.

But this is not true in the cases of polyalanine and polyleucine, for which the unitary expansion entropy is less than that calculated from the Singer theory. (This implies that the limiting area in the case of these peptides does not correspond to a rigid molecule, while the rigid molecule at limiting area is consistent for the proteins.)

The value of S_s calculated in this way may be considered the entropy of surface denaturation if the Singer model of copolypeptide monolayers is accepted and if the entropy of the reference state chosen is indeed the same as that of the native molecules. For example, the experimental data indicate that, for methemoglobin at the oil/water interface, $Z = 2.12$, corresponding to $S_s = +0.22$ eu, while for polyalanine and polyleucine one has $S_s = +1.6$ eu through use of Eq. (18).

Davies states that the values of s_s are similar for most proteins and makes a comparison with the thermal denaturation entropy of trypsin, which is +0.76 eu. The inference is that a number of linkages modified by thermal denaturation are not destroyed by surface denaturation. Other evidence leading to the conclusion that surface denaturation is not complete (found in sections titled "Lysozyme," "Ovalbumin," and "Structural Alteration of Spread Films") makes the argument more convincing, since the validity of this type of argument rests upon Singer's treatment, now known to be inadequate. This approach may be refined using the more satisfactory treatments of the theory of the force-area curve. At this stage of development, the treatment measured a quantity which may be more closely related to the free energy than to the entropy of the transition. We may cite some of the evidence which has been obtained by heating solutions and monolayers. Kaplan and Fraser (1953) found that fibers formed from protein monolayers were quite different when formed from native spread proteins instead of denatured ones, and that if proteins were heated prior to spreading the fibers formed were of the denatured type. Trapeznikov (1948) found a change in surface viscosity η_s at the temperature of bulk denaturation. These observations indicate that the properties of the film are dependent upon elements of structure which are affected by denaturation.

A second approach to the thermodynamic parameters of the polymeric monolayer is the study of the systems at different temperatures. Llopis and Albert (1959 a, b) have done this for bovine serum albumin and globulin. In both cases the enthalpy of spreading on acid at low temperature is negative, i.e., favors spreading. At higher temperatures the enthalpy term is positive. On alkaline substrates, cohesion always predominates. More work on the thermodynamics of the process is required for full understanding, but it is already clear that Singer's equation will have to be improved upon, as will the theory of Frisch and Simha, to take enthalpy effects into account. The theories of Motomura and Matuura (1963 a, b) and of Silberberg (1962 a, b) have not had sufficient application to be adequately judged.

These approaches are not adequate to evaluate the surface denaturation process, because they pertain to the already spread material. Comparisons based on Eq. (18) depend upon the assumption that the molecules at the point of minimum compressibility, or the collapse pressure, are in a state whose entropy corresponds to the solution or solid state. Although there are indications that this may be true (Benhamou (1954), MacRitchie (1963)) (as discussed later in the "Collapse" section), the issue is not settled. It would appear to be more suitable to measure the equilibrium involved in the adsorption from solution to the surface if such an equilibrium could be established. Guastalla and Benhamou (1961) attempted to evaluate the equilibrium parameters in such a situation and found that hemoglobin has too high a molecular weight to be suitable for the experiment; insulin, which is much smaller, appeared to be on the borderline of feasibility.* The data on the entropy and enthalpy of expansion of the already spread monolayer, in addition to the data from the adsorption and spreading steps, are required for a complete description of the surface denaturation process.

SURFACE PARAMETERS AND TECHNIQUES AT FINITE PRESSURES

Molecular weights are studied in the low-pressure region of the force-area curve, where properties of the molecules at the surface may be studied in a highly dilute state. In the highly expanded state, the theoretical attempts to describe molecular behavior have led to estimates of the intrinsic flexibility and size of the solute molecules in what is hoped to be a situation essentially free of intermolecular interaction. When one

*A study of this process with a series of polypeptides as a function of molecular weight would be useful.

compresses a film so that the molecules interact, a different type of information is forthcoming. Experimental parameters, other than the surface pressure will now be considered.

Interfacial Viscosity and Viscoelasticity

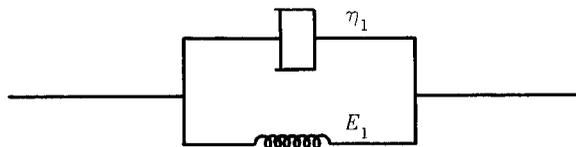
A review of interfacial viscosity and viscoelasticity was presented by Joly (1964). Polymer monolayers may be classified, rather empirically, by interfacial viscosity properties. One finds that condensed films and expanded films have quite different viscosity-area curves. In the case of the condensed film, one finds a high viscosity at areas larger than the limiting area found from a πA experiment. For expanded films, we find that the interface viscosity η_s drops outside the range of high surface pressures (Isemura and Hamaguchi (1954)).

A high surface viscosity would seem to imply intermolecular interaction. This may come about mechanically through chain entanglement (Davies (1953 c)) or through chemical interaction (Isemura and Hamaguchi (1954)), e.g., electrostatic salt linkage, hydrogen bonds. High surface viscosity at high area signifies that a network of some sort is formed by condensed films between molecules before compression sufficient to pack them tightly is reached. In contrast, expanded films show a large increase in interfacial viscosity only when the area is reduced to the limiting area of the force-area curve (Isemura and Hamaguchi (1954)). This implies that the repulsion effects which keep the film expanded also serve to inhibit chemical or mechanical intermolecular interactions at areas larger than the close-packed area.

Isemura and Hamaguchi (1954) emphasize the role of electrostatic interactions in producing surface viscosity, while Davies (1953 c) interpreted the increase of film viscosity of polypeptides at an oil/water interface as evidence that a loss of intramolecular cohesion allows sufficient interaction to occur at higher area, as chains are compressed. Increase of η_s at high charge may also be the result of expansion (see also Joly (1964)). A theory of viscosity based upon the general theory of rate processes has been formulated by Eyring and coworkers (Glasstone, Laidler, and Eyring (1941)). Conclusions reached from a study of activation areas are cited in the "Proline and Hydroxyproline" section. Ikeda and Isemura (1959) found that a strongly cohesive condensed polymer exhibits an area of activation which extends over the whole molecular area, while for expanded films with weaker cohesion, the area corresponds to only a small section of the molecule. Earlier, Joly (1948) had observed, also on the basis of the Eyring theory, that since all proteins seemed to have approximately the same viscosity coefficient at the onset of non-Newtonian viscosity, the kinetic subunits of the protein monolayer must be of similar area. Calculation of the activation energy of flow led to a value of $90A^2$ for this unit. Joly's treatment relies upon (a) an estimation of the contributions of the various components of the molecule to the interaction energy and (b) the deduction that the spacing of the kinetic units at the onset of non-Newtonian viscosity is such as to let the width of an assumed hexagonal pseudolattice be twice the diameter of the kinetic unit (assumed spherical). Ikeda and Isemura do not give the details of their calculation. The strongly cohesive polymers of poly(phenylalanine), polyleucine, etc., have a much higher density of nonpolar amino acids than the proteins.

An alternative method of analysis of viscoelasticity in films is shown in the series of works treating the rheology of materials as represented by a mechanical model. The mechanical analogy is a system of springs and dashpots; the springs represent elastic elements, and the dashpots, viscous elements. These elements may be combined in various ways, such as:

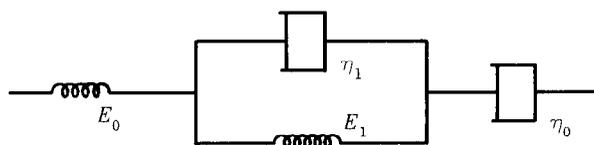
1. Voight Element:



2. Maxwell Element:



3. Four-Parameter Model:



In an appropriate mathematical analysis of the result of a stress-strain measurement, where strain is followed as a function of time, and the stress is permitted to decay, the parameters of such models, i.e., the E_i and η_i , may be evaluated. Such a treatment is given by Motomura and Matuura (1963 b), as well as by others. The relation between the type of model, the values of the constants, and what goes on in the actual film is still obscure. One may say that the existence of elastic elements implies the presence of a network of some kind in the film, and Tachibana and Inokuchi (1953) have found a relationship between kinks or plateaus in the force-area curve and the onset of instantaneous elasticity (i.e., nonzero E_0). They have also postulated that Hookean elasticity depends upon the strength and exchange rate of network bonds.*

The last paragraph of the paper by Motomura and Matuura indicates the type of conclusion which may be drawn. After showing that the viscoelastic parameters were larger for PMMA than for PMA, and were smallest for PVAc, they wrote: "In the preceding paper, the value of w/kT , which was related to the cohesive force between monomers, was estimated for the three polymers, and it was found that the order was PMMA > PMA > PVAc. The present result might show a parallelism between the values of viscoelasticity and w/kT . It seems proper from this point that linear polymer monolayers are made up of a two-dimensional network structure produced by extended polymer molecules and their rheological behavior is determined mainly by the cohesive force between segments"

Polymer films may, to some extent, be grouped according to the mechanical model describing them (Tachibana, Inokuchi, and Inokuchi (1959)); group A has an elastic element only. It is suggested that films of this type form a two-dimensional network and

*However, they have since shown that they can eliminate instantaneous elasticity without changing the force-area curve by use of an organic spreading solution (Tachibana, Inokuchi, and Inokuchi (1957)).

become elastic before the spread molecules are closely packed. Group A does not show viscoelasticity until the gel point is reached, while group B (the four-parameter model) gradually becomes viscous and begins to show viscoelasticity only when a multilayer is formed by compression.

It is important that large stresses not be imposed on the films during the measurement, since some interactions are very easily broken (Cheesman and Sten-Knudsen (1959), Tachibana and Inokuchi (1953)).

The molecular weight of flexible chain polymers, which cannot be derived from π - A relations, may be derived from the η_s - A curve on an empirical basis in the cellulose acetate, PVAc, and poly(vinyl alcohol) (PVA) series, where the relation

$$(\mu) = AM^\alpha \quad (19)$$

has been tested by Jaffe and Loutz (1958). They find, for PVA, $A = 10^{-7}$ and $\alpha = 1.2$; for PVAc, $A = 10^{-4.5}$ and $\alpha = 0.63$; for cellulose acetate, $A = 1.26 \times 10^{-7}$ and $\alpha = 0.97$. Here, (μ) is the limit at zero concentration (i.e., $\pi \rightarrow 0$) of the reduced surface viscosity coefficient

$$\mu = \left(\frac{\Delta_f}{\Delta_w} \right) \left(\frac{1}{C} \right). \quad (20)$$

In Eq. (20), Δ_f and Δ_w are the slopes of the plots of the log of the amplitude of an oscillating bob in the surface against the number of swings for film-covered and clean water surfaces, respectively. This is analogous to the Staudinger relation for bulk intrinsic viscosity. When a periodic disturbance is used, the motion of the sensor may be analyzed in terms of a complex modulus, whose real and imaginary parts correspond to the elastic modulus and viscosity (Tschoegl and Alexander (1960a, b)).

It was shown by Boyd and Vaslow (1958) that surface viscosity may be a sensitive detector of interaction in surface films. If one makes a mixed film which forms a two-dimensional solution, then

$$\log \Phi = X_1 \log \Phi_1 + X_2 \log \Phi_2 + X_1 X_2 \quad (21)$$

where $\Phi = 1/\eta_s =$ fluidity and X is an interaction parameter. This equation assumes approximate equality of molecular areas. In the case of protein interaction, Joly (1948, 1949) concluded that proteins when spread may be considered as aggregates of kinetic subunits, and the subunits were about $90A^2$ for all the cases considered. In such a case, the value of η_s may be a better indication of film interaction than the perturbation of force-area curves (Joly (1964)).

The measurement of surface viscosity is not a simple problem. The various instruments used do not in general yield results which are in agreement, and the instrumental setups to be used depend upon the nature of the film. A review of this topic was given by Jarvis (1962), and discussions are also presented by Davies and Rideal (1963, Chap. 5) and by Joly (1964).

The possible relationships of hysteresis of the force-area curve on rapid cycling, viscoelasticity, and the degree of spreading are discussed further in the "Ovalbumin" section.

Interfacial Potential

The measurement of interfacial potential is carried out with either a vibrating electrode or a radioactive electrode. The vibrating electrode functions as one plate of a capacitor, and the other plate is the interface in question. The capacity is determined by rapidly varying the distance between the electrode and the interface and measuring the current flow, or ac signal, across this element of the circuit. This procedure was pioneered by Yamins and Zisman (1933). Another method, which has become popular in recent years because of the increased availability of sources of ionizing radiation, employs the "radioactive electrode." In this case, air between a metal electrode and the interface is rendered conducting by ionizing radiation from a radioactive source in the metal electrode; the potential is measured with respect to a reference electrode immersed in the hypophase. In either case, the quantity of interest is the change in surface potential resulting when an interfacial layer is placed at the previously clean interface.

Interfacial potentials are measurable and stable in the instance where the concentration and mobility of charge on at least one side of the interface are sufficiently low that the interfacial potential is not screened from the sensing electrode by interposition of a net space charge between the electrode and the interface. Such a situation exists when the solubility of ionized material is too small to screen the interfacial charge, as at a gas/condensed phase interface or when a very nonpolar material such as paraffin oil is one bulk phase. In the case of more polar oils, one may not get a stable potential indication if the concentration of ionic species is large enough to reduce the thickness of the ionic double layer to a dimension of the order of the distance of the sensing electrode from the interface (Davies and Rideal (1963, Chap. 2)).

When a monolayer, or adsorbed layer, situates itself at an interface of the type described, the change in potential observed with respect to the potential of the clean interface may be considered to result from a number of components. Davies and Rideal consider convenient the following resolution of the potential into components. The observed potential change ΔV is related to the interfacial dipole moment by the Helmholtz relation for an uncharged monolayer:

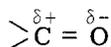
$$\Delta V = 4\pi n \mu_D \quad (22)$$

where n is the number of molecules of the film-forming substance per unit area of interface and μ_D is the vertical component of the dipole moment per molecule of such substance.

One may resolve μ_D into three components: μ_1 , corresponding to reorientation of solvent molecules caused by the presence of the material placed on the clean surface; μ_2 , corresponding to alignment of the dipoles of the polar end of the material; and μ_3 , corresponding to any unbalanced dipoles of the nonpolar or hydrophobic part of the molecule. The component μ_3 is included because even a nonpolar group, when lined up at an interface, may exhibit a permanent dipole resulting from the asymmetric condition existing at the end of the nonpolar portion of a molecule, e.g., as does the CH_3 group at the end of a paraffin chain. In addition, an ionized head group may contribute a component termed χ_0 caused by counter ions near the surface. The result is then

$$\Delta V = 4\pi n [(\mu_1 + \mu_2 + \mu_3) + \chi_0] = 4\pi n (\mu_D + \chi_0) \quad (23)$$

Study of the surface potential is helpful in determining the orientation of polar groups in the monolayer. The amide group contains the



dipole, which is the major contributor to μ_D of a nonpolar amino acid residue. If the oxygen atom points downward into the aqueous phase, we should therefore expect a positive value of μ_D .

Two papers have recently appeared which must be taken into consideration when performing or interpreting measurements of surface potential. Chambers (1963) has observed that a radioactive electrode will cause changes in the film adjacent to it; therefore, the electrode should not be used for any length of time at a single location. He has presented data showing the magnitude of the effect. Secondly, L. Ter Minassian-Saraga (1956) has pointed out in a review article that the presence of an electrode, even if not radioactive, will have an effect upon the film directly under it. She, therefore, recommends that when measuring ΔV in a transition region one should work quickly, as far as possible from the surface, and with an electrode as large as the film area, although this will eliminate detection of islands.

Microelectrophoresis

The microelectrophoretic technique (reviewed by Fraser (1957)) is of value in studying protein adsorption on solid particles or on droplets of emulsion. Studies of oil emulsions stabilized with lipids allowed evaluation of the charge on emulsion particles, which in turn allowed correlation of adsorption and clarification with charge as explained below (see "Emulsions"). Recently, Bull and co-workers, and Benhamou, de Mende, and Guastalla have examined several systems. One of the uncertainties in this field appears to be that adsorbed proteins have mobilities similar to those of dissolved proteins. Bull (1958) has shown, by means of the ionic strength dependence of the mobility of BSA and ovalbumin adsorbed on glass, that the effective radii of the particles correspond to very large particles and not to particles of the dimensions of the protein molecule. The apparent charge was found to be smaller than that calculated upon the basis of a proton titration, however. Bull reconciled results by postulating adsorption of the protein in a manner such that much of its charge points inward toward the highly polar glass surface, reducing the charge density on the outer surface.

Further studies of proteins adsorbed on various adsorbents have been made; however, microelectrophoresis does not appear to be on sufficiently solid footing as yet, either theoretically or experimentally, to make general statements concerning interpretation of the data within the scope of this review. The reader is referred to the papers of Barnett and Bull (1959), Chatteraj and Bull (1959), and Benhamou, de Mende, and Guastalla (1961).

Transferred and Adsorbed Monolayers on Solids

The Langmuir-Blodgett technique of transferring spread films from liquid surfaces to solid surfaces has proved to be useful and has led to a series of analyses which have helped to elucidate the structure and reactivity of monolayers. Descriptions of the technique have been given in recent reviews (Sobotka and Trurnit (1961), Rothen (1956)). Essentially, a layer from the liquid surface may be transferred to a metal or lipid-coated metal slide with an area which in some cases is very close to the area of the original monolayer; in other cases it is not, depending upon the surface pressure to which the film is subjected during transfer. The transfer of a monolayer may occur when a plate of glass, metal, mica, or a substance coated with paraffin or a fatty acid or other lipid film, is passed through the monolayer while the required surface pressure is maintained.

In the case of protein films, which are highly compressible and elastic, the thickness of a transferred film depends on the pressure to which the film is subjected during transfer. If the pressure is in the range of 2 to 6 dynes, the thickness of the transferred protein film is close to the thickness before transfer. For incompressible films, the transfer ratio is close to unity; for elastic films, such as proteins, the transfer ratio is variable with film pressure. Proteins may also be adsorbed directly from solution onto a plate immersed within the solution.

In either case, the plates may be coated prior to transfer or adsorption of the protein film with a layer of a dielectric material, most often barium stearate, which serves as an aid in determining the thickness of film and also may serve to modify the nature of the surface of the plate.

Films adsorbed from solution are usually thicker than transferred monolayers. Both the adsorbed and transferred films may be analyzed for enzymatic activity or susceptibility, and the optical thickness and refractive index may be measured. The case of bovine serum albumin has been analyzed in detail, and the results (summarized by Bateman and Adams (1957) and Bateman (1959)) indicate that the differences in the structures of adsorbed and of transferred films are reflected in the thickness and refractive index of the films:

| Type of Film | Refractive Index | Thickness (A) | Mass (mg/m ²) |
|--------------|------------------|---------------|---------------------------|
| Transferred | 1.5-1.6 | 9.1-9.6 | 1.1-1.2 |
| Adsorbed | 1.2-1.4 | 15-20 | 0.8-2 (pH 3-4) |

The contrasting optical properties of the two types of film are attributed to differences in structure. The transferred monolayers are thought to consist of a layer of nearly close-packed polypeptide chains lying in the plane of the film, with side chains vertical. The films formed by direct adsorption may consist of globular protein; there would be interstices, which might be filled (to raise the observed refractive index to 1.5 to 1.6) by adsorption at higher pH or salt concentration so that the amount of protein adsorbed rises to about 3 mg/m², with no change in thickness.

The protein layers so attached to slides may be studied in several ways. Film thickness and refractive index may be measured optically with an ellipsometer (Rothen (1956)) or by spectral analysis of reflected light (Bateman (1959, 1963)). A few studies of transferred films have raised the question as to whether monolayers may in some way recoil on transfer, so that small regions which would not affect the determination of average thickness may form which contain a thicker structure; the evidence is presented in "Structural Alteration of Spread Films."

These techniques, individually or in combination, are used in attempting to define the configuration of polypeptides and proteins at interfaces.

SYNTHETIC POLYPEPTIDES

General Considerations

A few studies of polypeptides were considered in a review by Cheesman and Davies (1954). Subsequently, much work has been done on a greater variety of polymers, especially in Japan by Isemura and co-workers.

The earlier studies of proteins showed that the area of close packing was, in most cases where good spreading was established, approximately 15A²/residue. This corresponds to the β type of structure, with side chain R groups alternating up and down. Since it was to be expected that hydrophobic groups would project upward into the nonpolar phase and hydrophilic groups downward into the aqueous phase, it seemed reasonable that the amino acid sequences of proteins would prove to be alternating hydrophobic and hydrophilic residues (Bull (1947)). The postulate soon foundered upon the facts of life as two important types of study were performed: first, the experimental elucidation of amino acid sequences, and second, studies on synthetic polypeptides. The sequence

studies showed that such sequences were not found in proteins, and the poly(amino acid) studies showed that such sequences were not required to lead to the observed results (Cheesman and Davies (1954)).

As a result of these studies, three general rules have emerged concerning the orientation of side chains (Davies (1953 b), Cheesman and Davies (1954)):

1. Polymers consisting only of nonpolar residues are folded at the air/water interface with the side chains alternately up into the air and down into the water. At the oil/water interface, the chains are all free to enter the oil phase.
2. Hydrocarbon side chains with ω -polar groups tend to lie flat on the air/water surface unless surrounded by hydrocarbon residues, in which case they are lifted up into the air. Isolated hydrocarbon side chains show the same effect at high areas. At the oil/water surface, the ω -polar groups tend to rise up into the oil when the film is compressed.
3. If part of a molecule is ionized, it can never completely leave the aqueous phase.

The question of secondary structure of polyamides and proteins is one to which much work has been addressed. In monolayers, the situation is as yet not completely understood.

The fact that the observed limiting areas of most polypeptides correspond to $\sim 15\text{A}^2$ /residue seems strongly indicative of the β type, or pleated sheet, structures, but there exist some data which seem to complicate the issue, as discussed below.

Nonpolar Polypeptides

Table 1 abstracts the data available to Cheesman and Davies at the time of their review in 1954, concerning the highly nonpolar amino acids, poly(DL-leucine), poly(DL-alanine), and poly(DL-phenylalanine). Using this data, they arrived at the following idea of the structure of nonpolar amino acids.

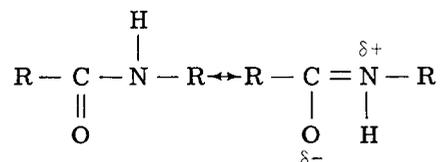
Since the molecular model studies show that the side chains have cross sections of about 30A^2 , the 17A^2 value of A_π indicates alternate up and down orientation (Davies (1953 a, b)). A similar observation had been made by Cumper and Alexander (1950) for poly(DL-phenylalanine). Since ΔV is higher at the acid air/water than at the oil/water interface, they deduce that the $>C=O$ dipoles are more nearly vertical at the air/water interface in acid. At pH 7 or 13, lower potentials are produced at the air/water interface. They postulate that this is caused by hydrogen bonds causing the $>C=O$ dipoles to become more horizontal. At the oil/water interface, cohesion is lost and flexibility increases. Cohesion now is not enough to maintain a hydrogen-bonded structure, and ΔV becomes almost independent of pH. All chains tend to be in the oil, so that ΔV becomes lower as the chains move into the oil and, as determined from studies of models, reorient the $>C=O$ groups from the vertical. The alternating side chain configuration no longer present, we cannot compress the poly(phenylalanine) monolayer beyond 25A^2 /residue without getting strong intermolecular interaction. This shows up in both the force-area curve and interfacial viscosity curve, while at the air/water interface one could compress poly(phenylalanine) to 14A^2 /residue. This configuration is called the "oil form." The postulated configuration of the polypeptides was deduced from studies of dipole moment and their change with pH. This change was attributed to a decreasing tendency to form hydrogen bonds between peptide groups as the subphase becomes more acid. Crisp's study (1946) of nylon, in which it was found that the usually extremely compact film became expanded at low pH, was apparently largely responsible for this idea. It was later found that other polyamide and polypeptide films behaved similarly (Davies (1953 b)). In studies of detergent binding to gelatin, Pankhurst (1949) and Yamashita and Isemura (1962)

Table 1
Surface Properties of Some Nonpolar Polypeptides

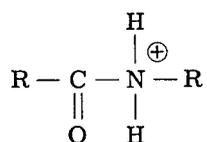
| Polymer | Inter- face | A_{η}^* (A^2 /residue) | | | A_{η}^* (A^2 /res) | μ (mD) | | | | Flexibility (%) | | Theoretical Area per Side Chain Cross Section (A^2) |
|--------------------|----------------|--------------------------------|------------------|-------|--|-------------------------------|-------------------------------|-------------------------------|------|-----------------|--|---|
| | | pH 2 | pH 6-8 | pH 13 | | pH 2 | pH 6-8 | pH 13 | pH 2 | pH 6-8 | | |
| Leucine | A/W | 19 | 17 | 17 | | 188 (at 20 A^2 /res) | 137 (at 20 A^2 /res) | 113 (at 20 A^2 /res) | | | | 30 |
| | O/W | | | | | 97 (at 20 A^2 /res) | 85 (at 20 A^2 /res) | 85 (at 20 A^2 /res) | 65 | | | |
| Phenyl- alanine | A/W | | 14 | | 14 (small η_s for $A > 25A^2$ /res) | 105 (at 100 A^2 /res) | 127 (at 100 A^2 /res) | 113 (at 100 A^2 /res) | | | | ~23 |
| | O/W | | | | 25 | | ~0 | | 65 | 25 | | |
| Alanine | A/W | | expanded | | 14.7 | | | | | | | |
| | O/W | | 23 (expanded) | | | | | | | | | 15 |

* A_{η} and A_{η} are limiting areas measured by surface pressure and surface viscosity respectively.

raised the possibility of peptide groups becoming protonated at low pH (about pH = 2). Pankhurst cites evidence from titration curves that anomalous proton uptake occurs at about pH 2. He recalled that Pauling suggested that protonation of the amide group could occur to destroy the resonance responsible for the double bond character and thus the stiffness of the peptide group. The resonance



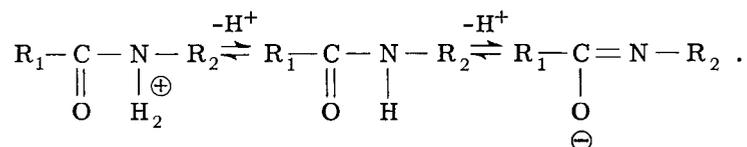
could be prevented by protonation, and



would be the stable form.

This would have three effects. First, the partial double bond character of the peptide bond would be reduced, so that the stiffness of the main chain would also be reduced. Second, the positive charge introduced would increase the already positive charge at this pH, which would increase the electrostatic repulsion effects, thus increasing π at constant area, or area at constant π , or increasing the flexibility. Third, a change of electric moment as the pH is lowered would also result. Pauling (1953) has stated that the degree of amide protonation is expected to be "very small and without significance." Klotz and co-workers (Hanlon and Klotz (1965), Hanlon, Russo, and Klotz (1963)) have obtained evidence for the protonation of the amide group by the IR spectral change of amides in acid; however, this has been done in organic acid solvents, and the pK in aqueous solution is expected to be much lower. At any rate, it appears that this phenomenon is only significant in the very acid range. The range of the change in properties with pH of nonpolar peptides (Davies (1953b), Yamshita and Isemura (1962)) seems too great to be explained solely on the basis of amide protonation, although there may be differences between pK at the interface and in the bulk solution.

At high pH, Schauenstein and Perko (1953) have shown that under certain circumstances the peptide group may become ionized by losing a proton, thus becoming negatively charged. The high pH effect is not completely elucidated; such factors as the amino acid content of the polymer and molecular weight seem to have a prominent effect upon the ionization. The peptide group is thus amphoteric, and the effect of pH upon the proteins and polypeptides must be considered from this point of view. According to these ideas, the range of possible ionization for the peptide group appears to be



The effect of ionization of this type upon the dipole moment of the monolayer has not yet been determined, to the reviewer's knowledge.

NRL Report 6318

Surface Chemistry of Proteins and Polypeptides

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November 30, 1965



**U.S. NAVAL RESEARCH LABORATORY
Washington, D.C.**

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ABSTRACT

This report is a review of recent literature in the field of polypeptide and protein surface chemistry, concentrating on the last decade. Theoretical approaches to polymers at liquid interfaces are considered, and surface measurement techniques are briefly outlined.

The various classes of polypeptides are considered, followed by a discussion of proteins in general and specific proteins in particular. Enzymatic activity, structure, and adsorption at various surfaces are considered also.

PROBLEM STATUS

This is an interim report; work on the problem is continuing.

AUTHORIZATION

NRL Problem C02-18A
RUDC-4B000/652-1/F101-99-01

Manuscript submitted June 30, 1965.

SURFACE CHEMISTRY OF PROTEINS AND POLYPEPTIDES

INTRODUCTION

This report is a review of certain aspects of the interfacial properties of proteins and synthetic polypeptides. These polymeric materials are of importance from a variety of viewpoints. To the biologist, they represent a class of natural products intimately associated with the vital processes of all viable organisms whose cells are of microscopic and submicroscopic dimensions. As each cell and organelle has its share of membranes, it is evident that the surface properties of natural products are important to the functioning of the organism. To the physical and polymer chemist, the fact that proteins and synthetic polypeptides may exist in well-defined conformations presents the opportunity to study the effects of the highly asymmetrical environment at an interface upon such structures. Since the amino acid residues found in proteins include highly nonpolar side chains combined with a polar backbone and other highly polar chains, it is to be expected, and indeed is found, that these polymers are highly surface active, making them important in technology for applications requiring stable foams and strong adsorption at interfaces. Conversely, their strong surface activity may hamper certain operations in which proteins are required but where adsorption and foaming are undesirable.

Previous reviews of this field have been published by Bull (1947), Rothen (1947, 1956), Cumper and Alexander (1951), Cheesman and Davies (1954), Fraser (1957), and in the very recent book of Davies and Rideal (1963) which contains some material dealing with protein surface chemistry. A description of some techniques is given by Sobotka and Trurnit (1961).

SURFACE PRESSURE, EQUATIONS OF STATE, AND MOLECULAR WEIGHTS AT INTERFACES

A fundamental parameter characterizing the interaction of a surface-active substance with the interface between two bulk phases is the change in surface tension produced at the interface by the presence of the surface-active compound. This difference in surface tension is measured in dynes/cm and may also be considered the surface pressure, because historically the similarity to a two-dimensional pressure is accentuated by the use of the Langmuir trough in its measurement. As surface tension is numerically equal to the surface free energy in ergs/cm², the surface pressure π is equal to the change in the surface free energy brought about by the presence of the surface-active agent.*

The surface pressure-area relation of compounds which form surface films may be analyzed by a method analogous to osmometry; an element of the apparatus confines a component of interest in a restricted region of the surface, while permitting other components free access to another region of the surface which is considered to be in a reference state. To expand the surface region on which the compound of interest is confined, we must place more diluent into that region, at the expense of the surface region which is free of the compound. The two regions may be separated, as in the case of the Langmuir trough, or the entire free surface may be covered, as is possible with the Wilhelmy balance, where the equilibrium is established with the bulk of liquid below the surface, whose state is considered to be in equilibrium with bulk liquid under a clean

*Surface pressure is often also denoted by F .

surface. Following Bull (1950) we may let the activity of the bulk solvent be A_0 and a be the activity on a clean surface in equilibrium with it. Then, for transfer of 1 mole of solvent from clean surface to film-covered area,

$$\begin{aligned}
 \pi A_1 &= A_1(\gamma_0 - \gamma_1) \\
 &= RT \ln \frac{a}{a_0} \approx -RT \ln \frac{N_1}{N_0} \quad (\text{if we may take } a_i \approx N_i) \\
 &= -RT \ln (1 - N_2) \\
 &= -RT \ln \left(1 - \frac{n_2}{n_1 + n_2} \right) \quad (1)
 \end{aligned}$$

where the subscripts 1 and 2 refer to solvent and film molecules respectively, n_i and N_i denote the number of moles and the mole fraction of component i in a surface of area A , A_1 is the molar area of solvent, N_0 (the mole fraction of solvent in the clean surface) is equal to unity, and γ is the surface tension. If we now let the film be very dilute, we may expand the logarithm, and assuming that the total area $A = A_1 n_1 + n_2 \delta_2$, where δ_2 is the area of film occupied by a mole of film-forming substance,

$$RT \ln n_2 = \pi A_1 (n_1 + n_2) \approx \pi A_1 n_1 = \pi (A - n_2 \delta_2),$$

and Eq. (1) becomes

$$\pi A = RT \ln n_2 + n_2 \delta_2 \pi. \quad (2)$$

If we plot πA versus π , the intercept on the πA axis determines n_2 and the slope determines δ_2 . Note that δ_2 is the apparent area based on the assumption that the area of a mole of water is invariant with surface concentration. This is not necessarily correct. Also, the activity may be proportional to concentration only in the case of molecules of circular section (Davies and Llopis (1955)).

This treatment holds in the region of infinite dilution and is used to justify extrapolation of the curve of πA versus π to $\pi = 0$ to find molecular weight. However, we have no thermodynamically rigorous theory at those higher pressures where measurements are possible. Therefore, theories which must be tested by experiment have been developed and applied to polymer and protein systems with the purpose of enabling extrapolations to be made with confidence, and also explaining the general behavior of the force-area curve.

Purely Entropic Theories

Singer's Theory - The theories applicable to protein systems which have been recently in use are those of Singer (1948) and of Frisch and Simha (1956). Singer's treatment is based upon the Flory-Huggins development of polymer theory. It assumes that there exists at the surface or interface of the polymer solution a quasi-lattice of sites, each of which may be occupied or vacant. The entropy of the polymeric system occupying these sites in a given manner may be calculated by statistical methods, and the free energy may be derived by assuming that there is no intermolecular attraction or repulsion (Davies (1954b)). The resulting equation is

$$\frac{\pi A_0}{kT} = \left[\ln \left(\frac{A}{A - A_0} \right) + \frac{(X-1)}{X} \frac{Z}{2} \ln \left(1 - \frac{2A_0}{A} \right) \right]. \quad (3)$$

In Eq. (3), A_0 is the limiting molecular area per residue, A is the available area per residue (or monomer unit) in the chain. The parameter Z is the lattice coordination number of the chain segments, i.e., the number of possible sites on which the neighboring residues may be situated. For a rigid chain $Z = 2$, and the chain configuration is fixed. Flexibility may be defined as $100 (Z - 2)/Z = \omega$. This assumes that the upper limit of Z is 4, i.e., a "square lattice." Here X is the degree of polymerization or DP , k is Boltzmann's constant, and T is temperature.

If $X = 1$ or $Z = 2$, Eq. (3) becomes

$$\pi = \frac{kT}{A_0} \ln \left(\frac{A}{A - A_0} \right). \quad (4)$$

If $Z = (2 + \epsilon)$ where ϵ is a small number,

$$\pi A = kT + 1/2 \pi A_0. \quad (5)$$

This is Bull's equation, except for the factor $1/2$.

Plots of πA versus π for polymers and proteins are often linear at the air/water interface where the cohesion is sufficient for low flexibility. If Z is not close to 2, Eq. (3) predicts that the force-area curve will be convex upward. In the oil/water interface $Z = 2$, so we expect curvature which prevents good extrapolation.

The value of Z depends on charge and ionic strength through their effect upon the net cohesive energy; thus, flexibility may be reduced by changes in pH or salt concentration. When cohesion is appreciable, the system is described by an equation relating electrostatic effects to surface pressure, presented by Davies and Llopis (1955); the equation has been derived on a different basis by Isemura, Hamaguchi, and Ikeda (1957). A discussion is presented by Davies and Rideal (1963) and by Payens (1960).

Frisch and Simha's Theory - It is possible that the polymer may not be completely in the interface but may loop into one or both phases forming the interface, especially when compressed. This consideration leads to the relation

$$\pi = \frac{X kT}{A_0} \left[\ln \left(\frac{A}{A - p A_0} \right) - \left(\frac{p^2 A_0}{A - p A_0} \right) \right] - J p^2 \frac{kT A_0^2}{A^2} \quad (6)$$

where A_0 = close-packed area, and J is a coefficient allowing for lateral entropic and enthalpic interaction between those residues fixed in the interface. In this equation,

$$p = 2\alpha / (\pi f_i X)^{1/2} \quad (7)$$

where f_i is a function of internal flexibility of the molecule, X is the DP , and α is called the intramolecular segment condensation coefficient, which expresses the interactions within the polymer molecule. At low π Eq. (6) becomes (since $A \gg A_0$)

$$\pi A = X p kT. \quad (8)$$

This implies that molecules with large DP can form loops more easily and return to the interface to have units act as "independent" kinetic units, lowering their apparent molecular weights. This is not verified experimentally (Davies and Rideal (1963)), since for several proteins and copolypeptides the proper molecular weights are obtained at air/water and oil/water interfaces when the Singer equation is used at very low π (<50 millidynes). This does not preclude its applicability to other experimental situations involving other polymers and interfaces or higher pressures. In practice, distinguishing

between applicability of the Singer or Frisch-Simha theories on the basis of πA experiments is difficult (Jaffe and Loutz (1964), Dieu (1956 a, b, c)). Jaffe and Loutz have shown that both equations may be cast into the form of the virial equation

$$\pi A = kT (1 + B_1 \pi + B_2 \pi^2 + \dots) \quad (9)$$

and that using either Eq. (3) or (6) one can fit the force-area curves reasonably well by adjusting the parameters available. In both the case of the Singer equation and that of Frisch and Simha, however, Jaffe and Loutz point out that the choice of parameters may be ambiguous and that there is no guarantee that any of the parameters chosen bears a simple relation to the property of the polymer it purports to describe. Davies and Rideal (1963) have also indicated that Z should be regarded as an empirical factor because of the many oversimplifications of the theory. Jaffe and Loutz propose to use the function of the first two "virial coefficients" (B_2/B_1^2) to describe the polymer, empirically establishing an assignment of flexibility to values of the ratio. This parameter is independent of a model; insight into details of molecular structure is lacking as yet.

It has also been emphasized (Dieu (1956 a, b, c)) that both Singer's and Frisch and Simha's treatments have neglected intermolecular enthalpy effects. A term in A_0^2/A^2 may be introduced in an attempt to account for enthalpy of mixing in Singer's treatment (Davies (1954 b), Davies and Llopis (1955), Ter Minassian-Saraga and Prigogine (1954)), and the enthalpy of interaction of adjacent residues in the "surface plane" is allowed by Frisch and Simha. There has been at least one case where the Frisch and Simha theory seems to fit the data better than Singer's equation, the case of polyvinyl alcohol at a benzene/water interface (Dieu 1956 a). Dieu points out, however, that even in cases where the Frisch and Simha theory fits better than Singer's theory, values of Z are obtained which do not seem to fit expected chain properties. This may, at the present state of development, be attributed to the lack of recognition of enthalpy effects in the treatment.

In general, one may say that for molecules of low flexibility at the air/water interface one may obtain a good determination of molecular weight (Davis and Rideal (1963), p. 241, Dieu (1956 a, b, c), Davies (1954 b), Davies and Llopis (1955), Bull (1947)). High flexibility (induced by the presence of an oil/water interface or high charge on the macromolecule) or extensive intermolecular association (as in polyleucine) renders extrapolations of the force-area curve uncertain. Jaffe and Loutz (1964) have shown that with flexible polymers, even in the extremely low pressure range below 0.04 dyne/cm, one cannot get a good extrapolation for determining molecular weights. The fact that πA versus π plots for many proteins are indeed linear at the air/water interface lends credence to the notion that not much intermolecular interaction occurs in these systems, although this is not conclusive. Surface rheology studies (Cheesman and Davies (1954), Imahori (1952 a-d), Davies (1954 a, b)) to be described later will also have a bearing on this point. A summary of results of molecular weight determination is given by Cheesman and Davies (1954) together with a discussion of elasticity determinations based on Singer's equation. Subsequent data are listed in papers by Dieu (1956 a-c), Jaffe and de Coene (1957), Harrap (1955 a, b), and Ter Minassian-Saraga (1956).

An illustration of the effect of these ambiguities is the study by Davies and Llopis (1955) of the copolymer 1:1:2 lysine-glutamic acid-leucine. They found that at neutral pH at the air/water interface they could easily get a linear πA versus π plot which gave the correct molecular weight. At extremes of pH, they were forced to very low pressures to overcome the curvature resulting from flexibility induced by charging the molecule. At the oil/water interface, they observed effects very much like the dissociations reported for proteins, and only when the measurements were carried to extremely low π was it clear that the correct molecular weight would be found at $\pi \rightarrow 0$. They point out that, because of the many approximations in the theory, no absolute significance should be attached to the values of Z .

The importance of taking enthalpy into consideration is strikingly illustrated by the fact that the temperature dependence of π is much greater than the proportionality to absolute temperature indicated by both theories (Dieu (1956 a-c), Llopis and Rebollo (1956), Llopis and Albert (1959 a, b)).

Davies and Llopis (1955) have indicated that an energy of interaction may be included in the value of the flexibility ω by writing $\omega = \omega_0 e^{-E/RT}$, which shows that flexibility may change with temperature. This is equivalent to an energy of interaction because it biases the coordination number, $Z = \omega + 2$, in favor either of a greater number of polymer-solvent contacts or of polymer-polymer contacts, depending on the value of E .

Another indication that the Singer and Frisch-Simha theories are not adequate is the observation by Bull (1950) and Dieu (1956 a-c) that the πA versus π curves for proteins are often neither linear nor monotone curves, but show two linear portions separated by a kink. Each linear portion may be extrapolated to yield the same molecular weight. This is taken as evidence of the existence of at least two surface states (Bull (1950), Dieu (1956 a-c)). Experiments on synthetic polypeptides (Isemura and Hamaguchi (1952, 1953)) also suggest this possibility, which will be discussed further when considerations of surface configuration are undertaken (see "Nonpolar Polypeptides"). However, Cheesman and Davies (1954) interpret the kink as evidence of an interaction, saying it shows "stickiness" of the molecules.

Theories Involving Energy of Interaction

Silberberg's Theory - Silberberg (1962) has developed a theory for adsorption of an isolated, flexible molecule at an infinite planar surface. It is assumed that for each segment placed at the surface the internal energy of the system is reduced by an adsorption energy wkT . It is then shown that the polymer molecule will adjust itself so that alternate runs of P_S segments are all in the surface and loops of P_B segments whose size is not a function of molecular weight are all out of the interface. For a molecule composed completely of adsorbable units, on a surface which is everywhere adsorbing, the loops are small, and even at small w the molecules stay close to the surface. Otherwise, the loops are much larger. Restrictions limiting the re-entry of an adsorption loop into the surface have a similar effect. The result is a thin bulk phase of high polymer concentration near the surface. The molecules of polymer are rather flattened, with about half of the segments in the surface phase in some cases. The inability to wash the polymer from the interface (i.e., apparent irreversibility), the similarity of its isotherm to that of Langmuir, and a slow approach to equilibrium are predicted. The surface equation of state derived covers the limiting cases in the literature. Although the treatment conforms with the Gibbs isotherm, the apparent irreversibility of polymer adsorption is explained by the prediction that the adsorption will be very strong even at extremely low solution concentration. The surface equation of state in the limit of low θ is

$$A_0 \pi = kT \left(\frac{\theta/p}{X} + \frac{\sigma^2}{2} \beta_s \right) \quad (10)$$

where X is the number of segments per polymer molecule, θ is fraction of surface sites occupied by polymer segments, β_s is a complicated function, and p is a function of polymer segments at the surface.

It is a consequence of the model that the ends of the molecule stay in the surface.

Motomura and Matuura's Theory - Motomura and Matuura (1963 a,b) have also recently derived a theory which takes the energy of interaction into account and which reduces to the Singer theory when the energy is negligible. This equation also predicts phase changes in the films if the temperature is low enough and energy large enough.

The force-area curve is almost independent of the degree of polymerization χ if χ is sufficiently large (i.e., 7300). Their equation is

$$\frac{\pi}{\pi_0} = \frac{Z}{2} \ln \left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \frac{A_0}{A} \right] - \ln \left[1 - \frac{A_0}{A} \right] - \frac{Z}{2} \ln \frac{\left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \frac{A_0}{A} \right] (\beta_M + 1) - 2 \left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \right] \frac{A_0}{A}}{\left(1 - \frac{A_0}{A} \right) (\beta_M + 1)} \quad (11)$$

where $\pi_0 = kT/A_0$, A_0 is the area occupied by a monomer unit, Z is the coordination number of a site in a two-dimensional surface lattice, χ is the degree of polymerization, and β_M is defined by the complex function

$$\beta_M^2 = \left\{ \frac{\left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \right] \left(1 - \frac{A_0}{A} \right) \frac{A_0}{A}}{\left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \frac{A_0}{A} \right]^2} (e^{2w/ZkT} - 1) + 1 \right\} \quad (12)$$

in which $-2w/Z$ is the energy of contact between neighboring monomer units.

These authors point out that the success of Singer's equation may be due to the fact that Z in Singer's theory behaves similarly to the energy term. They do not account for the possibility that some chains may loop into the bulk phases.

The quantity π/π_0 may exhibit a maximum and a negative minimum when plotted against A/A_0 . This is characteristic of a two-phase film and occurs when $w/kT > 0.864$.

Motomura and Matuura examined poly(vinyl acetate), poly(methyl acrylate), and poly(methyl methacrylate), i.e., PVAc, PMA, and PMMA. In the case of PVAc a value of w was obtained which allowed a good fit to the theory, assuming $Z = 4$, below 6 dynes/cm. Above this pressure, deviations occurred which were attributed to squeezing out of chain segments from the surface.

Molecules of PMA are less expanded as w/kT is higher, and the theory fits better. This is expected, because the lattice approximation should not be as valid for expanded films.

In both the PVAc and PMA cases, $w/kT < 0.864$, so that phase changes were not expected. For PMMA, $w/kT > 0.864$ and the film is of condensed type; it shows vanishingly small π at large areas, with π becoming measurable at ca. $30A^2/\text{monomer}$. The predicted curve becomes negative at $23.5A^2/\text{residue}$; below this the theory predicts a phase transitional region, which is supported experimentally by a fluctuation of the surface potential.

Studies of these films showed that the higher the value of w/kT , the more viscoelastic the film, as would be expected. A similar parallelism between viscoelasticity and cohesive energy was found in studies on bovine serum albumin (BSA) and gamma globulin. The ΔH of spreading and area of onset of non-Newtonian viscosity increased with pH in the case of BSA, while both parameters were independent of pH in the case of gamma globulin (Llopis and Albert (1959 a, b)).

While these results are very promising, especially since phase transitions may be handled, it is this reviewer's feeling that more data must be forthcoming before the newer theories may be fully evaluated.

Electrostatic Effects on the Force-Area Curve

The effect of charge upon the force-area curve of polymers may be separated into intermolecular and intramolecular effects. If the polymer molecules are charged, then repulsion of like charges will decrease the net cohesion of the polymer molecule.

If one writes the flexibility ω as

$$\omega = \omega_0 e^{-E/kT} \quad (13)$$

where ω_0 is the flexibility in the absence of cohesive effects (i.e., at the oil/water interface and at zero charge), electrostatic effects will appear in the parameter E , as will cohesive energy. At very high area where intermolecular effects may be neglected safely, the oil/water interface in the isoelectric region allows the establishment of a base curve for which $E = 0$; electrostatic (and cohesive) contributions to the force-area curve at the air/water interface may be obtained by subtraction. One thus obtains the "excess electrostatic surface pressure" (π_{el}) experimentally (Davies and Llopis (1955), Payens (1960), Davies and Rideal (1963), Chapter 5).

The interfacial potential Ψ may be calculated according to the Gouy theory, and one may write

$$\pi_{el} = \int_0^{\Psi} \sigma d\Psi' \quad (14)$$

where π_{el} is the electrostatic contribution to the surface pressure, and σ is the charge density of the monolayer. This leads to

$$\pi_{el} = 6.1 \sqrt{C} \left\{ \cosh \left[\operatorname{arc} \sinh \left(\frac{134}{\sqrt{C}} \right) \right] - 1 \right\} \quad (15)$$

(Payens (1960), Davies (1951)). Here, C is the concentration of 1:1 electrolyte. Intermolecular contributions, which may be evaluated at higher pressures, are evident since the apparent potentials decrease with area faster than indicated for isolated charges of the valency of the polymer. The contributions of the intermolecular and intramolecular effects may thus be considered as contributing to the effective potential. An effective charge may be derived by fitting such data.

The value of Z may be related to the electrostatic effect through the relation

$$Z - 2 = \omega = \omega_0 \exp \left(\frac{-E_{\text{cohesion}} + \pi_{el} A^*}{kT} \right) \quad (16)$$

and the value of A^* derived from the slope of a plot of π_{el} versus $\log \omega$. The value of A^* so obtained is considered to be the area of the minimum kinetic unit (Joly (1948)) in the polymer in the course of its molecular motion.

The effective charge perturbing the πA curve in this study was found to be 20 percent of the true value of the macromolecular charge and corresponds to the estimate of the charge expected to be at the periphery of the molecule, with the rest of the charge

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screened by counter-ions. The size of the kinetic unit appears to be that of five residues, a small fraction of the size of the molecule.

Thermodynamics of the Force-Area Curve

One may attempt to characterize the degree of disruption of molecular structure when a molecule is spread by the value of the free energy change on spreading. This is not a clear-cut analysis as yet, because it is not known how to resolve the observed change on spreading into its components corresponding to changes in solvation, polarization of the interfaces, changes in solution structure, and so on. Neglecting these matters, attempts have been made to compare the entropies of unfolding for various substances. Davies (1953 c) [Cheesman and Davies (1954)] used the following approach.

If one accepts the model of Singer (1948), one can calculate the entropy S_s of a spread polymer with respect to a reference state in which the polymer backbone chain is rigid from the high area portion of the force-area curve. This quantity was given by Davies (1953) as

$$S_s = \left(I - \frac{2}{X} \right) R \ln (Z - 1) \quad (17)$$

using the same notation as in the section titled "Singer's Theory."

The entropy change on compression of the film between any two areas can be calculated if one assumes that the free energy change has only an entropy term and no enthalpy term. Subtracting from this the entropy change which would be found for a completely rigid molecule undergoing expansion or compression between the same areas gives

$$\Delta S_s = \frac{1}{XT} \int_{A_1}^{A_2} \pi dA - \frac{1}{XT} \int_{A_1}^{A_2} \pi_r dA. \quad (18)$$

The entropy of the molecule at any area can be calculated relative to a reference state of the rigid main chain by evaluating π_r , the predicted value for a rigid chain (i.e., $Z = 2$). The Singer theory allows this to be done if the effect of side chains is neglected. Accepting this limitation, we may determine the entropy per residue at the limiting area relative to the rigid reference state.

It turns out for proteins (Davis (1953 c), Cheesman and Davies (1954)) that the experimental unitary entropy of expansion from the limiting area to high area is equal to the calculated unitary entropy of the molecule at high area relative to the rigid molecule reference state.

But this is not true in the cases of polyalanine and polyleucine, for which the unitary expansion entropy is less than that calculated from the Singer theory. (This implies that the limiting area in the case of these peptides does not correspond to a rigid molecule, while the rigid molecule at limiting area is consistent for the proteins.)

The value of S_s calculated in this way may be considered the entropy of surface denaturation if the Singer model of copolypeptide monolayers is accepted and if the entropy of the reference state chosen is indeed the same as that of the native molecules. For example, the experimental data indicate that, for methemoglobin at the oil/water interface, $Z = 2.12$, corresponding to $S_s = +0.22$ eu, while for polyalanine and polyleucine one has $S_s = +1.6$ eu through use of Eq. (18).

Davies states that the values of s_s are similar for most proteins and makes a comparison with the thermal denaturation entropy of trypsin, which is +0.76 eu. The inference is that a number of linkages modified by thermal denaturation are not destroyed by surface denaturation. Other evidence leading to the conclusion that surface denaturation is not complete (found in sections titled "Lysozyme," "Ovalbumin," and "Structural Alteration of Spread Films") makes the argument more convincing, since the validity of this type of argument rests upon Singer's treatment, now known to be inadequate. This approach may be refined using the more satisfactory treatments of the theory of the force-area curve. At this stage of development, the treatment measured a quantity which may be more closely related to the free energy than to the entropy of the transition. We may cite some of the evidence which has been obtained by heating solutions and monolayers. Kaplan and Fraser (1953) found that fibers formed from protein monolayers were quite different when formed from native spread proteins instead of denatured ones, and that if proteins were heated prior to spreading the fibers formed were of the denatured type. Trapeznikov (1948) found a change in surface viscosity η_s at the temperature of bulk denaturation. These observations indicate that the properties of the film are dependent upon elements of structure which are affected by denaturation.

A second approach to the thermodynamic parameters of the polymeric monolayer is the study of the systems at different temperatures. Llopis and Albert (1959 a, b) have done this for bovine serum albumin and globulin. In both cases the enthalpy of spreading on acid at low temperature is negative, i.e., favors spreading. At higher temperatures the enthalpy term is positive. On alkaline substrates, cohesion always predominates. More work on the thermodynamics of the process is required for full understanding, but it is already clear that Singer's equation will have to be improved upon, as will the theory of Frisch and Simha, to take enthalpy effects into account. The theories of Motomura and Matuura (1963 a, b) and of Silberberg (1962 a, b) have not had sufficient application to be adequately judged.

These approaches are not adequate to evaluate the surface denaturation process, because they pertain to the already spread material. Comparisons based on Eq. (18) depend upon the assumption that the molecules at the point of minimum compressibility, or the collapse pressure, are in a state whose entropy corresponds to the solution or solid state. Although there are indications that this may be true (Benhamou (1954), MacRitchie (1963)) (as discussed later in the "Collapse" section), the issue is not settled. It would appear to be more suitable to measure the equilibrium involved in the adsorption from solution to the surface if such an equilibrium could be established. Guastalla and Benhamou (1961) attempted to evaluate the equilibrium parameters in such a situation and found that hemoglobin has too high a molecular weight to be suitable for the experiment; insulin, which is much smaller, appeared to be on the borderline of feasibility.* The data on the entropy and enthalpy of expansion of the already spread monolayer, in addition to the data from the adsorption and spreading steps, are required for a complete description of the surface denaturation process.

SURFACE PARAMETERS AND TECHNIQUES AT FINITE PRESSURES

Molecular weights are studied in the low-pressure region of the force-area curve, where properties of the molecules at the surface may be studied in a highly dilute state. In the highly expanded state, the theoretical attempts to describe molecular behavior have led to estimates of the intrinsic flexibility and size of the solute molecules in what is hoped to be a situation essentially free of intermolecular interaction. When one

*A study of this process with a series of polypeptides as a function of molecular weight would be useful.

compresses a film so that the molecules interact, a different type of information is forthcoming. Experimental parameters, other than the surface pressure will now be considered.

Interfacial Viscosity and Viscoelasticity

A review of interfacial viscosity and viscoelasticity was presented by Joly (1964). Polymer monolayers may be classified, rather empirically, by interfacial viscosity properties. One finds that condensed films and expanded films have quite different viscosity-area curves. In the case of the condensed film, one finds a high viscosity at areas larger than the limiting area found from a πA experiment. For expanded films, we find that the interface viscosity η_s drops outside the range of high surface pressures (Isemura and Hamaguchi (1954)).

A high surface viscosity would seem to imply intermolecular interaction. This may come about mechanically through chain entanglement (Davies (1953 c)) or through chemical interaction (Isemura and Hamaguchi (1954)), e.g., electrostatic salt linkage, hydrogen bonds. High surface viscosity at high area signifies that a network of some sort is formed by condensed films between molecules before compression sufficient to pack them tightly is reached. In contrast, expanded films show a large increase in interfacial viscosity only when the area is reduced to the limiting area of the force-area curve (Isemura and Hamaguchi (1954)). This implies that the repulsion effects which keep the film expanded also serve to inhibit chemical or mechanical intermolecular interactions at areas larger than the close-packed area.

Isemura and Hamaguchi (1954) emphasize the role of electrostatic interactions in producing surface viscosity, while Davies (1953 c) interpreted the increase of film viscosity of polypeptides at an oil/water interface as evidence that a loss of intramolecular cohesion allows sufficient interaction to occur at higher area, as chains are compressed. Increase of η_s at high charge may also be the result of expansion (see also Joly (1964)). A theory of viscosity based upon the general theory of rate processes has been formulated by Eyring and coworkers (Glasstone, Laidler, and Eyring (1941)). Conclusions reached from a study of activation areas are cited in the "Proline and Hydroxyproline" section. Ikeda and Isemura (1959) found that a strongly cohesive condensed polymer exhibits an area of activation which extends over the whole molecular area, while for expanded films with weaker cohesion, the area corresponds to only a small section of the molecule. Earlier, Joly (1948) had observed, also on the basis of the Eyring theory, that since all proteins seemed to have approximately the same viscosity coefficient at the onset of non-Newtonian viscosity, the kinetic subunits of the protein monolayer must be of similar area. Calculation of the activation energy of flow led to a value of $90A^2$ for this unit. Joly's treatment relies upon (a) an estimation of the contributions of the various components of the molecule to the interaction energy and (b) the deduction that the spacing of the kinetic units at the onset of non-Newtonian viscosity is such as to let the width of an assumed hexagonal pseudolattice be twice the diameter of the kinetic unit (assumed spherical). Ikeda and Isemura do not give the details of their calculation. The strongly cohesive polymers of poly(phenylalanine), polyleucine, etc., have a much higher density of nonpolar amino acids than the proteins.

An alternative method of analysis of viscoelasticity in films is shown in the series of works treating the rheology of materials as represented by a mechanical model. The mechanical analogy is a system of springs and dashpots; the springs represent elastic elements, and the dashpots, viscous elements. These elements may be combined in various ways, such as:

NRL Report 6318

Surface Chemistry of Proteins and Polypeptides

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November 30, 1965



U.S. NAVAL RESEARCH LABORATORY
Washington, D.C.

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ABSTRACT

This report is a review of recent literature in the field of polypeptide and protein surface chemistry, concentrating on the last decade. Theoretical approaches to polymers at liquid interfaces are considered, and surface measurement techniques are briefly outlined.

The various classes of polypeptides are considered, followed by a discussion of proteins in general and specific proteins in particular. Enzymatic activity, structure, and adsorption at various surfaces are considered also.

PROBLEM STATUS

This is an interim report; work on the problem is continuing.

AUTHORIZATION

NRL Problem C02-18A
RUDC-4B000/652-1/F101-99-01

Manuscript submitted June 30, 1965.

SURFACE CHEMISTRY OF PROTEINS AND POLYPEPTIDES

INTRODUCTION

This report is a review of certain aspects of the interfacial properties of proteins and synthetic polypeptides. These polymeric materials are of importance from a variety of viewpoints. To the biologist, they represent a class of natural products intimately associated with the vital processes of all viable organisms whose cells are of microscopic and submicroscopic dimensions. As each cell and organelle has its share of membranes, it is evident that the surface properties of natural products are important to the functioning of the organism. To the physical and polymer chemist, the fact that proteins and synthetic polypeptides may exist in well-defined conformations presents the opportunity to study the effects of the highly asymmetrical environment at an interface upon such structures. Since the amino acid residues found in proteins include highly nonpolar side chains combined with a polar backbone and other highly polar chains, it is to be expected, and indeed is found, that these polymers are highly surface active, making them important in technology for applications requiring stable foams and strong adsorption at interfaces. Conversely, their strong surface activity may hamper certain operations in which proteins are required but where adsorption and foaming are undesirable.

Previous reviews of this field have been published by Bull (1947), Rothen (1947, 1956), Cumper and Alexander (1951), Cheesman and Davies (1954), Fraser (1957), and in the very recent book of Davies and Rideal (1963) which contains some material dealing with protein surface chemistry. A description of some techniques is given by Sobotka and Trurnit (1961).

SURFACE PRESSURE, EQUATIONS OF STATE, AND MOLECULAR WEIGHTS AT INTERFACES

A fundamental parameter characterizing the interaction of a surface-active substance with the interface between two bulk phases is the change in surface tension produced at the interface by the presence of the surface-active compound. This difference in surface tension is measured in dynes/cm and may also be considered the surface pressure, because historically the similarity to a two-dimensional pressure is accentuated by the use of the Langmuir trough in its measurement. As surface tension is numerically equal to the surface free energy in ergs/cm², the surface pressure π is equal to the change in the surface free energy brought about by the presence of the surface-active agent.*

The surface pressure-area relation of compounds which form surface films may be analyzed by a method analogous to osmometry; an element of the apparatus confines a component of interest in a restricted region of the surface, while permitting other components free access to another region of the surface which is considered to be in a reference state. To expand the surface region on which the compound of interest is confined, we must place more diluent into that region, at the expense of the surface region which is free of the compound. The two regions may be separated, as in the case of the Langmuir trough, or the entire free surface may be covered, as is possible with the Wilhelmy balance, where the equilibrium is established with the bulk of liquid below the surface, whose state is considered to be in equilibrium with bulk liquid under a clean

*Surface pressure is often also denoted by F .

surface. Following Bull (1950) we may let the activity of the bulk solvent be A_0 and a be the activity on a clean surface in equilibrium with it. Then, for transfer of 1 mole of solvent from clean surface to film-covered area,

$$\begin{aligned} \pi A_1 &= A_1(\gamma_0 - \gamma_1) \\ &= RT \ln \frac{a}{a_0} \approx -RT \ln \frac{N_1}{N_0} \quad (\text{if we may take } a_i \approx N_i) \\ &= -RT \ln (1 - N_2) \\ &= -RT \ln \left(1 - \frac{n_2}{n_1 + n_2} \right) \end{aligned} \quad (1)$$

where the subscripts 1 and 2 refer to solvent and film molecules respectively, n_i and N_i denote the number of moles and the mole fraction of component i in a surface of area A , A_1 is the molar area of solvent, N_0 (the mole fraction of solvent in the clean surface) is equal to unity, and γ is the surface tension. If we now let the film be very dilute, we may expand the logarithm, and assuming that the total area $A = A_1 n_1 + n_2 \delta_2$, where δ_2 is the area of film occupied by a mole of film-forming substance,

$$RT \ln n_2 = \pi A_1 (n_1 + n_2) \approx \pi A_1 n_1 = \pi (A - n_2 \delta_2),$$

and Eq. (1) becomes

$$\pi A = RT \ln n_2 + n_2 \delta_2 \pi. \quad (2)$$

If we plot πA versus π , the intercept on the πA axis determines n_2 and the slope determines δ_2 . Note that δ_2 is the apparent area based on the assumption that the area of a mole of water is invariant with surface concentration. This is not necessarily correct. Also, the activity may be proportional to concentration only in the case of molecules of circular section (Davies and Llopis (1955)).

This treatment holds in the region of infinite dilution and is used to justify extrapolation of the curve of πA versus π to $\pi = 0$ to find molecular weight. However, we have no thermodynamically rigorous theory at those higher pressures where measurements are possible. Therefore, theories which must be tested by experiment have been developed and applied to polymer and protein systems with the purpose of enabling extrapolations to be made with confidence, and also explaining the general behavior of the force-area curve.

Purely Entropic Theories

Singer's Theory - The theories applicable to protein systems which have been recently in use are those of Singer (1948) and of Frisch and Simha (1956). Singer's treatment is based upon the Flory-Huggins development of polymer theory. It assumes that there exists at the surface or interface of the polymer solution a quasi-lattice of sites, each of which may be occupied or vacant. The entropy of the polymeric system occupying these sites in a given manner may be calculated by statistical methods, and the free energy may be derived by assuming that there is no intermolecular attraction or repulsion (Davies (1954b)). The resulting equation is

$$\frac{\pi A_0}{kT} = \left[\ln \left(\frac{A}{A - A_0} \right) + \frac{(X-1)}{X} \frac{Z}{2} \ln \left(1 - \frac{2A_0}{A} \right) \right]. \quad (3)$$

In Eq. (3), A_0 is the limiting molecular area per residue, A is the available area per residue (or monomer unit) in the chain. The parameter Z is the lattice coordination number of the chain segments, i.e., the number of possible sites on which the neighboring residues may be situated. For a rigid chain $Z = 2$, and the chain configuration is fixed. Flexibility may be defined as $100 (Z - 2)/Z = \omega$. This assumes that the upper limit of Z is 4, i.e., a "square lattice." Here X is the degree of polymerization or DP , k is Boltzmann's constant, and T is temperature.

If $X = 1$ or $Z = 2$, Eq. (3) becomes

$$\pi = \frac{kT}{A_0} \ln \left(\frac{A}{A - A_0} \right). \quad (4)$$

If $Z = (2 + \epsilon)$ where ϵ is a small number,

$$\pi A = kT + 1/2 \pi A_0. \quad (5)$$

This is Bull's equation, except for the factor $1/2$.

Plots of πA versus π for polymers and proteins are often linear at the air/water interface where the cohesion is sufficient for low flexibility. If Z is not close to 2, Eq. (3) predicts that the force-area curve will be convex upward. In the oil/water interface $Z = 2$, so we expect curvature which prevents good extrapolation.

The value of Z depends on charge and ionic strength through their effect upon the net cohesive energy; thus, flexibility may be reduced by changes in pH or salt concentration. When cohesion is appreciable, the system is described by an equation relating electrostatic effects to surface pressure, presented by Davies and Llopis (1955); the equation has been derived on a different basis by Isemura, Hamaguchi, and Ikeda (1957). A discussion is presented by Davies and Rideal (1963) and by Payens (1960).

Frisch and Simha's Theory - It is possible that the polymer may not be completely in the interface but may loop into one or both phases forming the interface, especially when compressed. This consideration leads to the relation

$$\pi = \frac{X kT}{A_0} \left[\ln \left(\frac{A}{A - p A_0} \right) - \left(\frac{p^2 A_0}{A - p A_0} \right) \right] - J p^2 \frac{kT A_0^2}{A^2} \quad (6)$$

where A_0 = close-packed area, and J is a coefficient allowing for lateral entropic and enthalpic interaction between those residues fixed in the interface. In this equation,

$$p = 2\alpha / (\pi f_i X)^{1/2} \quad (7)$$

where f_i is a function of internal flexibility of the molecule, X is the DP , and α is called the intramolecular segment condensation coefficient, which expresses the interactions within the polymer molecule. At low π Eq. (6) becomes (since $A \gg A_0$)

$$\pi A = X p kT. \quad (8)$$

This implies that molecules with large DP can form loops more easily and return to the interface to have units act as "independent" kinetic units, lowering their apparent molecular weights. This is not verified experimentally (Davies and Rideal (1963)), since for several proteins and copolypeptides the proper molecular weights are obtained at air/water and oil/water interfaces when the Singer equation is used at very low π (<50 millidynes). This does not preclude its applicability to other experimental situations involving other polymers and interfaces or higher pressures. In practice, distinguishing

between applicability of the Singer or Frisch-Simha theories on the basis of πA experiments is difficult (Jaffe and Loutz (1964), Dieu (1956 a, b, c)). Jaffe and Loutz have shown that both equations may be cast into the form of the virial equation

$$\pi A = kT (1 + B_1 \pi + B_2 \pi^2 + \dots) \quad (9)$$

and that using either Eq. (3) or (6) one can fit the force-area curves reasonably well by adjusting the parameters available. In both the case of the Singer equation and that of Frisch and Simha, however, Jaffe and Loutz point out that the choice of parameters may be ambiguous and that there is no guarantee that any of the parameters chosen bears a simple relation to the property of the polymer it purports to describe. Davies and Rideal (1963) have also indicated that Z should be regarded as an empirical factor because of the many oversimplifications of the theory. Jaffe and Loutz propose to use the function of the first two "virial coefficients" (B_2/B_1^2) to describe the polymer, empirically establishing an assignment of flexibility to values of the ratio. This parameter is independent of a model; insight into details of molecular structure is lacking as yet.

It has also been emphasized (Dieu (1956 a, b, c)) that both Singer's and Frisch and Simha's treatments have neglected intermolecular enthalpy effects. A term in A_0^2/A^2 may be introduced in an attempt to account for enthalpy of mixing in Singer's treatment (Davies (1954 b), Davies and Llopis (1955), Ter Minassian-Saraga and Prigogine (1954)), and the enthalpy of interaction of adjacent residues in the "surface plane" is allowed by Frisch and Simha. There has been at least one case where the Frisch and Simha theory seems to fit the data better than Singer's equation, the case of polyvinyl alcohol at a benzene/water interface (Dieu 1956 a). Dieu points out, however, that even in cases where the Frisch and Simha theory fits better than Singer's theory, values of Z are obtained which do not seem to fit expected chain properties. This may, at the present state of development, be attributed to the lack of recognition of enthalpy effects in the treatment.

In general, one may say that for molecules of low flexibility at the air/water interface one may obtain a good determination of molecular weight (Davis and Rideal (1963), p. 241, Dieu (1956 a, b, c), Davies (1954 b), Davies and Llopis (1955), Bull (1947)). High flexibility (induced by the presence of an oil/water interface or high charge on the macromolecule) or extensive intermolecular association (as in polyleucine) renders extrapolations of the force-area curve uncertain. Jaffe and Loutz (1964) have shown that with flexible polymers, even in the extremely low pressure range below 0.04 dyne/cm, one cannot get a good extrapolation for determining molecular weights. The fact that πA versus π plots for many proteins are indeed linear at the air/water interface lends credence to the notion that not much intermolecular interaction occurs in these systems, although this is not conclusive. Surface rheology studies (Cheesman and Davies (1954), Imahori (1952 a-d), Davies (1954 a, b)) to be described later will also have a bearing on this point. A summary of results of molecular weight determination is given by Cheesman and Davies (1954) together with a discussion of elasticity determinations based on Singer's equation. Subsequent data are listed in papers by Dieu (1956 a-c), Jaffe and de Coene (1957), Harrap (1955 a, b), and Ter Minassian-Saraga (1956).

An illustration of the effect of these ambiguities is the study by Davies and Llopis (1955) of the copolymer 1:1:2 lysine-glutamic acid-leucine. They found that at neutral pH at the air/water interface they could easily get a linear πA versus π plot which gave the correct molecular weight. At extremes of pH, they were forced to very low pressures to overcome the curvature resulting from flexibility induced by charging the molecule. At the oil/water interface, they observed effects very much like the dissociations reported for proteins, and only when the measurements were carried to extremely low π was it clear that the correct molecular weight would be found at $\pi \rightarrow 0$. They point out that, because of the many approximations in the theory, no absolute significance should be attached to the values of Z .

The importance of taking enthalpy into consideration is strikingly illustrated by the fact that the temperature dependence of π is much greater than the proportionality to absolute temperature indicated by both theories (Dieu (1956 a-c), Llopis and Rebollo (1956), Llopis and Albert (1959 a, b)).

Davies and Llopis (1955) have indicated that an energy of interaction may be included in the value of the flexibility ω by writing $\omega = \omega_0 e^{-E/RT}$, which shows that flexibility may change with temperature. This is equivalent to an energy of interaction because it biases the coordination number, $Z = \omega + 2$, in favor either of a greater number of polymer-solvent contacts or of polymer-polymer contacts, depending on the value of E .

Another indication that the Singer and Frisch-Simha theories are not adequate is the observation by Bull (1950) and Dieu (1956 a-c) that the πA versus π curves for proteins are often neither linear nor monotone curves, but show two linear portions separated by a kink. Each linear portion may be extrapolated to yield the same molecular weight. This is taken as evidence of the existence of at least two surface states (Bull (1950), Dieu (1956 a-c)). Experiments on synthetic polypeptides (Isemura and Hamaguchi (1952, 1953)) also suggest this possibility, which will be discussed further when considerations of surface configuration are undertaken (see "Nonpolar Polypeptides"). However, Cheesman and Davies (1954) interpret the kink as evidence of an interaction, saying it shows "stickiness" of the molecules.

Theories Involving Energy of Interaction

Silberberg's Theory - Silberberg (1962) has developed a theory for adsorption of an isolated, flexible molecule at an infinite planar surface. It is assumed that for each segment placed at the surface the internal energy of the system is reduced by an adsorption energy wkT . It is then shown that the polymer molecule will adjust itself so that alternate runs of P_S segments are all in the surface and loops of P_B segments whose size is not a function of molecular weight are all out of the interface. For a molecule composed completely of adsorbable units, on a surface which is everywhere adsorbing, the loops are small, and even at small w the molecules stay close to the surface. Otherwise, the loops are much larger. Restrictions limiting the re-entry of an adsorption loop into the surface have a similar effect. The result is a thin bulk phase of high polymer concentration near the surface. The molecules of polymer are rather flattened, with about half of the segments in the surface phase in some cases. The inability to wash the polymer from the interface (i.e., apparent irreversibility), the similarity of its isotherm to that of Langmuir, and a slow approach to equilibrium are predicted. The surface equation of state derived covers the limiting cases in the literature. Although the treatment conforms with the Gibbs isotherm, the apparent irreversibility of polymer adsorption is explained by the prediction that the adsorption will be very strong even at extremely low solution concentration. The surface equation of state in the limit of low θ is

$$A_0 \pi = kT \left(\frac{\theta/p}{X} + \frac{\sigma^2}{2} \beta_s \right) \quad (10)$$

where X is the number of segments per polymer molecule, θ is fraction of surface sites occupied by polymer segments, β_s is a complicated function, and p is a function of polymer segments at the surface.

It is a consequence of the model that the ends of the molecule stay in the surface.

Motomura and Matuura's Theory - Motomura and Matuura (1963 a,b) have also recently derived a theory which takes the energy of interaction into account and which reduces to the Singer theory when the energy is negligible. This equation also predicts phase changes in the films if the temperature is low enough and energy large enough.

The force-area curve is almost independent of the degree of polymerization χ if χ is sufficiently large (i.e., 7300). Their equation is

$$\frac{\pi}{\pi_0} = \frac{Z}{2} \ln \left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \frac{A_0}{A} \right] - \ln \left[1 - \frac{A_0}{A} \right] - \frac{Z}{2} \ln \frac{\left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \frac{A_0}{A} \right] (\beta_M + 1) - 2 \left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \right] \frac{A_0}{A}}{\left(1 - \frac{A_0}{A} \right) (\beta_M + 1)} \quad (11)$$

where $\pi_0 = kT/A_0$, A_0 is the area occupied by a monomer unit, Z is the coordination number of a site in a two-dimensional surface lattice, χ is the degree of polymerization, and β_M is defined by the complex function

$$\beta_M^2 = \left\{ \frac{\left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \right] \left(1 - \frac{A_0}{A} \right) \frac{A_0}{A}}{\left[1 - \frac{2}{Z} \left(1 - \frac{1}{\chi} \right) \frac{A_0}{A} \right]^2} (e^{2w/ZkT} - 1) + 1 \right\} \quad (12)$$

in which $-2w/Z$ is the energy of contact between neighboring monomer units.

These authors point out that the success of Singer's equation may be due to the fact that Z in Singer's theory behaves similarly to the energy term. They do not account for the possibility that some chains may loop into the bulk phases.

The quantity π/π_0 may exhibit a maximum and a negative minimum when plotted against A/A_0 . This is characteristic of a two-phase film and occurs when $w/kT > 0.864$.

Motomura and Matuura examined poly(vinyl acetate), poly(methyl acrylate), and poly(methyl methacrylate), i.e., PVAc, PMA, and PMMA. In the case of PVAc a value of w was obtained which allowed a good fit to the theory, assuming $Z = 4$, below 6 dynes/cm. Above this pressure, deviations occurred which were attributed to squeezing out of chain segments from the surface.

Molecules of PMA are less expanded as w/kT is higher, and the theory fits better. This is expected, because the lattice approximation should not be as valid for expanded films.

In both the PVAc and PMA cases, $w/kT < 0.864$, so that phase changes were not expected. For PMMA, $w/kT > 0.864$ and the film is of condensed type; it shows vanishingly small π at large areas, with π becoming measurable at ca. $30A^2/\text{monomer}$. The predicted curve becomes negative at $23.5A^2/\text{residue}$; below this the theory predicts a phase transitional region, which is supported experimentally by a fluctuation of the surface potential.

Studies of these films showed that the higher the value of w/kT , the more viscoelastic the film, as would be expected. A similar parallelism between viscoelasticity and cohesive energy was found in studies on bovine serum albumin (BSA) and gamma globulin. The ΔH of spreading and area of onset of non-Newtonian viscosity increased with pH in the case of BSA, while both parameters were independent of pH in the case of gamma globulin (Llopis and Albert (1959 a, b)).

While these results are very promising, especially since phase transitions may be handled, it is this reviewer's feeling that more data must be forthcoming before the newer theories may be fully evaluated.

Electrostatic Effects on the Force-Area Curve

The effect of charge upon the force-area curve of polymers may be separated into intermolecular and intramolecular effects. If the polymer molecules are charged, then repulsion of like charges will decrease the net cohesion of the polymer molecule.

If one writes the flexibility ω as

$$\omega = \omega_0 e^{-E/kT} \quad (13)$$

where ω_0 is the flexibility in the absence of cohesive effects (i.e., at the oil/water interface and at zero charge), electrostatic effects will appear in the parameter E , as will cohesive energy. At very high area where intermolecular effects may be neglected safely, the oil/water interface in the isoelectric region allows the establishment of a base curve for which $E = 0$; electrostatic (and cohesive) contributions to the force-area curve at the air/water interface may be obtained by subtraction. One thus obtains the "excess electrostatic surface pressure" (π_{e1}) experimentally (Davies and Llopis (1955), Payens (1960), Davies and Rideal (1963), Chapter 5).

The interfacial potential Ψ may be calculated according to the Gouy theory, and one may write

$$\pi_{e1} = \int_0^{\Psi} \sigma d\Psi' \quad (14)$$

where π_{e1} is the electrostatic contribution to the surface pressure, and σ is the charge density of the monolayer. This leads to

$$\pi_{e1} = 6.1 \sqrt{C} \left\{ \cosh \left[\operatorname{arc} \sinh \left(\frac{134}{\sqrt{C}} \right) \right] - 1 \right\} \quad (15)$$

(Payens (1960), Davies (1951)). Here, C is the concentration of 1:1 electrolyte. Intermolecular contributions, which may be evaluated at higher pressures, are evident since the apparent potentials decrease with area faster than indicated for isolated charges of the valency of the polymer. The contributions of the intermolecular and intramolecular effects may thus be considered as contributing to the effective potential. An effective charge may be derived by fitting such data.

The value of Z may be related to the electrostatic effect through the relation

$$Z - 2 = \omega = \omega_0 \exp \left(\frac{-E_{\text{cohesion}} + \pi_{e1} A^*}{kT} \right) \quad (16)$$

and the value of A^* derived from the slope of a plot of π_{e1} versus $\log \omega$. The value of A^* so obtained is considered to be the area of the minimum kinetic unit (Joly (1948)) in the polymer in the course of its molecular motion.

The effective charge perturbing the πA curve in this study was found to be 20 percent of the true value of the macromolecular charge and corresponds to the estimate of the charge expected to be at the periphery of the molecule, with the rest of the charge

screened by counter-ions. The size of the kinetic unit appears to be that of five residues, a small fraction of the size of the molecule.

Thermodynamics of the Force-Area Curve

One may attempt to characterize the degree of disruption of molecular structure when a molecule is spread by the value of the free energy change on spreading. This is not a clear-cut analysis as yet, because it is not known how to resolve the observed change on spreading into its components corresponding to changes in solvation, polarization of the interfaces, changes in solution structure, and so on. Neglecting these matters, attempts have been made to compare the entropies of unfolding for various substances. Davies (1953 c) [Cheesman and Davies (1954)] used the following approach.

If one accepts the model of Singer (1948), one can calculate the entropy S_s of a spread polymer with respect to a reference state in which the polymer backbone chain is rigid from the high area portion of the force-area curve. This quantity was given by Davies (1953) as

$$S_s = \left(I - \frac{2}{X} \right) R \ln (Z - 1) \quad (17)$$

using the same notation as in the section titled "Singer's Theory."

The entropy change on compression of the film between any two areas can be calculated if one assumes that the free energy change has only an entropy term and no enthalpy term. Subtracting from this the entropy change which would be found for a completely rigid molecule undergoing expansion or compression between the same areas gives

$$\Delta S_s = \frac{1}{XT} \int_{A_1}^{A_2} \pi dA - \frac{1}{XT} \int_{A_1}^{A_2} \pi_r dA. \quad (18)$$

The entropy of the molecule at any area can be calculated relative to a reference state of the rigid main chain by evaluating π_r , the predicted value for a rigid chain (i.e., $Z = 2$). The Singer theory allows this to be done if the effect of side chains is neglected. Accepting this limitation, we may determine the entropy per residue at the limiting area relative to the rigid reference state.

It turns out for proteins (Davis (1953 c), Cheesman and Davies (1954)) that the experimental unitary entropy of expansion from the limiting area to high area is equal to the calculated unitary entropy of the molecule at high area relative to the rigid molecule reference state.

But this is not true in the cases of polyalanine and polyleucine, for which the unitary expansion entropy is less than that calculated from the Singer theory. (This implies that the limiting area in the case of these peptides does not correspond to a rigid molecule, while the rigid molecule at limiting area is consistent for the proteins.)

The value of S_s calculated in this way may be considered the entropy of surface denaturation if the Singer model of copolypeptide monolayers is accepted and if the entropy of the reference state chosen is indeed the same as that of the native molecules. For example, the experimental data indicate that, for methemoglobin at the oil/water interface, $Z = 2.12$, corresponding to $S_s = +0.22$ eu, while for polyalanine and polyleucine one has $S_s = +1.6$ eu through use of Eq. (18).

Davies states that the values of s_s are similar for most proteins and makes a comparison with the thermal denaturation entropy of trypsin, which is +0.76 eu. The inference is that a number of linkages modified by thermal denaturation are not destroyed by surface denaturation. Other evidence leading to the conclusion that surface denaturation is not complete (found in sections titled "Lysozyme," "Ovalbumin," and "Structural Alteration of Spread Films") makes the argument more convincing, since the validity of this type of argument rests upon Singer's treatment, now known to be inadequate. This approach may be refined using the more satisfactory treatments of the theory of the force-area curve. At this stage of development, the treatment measured a quantity which may be more closely related to the free energy than to the entropy of the transition. We may cite some of the evidence which has been obtained by heating solutions and monolayers. Kaplan and Fraser (1953) found that fibers formed from protein monolayers were quite different when formed from native spread proteins instead of denatured ones, and that if proteins were heated prior to spreading the fibers formed were of the denatured type. Trapeznikov (1948) found a change in surface viscosity η_s at the temperature of bulk denaturation. These observations indicate that the properties of the film are dependent upon elements of structure which are affected by denaturation.

A second approach to the thermodynamic parameters of the polymeric monolayer is the study of the systems at different temperatures. Llopis and Albert (1959 a, b) have done this for bovine serum albumin and globulin. In both cases the enthalpy of spreading on acid at low temperature is negative, i.e., favors spreading. At higher temperatures the enthalpy term is positive. On alkaline substrates, cohesion always predominates. More work on the thermodynamics of the process is required for full understanding, but it is already clear that Singer's equation will have to be improved upon, as will the theory of Frisch and Simha, to take enthalpy effects into account. The theories of Motomura and Matuura (1963 a, b) and of Silberberg (1962 a, b) have not had sufficient application to be adequately judged.

These approaches are not adequate to evaluate the surface denaturation process, because they pertain to the already spread material. Comparisons based on Eq. (18) depend upon the assumption that the molecules at the point of minimum compressibility, or the collapse pressure, are in a state whose entropy corresponds to the solution or solid state. Although there are indications that this may be true (Benhamou (1954), MacRitchie (1963)) (as discussed later in the "Collapse" section), the issue is not settled. It would appear to be more suitable to measure the equilibrium involved in the adsorption from solution to the surface if such an equilibrium could be established. Guastalla and Benhamou (1961) attempted to evaluate the equilibrium parameters in such a situation and found that hemoglobin has too high a molecular weight to be suitable for the experiment; insulin, which is much smaller, appeared to be on the borderline of feasibility.* The data on the entropy and enthalpy of expansion of the already spread monolayer, in addition to the data from the adsorption and spreading steps, are required for a complete description of the surface denaturation process.

SURFACE PARAMETERS AND TECHNIQUES AT FINITE PRESSURES

Molecular weights are studied in the low-pressure region of the force-area curve, where properties of the molecules at the surface may be studied in a highly dilute state. In the highly expanded state, the theoretical attempts to describe molecular behavior have led to estimates of the intrinsic flexibility and size of the solute molecules in what is hoped to be a situation essentially free of intermolecular interaction. When one

*A study of this process with a series of polypeptides as a function of molecular weight would be useful.

compresses a film so that the molecules interact, a different type of information is forthcoming. Experimental parameters, other than the surface pressure will now be considered.

Interfacial Viscosity and Viscoelasticity

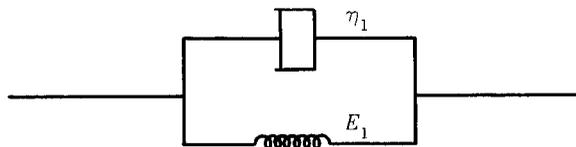
A review of interfacial viscosity and viscoelasticity was presented by Joly (1964). Polymer monolayers may be classified, rather empirically, by interfacial viscosity properties. One finds that condensed films and expanded films have quite different viscosity-area curves. In the case of the condensed film, one finds a high viscosity at areas larger than the limiting area found from a πA experiment. For expanded films, we find that the interface viscosity η_s drops outside the range of high surface pressures (Isemura and Hamaguchi (1954)).

A high surface viscosity would seem to imply intermolecular interaction. This may come about mechanically through chain entanglement (Davies (1953 c)) or through chemical interaction (Isemura and Hamaguchi (1954)), e.g., electrostatic salt linkage, hydrogen bonds. High surface viscosity at high area signifies that a network of some sort is formed by condensed films between molecules before compression sufficient to pack them tightly is reached. In contrast, expanded films show a large increase in interfacial viscosity only when the area is reduced to the limiting area of the force-area curve (Isemura and Hamaguchi (1954)). This implies that the repulsion effects which keep the film expanded also serve to inhibit chemical or mechanical intermolecular interactions at areas larger than the close-packed area.

Isemura and Hamaguchi (1954) emphasize the role of electrostatic interactions in producing surface viscosity, while Davies (1953 c) interpreted the increase of film viscosity of polypeptides at an oil/water interface as evidence that a loss of intramolecular cohesion allows sufficient interaction to occur at higher area, as chains are compressed. Increase of η_s at high charge may also be the result of expansion (see also Joly (1964)). A theory of viscosity based upon the general theory of rate processes has been formulated by Eyring and coworkers (Glasstone, Laidler, and Eyring (1941)). Conclusions reached from a study of activation areas are cited in the "Proline and Hydroxyproline" section. Ikeda and Isemura (1959) found that a strongly cohesive condensed polymer exhibits an area of activation which extends over the whole molecular area, while for expanded films with weaker cohesion, the area corresponds to only a small section of the molecule. Earlier, Joly (1948) had observed, also on the basis of the Eyring theory, that since all proteins seemed to have approximately the same viscosity coefficient at the onset of non-Newtonian viscosity, the kinetic subunits of the protein monolayer must be of similar area. Calculation of the activation energy of flow led to a value of $90A^2$ for this unit. Joly's treatment relies upon (a) an estimation of the contributions of the various components of the molecule to the interaction energy and (b) the deduction that the spacing of the kinetic units at the onset of non-Newtonian viscosity is such as to let the width of an assumed hexagonal pseudolattice be twice the diameter of the kinetic unit (assumed spherical). Ikeda and Isemura do not give the details of their calculation. The strongly cohesive polymers of poly(phenylalanine), polyleucine, etc., have a much higher density of nonpolar amino acids than the proteins.

An alternative method of analysis of viscoelasticity in films is shown in the series of works treating the rheology of materials as represented by a mechanical model. The mechanical analogy is a system of springs and dashpots; the springs represent elastic elements, and the dashpots, viscous elements. These elements may be combined in various ways, such as:

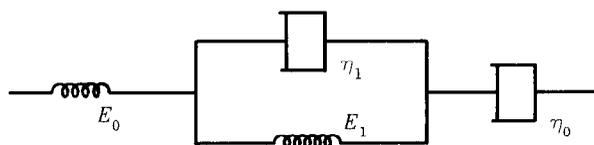
1. Voight Element:



2. Maxwell Element:



3. Four-Parameter Model:



In an appropriate mathematical analysis of the result of a stress-strain measurement, where strain is followed as a function of time, and the stress is permitted to decay, the parameters of such models, i.e., the E_i and η_i , may be evaluated. Such a treatment is given by Motomura and Matuura (1963 b), as well as by others. The relation between the type of model, the values of the constants, and what goes on in the actual film is still obscure. One may say that the existence of elastic elements implies the presence of a network of some kind in the film, and Tachibana and Inokuchi (1953) have found a relationship between kinks or plateaus in the force-area curve and the onset of instantaneous elasticity (i.e., nonzero E_0). They have also postulated that Hookean elasticity depends upon the strength and exchange rate of network bonds.*

The last paragraph of the paper by Motomura and Matuura indicates the type of conclusion which may be drawn. After showing that the viscoelastic parameters were larger for PMMA than for PMA, and were smallest for PVAc, they wrote: "In the preceding paper, the value of w/kT , which was related to the cohesive force between monomers, was estimated for the three polymers, and it was found that the order was PMMA > PMA > PVAc. The present result might show a parallelism between the values of viscoelasticity and w/kT . It seems proper from this point that linear polymer monolayers are made up of a two-dimensional network structure produced by extended polymer molecules and their rheological behavior is determined mainly by the cohesive force between segments"

Polymer films may, to some extent, be grouped according to the mechanical model describing them (Tachibana, Inokuchi, and Inokuchi (1959)); group A has an elastic element only. It is suggested that films of this type form a two-dimensional network and

*However, they have since shown that they can eliminate instantaneous elasticity without changing the force-area curve by use of an organic spreading solution (Tachibana, Inokuchi, and Inokuchi (1957)).