SURFACE PROPERTIES OF PROTEINS

by

SHU-HSIEN CHEN

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1976

This manuscript has been read and accepted for the Graduate Committee in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

MAY 17, 1976
date

Henri L. Rosano
Chairman of Examining Committee

5/18/76
date

Leonard H. Schwartz
Executive Officer

Bernard J. Bulkin
Dr. Bernard Bulkin

Henri L. Rosano
Dr. Henri L. Rosano

Arthur E. Woodward
Dr. Arthur E. Woodward

Supervisory Committee

The City University of New York

Acknowledgements

My most sincere thanks go to my mentor, Professor Henri L. Rosano for his untiring guidance, immeasurable encouragement and advice. I have learned much and benefited greatly from his broad knowledge and experience in surface chemistry.

I would like to thank Dr. G. Colacicco, of Albert Einstein College of Medicine, for stimulating discussion. I would like also to thank my colleagues Dr. William Gerbacia and Dr. James Whittam for their helpful comments and suggestions. Thanks are also due to E. La Gamma, for showing me to use the surface balance.

I wish to express my gratitude to Dr. B.Bulkin and Dr. A. Woodward, for their generosity in offering their time to serve on my guidance and examining committee.

I must also acknowledge Mr. H. Schimatz for his help in designing glass equipments used in this work, and Mrs. C. Silver for her inestimatable secretarial help throughout my stay at the college.

Finally, I would like to express my deepest gratitude to my friends Dr. R. Colon and S. Schechter for correcting my grammar, Dr. D. Chen for helpful discussion, C. S. Chuang for his technical assistance, and K. H. Hsu for drawing some of the figures presented in this dissertation.

CONTENTS

TABLES

ILLUSTRATIONS

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Introduction

Most of the reactions occuring in living organisms take place at interfacial surfaces. In addition, the dispersion of one phase in another phase e. g., as the formation of emulsions or foams are greatly influenced by the presence of amphipatic molecules oriented at the interface. The following work deals with the study of the surface properties of proteins which are of both biological and physical importance.

Three proteins -- bovine serum albumin, β-casein and gelatin are selected for the study mainly because they are available in relatively pure form. Furthermore, protein-lipid interactions which are of great practical importance, are also investigated, using the following model systems: bovine serum albumin - sodium eicosyl sulfate, bovine serum albumin -fatty acid (C_{20}, C_{22}), gelatin - sodium eicosyl sulfate and gelatin -fatty acid (C_{20}, C_{22}).

In view of the fact that a model was required, a monomolecular film was utilized. This molecular layer provided an amphipatic substance which was oriented at an interface of two bulk phases, thus proving itself convenient and simple for the study of the interfacial film.

Several methods were utilized to obtain a monolayer and are outlined as follows :

1. Spreading of the surface active molecules from the air phase. (Used throughout the following work for preparing insoluble monolayers.)

2. Adsorption of the surfactant from the solution to the interface. (Since the surfactant is introduced by injecting or pipeting into the

bulk of the substrate, the molecules are in equilibrium between the solution and the interfacial phase. This technique is employed in the adsorption and penetration experiments presented in chapter 5.)

3. A combination of the above. (This technique provides an excellent method to combine two different substances into an interface from two adjacent bulk phases, thus allowing one to study the course of interaction of the substances at the interface. The interaction experiments summarized in chapter 5 are conducted by this technique.)

A few investigators (1), using stretched-thread surface micromanometer, have been able to determine very low surface pressure necessary for the estimation of molecular weight and co-surface of molecules in the monolayer. This technique was utilized in our procedure to estimate the molecular weights and co-surfaces of bovine serum albumin and β-casein spread on various pH substrates and will be presented in chapter 1.

In the following chapter a statistical mechanical partition function and thermodynamic equations are used to estimate surface pressures which are then compared with those determined by monolayer technique.

Proteins which are not very surface active and often soluble in aqueous substrate, are not easily spread on aqueous surfaces. When they are spread, they can assume different conformations at different conditions and consequently a reproducible monolayer is difficult to obtain. However, by using a specific spreading agent under controlled conditions an expanded monolayer can be obtained. The effects of concen-

tration of various spreading agents (n-pentanol, n-propanol and ethanol), and the pH and ionic strength of substrates on the spreading of bovine serum albumin over aqueous surface were investigated and will be discussed later on.

Surface pressures caused by a small mechanical perturbation were measured at various distances from the movable barrier. The purpose was to obtain the surface viscosities of bovine serum albumin and β-casein monolayers which were spread on various substrates. These will be thoroughly discussed in chapter 4.

CHAPTER ONE

Molecular Weight and Co-Surface of Bovine Serum Albumin and β-Casein

I. Background

The use of monolayers for molecular weight determinations was first suggested by Guastalla in 1938 (1). It involves the study of very expanded " gaseous " films which exert very low surface pressures (π). By kinetic theory analogous to the three-dimensional ideal gas, a gaseous monolayer of area A follows a two-dimensional ideal gas law

$$\pi A = n R T$$

where n is the number of mole. For a substance of unknown molecular weight M, $n = \frac{W}{M}$, where w is the weight of film spread on the area A, then

$$\pi A = \frac{W}{M} R T$$

In a real case ideality is not attainable and the deviation has been accounted for by introducing the Van der Waal's attractive force and co-surface correction (2).

$$(\pi - \pi_o)(A - A_o) = n R T.$$

At extremely low concentration the molecules are far apart from each other and move independently, thus π_o has been assumed to be equal to zero. The equation becomes

$$\pi (A - A_o) = n R T = \frac{W}{M} R T.$$

Substituting concentration C for $\frac{1}{A}$ to the above equation

$$\frac{\pi}{C}(1 - A_o C) = \frac{W}{M} R T$$

and geometrically extrapolating C/π vs. C curve to infinite dilution, Guastalla has calculated the molecular weight and co-surface of protein (1b). This method has been applied to determine the molecular weights and co-surfaces of oxyhemoglobin (3), polyester (4), lipopolysaccharide

(5) and fatty acid (6).

Assuming an insoluble monolayer to be a perfect osmometer and following a thermodynamic approach, Bull by plotting πA vs. π and calculating the intercept of the curve to the y axis by the least square method, determined the molecular weight from the intercept. The molecular area was calculated from the film area at minimum compressibility. From this approach Bull arrived at the same equation (7) derived by Guastalla.

Allan and Alexander (8) have used Bull's method to determine the molecular weights of several proteins and found that the surface pressure against film area relationships of the gaseous films are affected by molecular cohesive forces at the air/ liquid interface. Therefore, they concluded that molecular weight can not be estimated reliably from the following equation: $\pi A = \pi A_o + n R T$. On the other hand, they suggested that the method may be employed to distinguish between proteins and their dissociation products.

To obtain a correct molecular weight of a protein, Harrap (9) has shown that a very low surface pressure due to a very low surface concentration of the protein has to be measured, because association occurs when the surface concentration is increased. It is also found that the molecular weight is affected markly by pH and the ionic strength of the substrate (9) e. g., at low pH, insulin was found to have a molecular weight of 6 000 which is in agreement with Frederieq's value(10). At higher pH, the molecular weight calculated by Bull's method increases with the surface concentration, unless the experiment is carried out at very low surface concentration. An increase in ionic strength has a dissociation effect at the isoelectric point of the protein, and an association effect at pH values removed from the isoelectric point. The effects have

been explained in terms of the Coulombic and non-Coulombic forces operating in the system.

Fowkes has proposed an alternate method for the calculation of molecular weight by monolayer technique (11). By considering the monolayer as a semipermeable membrane, the surface pressure as an osmotic pressure (π) and assuming activity coefficient of solvent molecules in the monolayer to be unity, a thermodynamic relationship between the surface pressure and mole fraction of solvent is obtained:

$$\pi = - \frac{k T}{\sigma_2} \ln X_2$$

where X_2 and σ_2 are the mole fraction and the partial molecular area of the solvent molecules in the monolayer. From the equation the ratio of the number of moles of the solvent to that of the film forming molecules $\frac{n_2}{n_1}$ is calculated and plotted against the film area A. By assuming σ_2 to be equal to 9.7 Å^2, the molecular weight and the partial molecular area of the film forming molecules are determined respectively from the slope and the intercept of the A vs. n_2 / n_1 curve. It has been claimed that this method gives a better estimation of molecular weight than Bull's method, because the molecular weight is calculated from the more easily determined slope of the A vs. n_2 / n_1 curve.

Since Guastalla's method enables one to measure a very low surface pressure which is necessary for the determination of the molecular weight of a protein correctly, the method was used in the following experiment to estimate the molecular weights and the co-surfaces of bovine serum albumin and β-casein molecules spread on aqueous substrates of different pH values.

II. Theory

A very dilute monolayer where the molecules are far apart and move independently, behaves as a two- dimensional ideal gas. The film molecules move with an average translational kinetic energy 1/2 k T for each degree of freedom. For the two-dimensional surface, the total kinetic energy is k T, which is assumed to produce the surface pressure π

$$\pi a = k T$$

where a is the surface area per molecule. In a real system, deviations from ideality were observed and have been treated by adopting co-surface correction similar to Van der Waal's co-volume correction in the three-dimensional real gas. The equation for one mole of protein becomes (12)

$$\pi (A - A_\omega) = R T$$

and for one gram:

$$\pi (\frac{1}{C} - \frac{1}{C_\omega}) = \frac{R T}{M}$$

where A_ω is the co-surface correction, C is the surface concentration in gram / cm^2, M is the molecular weight and R is the gas constant (8.31×10^7 erg / mole, deg.). The above equation can be used to calculate the molecular weight and the co-surface of a protein by extrapolating to infinite dilution.

$$\frac{\pi}{C} (1 - \frac{C}{C_\omega}) = \frac{R T}{M}$$

or

$$(1 - \frac{C}{C_\omega}) = \frac{R T}{M} \frac{C}{\pi}$$

Since C is a linear function of C/π, at the limit of $C \rightarrow 0$,

$$M = R T (\frac{C}{\pi})_{C \rightarrow 0}$$

Similarly, at $\left(\frac{C}{\pi}\right) \rightarrow 0$, $C_o = C$, thus the co-surface per molecule is

$$a_o = \frac{M}{C_o} \frac{1}{6.02 \times 10^{23}}$$

III. Experimental

1. Apparatus and Procedure

Guastalla's " Surface micromanometer " (Figure 1.1) was used to measure low surface pressure down to millidyne / cm. It consisted of a pyrex glass trough filled with water or solution, a floating paraffin coated mica frame devided into two equal area compartments by a fine silk thread which was lightly coated with paraffin wax, a torsion wire attached to the silk thread through a wooden stick and a paraffin coated needle, a light source,and a lens-mirror system. The instrument was enclosed in a Faraday box which protected the measurement from the disturbance of breeze and dust.

A known volume of protein solution was deposited with an Agla micrometer syringe (Burroughs Wellcome Co., Tuckahoe, N.Y.) on one side of the thread, and an equal volume of solvent was placed on the other side. The surface pressure exerted by the film caused a deflection of the thread. Since equal volume of solvent was placed on each side of the thread, the surface pressure exerted by trace surface active impurity in the solvent would be the same on each side and the corresponding deflection of the same size but in opposite direction would cancel each other. The displacement of the center of the silk thread, corresponding to the surface pressure exerted by the protein film only, was observed through the lens-mirror system as a function of time. A linear relationship was obtained by plotting the displacement vs. the square root of time (13). The dis-

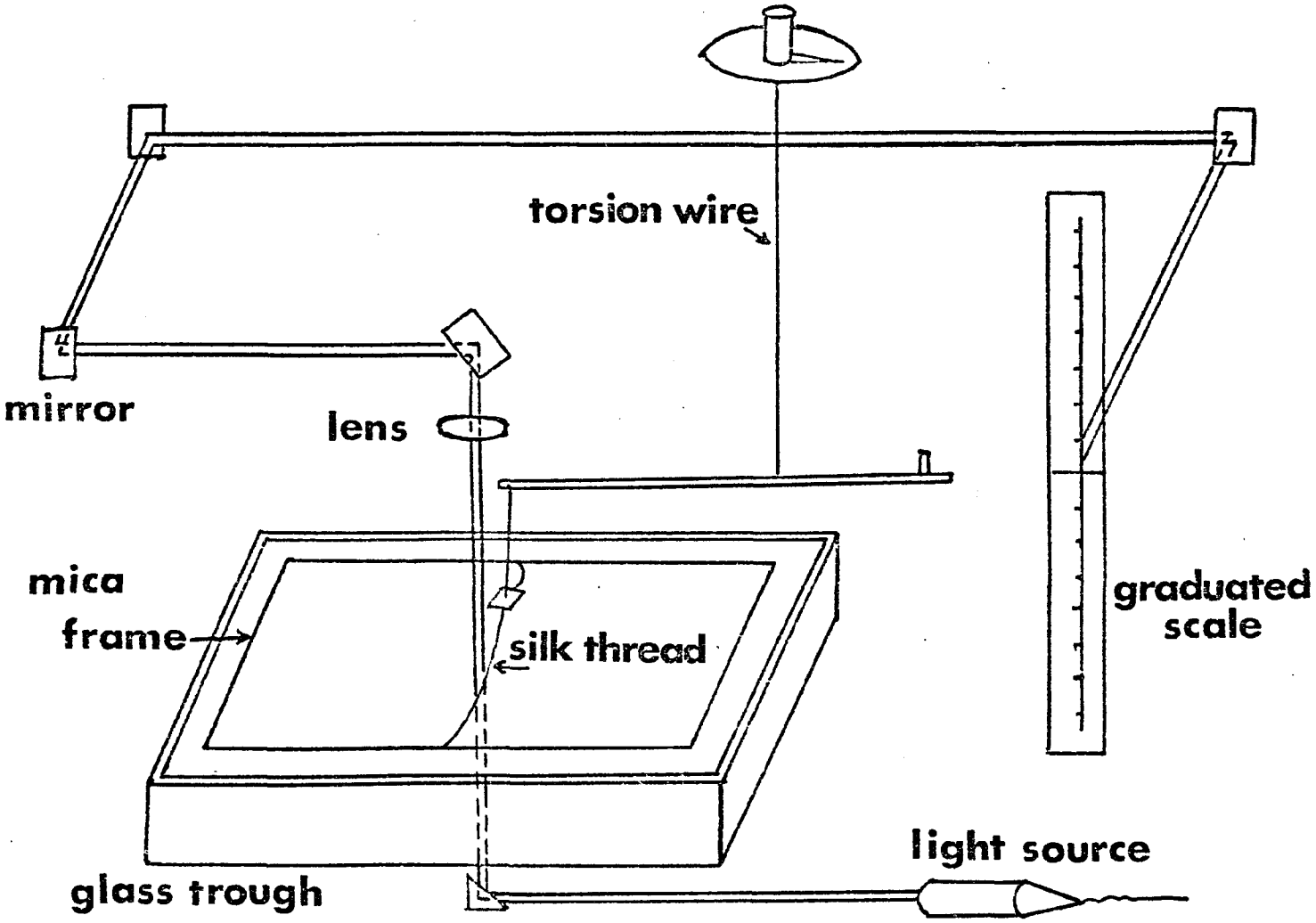

torsion wire
mirror
lens
mica
frame
silk thread
graduated
scale
light source
glass trough

Figure 1.1

placement at zero time was then used to calculate the surface pressure which was related to the displacement of the thread by the torque applied to the torsion wire.

The surface was cleaned by dusting calcinated talcum powder onto it and removing it with the aid of a hollow glass tip connected to an aspirator.

2. Material

Bovine serum albumin was perchased from the Nutritional Biochemicals Corporation, Cleveland, Ohio. β-casein was provided by Dr. V. Moreno of the General Food Corporation, Tarrytown, N. Y.. Sodium hydroxide, hydrochloric acid and 1-pentanol were obtained from the Fisher Scientific Co., Fair Lawn, N. J.. All the substances were used directly without further purification.

Spreading solutions were prepared by dissolving crystal protein in freshly distilled water containing 0.5 % 1-pentanol. The protein concentration in the solution was about 1 mg / ml and the quantity spread was of the order of one microgram over 200 cm^2 of aqueous surface. The substrates were prepared by mixing 0.1 N NaOH solution with 0.1 N HCl solution to a desired pH.

Trace surface active impurity in the substrate was removed by foaming the substrate solution in a 500 ml medium porosity sintered glass funnel. The foam was swept away with a spatula.

IV. Results and Discussion

1. Molecular Weight and Co-surface

The determinations of molecular weights and co-surfaces of the bovine

serum albumin and β-casein are shown in figure 1.2. The C/π vs. C curve has a negative deviation from the ideal horizontal line of zero slope. It is explained as the result of the presence of a large net repulsive force in the monolayer (4). A large positive value of co-surface correction which is the characteristic of the large and complex protein molecule, was observed for each protein. The co-surfaces were found to be 12000 $\mathring{A}^2$ and 5600 $\mathring{A}^2$ respectively for the BSA and the β-casein. The value of 12000 $\mathring{A}^2$ for the BSA was in agreement with those determined by Bull (7d), by Muramatsu and Sobotka (25).

The molecular weights determined are 67 000 and 27 000 respectively for the BSA and the β-casein . They agree with the data determined by osmotic pressure (14), sedimentation equilibrium (15,17, 19), light scattering (16), Archibald's method (18, 20), sedimentation diffussion (21) and chemical method (22, 23, 24) as well as monolayer technique reported by Bull (7d), Muramatsu and Sobotka (25). Therefore, the determination of molecular weight by Guastalla's monolayer technique at infinite dilution on isoelectric substrate, is considered to be valid.

2. The Effect of pH of the Substrate

When the pH of the substrate is removed from the isoelectric point of the protein, the apparent molecular weights and the apparent co-surfaces of the BSA and the β-casein calculated by Guastalla's method become smaller than the values at the isoelectric pH (Table I.1, Figures 1.3 and 1.4). Since it is known that both BSA and β-casein do not dissociate in acidic and basic conditions, the calculated apparent molecular weights and co-surfaces can not be the true molecular weights and the co-surfaces of the proteins. In other words, gaseous equation employed in Guastalla's

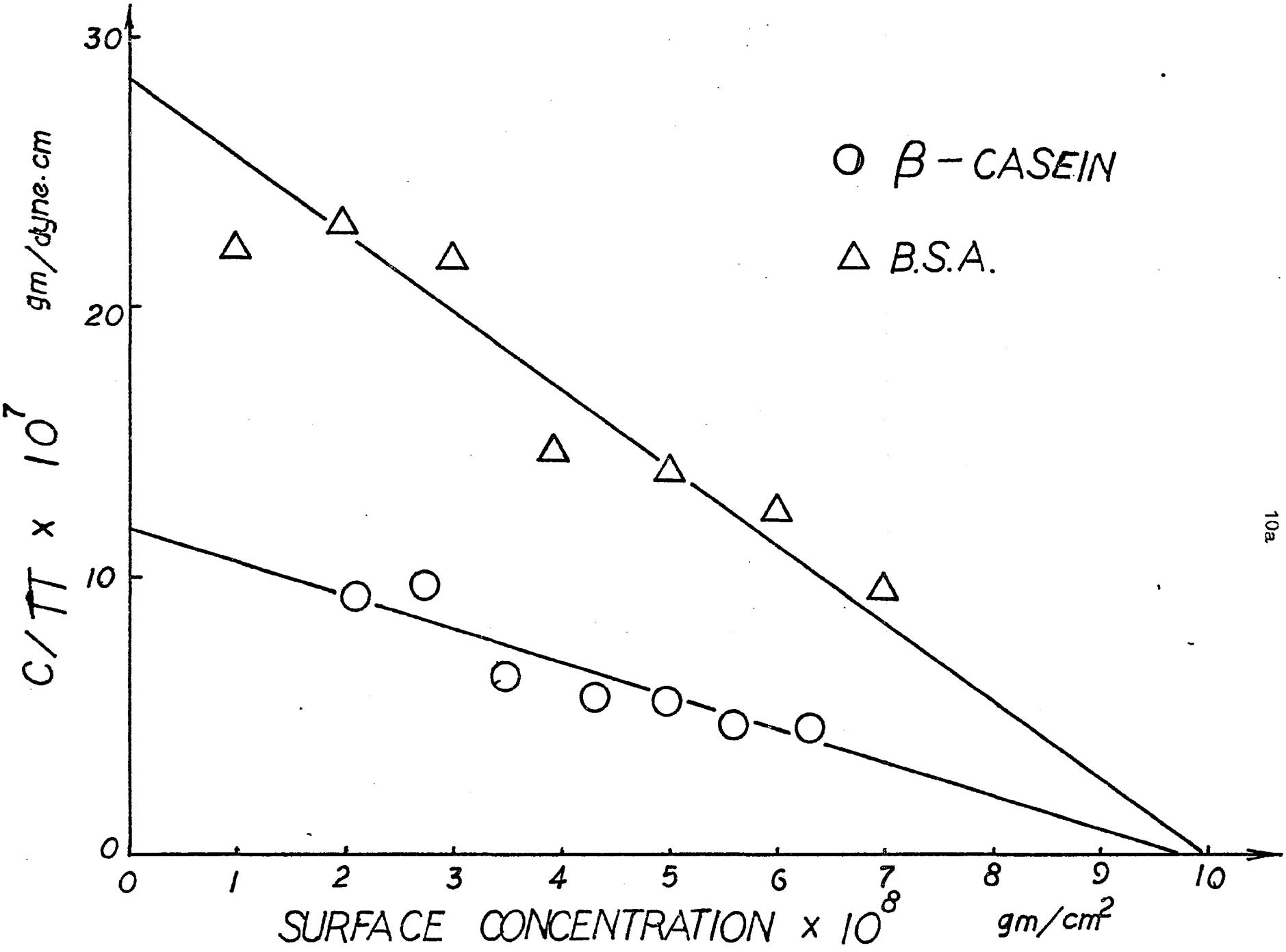

30
20
10
0
C/TT x 10^7
gm/dyne.cm
O β-CASEIN
Δ B.S.A.
0 1 2 3 4 5 6 7 8 9 10
SURFACE CONCENTRATION x 10^8
gm/cm^2

Figure 1.2

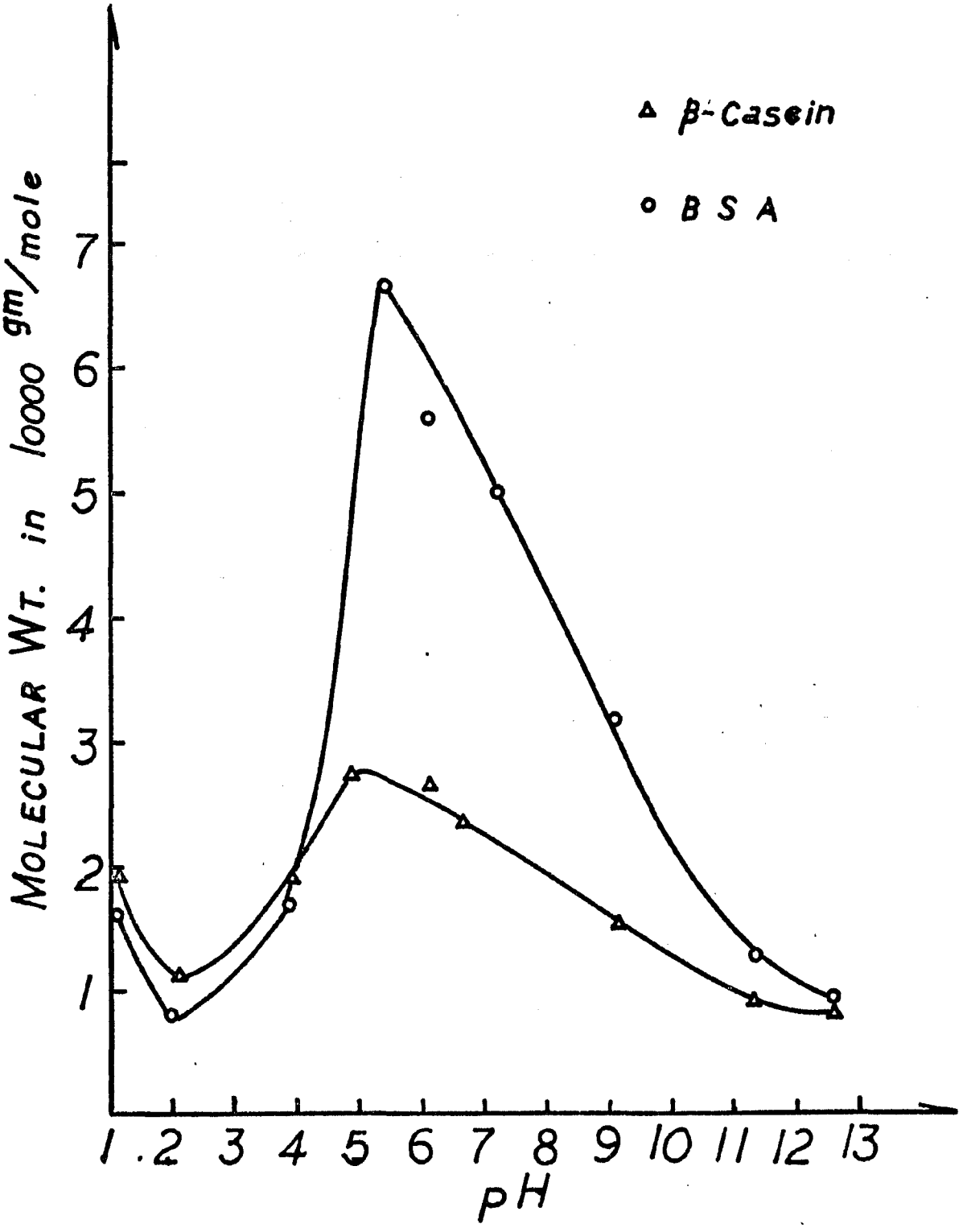

β-Casein
B S A
Molecular Wt. in 10000 gm/mole
1
2
3
4
5
6
7
1 .2 3 4 5 6 7 8 9 10 11 12 13
pH

Figure 1.3

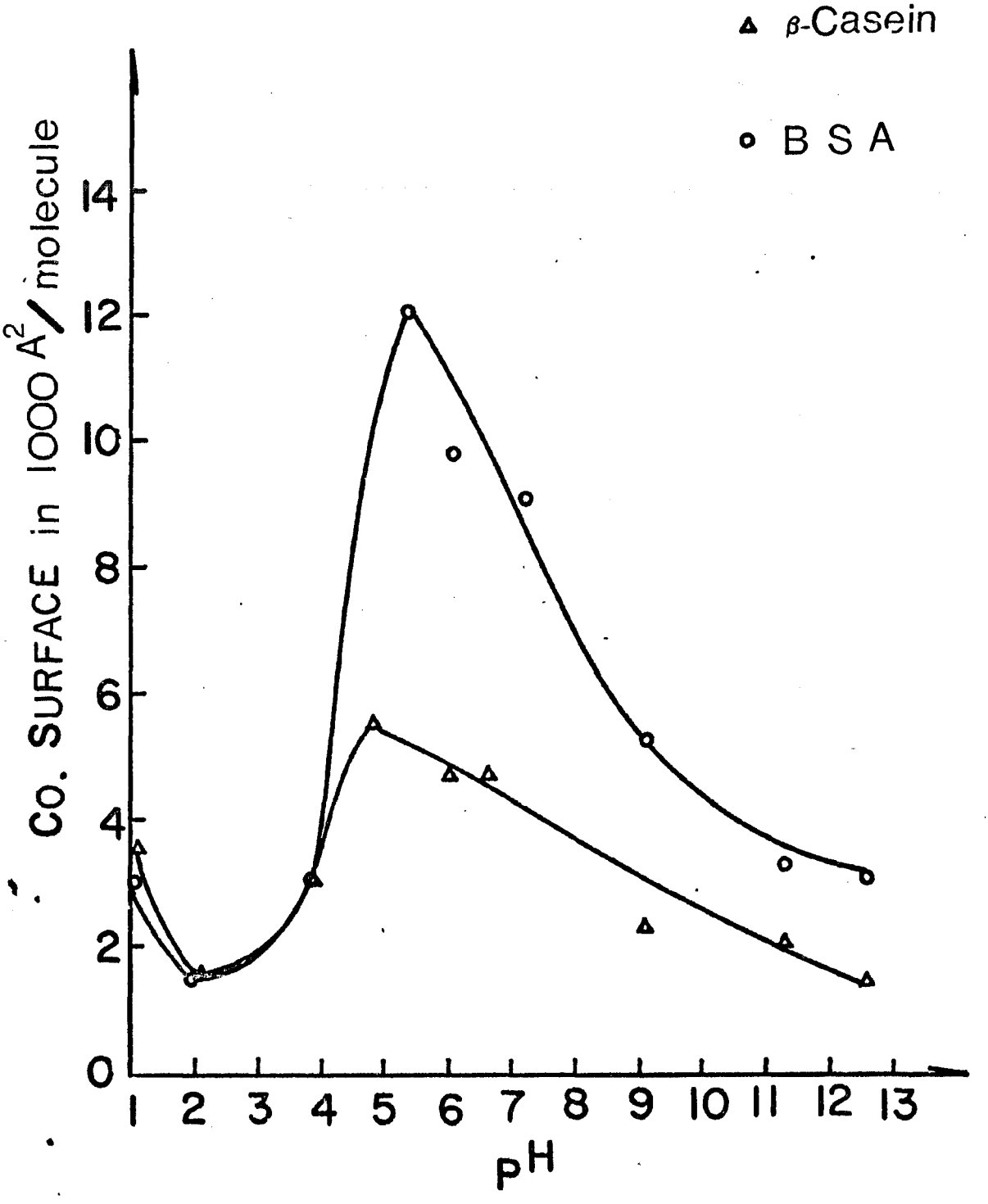

β-Casein
B S A
Co. SURFACE in 1000 A²/ molecule
0
2
4
6
8
10
12
14
1 2 3 4 5 6 7 8 9 10 11 12 13
pH

Figure 1.4

Table I.1

Bovine serum albumin

pH	Molecular Weight (g / mole)	Co-Surface ($\mathring{A}^2$ / molecule)
1.1	15900	3070
2.0	8200	1540
3.9	17300	3040
5.35	66600	12000
6.05	56300	9840
7.2	50200	9130
9.1	31800	5280
11.3	12500	3330
12.55	9900	3080

β-casein

pH	Molecular Weight (g / mole)	Co-Surface ($\mathring{A}^2$ / molecule)
1.1	19300	3490
2.1	11500	1540
3.9	19200	3010
4.8	27200	5570
6.05	26400	4740
6.6	23600	4730
9.1	15200	2280
11.3	9070	2080
12.55	8150	1380

method for the determination of molecular weight and co-surface is not applicable to acidic and basic monolayers.

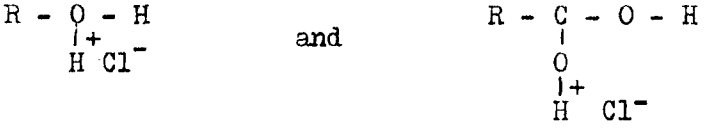
In order to interpret the above results, Bull's method has been used, πA is plotted against π and extrapolated to $\pi = 0$. All πA vs. π curves are parallel to each other except those above pH11.3 (figures 1.6 and 1.5). The parallel lines may be described by the equation:

$$\pi A - b = n_2 R T + n_2 \sigma_p \pi$$

where b is the distance from the πA vs.π curve at the isoelectric pH to the curve at the other pH. It represents the repulsive forces between the molecules in the monolayer. The value increases as the pH of the substrate is farther from the isoelectric pH, because the net charge of the protein molecule increases.

Under very alkaline condition, the deviation of the πA vs.π curve from parallelism indicates that the molecular area of the protein at this condition is different from those at other pH. The molecular area becomes larger because of the further unfolding of the protein molecule due to the breaking of hydrogen bonds owing to the ionization of phenolic hydroxyl groups. This interpretation is supported by the result that the deviation from parallelism is much larger for the bovine serum albumin which has 14 tyrosine residues than for the β-casein which has only 4 phenolic hydroxyl residues.

It is not understood why the value of b decreases below pH 2. A most probable explaination is that oxonium compounds :



are formed below pH 2 (26), hence the repulsive force between the positive charged groups ($-NH_3^+$) is minimized by the presence of these

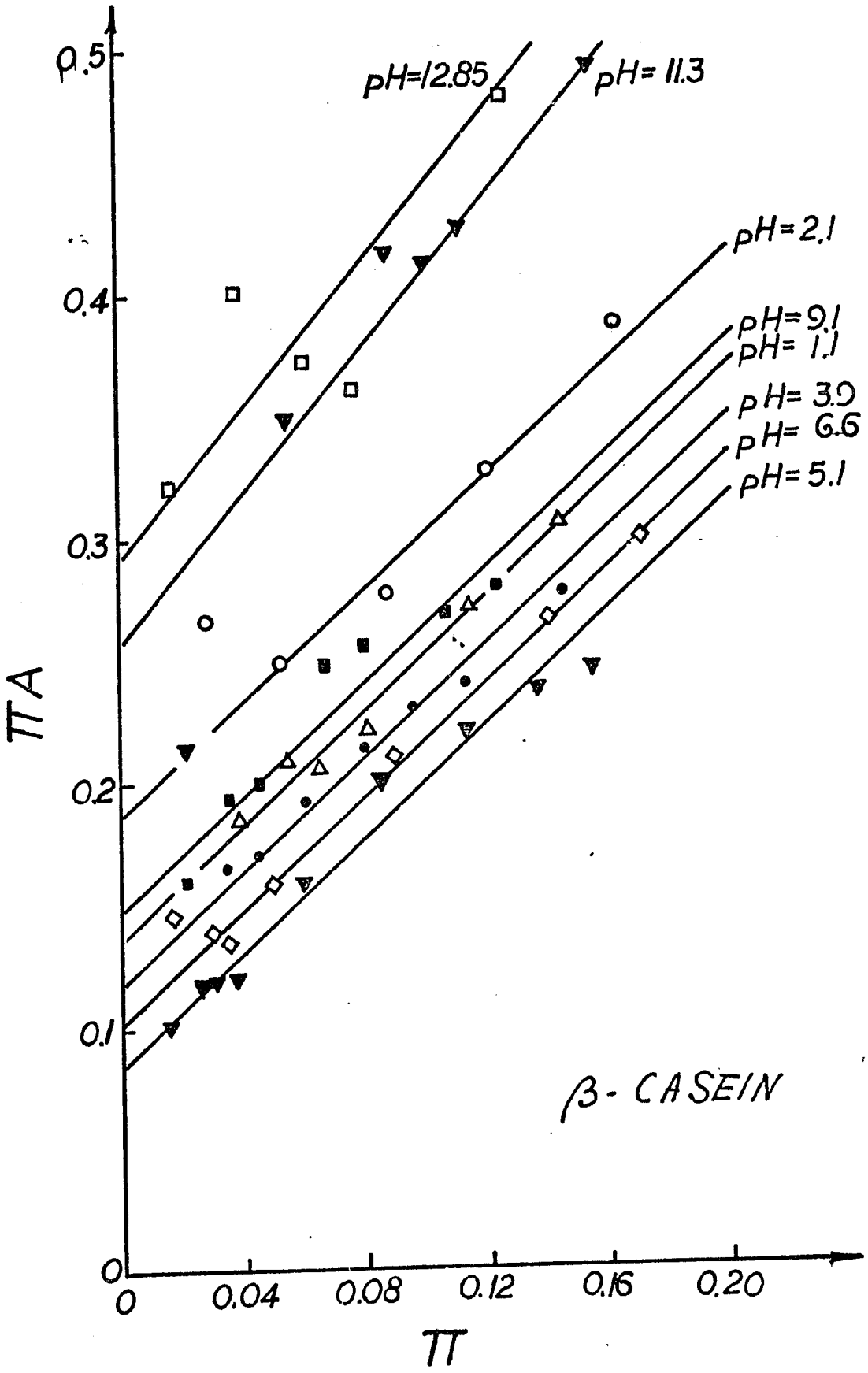

0.5
0.4
0.3
0.2
0.1
0
0
0.04
0.08
0.12
0.16
0.20
ΠA
Π
pH=12.85
pH= 11.3
pH=2.1
pH=9.1
pH= 1.1
pH= 3.0
pH= 6.6
pH=5.1
β-CASEIN

Figure 1.5

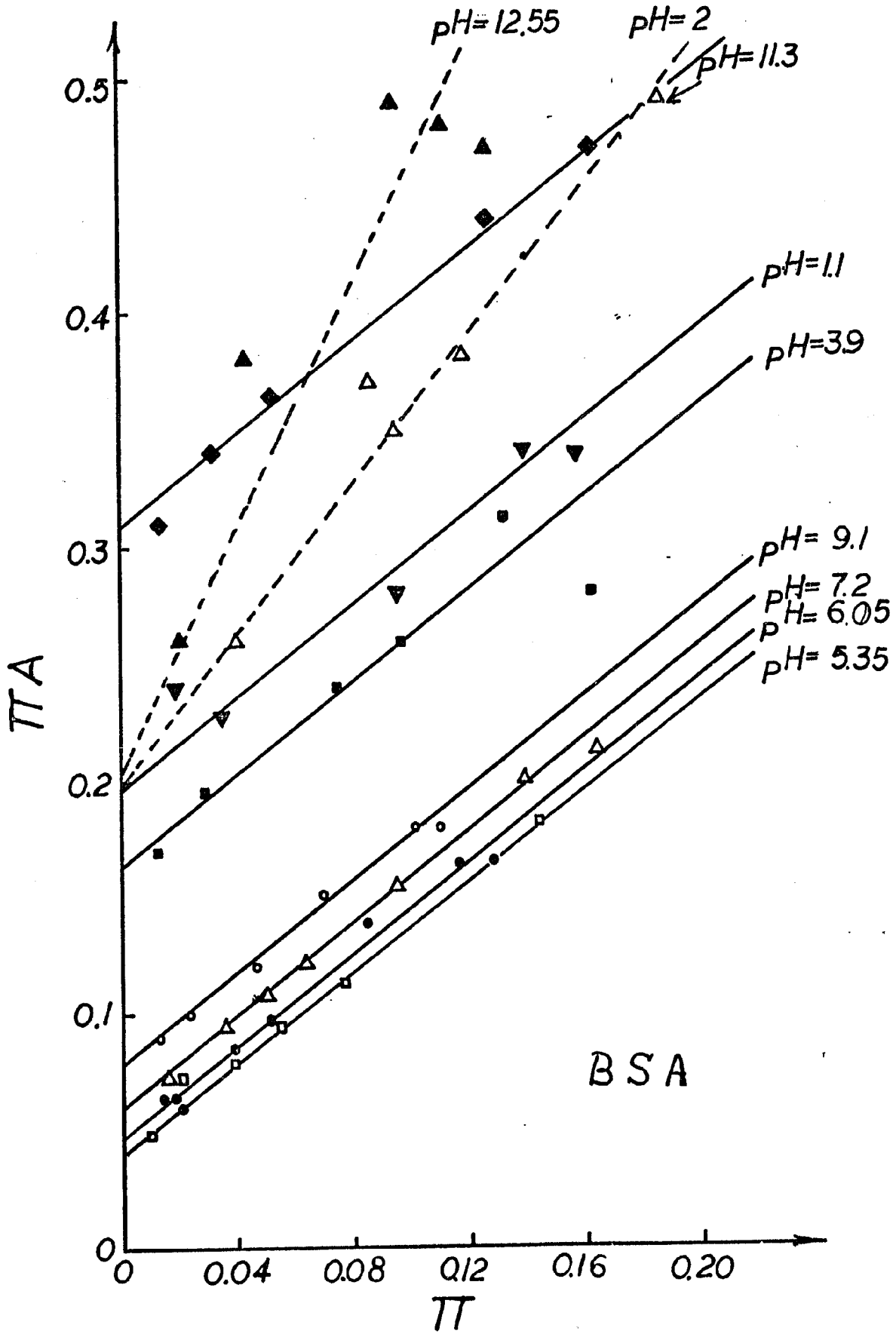

pH= 12.55
pH= 2
pH= 11.3
pH= 1.1
pH= 3.9
pH= 9.1
pH= 7.2
pH= 6.05
pH= 5.35
BSA
π/A
π
0
0.1
0.2
0.3
0.4
0.5
0.04
0.08
0.12
0.16
0.20

Figure 1.6

compounds.

V. Conclusion

The molecular weights and the co-surfaces of the bovine serum albumin and the β-casein spread at their isoelectric points are found to be in good agreement with those determined by other methods reported in the literature.

The agreement of the molecular weight and the co-surface calculated by Guastalla's method with those determined by various other methods, confirms the validity of the gaseous equation in describing the very dilute monolayer of protein at its isoelectric point. However, the gaseous equation is not satisfactory to represent the protein monolayer spread on a substrate of pH other than the isoelectric point of the protein. This is due to the fact that each molecule has net positive or negative charge, thus deviating from ideal gas behavior. Therefore, the molecular weight and the co-surface calculated from the gaseous equation under this condition can not represent the true values.

In order to interpret the experimental results, an empirical equation has been proposed to describe the protein monolayer at various pH values.

$$\pi A - b = n_2 R T + n_2 \sigma_p \pi$$

where b is a variable which changes with the pH of the substrate and may be related to the repulsive force between the molecules in the monolayer. Below pH 2, the molecular charge is shielded by the presence of the oxonium compounds, thus b decreases with the pH. Under very alkaline conditions, because of further unfolding of the protein molecules, the monolayer deviates from the above equation.

CHAPTER TWO

Calculation of Surface Pressure Using Statistical Mechanical Approach

I. Introduction

Equations of state for monolayers have been developed using two main approaches: a) molecular and b) thermodynamic models. The agreement between these models and experimental results has been limited because of several fumdamental factors. In the case of molecular model (1), the steric picture of the interface is usually simplified to permit the model to be handled mathematically, but this approach limits the applicability of the model. In order to further generalize the model, more approximations are introduced, but the resultant equations become difficult to solve exactly, and resort to approximate solutions becomes necessary. In the case of thermodynamic models (2-6), the necessity of estimating difficult parameters such as activities in the interfacial regions as a function of surface concentration makes this approach objectionable.

Statistical mechanics provides the needed bridge between mechanics and thermodynamics, and allows a theoretical calculation of thermodynamic properties from information about individual molecules (7,8). For simple systems, such as two-dimensional gases, the calculations are not complex, and values of thermodynamic properties so obtained are often more accurate than ones measured directly.

As in three-dimensional fluids, the extremely dilute or "gaseous" region of the monolayer is the least complex region to investigate theoretically. Moreover, the gaseous region in a surface isotherm of a monolayer obeys a two-dimensional ideal gas equation at the limit of infinite surface dilution. When the surface concentration increases, positive and negative deviations from ideality are observed, as with three-dimensional gases.

An equation relating the surface pressure to the translational partition function of the two-dimensional gas molecules has been derived (7,8). Surface pressures of bovine serum albumin and β-casein at various surface concentrations were calculated using the above equation. Experimental to calculated surface pressures versus surface concentration curves were plotted and will be discussed later.

II. Theory

In a two-dimensional film, at very low surface concentrations where the surfactant is considered to be in a "gaseous" state, the translational partition function for the surface molecules can be determined by statistical mechanics and used to calculate a "surface pressure" as a function of surface concentration. By analogy to real gases, the Helmholtz free energy of a two-dimensional gas is given by:

$$A = - N K T \ln Q$$

The equation of state which can be obtained by differentiating the work function partially with respect to the area, can be expressed by the equation:

$$\Pi = - \left(\frac{\partial A}{\partial \sigma} \right)_T = N K T \left(\frac{\partial \ln Q}{\partial \sigma} \right)_T \qquad (1)$$

where Π is the surface pressure, σ is the molar area, N is the Avogadro's number, K is Boltzmann's constant, T is the absolute temperature and Q is the two-dimensional molecular partition function. Q is given by:

$$Q = \left(\frac{2 \pi m K T}{h^2 \delta} \right) \qquad (2)$$

where m is the mass of the "gaseous " molecule in the film, h is Planck's constant and δ is the surface concentration expressed in molecules per cm^2.

III. Experimental

The gaseous surface isotherms of bovine serum albumin and β-casein were established using the surface micromanometer technique described in chapter one. The two-dimensional molecular partition functions of the two proteins were estimated from experimentally determined values of m and δ with equation 2. And the surface pressures at various surface concentrations were calculated with equation 1.

Table II.1 shows the ratio of experimental to calculated surface pressures, termed the " z factor", versus pH of the aqueous substrate. In the last two columns, the calculated values of surface pressure are respectively obtained from the Boyle's Law expression and from the assumption of osmotic equilibrium using the Fowkes' equation of state (4,9).

Figure 2.1 shows the plot of z vs. surface concentration for bovine serum albumin film on distilled water and β-casein film on 0.5 M NaCl solution, using the partition function approach and the Boyle's Law approach. Figure 2.2 and 2.3 show similar plots for the proteins monolayers at various pH of the substrate.

Figure 2.4 shows the determination of the z factor, using the Fowkes' equation of state. This z is assumed to be the number of particles per molecule.

IV. Discussion

1. Validity of the Partition Function Approach

Table II.1 and figure 2.1 to 2.3 demonstrate that the partition function approach leads to calculated surface pressures that are in

Table II.1

Substrate	°C	pH	$Z = \pi_{exp.} / \pi_{calc.}$		
			$\frac{\pi\ exp.}{RT(\partial \ln Q/\partial \sigma)_T}$	$\frac{\pi exp.}{\delta KT}$	$\frac{\pi\ exp.}{\pi_{calc.}(ref.4)}$
			Extrapolated to C = 0		
Bovine serum albumin					
H_2O	24		1.0	1.0	1.01
0.1 N NaOH + 0.1 N HCl	26	1.1	1.0	1.0	1.00
"	22	2.0	1.0	1.0	1.01
"	24	3.9	1.0	1.0	1.00
"	26	5.35	1.0	1.0	1.05
"	24	6.05	1.0	1.0	0.97
"	23	7.2	1.0	1.0	1.04
"	21	9.12	1.0	1.0	0.99
"	26	11.3	1.0	1.0	1.01
"	24	12.55	1.0	1.0	1.01
β - casein					
0.5 M NaCl	25	6.4	1.0	1.0	0.99
0.1 N NaOH + 0.1 N HCl	26	1.1	1.0	1.0	1.01
"	22	2.1	1.0	1.0	0.98
"	24	3.9	1.0	1.0	1.01
"	24	4.8	1.0	1.0	1.05
"	24	5.1	1.0	1.0	1.04
"	23	6.6	1.0	1.0	1.05
"	21	9.1	1.0	1.0	1.04
"	26	11.3	1.0	1.0	0.98
"	24	12.55	1.0	1.0	0.98

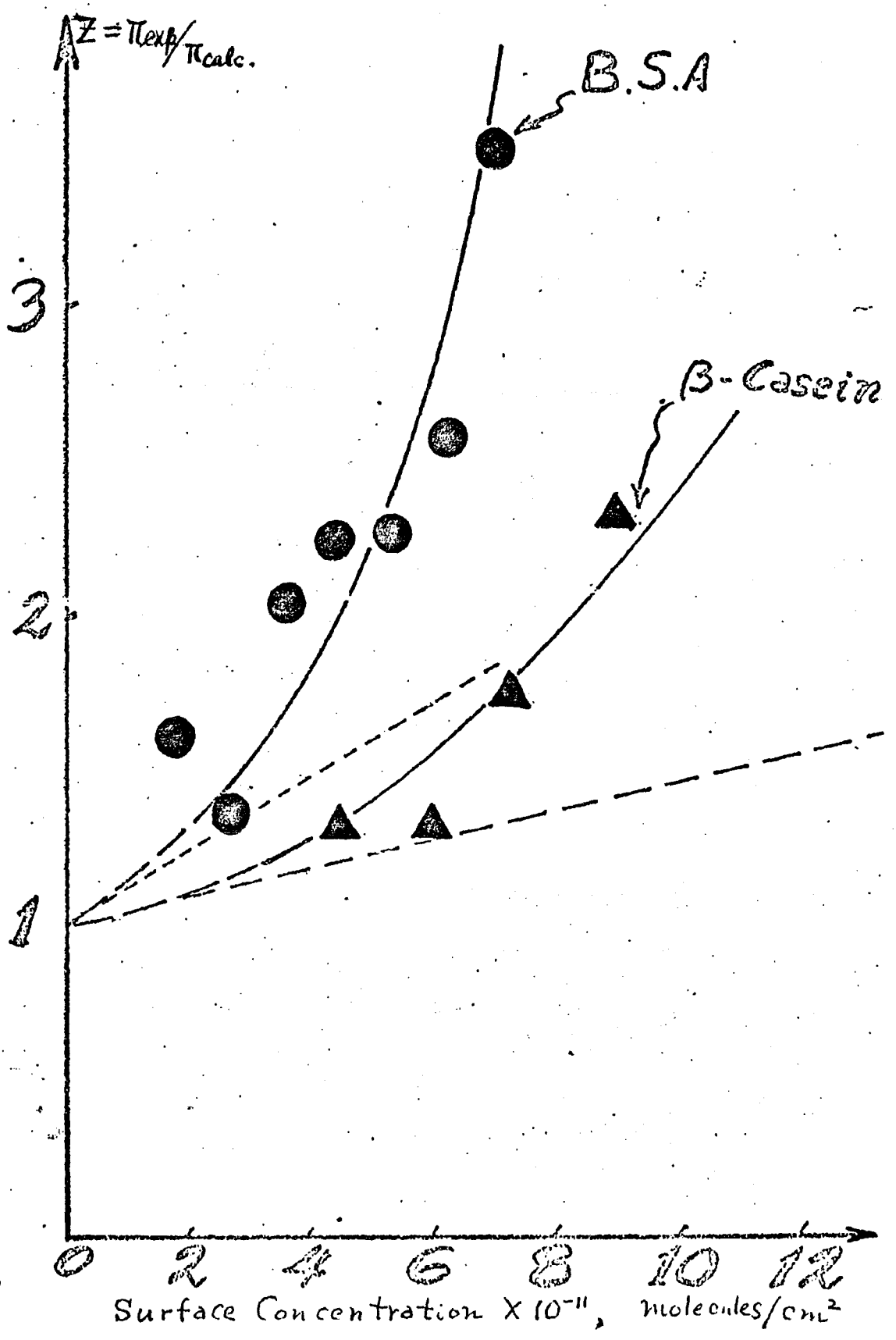

Z = πexp/πcalc.
B.S.A
β-Casein
3
2
1
0
2
4
6
8
10
12
Surface Concentration X 10⁻¹¹, molecules/cm²

Figure 2.1

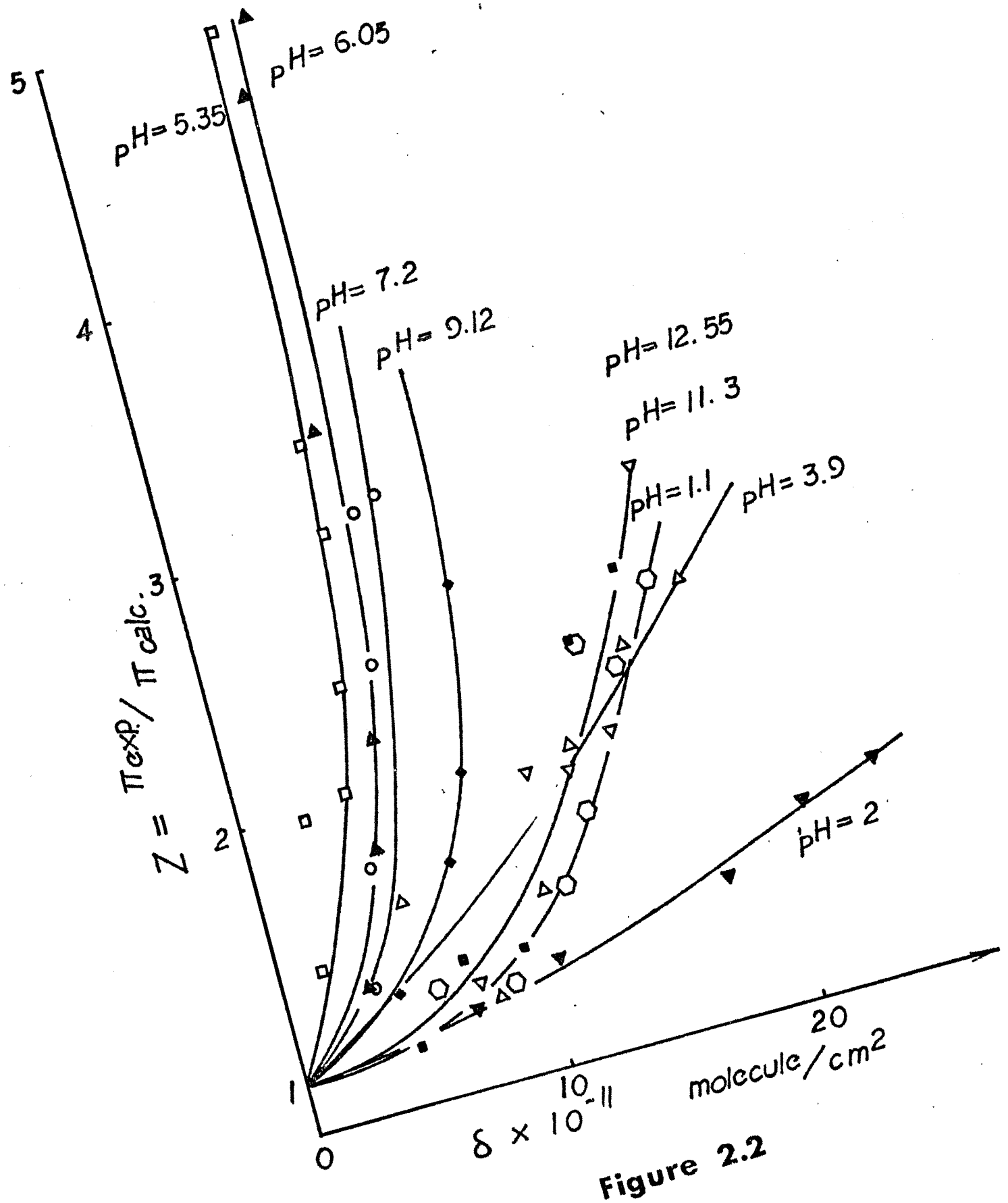

pH= 6.05
pH= 5.35
pH= 7.2
pH= 9.12
pH= 12.55
pH= 11.3
pH= 1.1
pH= 3.9
pH= 2
5
4
3
2
1
0
Z = π exp./ π calc.
10
20
molecule/cm²
δ x 10⁻¹¹

Figure 2.2

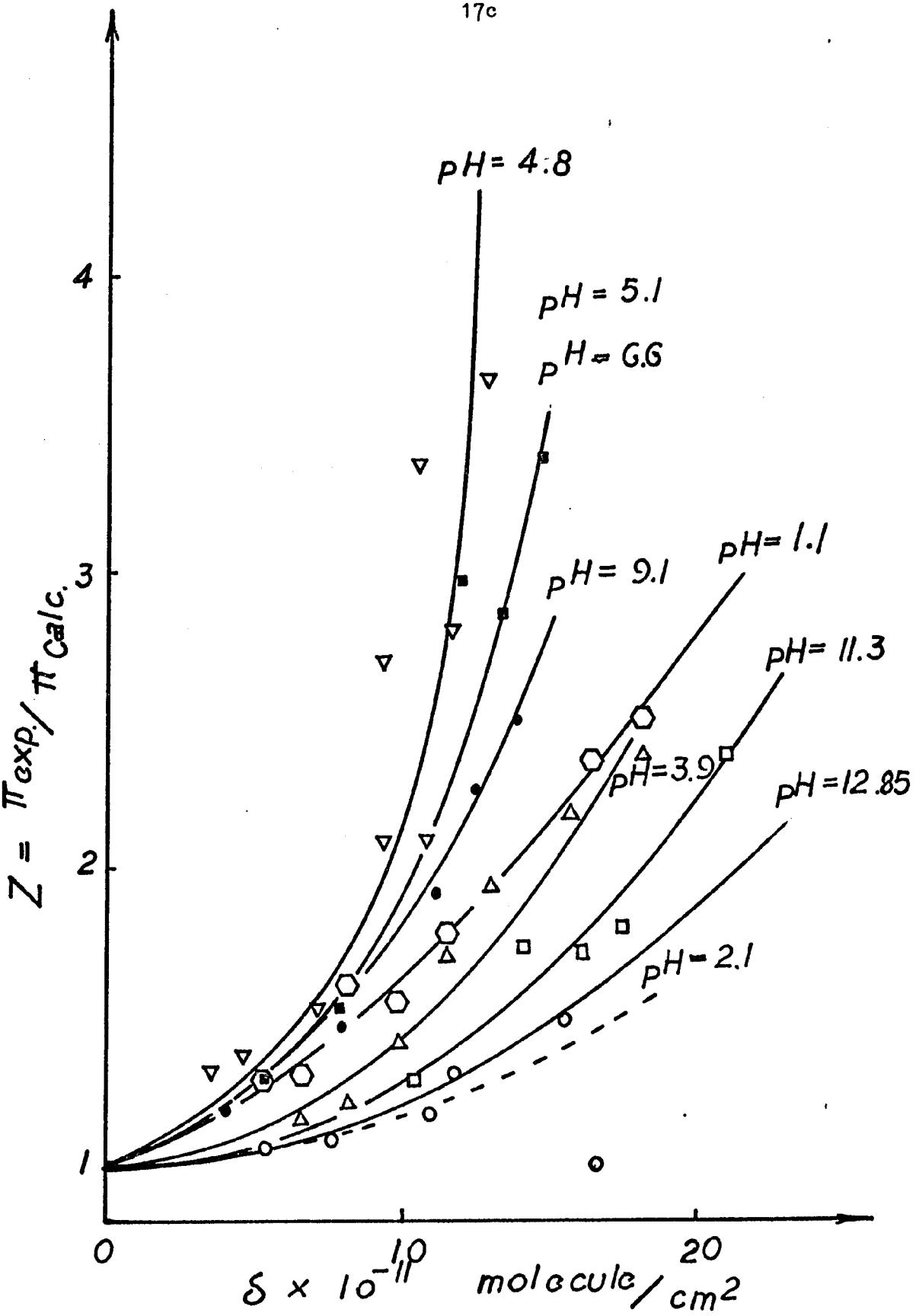

pH= 4.8
pH = 5.1
pH = 6.6
pH = 9.1
pH= 1.1
pH= 11.3
pH=3.9
pH =12.85
pH = 2.1
Z = π exp./ π Calc.
1
2
3
4
0
20
δ x 10^-10 molecule/cm2

Figure 2.3

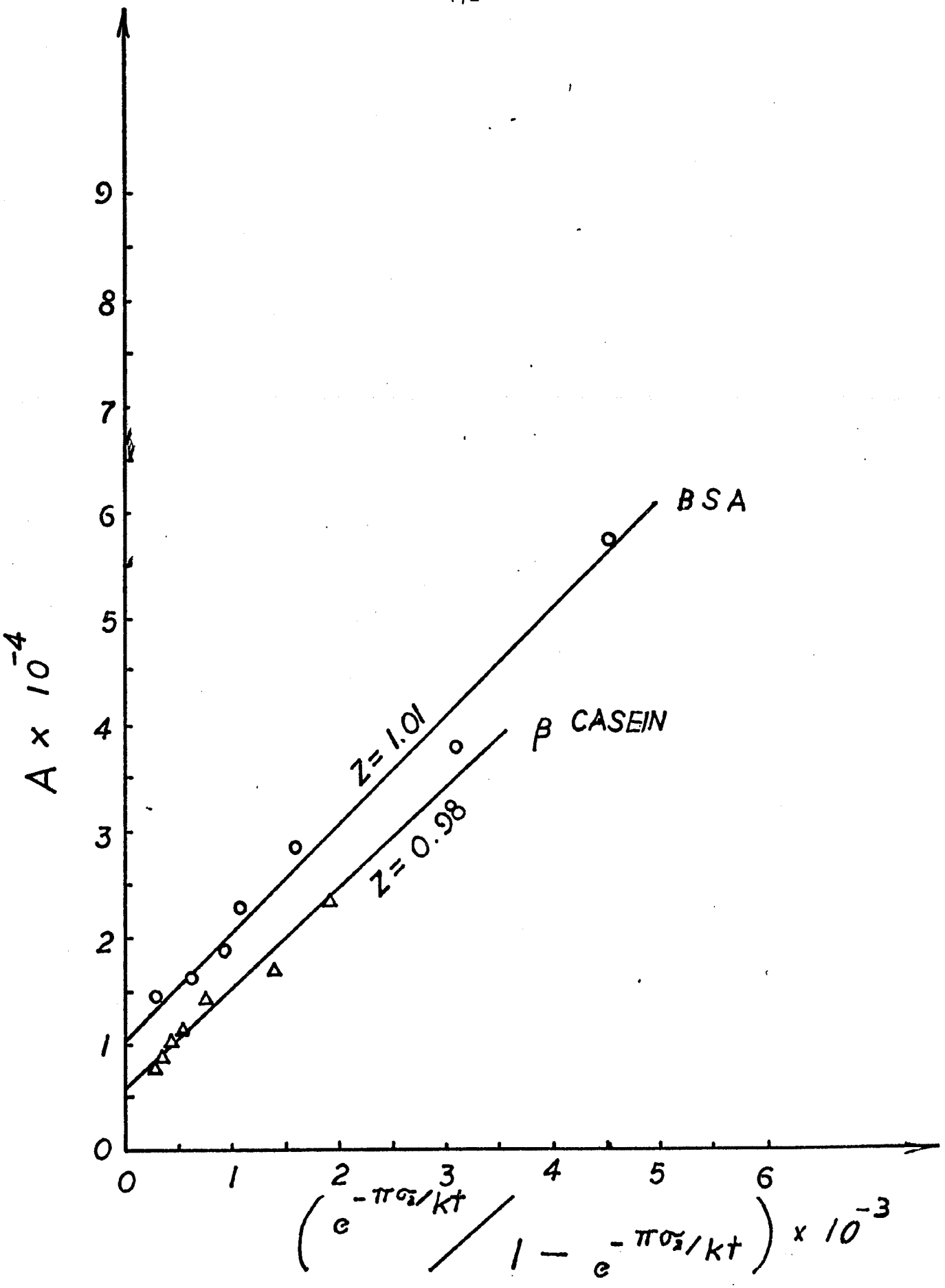

A x 10^-4
0
1
2
3
4
5
6
7
8
9
BSA
β CASEIN
Z=1.01
Z=0.98
0
1
2
3
4
5
6
(e^-πσi/kt / 1 - e^-πσi/kt) x 10^-3

Figure 2.4

agreement with those calculated using Boyle's Law and Fowkes' thermodynamic equation. The two-dimensional partition function approach, therefore, gives a good representation of the gaseous " pressure ", similar to the case of three-dimensional gases. Also, the monatomic translational partition function is shown to be a good approximation of the interactions experienced by the polyatomic monolayer molecules.

2. Co-Surface and Repulsion Effects

The calculation of surface pressure, using the partition function approach (equation 1), results in the observation that the $\left(\frac{\partial \ln \Omega}{\partial \sigma} \right)_T$ ratio for the two proteins increases with increasing repulsive interaction, because the co-surface effect leads to measurement of a higher concentration than actual. As a result, the " z factor " for the proteins increases with the surface concentration, as noted in Figures 2.1, 2.2, and 2.3. If the surface concentration is expressed in number of molecules (n) per cm^2 of surface available (1 cm^2 - n x σ; σ being the co-surface obtained by Guastalla's method), the z vs. surface concentration curves will be represented by the dotted lines in Figure 2.1.

These dotted lines have positive slopes indicating that in addition to co-surface effect repulsive forces have also to be considered. The existent of repulsive forces in the protein monolayers has been indicated by the negative deviation of the C / π vs. C curve (Figure 1.2 of chapter one) from the ideal horizontal line of zero slope (9-11).

3. Desorption of Protein at Other pH than Its Isoelectric Point

Figure 2.5 shows that after co-surface effect is corrected, the

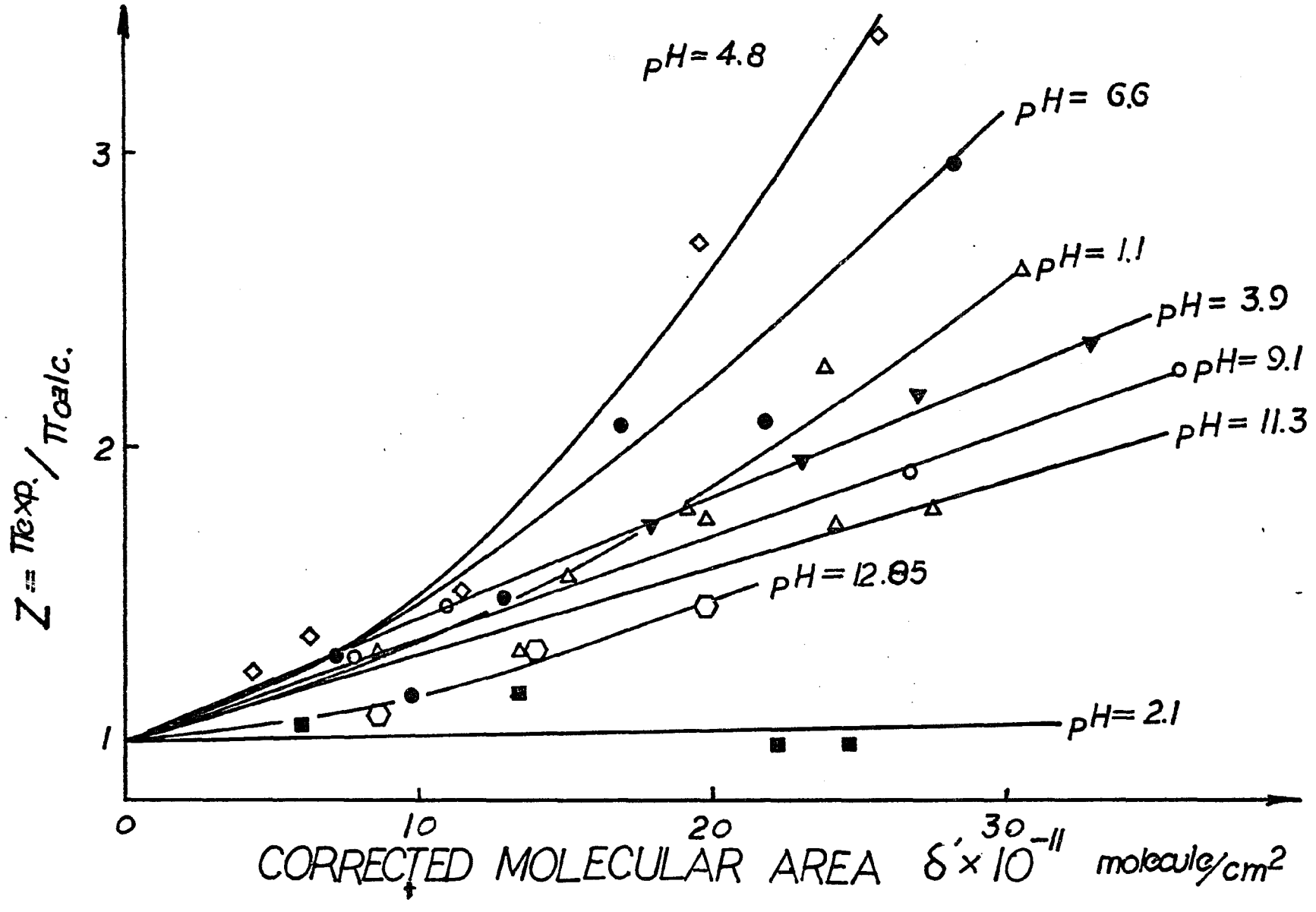

pH= 4.8
pH= 6.6
pH= 1.1
pH= 3.9
pH= 9.1
pH= 11.3
pH= 12.85
pH= 2.1
Z = πexp./πcalc.
1
2
3
0
10
20
30
CORRECTED MOLECULAR AREA δ' x 10⁻¹¹ molecule/cm²

Figure 2.5

deviation of the z vs. surface concentration curve from ideal horizontal line decreases with increasing net charge of the protein. This is interpreted to be the result of increasing desorption of protein into the substrate with increasing net charge.

The z vs. surface concentration curve at pH 1.1, however, has a larger positive deviation from the ideal horizontal line than that of the curve at pH 2.1. This abnormal behavior of the monolayer at pH 1.1 is probably due to the presence of very large repulsive force which surpasses the desorption effect of the protein into the substrate.

It is also interesting to note that at infinite surface dilution, the "z factors"for both bovine serum albumin and β-casein monolayers spread on various pH of substrates tend to a unique value "one", indicating that the proteins do not dissociate into smaller segments or associate into a larger molecule as the pH of the substrate is varied. The same conclusion is obtained from the calculations of the "z factor" for the protein, using the Fowkes'thermodynamic equation of state (see Table II.1).

V. Conclusions

The use of surface pressure described as a function of a two-dimensional translational partition function is justified in the case of a two-dimensional gaseous protein monolayer which obeys Boyle's Law. Deviations from ideality can probably be explained by taking into consideration co-surface effects, repulsion effects and desorption of charged proteins into the substrate.

In addition, the monolayer approach permits the verification of the statistical mechanical approach to thermodynamics.

CHAPTER THREE

Condensed Monolayer of Bovine Serum Albumin

I. Introduction

In the study of protein monolayers, the first thing encountered is the formation of the monolayer. To produce an evenly distributed well spread protein monolayer on an aqueous surface two methods have been used: 1) applying a minute quantity of protein crystals directly on the aqueous surface (1,2); and 2) deposition of droplets of an aqueous protein solution (3-9).

The first method is tedious, it involves the handling of minute quantities of protein and spreading is often incomplete. The second method is more practical, but has the disadvantage in that there is a loss of protein into the aqueous substrate unless a spreading agent is added to the spreading protein solution. The additive, by lowering the surface tension of the protein solution, insures the spreading and formation of the protein monolayer. After monolayer formation, the additive desorbs rapidly into the aqueous substrate. Various additives such as ethanol (5), n-propanol (9) and n-pentanol (3,8,10) have been used by different workers and the quantity used varies from one investigator to the other.

Based on the idea that the spread protein needed a certain time to reach an equilibrium, some workers (5,12) have allowed the monolayer to stand for five minutes before compression, while other workers (7,11) have suggested a longer waiting time. It appeared that a consistent condition for spreading protein monolayer was not present. Therefore, it was important to study the effect of waiting time as well as the effects of additives on the spreading of protein over an aqueous surface. Bovine serum albumin, because of its availability in pure form and its well-known characterization was used in this study.

In addition, the influences of pH and ionic strength of the substrate on the surface properties of bovine serum albumin monolayer were also investigated.

II. Experimental

The apparatus consisted of a paraffin coated fused silica trough (31.2x 13.8 x 2.5 cm) equipped with a movable barrier which was driven by a reversible motor, a surface tension and a surface potential measuring devices. The fused silica trough was used to retain the liquid substrate and the film which was formed by depositing, with an Agla micrometer syringe (Burroughs Wellcome Co., Tuckahoe, N. Y.), droplets of aqueous protein solution on the surface of the substrate. The temperature of the substrate was regulated by circulating water from a constant-temperature bath through a glass cooling coil submerged in the substrate. Temperature control was good within $\pm$ 0.5°C.

The surface pressures were determined from surface tension measurements which were made by suspending a wettable sand blasted platinum blade from a microforce transducer-amplifier system (model 311A, The Sanborn Co., Waltham,Mass.). The transducer output was recorded continuously on a two channel recorder (Sargent-Welch, model DSRG, Sringfield, N. J.). The surface tensions were reproducible within $\pm$ 0.1 dynes.

The surface potentials were measured with an air electrode (13) (a radium-226 source, U. S. Radium Corp., Morristown, N. J.) placed 1 to 2 mm above the surface of the liquid substrate and connected to a precision potentiometer (Model 2730, Honeywell, Denver Division), a high-input resistance electrometer (Model 610 B, Keithley Instruments, Inc., Cleveland, Ohio), and a trough electrode (Ag/ AgCl)dipped into

the bulk of the aqueous substrate. The radioactive electrode was connected to the input terminal of the electrometer with Amphenol low-noise graphitized shield cable and connectors. The entire circuit was grounded. The e. m. f. of the cell composed of the radioactive electrode, trough electrode, potentiometer and electrometer, all connected in series, was measured immediately after cleaning the surface of the aqueous substrate (V_o), and compared with the e. m. f. obtained after spreading a film on the surface (V). The difference between the two e. m. f.'s ($V - V_o$) is the surface potential. The potentiometer opposed a convenient fraction of the cell e. m. f. and the electrometer output was recorded continuously on the same recorder used for surface tension measurements. Sensitivity of the surface potential measurements was about $\pm$ 1 mv., and reproducibility of the data was within $\pm$ 5mv.

Surface pressure and surface potential measurements were made simultaneously and continuously at 30° C while the monolayer was compressed at a constant rate of 2.90 cm / min. The experimental results were presented as surface pressure- surface area isotherms (π -σ) and surface potential- surface area isotherms (ΔV- σ).

The spreading solutions were prepared by dissolving crystalline bovine serume albumin (Nutritional Biochemicals Corporation, Cleveland, Ohio) in freshly distilled water containing various concentration of 1-pentanol, 1-propanol or ethanol. The protein concentration in the spreading solution was 0.8-0.9 mg /ml and the quantity spread was of the order 0.018 mg over 380 cm^2 of aqueous surface such that the initial surface pressure would not exceed 0.2 dynes / cm.

Two kinds of aqueous substrates were used. One was distilled water to which 10^{-3} M versene [disodium (ethylene dinitrilo) tetracetate, reagent grade, Fisher Scientific Co.] was added to sequester any poly-

valent cations present. The pH of the water was adjusted by adding concentrated NaOH or HCl. The other substrates were NaCl solutions at an ionic strength of 0.01 and 0.5. In these solutions the pH was adjusted by adding 0.01 N HCl or 0.01 N NaOH to the 0.01 N NaCl solution and 0.5 N HCl or 0.5 N NaOH to the other NaCl solution.

Concentrated HCl, NaOH and NaCl crystals were reagent grade and used without further purification.

All aqueous substrates were foamed in a 500 ml medium porosity sintered glass funnel, the foam was removed several times by sweeping the surface to remove any traces of surface active impurities. This operation provided a satisfactory clean water surface (14).

III. Results and Discussion

1. Surface Tension of Aqueous Bovine Serum Albumin Solution

The surface tension of the protein solution (0.7744 mg / ml aqueous BSA solution) was measured at 26° C and found to increase slowly with time. The surface tension, extrapolated to zero time, was 65.15 dynes/cm which was 16.66 dynes/cm less than the surface tension of distilled water at the same temperature. It was apparent that the crystalline BSA contained surface active impurity, probably trace of decanol entrapped in the crystals of the BSA (15).

2. Effect of the Concentration of 1-pentanol on the Spreading of the Aqueous Protein Solution

It was observed that the π -α isotherms obtained from 0.01 N NaOH (pH 6.15) substrate with 0.1%, 0.5 % and 1% aqueous 1-pentanol spreading solution were almost identical. Each isotherm was found to be

slightly more expanded than that obtained without the spreading agent. The optimum concentration of 1-pentanol was 0.5%, above this concentration a less expanded film was obtained. The ΔV-σ curves were also almost the same. It was noted that the BSA solution spread even in the absence of 1-pentanol. This was attributed to the trace amounts of decanol which was used in the preparation of crystalline BSA.

3. Effects of Various Spreading Agents

As the concentration of ethanol and 1-pentanol in the spreading solution increased (0.5%, 10%, 25%), the π-σ isotherms became less expanded and the ΔV-σ curves shifted toward lower surface potential. A probable reason is that the alcohol dissolved in the substrate and carried the protein along. The higher the concentration of the alcohol the more protein was carried along. These results with 1-pentanol were in contradiction to that obtained by Minõnes et al. (12). The different substrates used in this experiment and in their experiment (a buffer solution of acetic acid-sodium acetate) may account for this divergence.

4. Effect of the Elapsed Time between Spreading and the Start of Compression

When the monolayer was maintained at low surface pressure for more than 5 minutes to the air before compression, no reproducible isotherm was obtained. This was attributed to the emergence of the surface active impurities.

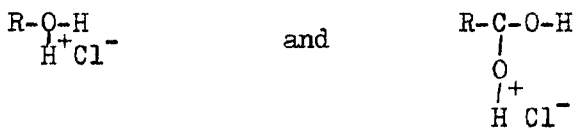
5. Effect of pH and ionic Strength of Substrate

Figure 3.1 describes the effect of pH on the π-σ and ΔV-σ

isotherms of BSA monolayers spread on dstilled water. Around the isoelectric point (pH 4.9), the BSA monolayers are more expanded than those at the other pH regions. Beyond pH 11.95 markedly expanded films are observed. The ΔV-σ curves are all positive and increase with compression.

Around the isoelectric point, a BSA molecule has zero or a small net charge because of having nearly equal numbers of negative charged groups ($-COO^-$) and positive charged groups ($-NH_3^+$). Since it is relatively less soluble in an aqueous solution, the molecule would stay on the surface of the substrate when the monolayer was compressed. As a result, an expanded film and a gradual rise ΔV-σ curve were observed. In the case of monolayer spread on a more acidic or basic substrate, the BSA molecules, having a large net positive or negative charge, would be easily pushed into the substrate during the compression, because of their higher solubility in the aqueous substrate. Therefore condensed films were observed on either side of the isoelectric point of the protein. As the molecule was pushed partially out of the liquid surface, the charge groups were reoriented to more vertical positions to the surface, leading to a rapid increase in surface potential with compression.

A better spread monolayer obtained on a substrate of more acidic than pH 2.85 may be attributed to the formation of oxonium compound (15) :



The markedly expanded films formed above pH 11.95 are probably due to the breaking of hydrogen bond owing to the ionization of hydroxyl

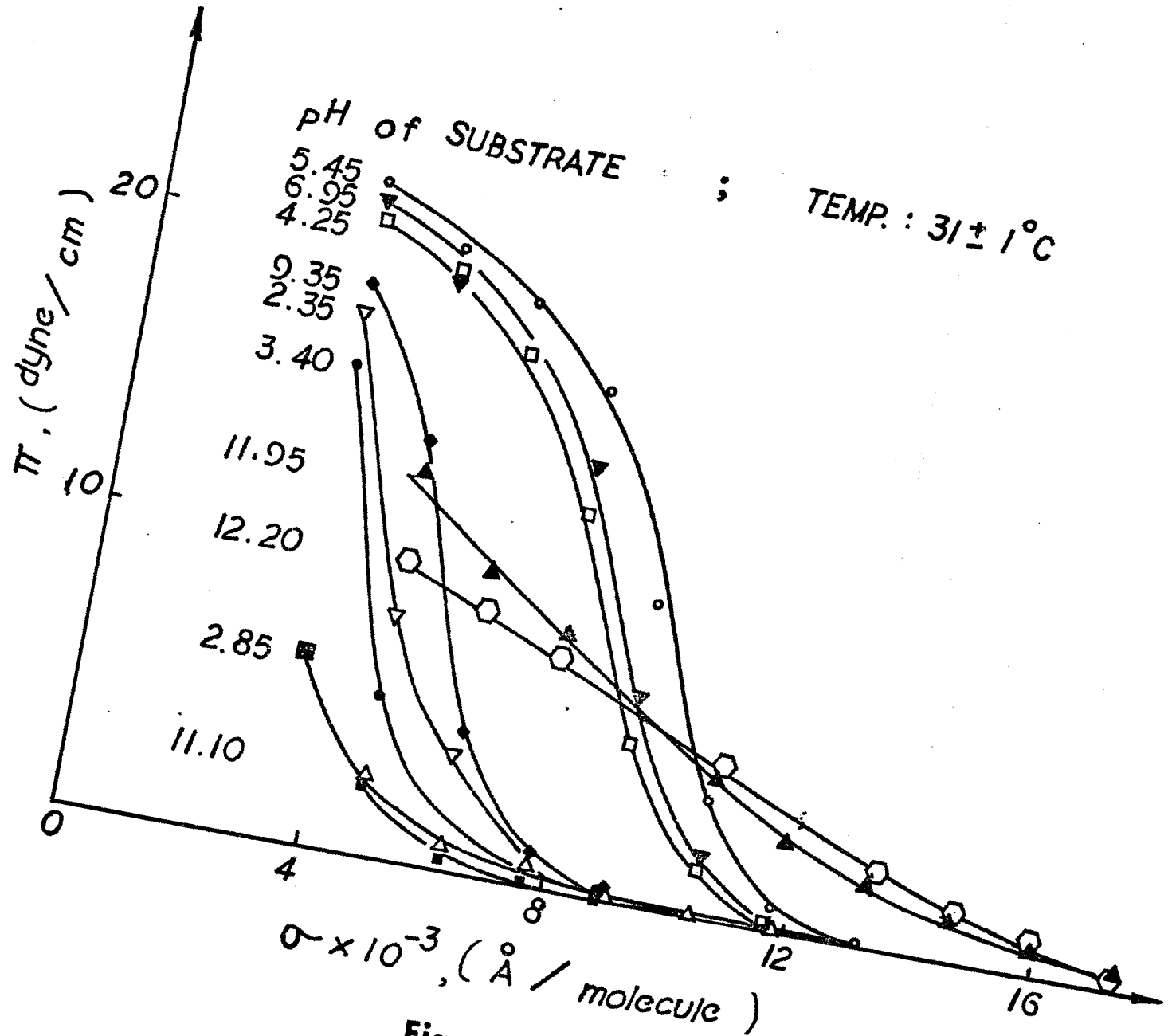

pH of SUBSTRATE ; TEMP. : 31 ± 1°C
5.45
6.95
4.25
9.35
2.35
3.40
11.95
12.20
2.85
11.10
π, (dyne / cm)
20
10
0
4
8
12
16
σ x 10⁻³, (Å / molecule)

Figure 3.1a

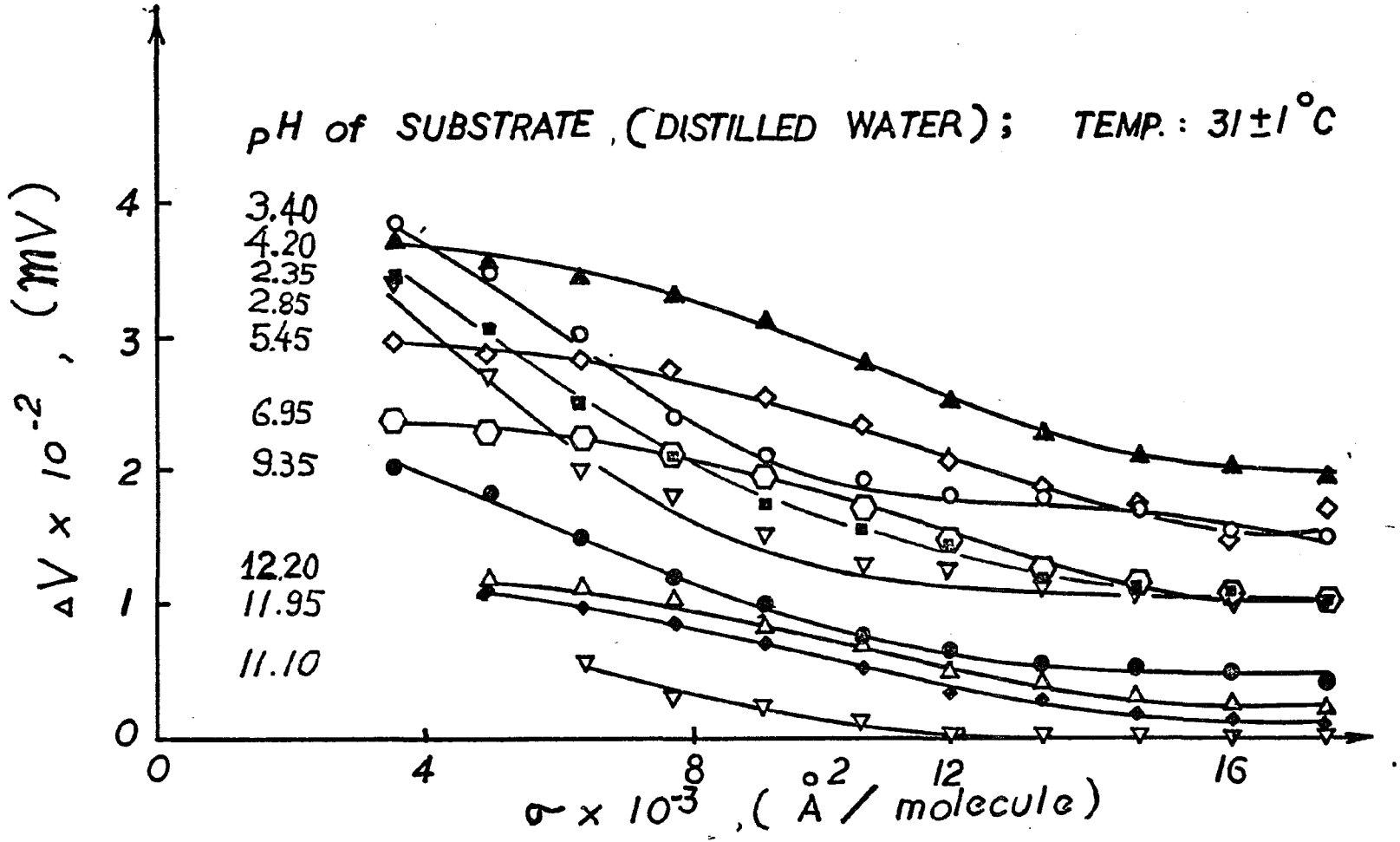

pH of SUBSTRATE (DISTILLED WATER); TEMP. : 31±1°C
3.40
4.20
2.35
2.85
5.45
6.95
9.35
12.20
11.95
11.10
ΔV x 10^-2 , (mV)
σ x 10^-3 , (Å^2 / molecule)
0
1
2
3
4
0
4
8
12
16

Figure 3.1b

group (15) and the penetration of the counter ions (Na^+) into the plane of the interfacial head group (16). Furthermore, the repulsive force between ionized carboxyl groups is also in effect. The small and slow rise in surface potential during compression indicates that the negative charged carboxyl groups are almost neutralized by the penetrated counter ions.

Figure 3.2 demonstrates that the presence of NaCl in the substrate minimizes the effect of the substrate. An interpretation is given that Na^+ and Cl^- ions adsorb to the monolayer and somehow neutralize the charged protein, thus the protein behaves as it was at the isoelectric point. In addition, under the monolayer the adsorbed ions form a double layer which screens the effect of the substrate. When the concentration of the NaCl increases, a better screening effect is observed.

IV. Summary

The influences of the spreading agent and the condition of substrate on the spreading of the bovine serum albumin over aqueous solution have been examined. It is concluded that 1-pentanol was a good spreading agent for BSA and the optimum concentration was 0.5%. There was a marked effect of the pH of the substrate on the spreading. A well spread monolayer was formed on the isoelectric substrate. At high pH an abnormally expanded monolayer was noticed, it was attributed to the breaking of hydrogen bond owing to the ionization of the hydroxyl group. The presence of NaCl in the substrate was seen to screen the effect of pH, the screening effect being increased with the concentration of NaCl.

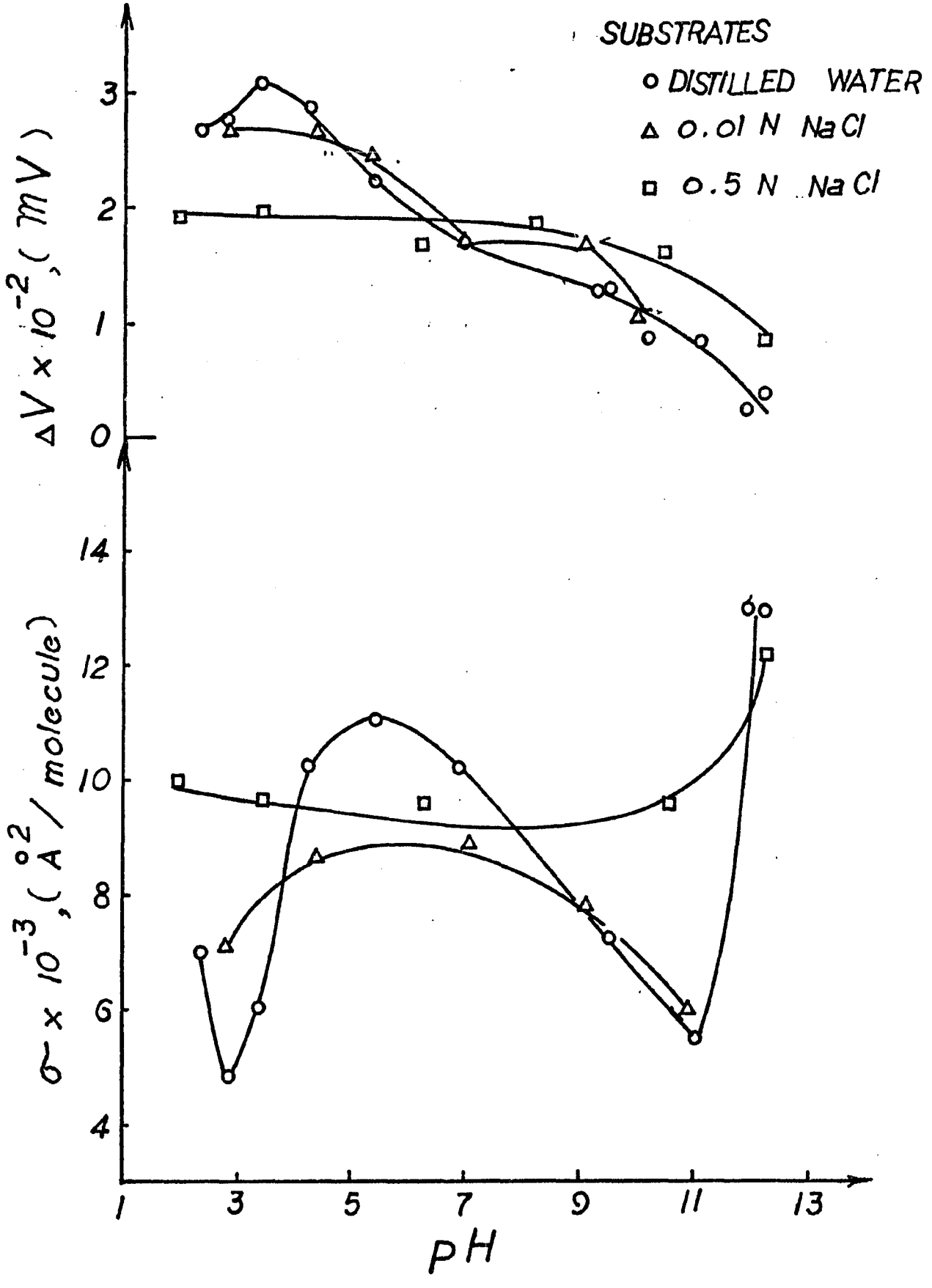

SUBSTRATES
o DISTILLED WATER
Δ 0.01 N NaCl
□ 0.5 N NaCl
ΔV x 10^-2, (mV)
σ x 10^-3, (Å^2 / molecule)
pH
0
1
2
3
4
6
8
10
12
14
1
3
5
7
9
11
13

Figure 3.2

CHAPTER FOUR

Role of the Bovine Serum Albumin Subphase in Relation to Surface Viscosity and Film Transfer

I. Introduction

The concept of surface viscosity was first introduced by Boussinesq (1) in order to account for certain mechanical properties of liquid interfaces. It is assumed that at equilibrium, in the absence of all external stresses, thermal agitation equalizes the mean molecular distances in all directions within the plane of the liquid surface phase. In addition, a static isotropy exists at every point normal to the surface. Under the influence of a mechanical stress, the plane of the liquid surface is deformed. Two types of surface deformation--dilatation and shear have been considered, and respectively coefficients of surface dilatational viscosity and surface shear viscosity have been defined.

Various techniques for measuring surface viscosity have since been developed and refined (2-13). However, each technique because of its limitations can only be applied to measure the viscosity of a certain type of fluid surface or a limited viscosity range of liquid surface (14, 15).

Proteins are often used in emulsion and foam formulations because of their stabilizing properties. A monolayer can be considered a model of half a foam lamella. Therefore the monolayer technique is applicable to the above problem. In previous experiments (16-18), it has been observed that a moving monolayer can drag along a considerable amount of substrate. This phenomenon is suspected to play a role in the surface viscosity which, in turn, relates to emulsion and foam stability. The purpose of this study is to investigate how a monolayer and its subphase are affected when the monolayer is submitted to a mechanical perturbation.

Panaiotov measured simultaneously two surface pressures of a locally perturbed myristic acid monolayer (19). He found that a local surface pressure perturbation applied in the plane of a monolayer propagated to the rest of the monolayer and time lags were detectable at different distances from the point of perturbation. The explanation given was that when a monolayer is perturbed, energy dissipation occurs inside the monolayer as well as in the underlying substrate which is mechanically coupled to the monolayer. This energy dissipation was accomplished via three different processes; i. e.,1) relaxation process due to adsorption-desorption exchange of soluble film forming molecules, 2) elastic dissipation process where the change in surface area was proportional to the change in surface pressure , and 3) viscous dissipation processes which depended on the viscosities of the monolayer and the substrate. For an insoluble monolayer, the first process is absent. By comparing the propagation rate of a surface pressure perturbation in an elastic film with that in a viscoelastic film, he analyzed the viscous dissipation processes and separated out the viscous contribution of the monolayer from that of the substrate (20). It appeared to us that if Panaiotov's assumptions are correct, his method would provide us a means to study the roles played by monolayer and substrate.

In the first phase of this study, Panaiotov's experiment was tried, but the barriers for measuring surface pressures were replaced by wettable platinum blades which measured surface tensions. It was found that Panaiotov's vertical glass plate which was used to perturb the monolayer, did not give consistent results, therefore in the second phase of this study, the glass plate was replaced by a movable barrier which perturbed, at constant speed, the monolayer horizontally.

II. Experimental

1) Compression and Expansion Studies

The experimental apparatus for measuring surface pressures consists of a paraffin coated Langmuir metal trough (65 x 14 x 2 cm) equipped with a movable barrier driven by a reversible motor, and a sand-blasted platinum blade suspended from a microforce transducer-amplifier system (Model 311 A, The Sanborn Co, Waltham, Mass.). The sand-blasted platinum blade may be placed at any distance from the movable barrier such that the surface tension of a film at that position may be measured. The transducer output is sent continuously to an x-y recorder (Model 370, Keithley Instruments, Inc., Cleveland, Ohio 44139). The surface tensions are reproducible within $\pm$ 0.1 dyne.

The compression and expansion rates were varied between 0.009 and 0.10 cm/sec.. Isotherms were determined by compressing (and expanding) for short duration of time and then allowing the system to reach equilibrium.

An aqueous protein solution containing 0.5% 1- pentanol or surfactant in n- hexane was deposited on the aqueous substrate with an Agla micrometer syringe (Burroughs Wellcome Co., Tuckahoe, N. Y.). The substrate and the film were retained in the paraffin coated trough. The surface tensions at different positions on the monolayer were measured continuously as a function of time while the barrier was moving at a constant rate.

The surface was cleaned by dusting calcinated talcum powder on the substrate and removing it with the aid of a hollow glass tip connected to an aspirator.

2) Film Transfer Study

An apparatus originally developed by Abribat, Rosano and Vaillet (21) for studying the transfer of film (distilation isotherme superficielle) was used to determine if the subphase was also being dragged with the surface film. It consisted of two troughs of water connected by a set of glass rods bent at right angles and in parallel contact (figure 4.1). To determine the amount of substrate dragged, a monolayer was spread on the substrate in trough 1. For oleic acid, decyl alcohol and methyl laurate the excess material remained as a lens. For bovine serum albumin, the protein deposited on the surface was a very small quantity such that only one monolayer of approximately 16 dynes per cm was formed. Since trough 2 was continuously flushed with water to clean the surface, a pressure differential existed between the two troughs which caused the monolayer to transfer from the higher surface pressure trough 1 over the glass rods to trough 2 (zero surface pressure). The surface pressures were determined by the Wilhelmy wettable blade technique previously described. The change in height of the substrate level in trough 1 was measured with a cathetometer which could read to $\pm$ 0.02 mm. Since the levels of water in the two troughs were the same, hydrostatic pressure was negligible. A correction for the evaporation of substrate was made.

3) Reagents

The aqueous protein solutions (bovine serum albumine and β-casein) were prepared in the same way as outlined in chapter 3. The fatty acid solutions were made by dissolving about 25 mg of the fatty acid (arachidic acid from Nutritional Biochemical Corporation, Cleveland, Ohio;

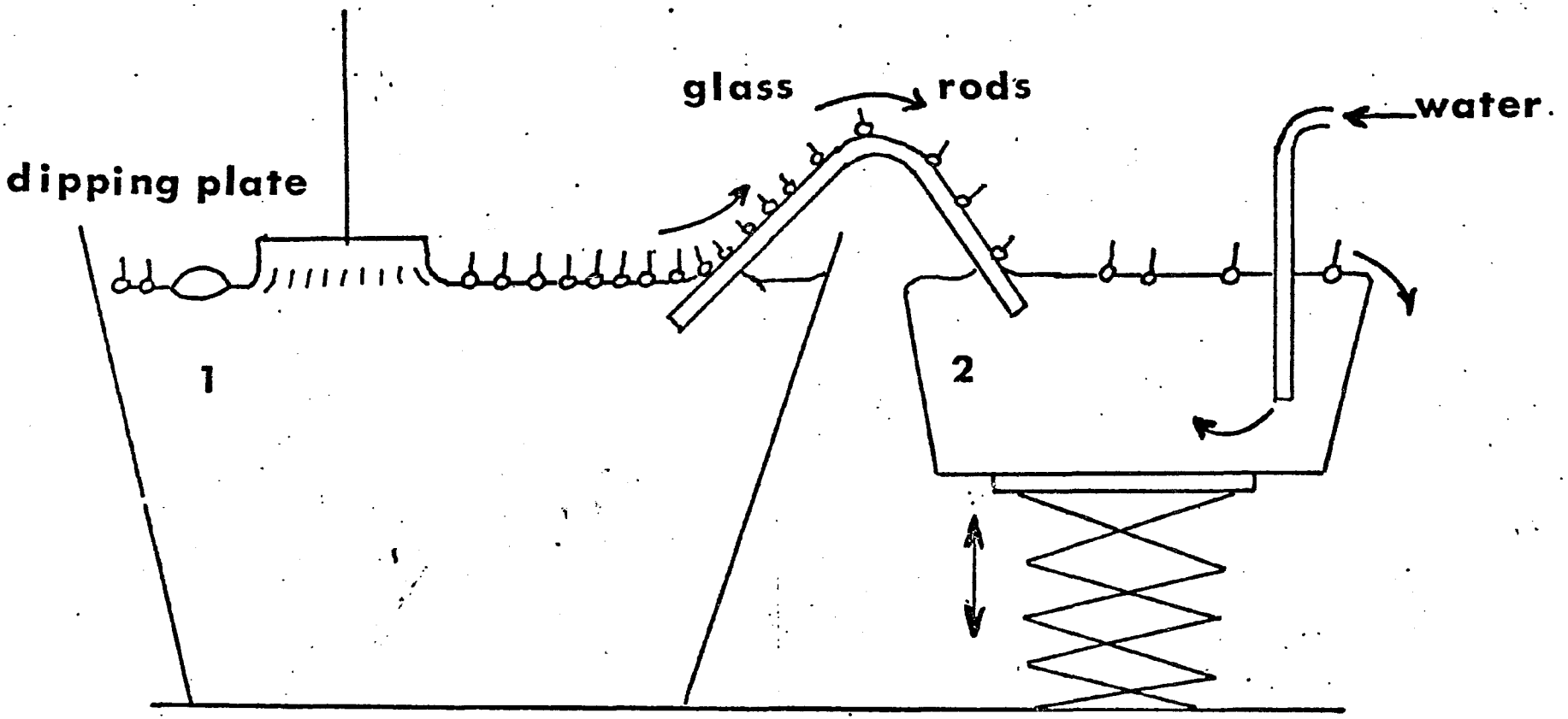


Figure 4.1

myristic acid from Eastman Organic Chemical, Rochester, N. Y.) per 25 ml spectrograde n- hexane (Fisher Scientific Co.). The sodium eicosyl sulfate solution was obtained by dissolving about 16 mg of sodium eicosyl sulfate (Schuchardt, München, Germany) in 50 ml of mixture of n- hexane, 2- propanol (Allied Chemical, Morristown, N. J.) and water (34 : 15 : 1ml).

The substrates of constant ionic strength (0.1) were prepared by mixing 0.1 N HCl solution with 0.1 N NaOH solution to the desired pH values. Other substrates used for studying the influences of ionic strength of substrate were 6 N urea, 0.1 N NaCl, 0.01 N NaCl and 0.001 N NaCl solutions. For the arachidic acid and the sodium eicosyl sulfate monolayers, 0.1 N 2-amino-1-butanol (AB) and 0.1 N 2-amino-2-methyl-1-propanol (AMP) were used as the substrates. Both AB and AMP were perchased from Commercial Solvents Co., N. Y.,N. Y. .

The proteins, fatty acids and all other reagents were used without further purification. The substrate solutions and the distilled water for the subatrates were foamed in a 600 ml medium porosity sintered glass funnel, the foam was removed several times by sweeping the surface.

For film transfer experiment, solution of oleic acid (Nutritional Biochemicals Corporation, Cleveland, Ohio) was prepared by diluting 1 ml of the oleic acid with 49 ml of absolute alcohol (Commercial Solvents Corporation, Terre Hante, Indiana). Pure n-decyl alcohol (Brothers Chemical Co., Orange, N. J.) and Methyl laurate were used without dilution.

III. Results

1) Compression and Decompression Studies

Bovine serum albumin was spread on an isoelectric substrate (pH 5.3) and initially compressed to a pressure of 5 dynes/cm. The moving barrier compressed the monolayer at a constant rate of 1.96×10^{-2} cm/sec. for 60 seconds while the surface pressure (π) was recorded at various positions in the trough. Figure 4.2 describes the results of π vs. time at various distances from the moving barrier. The surface pressures depend on the distance between the wettable blade and the moving barrier - i. e., the further from the barrier the pressure is recorded, the small is the slope ($\partial \pi / \partial T$).

After the barrier was compressed for 60 sec. and then stopped, essentially horizontal lines result (figure 4.2); thus, the pressure was constant at each position. At small distances from the moving barrier (x= 5 and 10 cm) a slight decrease in pressure occured, but shortly leveled out and remained constant during our observations (10 to 15 min.). The system never reached one equilibrium pressure throughout the trough.

A hysteresis phenomenon was observed when the monolayer was expanded after 60 sec. of compression. Increasing the rate of compression yields a set of π vs. time curves similar to those in figure 4.2 but with larger slopes $\partial \pi / \partial T$. The rate of compression had little or no effect on the pressure when measured at a fixed distance providing the monolayer was compressed to the same area.

Figure 4.3 describes the effect of pH on the surface pressure (π) of bovine serum albumin monolayers at various distances from the moving barrier. Each reading was taken after the barrier was compressed at 1.96×10^{-2} cm/sec. for 60 seconds. The initial pressure of the system was 5 dynes/cm. The pH was altered with 0.1 N HCl and 0.1 N NaOH. Although the pH was varied the ionic strength was kept constant. When the

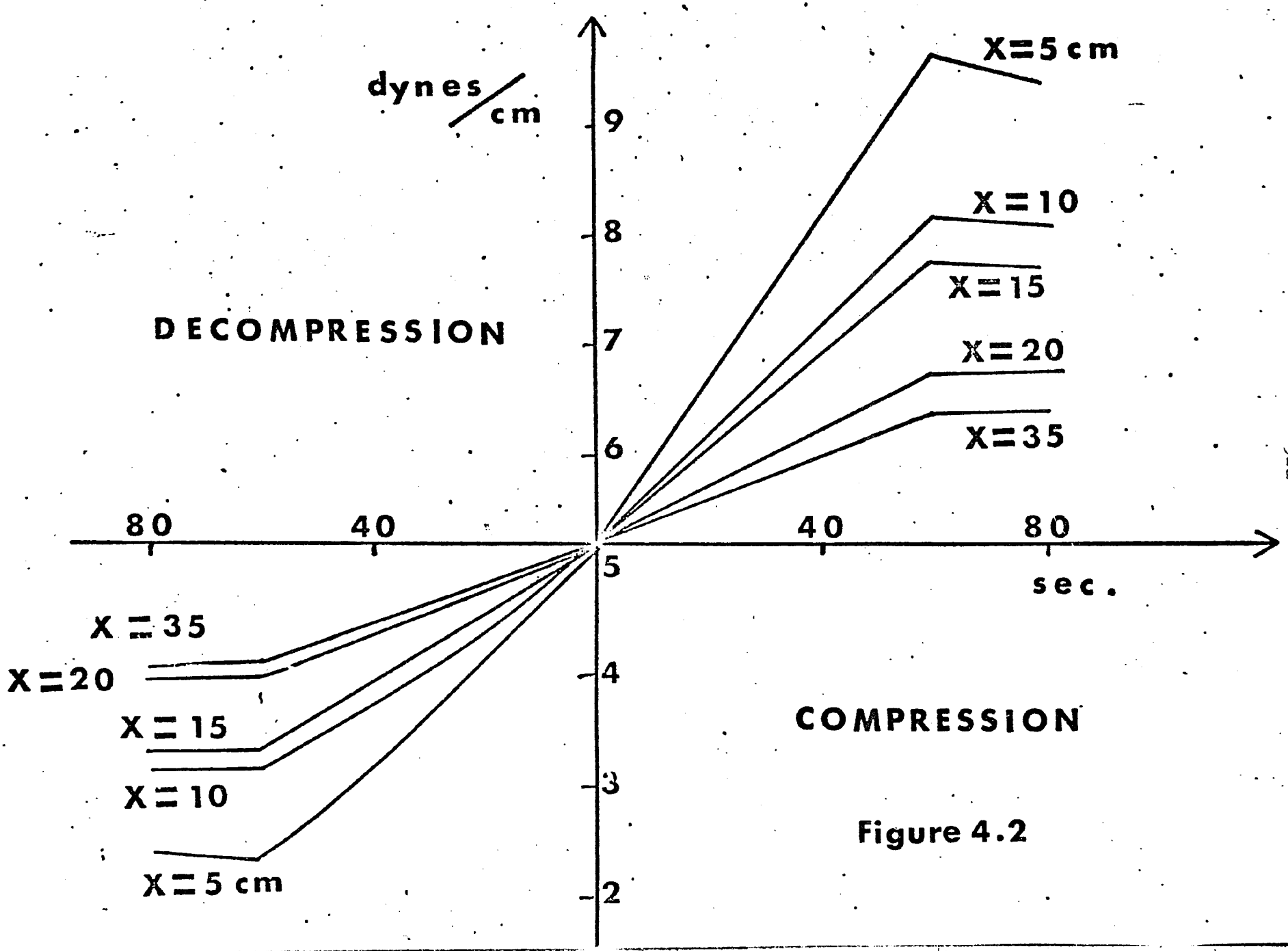

dynes/cm
9
8
7
6
5
4
3
2
X=5 cm
X=10
X=15
X=20
X=35
DECOMPRESSION
80
40
40
80
sec.
X=35
X=20
X=15
X=10
X=5 cm
COMPRESSION

Figure 4.2

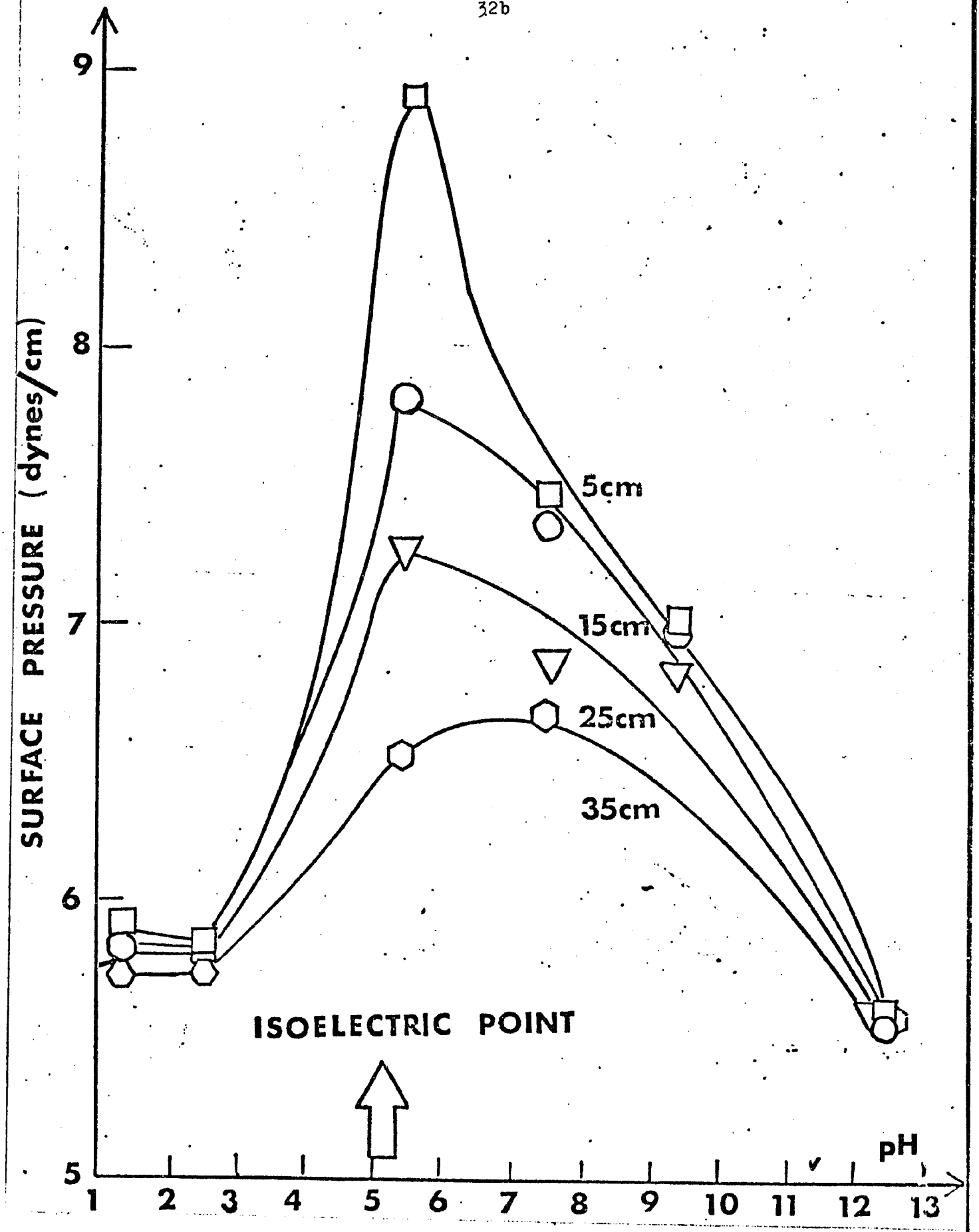

SURFACE PRESSURE (dynes/cm)
9
8
7
6
5
5cm
15cm
25cm
35cm
ISOELECTRIC POINT
pH
1
2
3
4
5
6
7
8
9
10
11
12
13

Figure 4.3

ionic strength of the aqueous substrate varied from 0.001 to 0.1 (by adding NaCl), the effect was obvious in all cases; the greatest effect appeared at the lowest ionic strength.

Similar effects were observed when β-casein spread on 0.001 N NaCl solution (pH 5.05, 24.5 °C; figure 4.4) or distilled water (pH 5.5, 23.5 °C), but the effects were relatively smaller than those observed for the bovine serum albumin monolayer. Also as for the bovine serum albumin monolayer, the effects become smaller as the ionic strength of the substrate increased (0.001 to 0.1). No effect was observed when the substrate was 6 M urea (pH 6.5, 24.5 °C). Figure 4.5 shows the effect of pH of the substrate on the surface pressure (π) of β-casein monolayers.

When a monolayer of myristic acid spread on 0.01 N HCl was used over the same pressure range (5 dynes/ cm), the change in surface pressure on compression and expansion was independent of the position of the wettable blade from the moving barrier.

Addition experiments were conducted on sodium eicosyl sulfate and arachidic acid monolayers spread over 1). 0.1 N 2-amino-2-methyl-1-propanol (pH 9.65, 24.5 °C) and 2). 0.1 N 2-amino-1-butanol solution (pH 8.5, 24.5 °C). In all cases of compression or decompression, the measured surface pressures were independent of the distance between the measuring device and the compression barrier.

2) Transfer of Monolayer Subphase

Figure 4.6 describes the changes in surface pressure and substrate level as a function of time for various monolayers (Four glass rods of o. d. 4 mm and total length from trough 1 to trough 2 of 6.94 cm were used.). For oleic acid, decyl alcohol and methyl laurate the pressure

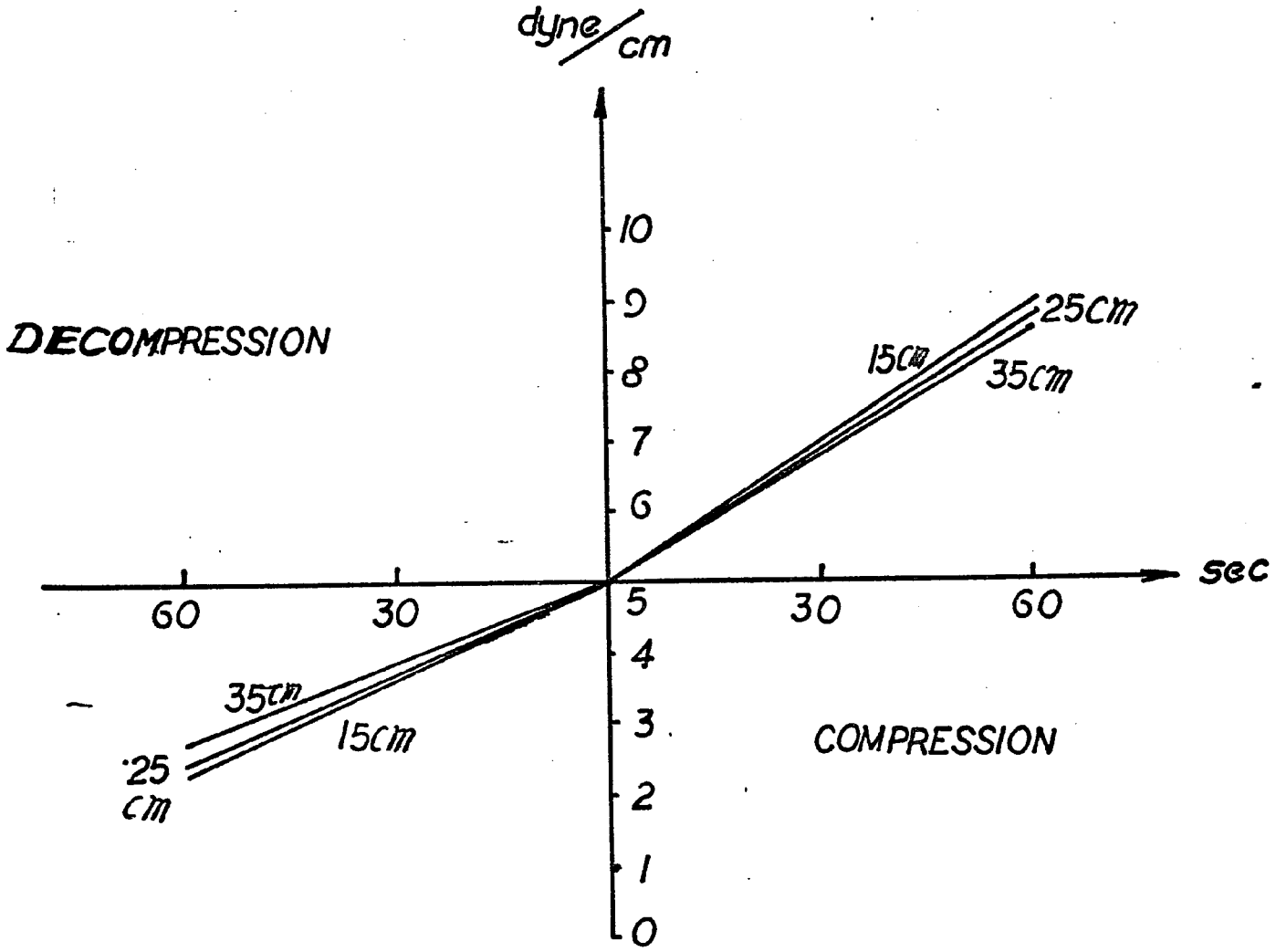

dyne/cm
10
9
8
7
6
5
4
3
2
1
0
DECOMPRESSION
COMPRESSION
sec
60
30
30
60
25cm
35cm
15cm
35cm
15cm
25
cm

Figure 4.4

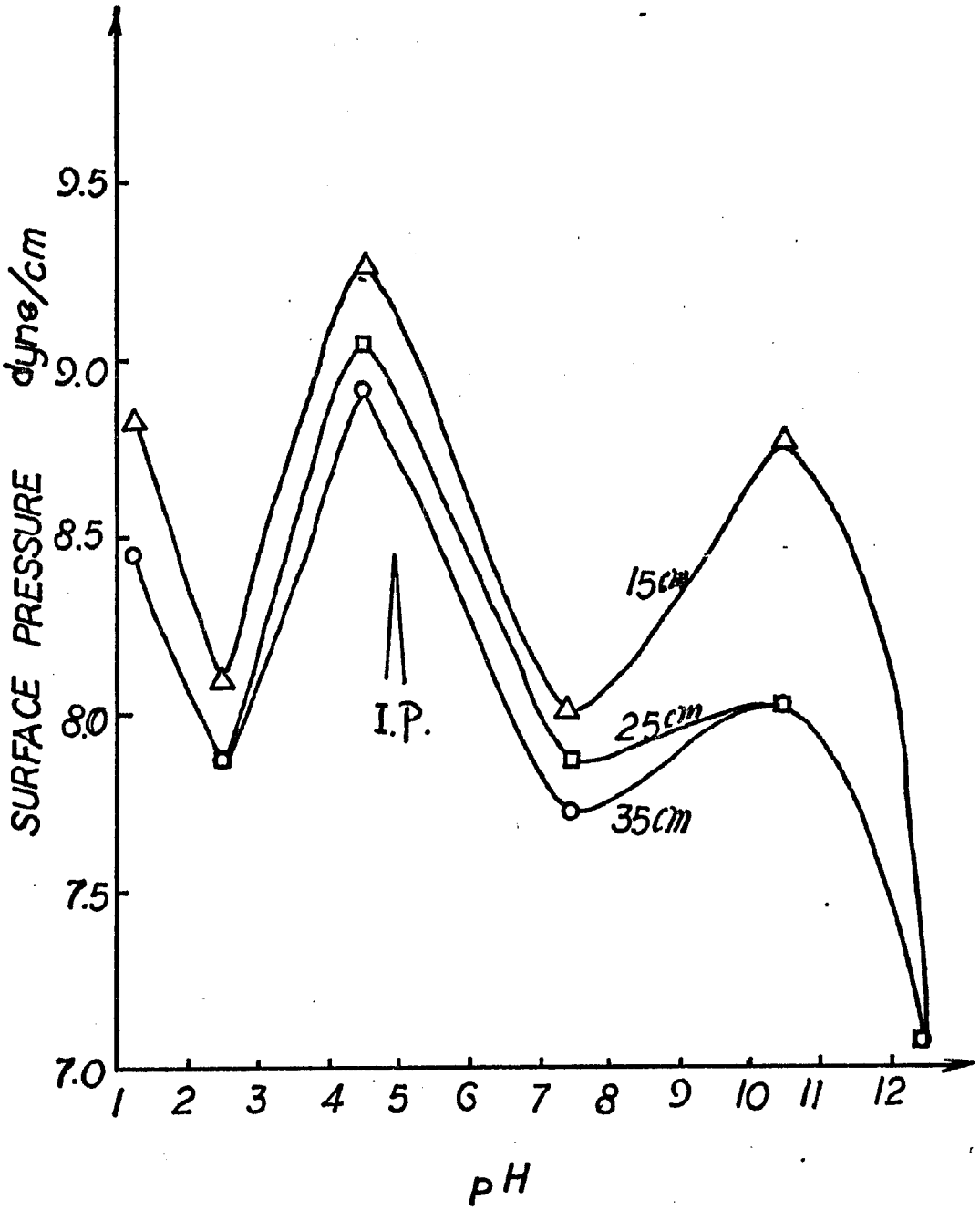

SURFACE PRESSURE dyne/cm
9.5
9.0
8.5
8.0
7.5
7.0
1
2
3
4
5
6
7
8
9
10
11
12
pH
I.P.
15cm
25cm
35cm

Figure 4.5

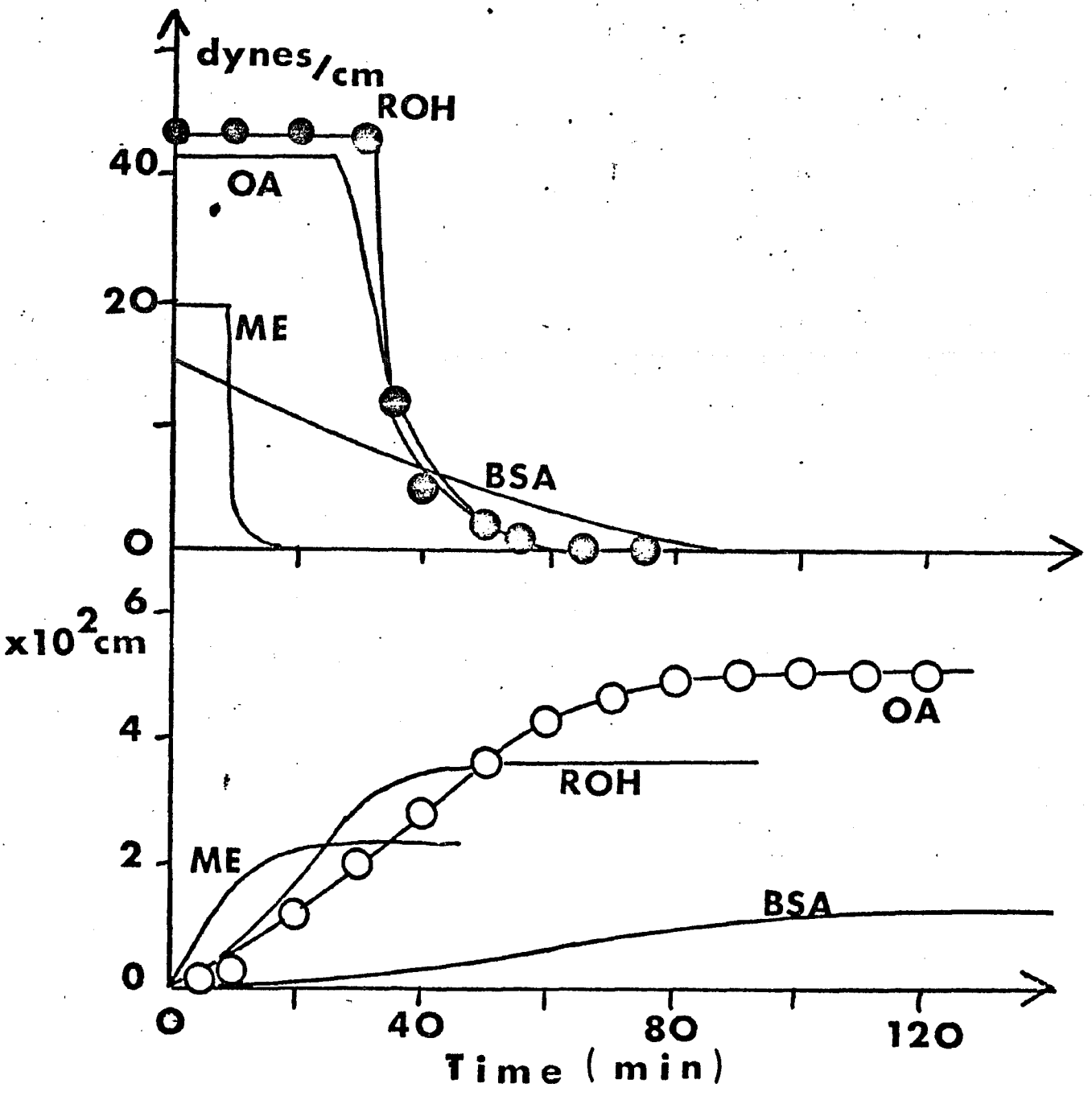

dynes/cm
ROH
40
OA
20
ME
BSA
0
6
x10² cm
4
OA
ROH
2
ME
BSA
0
0
40
80
120
Time (min)

Figure 4.6

remained constant and equal to the collapse pressure of the monolayer as long as the lens was present. Thereafter the surface pressure decreased as the monolayer was transfered from trough 1. For the protein monolayer which is somewhat soluble, no plateau region was observed, but the change in surface pressure was gradual and fairly constant.

IV. Interpretation

The surface properties of macromolecules most likely arise from the unfolding of the long chains in the surface region. Flory and Huggins (22-24) developed a theory in 1942 for polymer solution, and Singer (25) later applied it to surface films. Since then Frisch and Simba (26) and Silberberg (27) proposed additional models which account for those portions of the polymer chain that are pushed into the bulk phase, or even lifted off the surface, upon compression.

In proteins the unfolding and coiling of the chains are known as denaturation. The denaturation effect is most likely responsible for the results in figures 4.2 and 4.4. As the monolayer is compressed, the long chains of the protein reorient themselves in the monolayer, thereby affecting the surface viscosity. Since the greatest reorientation is near the barrier, the most dramatic change in the viscosity coefficient is in this region.

For clarity consider each individual protein molecule as an elastic spring and a dashpot in parallel (Kelvin model). As the barrier is compressed a force developed on the mechanical model. The dashpot compresses and exerts a resisting force --i.e., analogous to the surface viscosity force between the protein and bulk phase. As the piston is compressed the spring will also supply a force --i.e., analogous to the vis-

cosity force developed within the protein molecule as it coils. Finally as the entire " Kelvin solid" is moved, the force will be transferred to the models behind it--i.e., analogous to the viscosity within the monolayer produced by intensity models. With this type of model the surface pressure will depend on position. This model is also in agreement with the results of Blank (28) who observed that bovine serum albumin monolayers had a plastic component as part of its rheological behavior.

From the above model, it may be assumed that the resistance to the moving barrier is caused by a viscosity effect which is developed in the following ways:

1) The viscosity within the monolayer itself which can be attributed to the coiling of the molecule and protein-protein interaction.

2) The viscosity developed between the sheath of water molecules bound to the monolayer and the bulk phase (surface drag viscosity).

Numerous authors studied this phenomenon by determining the amount of substrate carried by a moving monolayer--e.g., D. J. Crisp (18) and C. Y. Pak and N. L. Gershfeld (17). J. H. Schulman and T. Teorell (16) estimated that the thickness of the aqueous bulk layer dragged with a moving monolayer of oleic acid is around 0.03 mm. H. L. Rosano (29) determined for a 0.07 M $NaSO_4$/1-butanol/water cell that the diffusion layer at the interface is on the order of 300 microns. With this concept in mind the following mathematical model is proposed to explain if the phenomenon is caused by the protein monolayer or the surface drag viscosity effect.

A small and local pressure change is produced in a monolayer by compression at a constant rate. The propagation of the surface pressure is detected at various distances from the point of surface perturbation.

Consider a small element of the monolayer (length M; width ∂x) perpendicular to the ox axis. At the time t =0 the perturbation is produced by the moving barrier. At time t the perturbation has reached the small element of the monolayer M∂x (where M is also the width of the trough). Let $\partial\pi$ be the difference in surface pressure on each side of the surface element M∂x and u the velocity of the perturbation. The forces acting on this small element of surface are: the force caused by the surface pressure,

$$\partial f_1 = \partial\pi M \tag{1}$$

and the force caused by the two-dimensional surface viscosity and the friction between the surface film and the subphase (∂f_2). These two forces equal the inertial force of the surface element of mass ∂m.

$$\partial f = \partial m \frac{\partial u}{\partial t} \tag{2}$$

t = time. Therefore,

$$\partial f_1 - \partial f_2 = \partial f \tag{3}$$

substituting and dividing by M∂x

$$\frac{\partial \pi}{\partial x} - \frac{\partial f_2}{M\partial x} = \frac{\partial m}{M\partial x}\frac{\partial u}{\partial t}$$

but $\partial m/M\partial x$ equals the surface density (δ); thus

$$\frac{\partial \pi}{\partial x} - \frac{\partial f_2}{M\partial x} = \delta \frac{\partial u}{\partial t} \tag{4}$$

Let $\partial f_2/M\partial x = Y$

$$\frac{\partial \pi}{\partial x} - Y = \delta \frac{\partial u}{\partial t}$$

or

$$Y = \frac{\partial \pi}{\partial x} - \delta \frac{\partial u}{\partial t} \tag{5}$$

From the continuity equation it can be shown that

$$\frac{\partial \delta}{\partial t} + \frac{\partial(\delta u)}{\partial x} = 0$$

$$\frac{\partial \delta}{\partial t} + \frac{\partial \delta}{\partial x} u + \delta \frac{\partial u}{\partial x} = 0$$

for a small perturbation the surface film density remains practically constant and

$$\frac{\partial \delta}{\partial t} + \delta \frac{\partial u}{\partial x} = 0$$

or

$$\frac{\partial \ln \delta}{\partial t} = - \frac{\partial u}{\partial x} \qquad (6)$$

Gibbs (30) noted an elasticity associated with a liquid film if the surface tension varies with the area of the surface; for a thin liquid film of area s, the Gibbs elasticity is given by

$$E = \sigma \frac{\partial \gamma}{\partial \sigma}$$

where E is the film elasticity, σ the molecular area, γ the surface tension, and δ the surface density. Since

$$\sigma = 1 / \delta$$

$$\partial \sigma = \frac{-1}{\delta^2} \partial \delta$$

$$E = - \frac{1}{\delta} \delta^2 \frac{\partial \gamma}{\partial \delta} = - \delta \frac{\partial \gamma}{\partial \delta}$$

but

$$\partial \gamma = - \partial \pi$$

Therefore

$$E = \partial \pi / \partial \ln \delta$$

By introducing Gibbs' elasticity coefficient E, which equals $\partial \pi / \partial \ln \delta$ in equation 6, one obtains

$$\frac{1}{E} \frac{\partial \pi}{\partial t} = - \frac{\partial u}{\partial x} \qquad (7)$$

Therefore,

$$\Delta u = - \int_{X_o}^{X_1} \frac{1}{E} \frac{\partial \pi}{\partial t} \partial x$$

Experimental Determination of Y (Table IV.1)

The elasticity coefficient (E) and the surface density (δ) are determined directly from the compression isotherm (π vs. σ). The monolayer is compressed from a given surface pressure with the position of the wettable blade at distance x from the compression barrier--i.e., compressed from 5.0 to 5.5 dynes / cm. Graphs of π vs. time are plotted for various positions of the blade (distance x) compressing over the same area. Figure 4.2 and 4.4 represent a family of curves for bovine serum albumin and β-casein respectively. Curve fitting of the results of BSA leads to

$$\pi (X, t) = (.264) + X^{(-.152)}$$

which can be used to solve for

$$\frac{\partial \pi}{\partial t}, \quad \frac{\partial \pi}{\partial x} \quad \text{and} \quad \frac{\partial u}{\partial t}$$

Solving Equation 5 for the frictional shear gives:

$$Y = - .0401 + X^{(-1.152)} - \frac{\delta (0.264)}{E} \frac{\partial x}{\partial t} X^{-.152}$$

If it is assumed that the local velocity gradient decreases linearly perpendicular to the interface (Newtonian profile at each point x), the average three-dimensional viscosity for a film thickness of E = 50 Å and E = 5 x 10^{+6} Å may be compared. In the former case, it is assumed that the monolayer and oriented substrate are only 50 Å thick; the latter number is based on a much larger oriented subphase (16, 29). In the first case the calculated viscosity varies between 7.07 x 10^{-5} and 5.41 x 10^{-4} cps; in the second case it varies from 54 to 8 cps. (with increasing distance from the barrier). From the calculation of viscosity, the assump-

Table IV.1 Bovine Serum Albumin on Water (pH 5.2, 24.0°C).

Initial Pressure, 9.3 dynes/cm

X	$\frac{\partial \Pi}{\partial x}$	$\zeta \frac{\partial u}{\partial t}$	Y	$\eta_E = 50\text{Å}$	$\eta_E = 5 \times 10^{-2}$cm
(cm)	(dyne/cm^2)	$\frac{\text{dynes}}{\text{cm}^2}$	(dyne/cm^2)	(cps)	(cps)
6.4	-6.16×10^{-2}	10^{-11}	6.16×10^{-2}	5.41×10^{-4}	54.1
10.7	-3.81×10^{-2}	10^{-11}	6.16×10^{-2}	3.44×10^{-4}	34.1
18.5	-7.84×10^{-3}	10^{-11}	7.84×10^{-3}	8.83×10^{-5}	8.83
27.7	-4.45×10^{-3}	10^{-11}	4.45×10^{-3}	7.07×10^{-5}	7.07

tion that $E = 5 \times 10^{+6}$ Å is more reasonable.

V. Discussion

In light of the experiments on the compression and expansion of the monolayer and the calculation on the depth of the dragged substrate, the following conclusions are suggested. First the structure of the sheath of bound water below the monolayer and the extent to which it is bound to the monolayer affect the surface viscosity. Secondly the coiling of the protein monolayer is an important observed effect. Thus the following hypothesis of a non-structured and structured subphase is advanced.

For example, in the case of myristic acid and eicosyl sodium sulfate spread on aqueous substrates and substrates containing alkyl amines, the subphase is oriented because of dipole-dipole interaction but still is in the liquid state. This orientation has been accounted for in many monolayer studies concerned with surface potential (31-34). Upon compression, the substrate molecules, although oriented, can slip or be squeezed into the bulk; thus the transmission of the surface pressure will be instantaneous and independent of the position of the device for measuring the surface pressure. In the case of the protein monolayers the subphase is bound strongly to the protein(35) and produces a " pseudo gel " phase as described by Colacicco et al. (36). This helps explain our observed phenomenon, since on compression the gel will deform and drag along with the monolayer. This idea of a structured subphase explains why protein solutions, although not necessarily good foaming agents, are usually good foam stabilizers. Blank and Lee (37) also reported surface tension gradients when studying films of lung extracts.

The observation of a larger viscosity effect for bovine serum albumin is in agreement with Boyd's measurement that BSA monolayer is considerably more viscous than β-casein monolayer (38). It appears that the presence of a continuous two-dimensional network in a BSA monolayer (36, 38) may cause maximal interaction between the monolayer and water, and enhance the formation of continuous structured water underneath the monolayer.

In addition the decrease in the effect with bovine serum albumin above and below the isoelectric point indicates that as the protein becomes ionized, the gel subphase breaks down and becomes similar to that of the myristic acid subphase.

To further substantiate the theory on the role of the subphase structure and surface viscosity, the results of the isothermal surface distillation technique must be examined (Figure 4.6). For oleic acid, decyl alcohol and methyl laurate more than one monolayer was used (since a lens formed on the surface) to drag the substrate from trough 1 to trough 2. The thickness of the substrate dragged by one monolayer can be determined readily since the total number of molecules placed on the surface and the area per molecule at the collapse pressure are known. Considering this correction, the thickness of substrate-dragged per monolayer is: oleic acid = 3.28×10^{-3} cm, decyl alcohol = 2.15×10^{-4}cm, methyl laurate = 1.28×10^{-3} cm. For the B S A, the result is taken directly from Figure 4.6: 1.25×10^{-2} cm since only one monolayer was formed and the operating pressure was low enough to prevent desorption. Thus, it is obvious that the protein drags a considerable amount of substrate compared with the smaller molecules studied although each monolayer studied will drag the subphase.

VI. Conclusion

A phenomenon has been observed on only protein monolayers. A hypothesis is proposed that not only is the protein monolayer responsible for the effect but the subphase structure also plays an important role.

Film transfer experiments demonstrate that there is always a sheath of subphase associated with the monolayer. However, when the monolayer is compressed or decompressed in an enclosed area, there will be a certain degree of slippage at the monolayer/ subphase interface. When the subphase water molecules are strongly bound to the monolayer, as for a protein monolayer spread on an aqueous substrate at its isoelectric point, little slippage exists, and the phenomenon in Figure 4.2 and 4.4 are observed. For monolayer systems where there is substantial slippage at the monolayer /subphase interface the effect will not be observed during compression or decompression experiments. This effect helps explain why proteins are used with other surfactants to stabilize emulsion and foam formulations (28, 39).

Nevertheless, no matter how one interprets the theory, the implications of this experiment are more important and should be understood and used for future work on macromolecular monolayer systems. It is suggested that an additional variable-- the distance of the surface pressure measuring device from the point of perturbation is to be included in macromolecule monolayer work to ensure accuracy and reproducibility.

CHAPTER FIVE

Surface Interaction of Eicosyl Sodium Sulfate Monolayers with Gelatins and Bovine Serum Albumin

I. Background

The study of fatty acid-protein binding is important in the understanding of the properties and behavior of many foods (1), lipid transport processes (2-4), and the structure and function of biological membrane (5-9). However, the nature of the above interactions is still imperfectly understood.

Various techniques have been used: Putnam and Neurath (12-14) have employed viscosity, electrophoresis and diffusion methods to investigate the interaction between protein and sodium dodecyl sulfate. Elkes, Frazer, Schulman and Steward (16) used an emulsion technique which estimated the amount of protein adsorbed at the surface of drops. These workers have attributed the protein complex formation to electrostatic force between oppositely charged groups. Boyer, Ballon and Luck (10, 11) by means of electrophoretic mobility and viscosity measurements, have observed the stability effect of fatty acid on protein against denaturation increases with increasing chain length of the fatty acid, and concluded that the specific affinities of the fatty acids to proteins could not be attributed to electrostatic association alone. Applying an analytical method, Pankhurst has studied the composition of complexes formed between gelatin and sodium dodecyl sulfate (15). Their study attributed the complex formation to ion-dipole and electrostatic association.

In 1935, Schulman and Hughes (17) developed a monolayer penetration technique which has since been applied to study the interactions of various proteins with surfactants (18-20, 5-7).

Matalon and Schulman (20) performed penetration experiments at

constant area as well as at constant surface pressure. In the case of constant area, the spread insoluble surfactant monolayers were kept at constant area and a protein previously dissolved in a buffer solution was injected into the substrate under the monolayer. When the charge of the insoluble monolayer had an opposite sign to that of the soluble protein, enhanced adsorption of protein into the insoluble monolayer was noted. These adsorbed protein molecules desorbed as the sign of their net charge reversed by changing the pH of the substrate.

In the constant pressure penetration experiment, the proteins, having opposite signs to the insoluble monolayer, were injected into a substrate kept at constant surface pressure. The penetration was observed as an increase of surface area and a change in surface potential. Applying the above methods to study the interaction of surfactants like cardiolipin, $C_{22}H_{45}SO_4Na$ and Stearylcholin with proteins, Matalon and Schulman have concluded that protein-surfactant interactions are mainly electrostatic.

Eley and Hedge (5,6) investigated quantitatively the interactions of stearic acid, cholesterol and other more complex lipids like distearin, cephalin and lecithin with bovine serum albumin and insulin. In these studies various quantities of protein solutions were injected under lipid monolayers at constant area. The established surface isotherms showed two marked discontinuities which were interpreted as corresponding to the formation of the first sublayer of adsorbed denature proteins and the second sublayer of adsorbed native proteins. It was described that the first sublayer of adsorbed proteins combined with stearic acid, cholesterol, distearin and cephalin molecules through

the peptide bonds, probably by means of hydrogen bonding or ion-dipole association. However, with lecithin which has bulky charged groups the interaction was mainly ionic.

Following the same monolayer approach and studying lipid-polypeptide interactions, Chatelain, et al. (21) concluded that the adsorption of polypeptides to a lipid monolayer is due to electrostatic interaction, while the interaction between lipid and polypeptide molecules in the plane of the interface is essentially hydrophobic. In addition, he concluded that the adsorption of polypeptide with helical conformation to the surface was hindered by the presence of a lipid monolayer and the lipid-water interface did not induce a change of the conformation of the adsorbed polypeptide.

In spite of these studies , the following problems are still not completely explained:

1) Does the protein and surfactant form a molecular association or simply a two-dimensional solution?

2) Is the kinetics of the interaction primarily caused by solubility or electrostatic factors ?

To obtain a better understanding of the above problems, protein-surfactant interactions were carried out at air/liquid interfaces, using monolayer penetration technique. Surfactant molecules such as arachidic acid, behenic acid and sodium eicosyl sulfate were spread on a liquid surface and compressed to a specific surface pressure While keeping the surface pressure constant, an aqueous solution of bovine serum albumin or gelatin was injected under the monolayer. The interactions were recorded as the rate of change in surface area and surface potential. The ionic properties of the various proteins and

surfactants were monitored by changing the pH of the substrates.

In order to determine the nature of the penetration products, the penetrated surfactant monolayer was subsequently compressed, yielding surface isotherms of the protein-surfactant film. These surface isotherms were then compared with those of the components.

Blank et al. (22), studying the adsorption kinetics of ovalbumin monolayers, found that the adsorption rate changed with the pH of the substrate and was maximum at the isoelectric point of the protein. In the case of our study, the adsorption rate of the bovine serum albumin or the gelatin to the air/water interface probably play a role in the kinetics of surface interactions with the monolayer. To clarify this point, adsorptions of gelatin and bovine serum albumin to air/water interfaces were carried out at substrates of different pH values.

II. Experimental

1. Apparatus and Procedure

The apparatus for measuring surface pressure and surface potential has been described in chapter 3. To maintain a constant surface pressure throughout the experiment, the microforce transducer amplifier system which measured surface tensions, was replaced by a Rosano tensiometer (Biolar Corp. North Grafton, Mass.,) equipped with a platinum mercury contact that would activate a reversible motor to drive a barrier forward or backward when it was closed. This allowed the surface area to contract or expand automatically.

The penetration was carried out in a fused silica trough (31.2 x 13.0 x 2.6 cm) which contained an aqueous substrate. A surfactant

solution was deposited on the aqueous surface with an Agla micrometer syringe (Burroughs Wellcome Co., Tuckahoe, N. Y.). After the insoluble monolayer was compressed to a desired surface pressure, a small volume of a protein solution was injected into the substrate under the monolayer with a pipet. The substate was stirred carefully by withdrawing and blowing the substrate with the pipet for a few times. The penetration of the protein into the insoluble monolayer was recorded as the change in film area and surface potential vs. time until the area remained practically constant.

Adsorption experiments of proteins were performed in a 400 ml crystallizing dish (50 x 100). 250 ml aqueous substrate was retained in the dish and an aqueous protein solution containing 1.224 mg BSA or 4.782 mg pigskin gelatin or 5.110 mg calfskin gelatin was delivered into the substrate from a buret with its end immersed in the substrate and extended to the bottom of the dish. The substrate was stirred gently with a magnetic stirrer during the addition of protein solution. The adsorption was determined from the surface tension measurement which was made continuously for half an hour.

2. Reagents

The bovine serum albumin crystals, arachidic acid and behenic acid were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio. The purified pigskin gelatin and calfskin gelatin were from Eastman Kodak Co., Rochester, N. Y., and the eicosyl sodium sulfate was from Schuchardt, Munich, West Germany. All the reagents were used without further purification.

The aqueous bovine serum albumin solution was prepared by dissolving

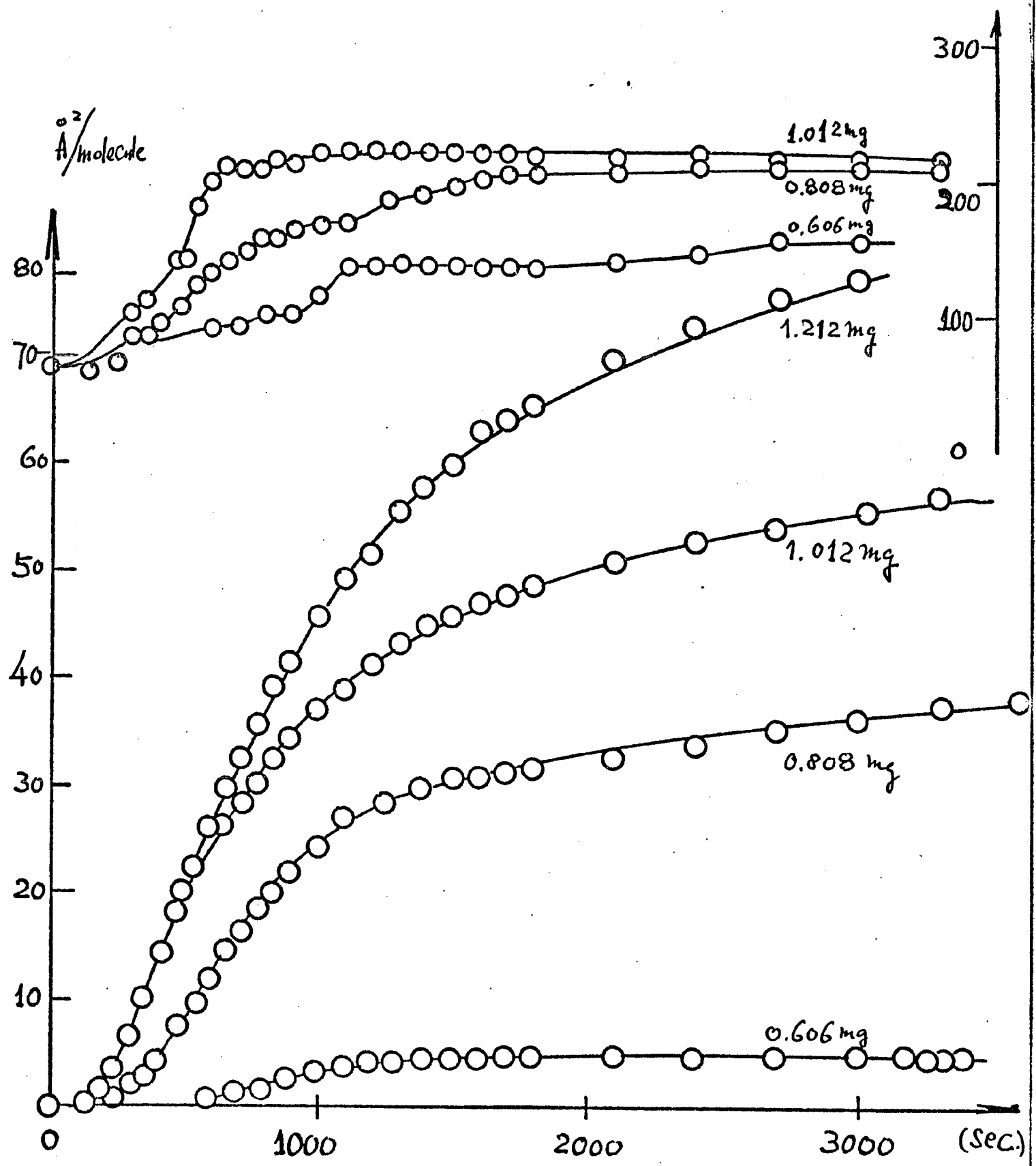

ΔV(mV)
300
200
100
0
Å²/molecule
80
70
60
50
40
30
20
10
1.012 mg
0.808 mg
0.606 mg
1.212 mg
1.012 mg
0.808 mg
0.606 mg
0
1000
2000
3000
(Sec.)

Figure 5.1

bovine serum albumin crystals in freshly distilled water. In preparing aqueous gelatin solution, the solution was heated to about 60° C. The surfactant solutions were made by dissolving 25 mg of fatty acid in 25 ml n-hexane and dissolving 5 mg of eicosyl sodium sulfate in 3:2 mixture of n-hexane and 2-propanol with a few drops of distilled water.

III. Results

1. Effect of Concentration of Protein on Penetration

Figure 5.1 describes the results of the penetration of bovine serum albumin into eicosyl sodium sulfate monolayers when various amounts of BSA solutions were injected into the substrate at the isoelectric point (4.7) of the protein. The film was kept at a constant surface pressure of 20 dynes/cm. It appeared that the increase in surface potential and the expansion of the monolayer were proportional to the amount of the protein injected. Similar results with a smaller expansion were observed when pigskin gelatin solutions were injected under the eicosyl sodium sulfate monolayers at the same condition (20dynes/cm, pH 9.1). In addition, the penetrations of proteins into behenic acid monolayers were found to be more difficult even at a lower surface pressure.

2. Effect of Surface Pressure on Film Penetration

Figure 5.2 demonstrates that the penetration of pigskin gelatin into the eicosyl sodium sulfate monolayer decreases considerably with increasing the surface pressure from 15 to 20 dynes/cm. The same effect was observed when behenic acid monolayers were penetrated by the bovine

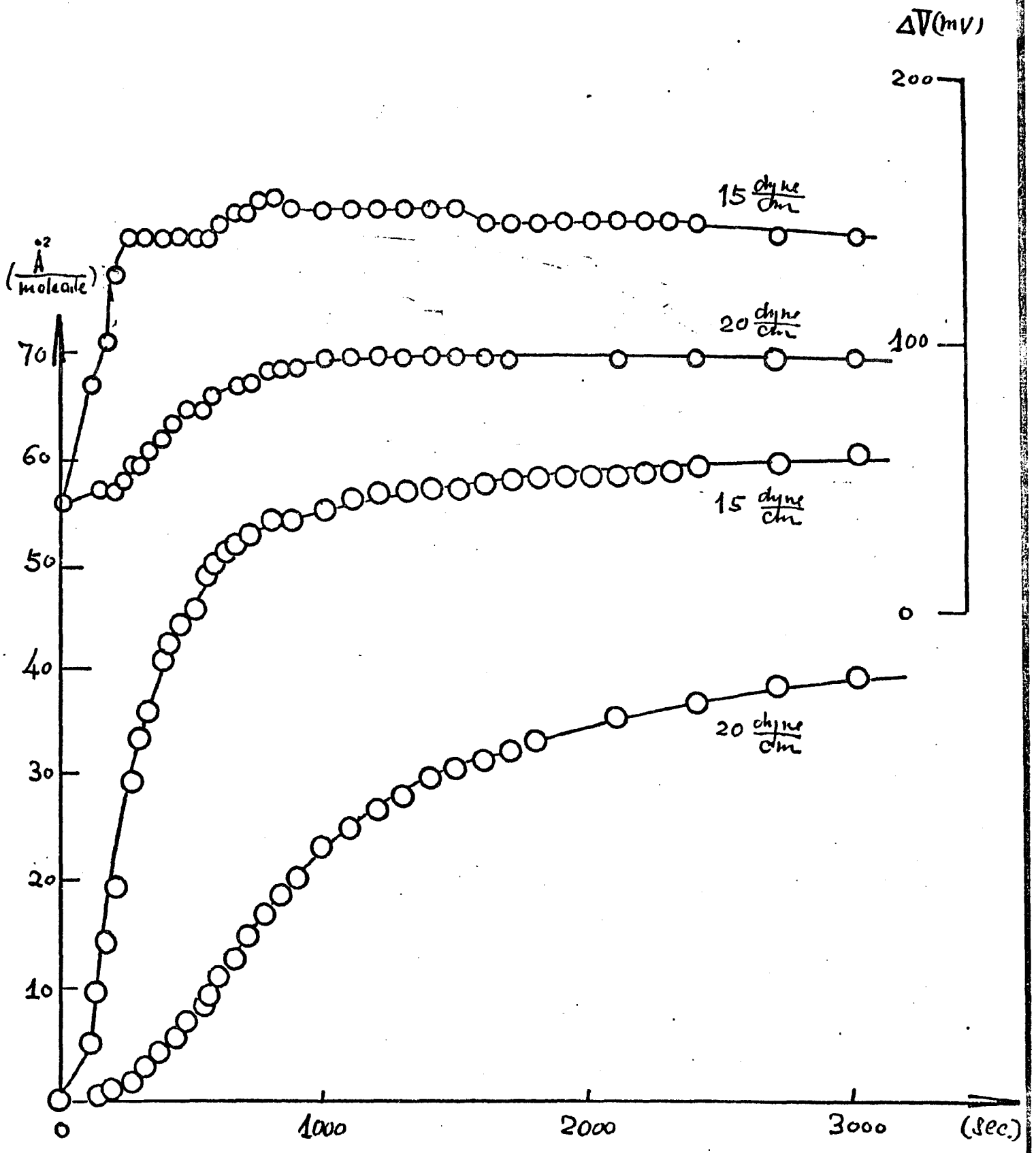

ΔV(mV)
200
100
0
15 dyne/cm
20 dyne/cm
15 dyne/cm
20 dyne/cm
Å²/molecule
70
60
50
40
30
20
10
0
1000
2000
3000
(sec.)

Figure 5.2

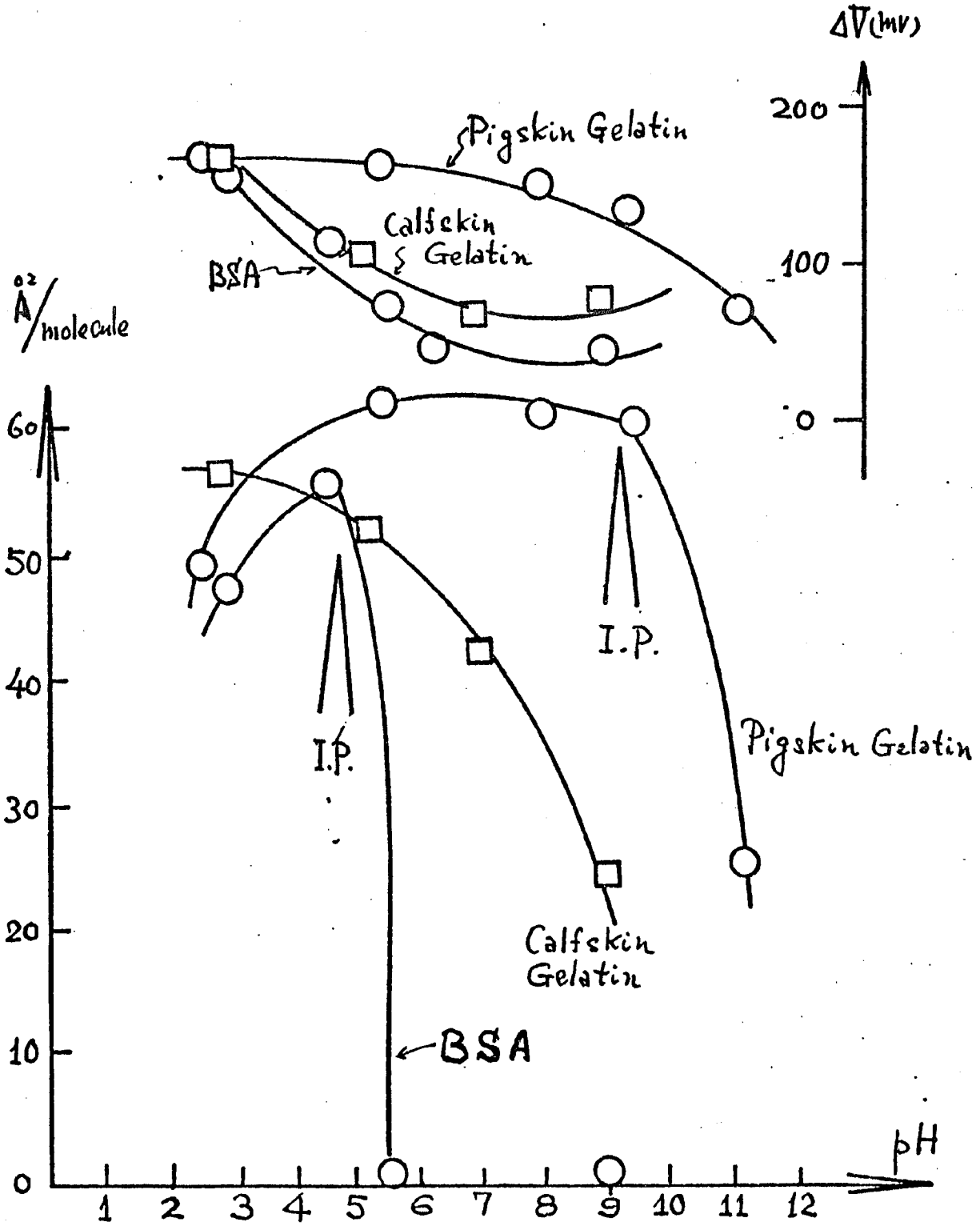

ΔV(mv)
200
100
0
Pigskin Gelatin
Calfskin
Gelatin
BSA
Å²/molecule
60
50
40
30
20
10
0
I.P.
I.P.
Pigskin Gelatin
Calfskin
Gelatin
BSA
pH
1
2
3
4
5
6
7
8
9
10
11
12

Figure 5.3

serum albumin or pigskin gelatin.

3. Effect of Bulk PH on Film Penetration

Penetration curves at various pH were essentially similar in shape to those shown in Figures 5.1 and 5.2. The effect of pH on penetration of bovine serum albumin and gelatin into eicosyl sodium sulfate monolayers is shown in Figure 5.3. The surface potential and the extension of the film at each pH were taken after the film was penetrated for 50 minutes. The pH was altered with 0.1 N NaOH and 0.1 N HCl. Although the pH was varied, the ionic strength was kept constant.

4. Surface Isotherm of Penetrated Monolayer

The compression surface isotherms of bovine serum albumin, eicosyl sodium sulfate and eicosyl sodium sulfate after penetration by bovine serum albumin for 30 minutes are shown in Figure 5.4. Upon compression, the penetrated monolayer was stable and able to sustain a surface pressure of 39 dynes/cm before collapse. At the collapse point, the surface potential of the film was about 100 mV above that of the pure eicosyl sodium sulfate monolayer, indicating the bovine serum albumin was somehow associated to the insoluble monolayer.

Figure 5.5 describes the compression of penetrated arachidic acid monolayer. In this case, the bovine serum albumin was squeezed out of the monolayer at the collapse pressure of the protein, then the isotherm overlaped with that of the pure arachidic acid monolayer. Similar result was noted from the compression of pigskin gelatin-arachidic acid monolayer system.

When the eicosyl sodium sulfate monolayer penetrated by pigskin gelatin was compressed, the monolayer sustained a surface pressure of

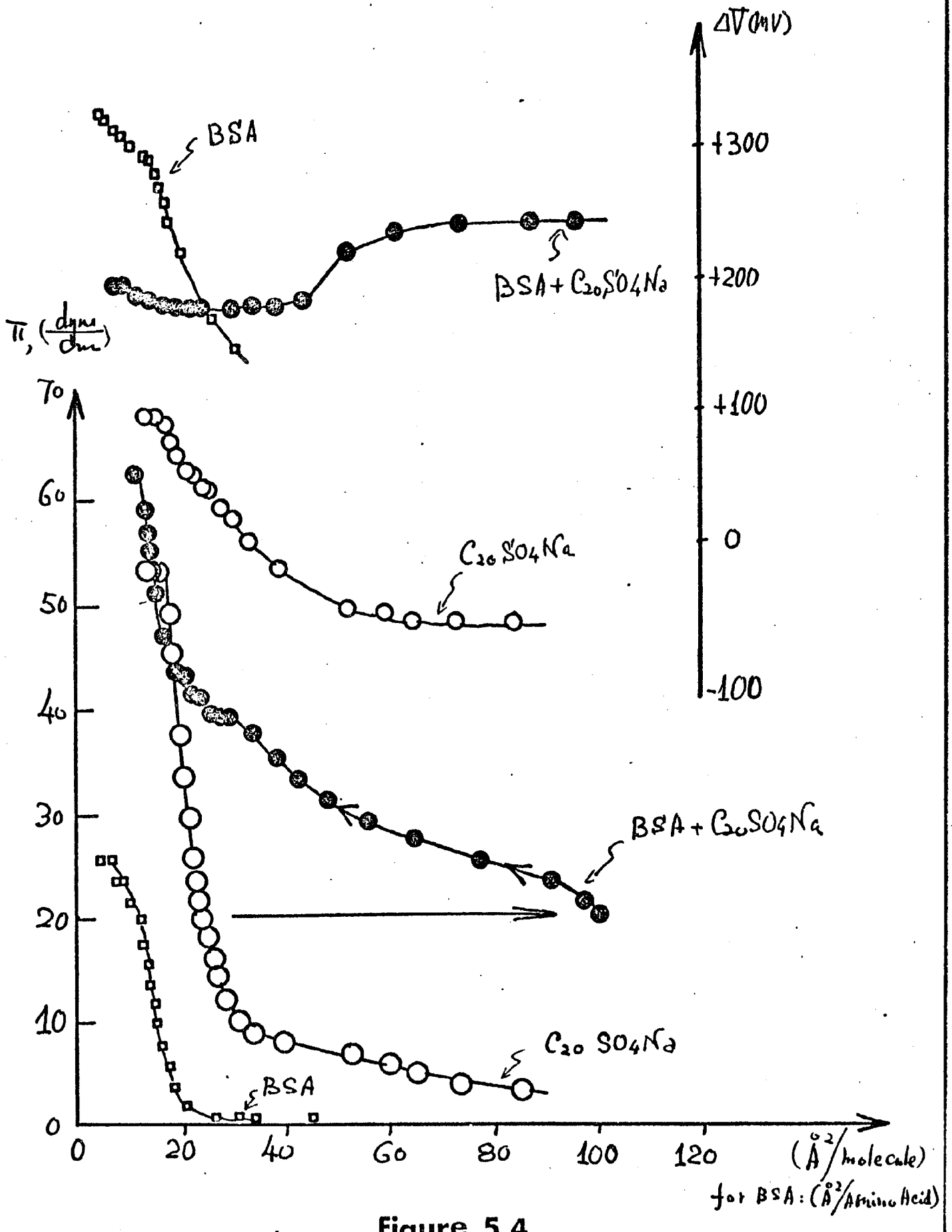

ΔV (mV)
+300
+200
+100
0
-100
BSA
BSA + C20SO4Na
π, (dyne/cm)
To
60
50
40
30
20
10
0
C20SO4Na
BSA + C20SO4Na
C20 SO4Na
BSA
0
20
40
60
80
100
120
(Å²/molecule)
for BSA: (Å²/Amino Acid)

Figure 5.4

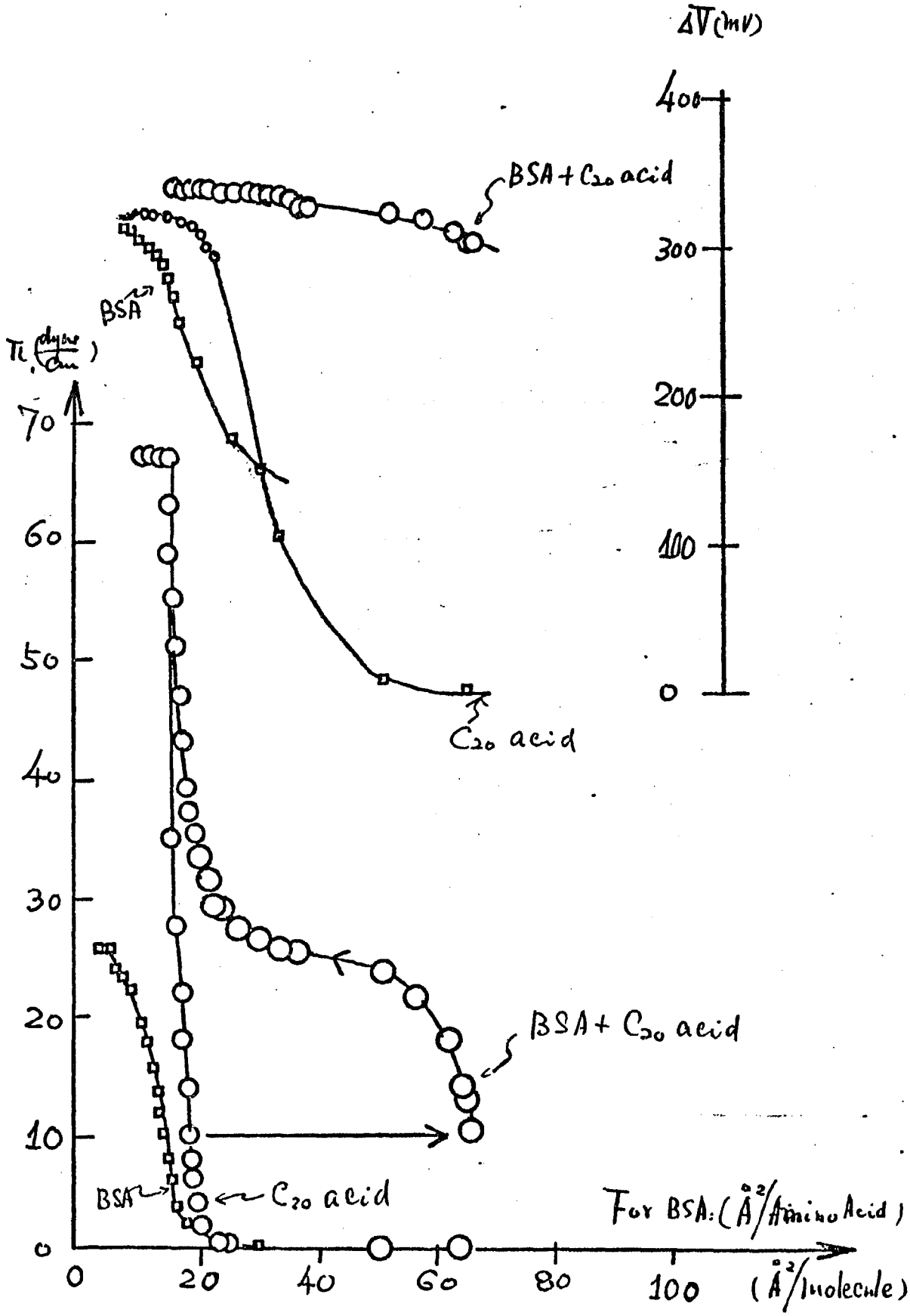

ΔV (mV)
400
300
200
100
0
BSA + C20 acid
BSA
π (dyne/cm)
70
60
50
40
30
20
10
0
C20 acid
BSA + C20 acid
BSA
C20 acid
For BSA: (Å²/Amino Acid)
0
20
40
60
80
100
(Å²/molecule)

Figure 5.5

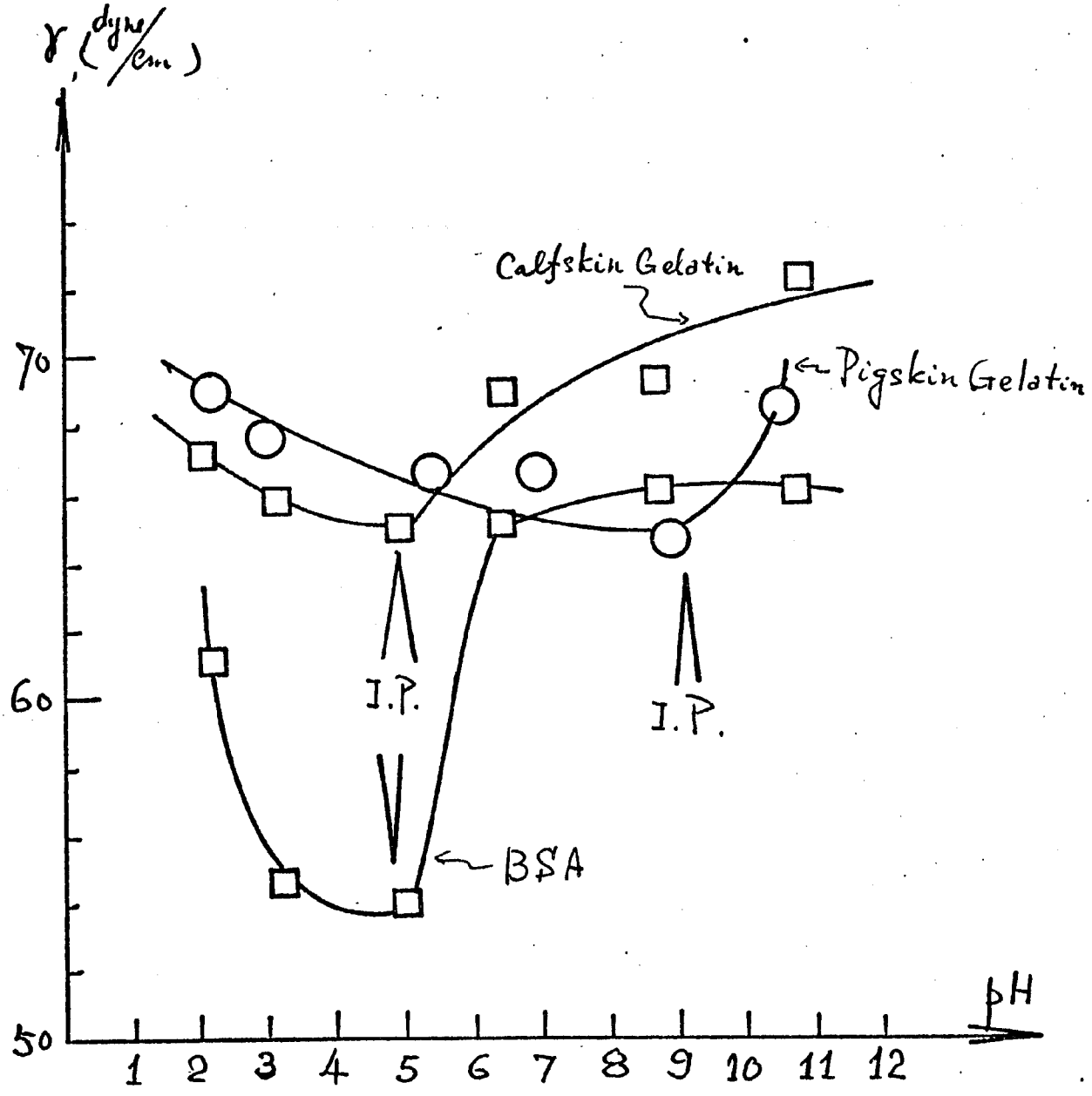

γ (dyne/cm)
70
60
50
Calfskin Gelatin
Pigskin Gelatin
I.P.
I.P.
BSA
pH
1 2 3 4 5 6 7 8 9 10 11 12

Figure 5.6

69 dynes/cm and became a gel.

5. Adsorption of Protein

The surface tensions of various solutions of bovine serum albumin, pigskin gelatin and calfskin gelatin, 30 minutes after injection of protein are plotted on Figure 5.6. For each protein, maximum adsorption occured around its isoelectric point.

IV. Discussion

From the above experimental results film penetration proceeds probably through the following steps:

1) Adsorption of protein onto the charged monolayer.

This adsorption is enhanced by the electrical attraction between the monolayer and the adsorbed protein which is positively charged below its isoelectric point. Above the isoelectric point, the adsorption is hampered by the electrical repulsion between the negatively charged protein and monolayer.

2) Electrical neutralization of the monolayer.

The adsorbed protein reacts with the monolayer molecules. At the same time the protein unfolds and exposes its hydrophobic side chains which penetrate into the interface.

3) Further adsorption of protein.

Generally the protein injected into the substrate is in excess, thus further adsorption of protein to the surface is possible. However, the presence of the first adsorbed protein layer may set up an energy barrier and made the further adsorption of protein difficult. As a

consequence, hydrophobic side chains of protein penetrate with difficulty into the monolayer.

The ability of protein to penetrate into an insoluble monolayer seems to depend on the availability of nonpolar side chains in the protein. For example, gelatin which is less surface active then bovine serum albumin, but composed of more than 50 percent non-polar residues, penetrates into the eicosyl sodium sulfate monolayer as easily as the bovine serum albumin.

Once the protein penetrates into the monolayer, they may form strong or weak association which appears to depend mainly on the chemical properties of the monolayer molecules. For instance, the eicosyl sodium sulfate bound both penetrated bovine serum albumin and gelatin strongly. Although in the case of the arachidic acid which contained also 20 carbon atoms, strong association was not observed.

A monolayer of a long chain sulfate remains ionized and negatively charged over a wide pH range. If long chain sulfate/protein interaction is primarily electrostatic in nature, interaction will be maximum below the isoelectric point of the protein when the protein is still positively charged. However, it was observed that interaction was maximum at the isoelectric point which leads us to another possible explanation. At the isoelectric point the rate of adsorption of the protein is maximum and electrostatic interaction are still possible. Below the isoelectric point the protein becomes more soluble, the rate of adsorption decreases, but the electrostatic interaction increases. Above the isoelectric point the protein solubility increases, but the adsorption and electrostatic interaction both decrease. Therefore, it seems that the

adsorption of protein into the monolayer is more important in causing the protein/surfactant interaction.

Matalon and Schulman (20) found that the desorption of protein from a penetrated monolayer at constant area and the ejection of protein from a penetrated monolayer at constant pressure, by reversing the charge of the protein, were always incomplete. These results are not surprising, since the interaction between the protein and monolayer, is not mainly due to the electrostatic factor as they have suggested, but is mainly due to the solubility of the protein and the hydrophobic bond between the protein and the spread monolayer. Our conclusions are closer to Eley and Hedge's (5). These investigators found that hydrophobic but not electrostatic factor contributed primarily to the protein/lipid interaction.

In the complex formation between the sodium dodecyl sulfate and gelatin, Pankhurst has attributed the adsorption of sodium dodecyl sulfate to gelatin above the isoelectric point to an ion-dipole mechanism through the keto-imide groups in the backbone of the polypeptide chains. He explained that less adsorption below the isoelectric point was due to the presence of the H^+ ion inhibiting the formation of the keto-imide resonance necessary for the formation of the dipole. This is not like in our case, since the penetration of the protein into the insoluble monolayer is considerably larger below the isoelectric point of the protein.

To check our theory which emphasizes rate of adsorption and electrical interaction a mixture of 5 g/l pigskin gelatin (IEP=9.1) and 1 g/l sodium dodecyl sulfate solutions at various pH were foamed.

Maximum foamability was observed at pH = 6.6 . This result can be explained as follows: it is assumed that completely ionized sodium dodecyl sulfate molecules interact with cationic groups on the gelatin. The effective isoelectric point of the gelatin will be shifted toward lower pH (from pH = 9.1 to pH = 6.6).

CHAPTER SIX

General Conclusions

Interfacial properties of bovine serum albumin, β-casein and gelatin can be studied by monolayer techniques. An air-liquid interface provides a simple and manageable interfacial region where a gaseous or condensed protein monolayer may be formed easily by spreading an aqueous protein solution from the air phase. The measurements of surface physical parameters e. g., surface tension, surface pressure and surface potential allow one to characterize the protein monolayer, determine surface properties and study various surface phenomena.

The charge of the protein in the monolayer may be varied conveniently by changing the pH of the substrate, thus the ionic or electrostatic properties of the protein at the interface can be investigated. Generally surface properties of proteins are markedly affected by the sign and magnitude of the net charges of the protein molecules in the monolayer.

1. Molecular Weight and Co-surface

Molecular weights and co-surfaces of bovine serum albumin and β-casein have been estimated from the surface concentrations and the surface pressures of the gaseous protein monolayers, using Guastalla's two-dimensional gaseous equation. The estimated values vary with the pH of the substrate on which the protein is spread. Only the values at the isoelectric point of the protein are in agreement with those values obtained by other methods which are reported in the literature. Therefore it is concluded that the two-dimensional gaseous equation satisfactorily describes the protein monolayer at its isoelectric point and may be used to calculate the molecular weight and the co-surface of protein at that condition. At other pH than the isoelectric point

of protein, the Guastalla's method of molecular weight and co-surface estimation is not valid, since the two-dimensional gaseous equation does not satisfactorily describe the protein monolayer due to the existence of the repulsive force between charged protein molecules.

2. Spreading

Spreading of protein depends on the quantity and nature of surface active agents such as n-pentanol, n-propanol and ethanol, added into the spreading protein solution. The initial surface area, the pH and ionic strength of the substrate on which the protein is spread, also affect the spreading of protein. A relatively expanded monolayer can be obtained by spreading an aqueous protein solution containing 0.5% n-pentanol on a sufficiently large surface area of aqueous substrate. Desorption of protein into the substrate during and after spreading may be avoided or minimized by using an aqueous substrate at the isoelectric point of the protein. A salt solution can also be used as a substrate to minimize the desorption of protein.

3. Surface Viscosity

It has been observed that surface tension of a protein monolayer varies with the distance between the measuring device and the compression barrier; the closer is the measuring device to the barrier, the smaller is the surface tension. A hypothesis is proposed that not only is the protein monolayer responsible for the effect, but the subphase structure also plays an important role.

It is found that there is always a sheath of subphase associated with the monolayer. However, when the monolayer is compressed or de-

compressed in an enclosed area, a certain degree of slippage at the monolayer / subphase interface will occur. When the subphase water molecules are strongly bound to the monolayer, as for a protein monolayer spread in an aqueous substrate at its isoelectric point, little slippage exist, and the phenomenon described in the last paragraph is observed. For monolayer system where there is substantial slippage at the monolayer / subphase interface the effect is not observed during compression and decompression experiments. This effect helps explain why proteins are used with other surfactants to stabilize emulsion and foam formulations.

4. Protein-Surfactant Interaction

Surface interaction between protein and surfactant can be investigated by monolayer penetration technique. It is found that the penetration of protein into an insoluble surfactant monolayer depends on the solubility and hydrophobicity of the protein, plus an electrostatic factor. Among these factors, the solubility of protein (or the adsorption of protein onto the interface) is found to be the most important factor in determining the kinetics of film penetration.

The nature of the penetrated monolayer can be examined by compressing the monolayer. When the protein is able to form a strong association with the insoluble monolayer molecule, the penetrated monolayer will sustain a higher surface pressure than the collapse pressure of protein; such phenomenon was observed in both the BSA-eicosyl sodium sulfate and pigskin gelatin-eicosyl sodium sulfate system. On the other hand, when the protein does not bind or only weakly binds to the insoluble monolayer molecule, the protein will be squeezed out at its collapse pressure on compression; this was observed in protein-arachidic acid system.

Appendix I

Table A-I Amino Acid Composition of Bovine Serum Albumin, β-casein and Gelatin

Amino Acid Residue	B S A[1]	β-casein[2]	Gelatin[3]
Alanine	39	5	10
Asp or Asn	8		
Cysteine	32	0	0.1
Aspartic acid	37	4	8.5
Glutamic acid	58	17	5.4
Phenylalanine	20	9	2.0
Glycine	12	5	23.6
Histidine	14	5	1.0±0.1
Isoleucine	12	10	1.7
Lysine	65	11	43.0±0.2
Leucine	51	22	3.7±0.5
Methionine	4	6	0.8
Asparagine	6	5	
Proline	27	35	15.3±0.4
Glutamine	11	22	
Arginine	17	4	7.6±0.1
Serine	26	11	1.5
Threonine	32	9	3.3
Valine	29	19	2.5
Tryptophan	1	1	0.0
Tyrosine	14	4	0.2
Glu or Gln	14		
Phosphoserine		5	
Hydroxy Proline			13.0
Total Amino Acid	529	209	104.5%

3. Calculated to 16.0% Nitrogen

Appendix II

1. Torsion modulus (E_r) and moment of inertia (I_r) of torsion wire[4a]

The torsion modulus of the torsion wire is a function of the length, diameter and nature of the wire. If a torsion pendulum oscilates in air, the period of the system may be related to the torsion modulus and moment of inertia of the system.

$$t_a = 2\pi \left(\frac{I_a}{E_r} \right)^{1/2} \qquad 1$$

By adding an inertial element to the system, the equation becomes

$$T_a = 2\pi \left(\frac{I_a + I_a'}{E_r} \right)^{1/2} \qquad 2$$

where t_a : period of the system without added inertial element, in air.

I_a : moment of inertia of the system without added inertial element, in air.

T_a : period of the system with added inertial element, in air.

I_a': moment of inertia of added element.

E_r : torsion modulus of the wire.

Solving equations 1 and 2 and eleminating E_r

$$I_a = I_a' \left[\frac{t_a^2}{T_a^2 - t_a^2} \right] \qquad 3$$

By eleminating I_a

$$E_r = \frac{4\pi^2 \, I_a'}{T_a^2 - t_a^2} \qquad 4$$

or from equation 1

$$E_r = 4\pi^2 \frac{I_a}{t_a^2} \qquad 5$$

In the above equations, it was assumed that any contribution by the water substrate to I_r and E_r was considered negligible (i.e., $I_a \approx I_r$).

2. Sample calculations of torsion modulus (E_r) and moment of inertia (I_r) of torsion wire

Equal weights are placed at the ends of a 10 cm bar suspended from a torsion wire which is twisted into rotary harmonic motion. If $I_a' = m\,r^2$, and m is the total weight suspended in grams, E_r is calculated from equation 4.

m	$I_a' = m\,r^2$	T_a	Ta^2	$T_a^2 - t_a^2$	E_r
(gram)	($g\ cm^2$)	(sec.)	($sec.^2$)	($sec.^2$)	($g\ cm^2/sec.^2$)
0	0	11.4	130.0($=t_a^2$)		
3	300	14.2	201.6	71.6	165.3
5	500	15.7	246.5	116.5	169.6
10	1000	19.1	364.8	234.8	168.0
20	2000	24.4	595.4	465.4	169.9
30	3000	29.1	846.8	716.8	165.5
40	4000	32.5	1056.0	926.0	170.4

The average value of E_r in the above example is 168.1 $g\ cm^2/\ sec.^2$

If T_a^2 is plotted against I_a' and extrapolated to $T_a^2 = 0$, $(I_a)_o = -\ 550\ g\ cm^2$.

Appendix III

Surface micromanometer

1. Characteristic of apparatus

The tension of the curved thread is $\pi_s r$. This tension is equal, in absolute value, to the applied tension t.

$$t = \pi_s r$$

where π_s : surface pressure

r : radius of circle. (figure A.1)

In a circle whose arc is the thread, from geometry,

$$K = 2\left[r^2 - (r - f)^2\right]^{1/2}$$

or

$$f (2r - f) = \frac{K^2}{4} \qquad 1$$

where f is the displacement of the center of the thread. For small curvature, f is negligible compared to the diameter 2r and the length of the silk thread $S \simeq K$. Therefore,

$$f (2r) = \frac{S^2}{4} \qquad 2$$

or

$$r = \frac{S^2}{8f}$$

and

$$\pi_s = \frac{t}{r} = \frac{8 f t}{S^2} \qquad 3$$

From equation 1, the exact value of r may be calculated as :

$$r_{exact} = \frac{1}{2f}\left[f^2 + (\frac{K}{2})^2\right]$$

From the simplified relation, equation 2, the approximate value of r is:

$$r_{approx.} = \frac{1}{2f} (\frac{S2}{4})$$

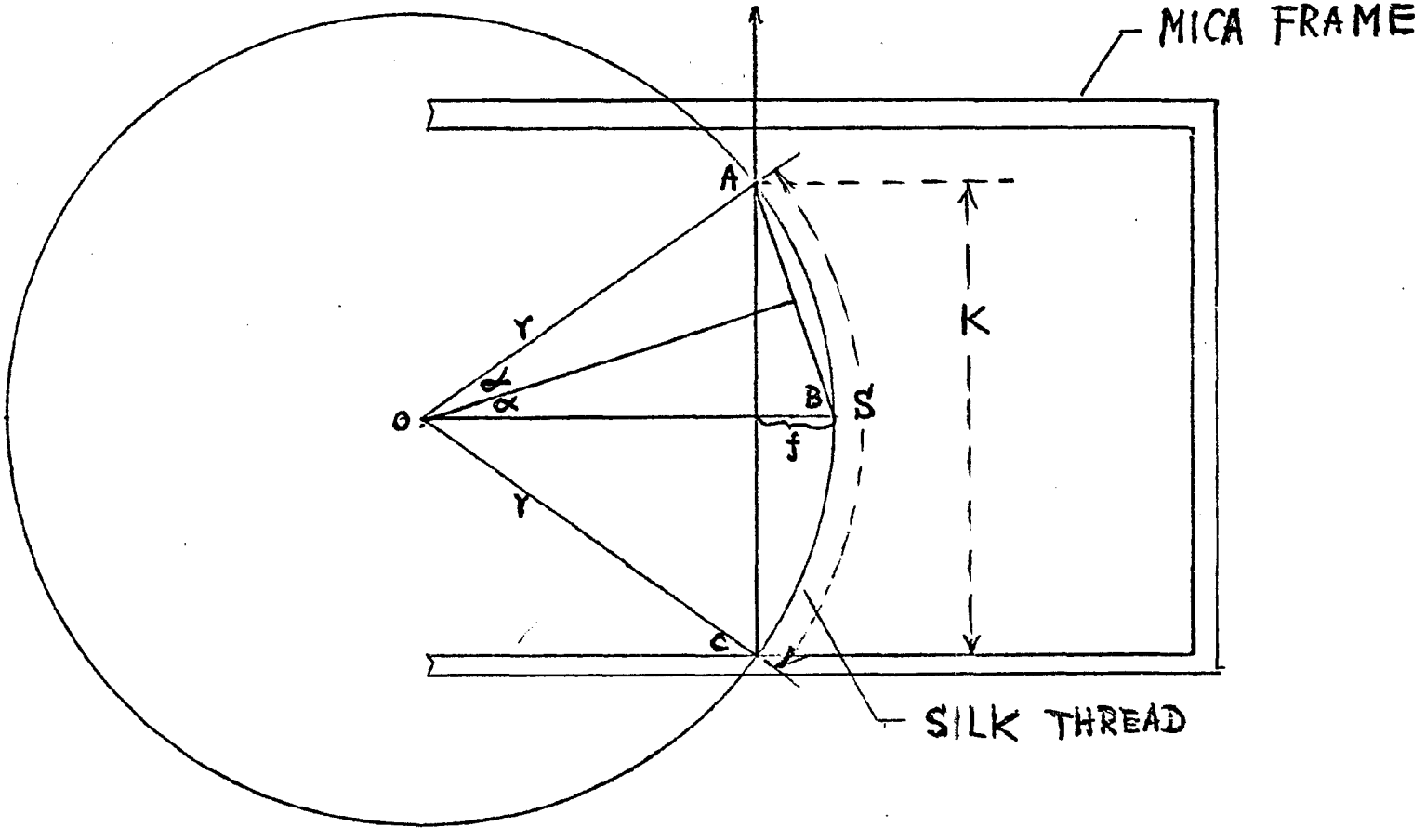


Figure A-1

For a given surface pressure, the ratio between the surface pressure read on the apparatus and the true surface pressure will be equal to:

$$\frac{\Pi s_{approx.}}{\Pi s_{exact}} = \frac{r_{exact}}{r_{approx.}} = \frac{f^2 + \frac{K^2}{4}}{\frac{S^2}{4}} = \frac{(\text{chord A B}_o)^2}{(\text{arc A B})^2}$$

Since Chord A B $= 2r \sin \alpha$

arc A B $=$ arc $(2r\alpha) = 2r$ arc α

therefore $$\frac{\Pi s_{approx}}{\Pi s_{exact}} = \frac{(\sin \alpha)^2}{(\text{arc} \alpha)^2} \qquad 4$$

where α is one quarter of the angle AOC (figure A.1). Assuming $\Pi s_{approx} / \Pi s_{exact}$ will not be less than 0.99, which means that the simplified assumption of equation 2 does not lead to an error greater than 1 %, then

$$\frac{(\sin \alpha)}{(\text{arc } \alpha)} = 0.995$$

Consequently, $\alpha \leq 10°$. For $\alpha = 10°$, the rise of the thread, f, is equal to: $f = r - r \cos 2\alpha = 0.06\ r$ and the length of an arc of $4\alpha = 40°$ is $S = 0.70\ r$. Thus the error never exceeds 1 % if the ratio $(\frac{f}{s})$ remains less than $(\frac{0.06}{0.70})$. The length of the thread used with this device in chapter 1, is 13.8 cm. The displacement of the center of the silk thread is usually below 10.6 mm, therefore the error does not exceed 0.9 %.

2. Sensitivity of apparatus

The apparatus was adjusted so that one millidyne / cm, was measured

by a displacement of one millimeter on the scale(figure 1.1). The sensitivity was obtained by applying a tension to the silk thread. The tension is related to the torsion angle (Ω_r) in radians by:

$$t = \frac{E \Omega_r}{b} \qquad 5$$

where b = the length of the horizontal torsion bar

E = the torsion modulus of wire

t = applied tension

Combining equations 3 and 5:

$$\Pi_s = \frac{8f\, E \Omega_r}{b\, S^2}$$

The radian measured is converted to 100 units per 2π radians, as follows:

$$\Omega = \frac{\Pi_s\, S^2\, b}{f8\, E} \left(\frac{100}{2\pi} \right) \text{ units} \qquad 6$$

To obtain f, measure the amplification factor:

actual width of a glass rod = 5.91 mm

amplified width of the glass rod = 56.0 mm

amplification factor = 56.0 /5.91 = 9.48

Therefore, displacement of thread (f) observed as one millimeter is equivalent to 1 / 94.8 cm.

For a silk thread length of 13.8 cm, b =24.5 cm, E_r = 168.1 g cm^2/sec^2, equation 6 gives:

$$\Omega = \frac{(10^{-3})(13.8)^2(24.5)(100)}{(8)(1/94.8)(168.1)(2)(3.14)}$$

Ω = 5.24 units

Appendix IV

1. Fowkes' Equation of State[5]

1.1 For non-ionized gaseous monolayers

The molecules of insoluble monolayer (1) spread on water are considered to be localized by means of a semipermeable membrane, but the molecules of water (2)are free to diffuse through the membrane. The Gibbs free energy of water molecules entering the surface, G_2^s, can be described as

$$d\,G_2^s = K\,T\,d\,\ln x_2 \phi_2 - \sigma_2 d\,\gamma \qquad 1$$

where the second term on the right is the work required to extend the surface. Similarly, for the bulk phase

$$d\,G_2 = K\,T\,d\,\ln c_2\,f_2 \qquad 2$$

where γ = surface tension, $d\gamma = -d\pi = -(\gamma_o - \gamma)$

x_2, c_2 = mole fractions of water molecules in the surface and the bulk phase respectively.

ϕ_2, f_2 = activity coefficients of water molecules in the surface and the bulk phase respectively.

σ_2 = partial molecular area of water in surface = $\left(\frac{\partial A}{\partial n_2}\right)_{n_1}$

A = total area of system

n_1 = molecules of insoluble monolayer

n_2 = molecules of water in surface

γ_o = surface tension without insoluble monolayer

π = surface pressure

At equilibrium for the water molecules

$$d\,G_2^s = d\,G_2$$

or $$K\ T\ d\ \ln x_2 \phi_2 + \sigma_2\ d\pi = K\ T\ d\ \ln c_2\ f_2 \qquad 3$$

For dilute solution of surfactant in water (insoluble gaseous monolayer), the term ($K\ T\ d\ \ln c_2\ f_2$) is assumed to be negligibly small. Further, Fowkes assumes ideal dilute surface solution, so that $\phi_2 = 1$.

Therefore $$K\ T\ d\ \ln x_2 + \sigma_2\ d\pi = 0$$

where $$\sigma_2 = \sigma_2(\pi).$$

By integration, after defining the standard state π_2° , x_2° :

$$\pi = \frac{-K\ T}{\bar{\sigma}_2} \ln x_2$$

where $\bar{\sigma}_2 = \dfrac{\int_0^{\pi} \sigma_2(\pi)\ d\pi}{\pi - 0}$ is the average value.

For water σ_2 is virtually independent of film pressure, so that σ_2 (9.7 $\mathring{A}^2$) may be used for $\bar{\sigma}_2$.

$$\pi = \frac{-K\ T}{\sigma_2} \ln x_2 \qquad 4$$

To derive the perfect gas equation, the $\ln x_2$ term is expanded; for a binary solution film :

$$\ln x_2 = \ln (1 - x_1)$$

and the series expansion, using only the first term if x_1 is very small (for gaseous monolayer) leads to :

$$\ln (1 - x_1) \simeq - x_1$$

Since

$$x_1 = \frac{n_1}{n_1 + n_2} \quad , \quad x_2 = \frac{n_2}{n_1 + n_2}$$

by substitution into equation 4,

$$\pi \simeq \frac{K\ T}{\sigma_2} x_1 = \frac{K\ T}{\sigma_2} \left(\frac{n_1}{n_1 + n_2} \right)$$

Rearrange

$$\frac{(n_1 + n_2)\sigma_2}{n_1} = (\sigma_2 - \sigma_1) + \frac{n_1\sigma_1 + n_2\sigma_2}{n_1}$$

and define A_1 = molecular area of the surfactant,

$$A_1 = \frac{n_1\sigma_1 + n_2\sigma_2}{n_1} = \frac{A}{n_1} \qquad 5$$

then

$$\frac{(n_1 + n_2)\sigma_2}{n_1} = A_1 - (\sigma_1 - \sigma_2)$$

or

$$\pi\left[A_1 - (\sigma_1 - \sigma_2)\right] = K\,T \qquad 6$$

For very dilute monolayers, $(\sigma_1 - \sigma_2)$ becomes very small relative to A_1, thus equation 6 reduces to the ideal case

$$\pi A_1 = K\,T \qquad 7$$

The Fowkes equation of state can be used to obtain molecular weight of the film molecule in the gaseous monolayer. If m is the mass of the solute spread on surface area A:

$$\frac{A}{m} = \frac{n_1 A_1}{m} = \frac{n_1\sigma_2}{m}\left(\frac{n_2}{n_1}\right) + \frac{n_1\sigma_1}{m} \qquad 8$$

If M is the molecular weight, and N is the Avogadro's number,

$$n_1 = \frac{N\,m}{M}$$

Since $x_1 = \dfrac{n_1}{n_1 + n_2} = \dfrac{1}{1 + \dfrac{n_2}{n_1}} = 1 - x_2$, equation 4 may be rearranged into

$$\pi = \frac{-K\,T}{\sigma_2}\ln\left(1 - \frac{1}{1 + \dfrac{n_2}{n_1}}\right) \qquad 9$$

For each value of π, a value of n_2 / n_1 can be calculated from equation

9 and plotted vs. A/ m. From equation 8, the slope ($n_1 \sigma_2 / m$) = σ_2 N/ M allows a calculation of M, and the intercept $n_1 \sigma_1 / m = \sigma_1 (N / M)$ gives a value for σ_1. Fowkes claims that this method of calculation of molecular weight is superior to the method used by Bull and Guastalla, since a slope is more accurately measured than an intercept. Also, information on n_2 / n_1 is valuable, since it indicates the maximum surface pressure at which solute molecules can be separated by solvent molecules.

1.2 For ionized monolayer

The Fowkes treatment can be extended to ionized gaseous monolayers by defining" z " as the number of dissociated particles per molecule of solute. Thus equation 5 becomes

$$A_1 = \sigma_1 + \left(\frac{n_2}{n_1 z} \right) z \sigma_2 \qquad 10$$

From equation 4, solving for x_2

$$x_2 = e^{\frac{-\pi \sigma_2}{K T}} = 1 - x_1$$

$$\frac{x_2}{x_1} = \frac{x_2}{1 - x_2} = \frac{e^{-\pi \sigma_2 / K T}}{1 - e^{-\pi \sigma_2 / K T}} = \frac{\frac{n_2}{n_1 z + n_2}}{\frac{n_1 z}{n_1 z + n_2}} = \frac{n_2}{n_1 z} \qquad 11$$

The isotherm then becomes:

$$A_1 = \sigma_1 + z \sigma_2 \left(\frac{e^{-\pi \sigma_2 / K T}}{1 - e^{-\pi \sigma_2 / K T}} \right) \qquad 12$$

This equation is used to estimate " z " and σ_1 from the slope and the intercept of A_1 vs. $n_1 / n_1 z$ plot.

Combine equation 10 and 11, and solve for x_2

$$\frac{1}{x_2} = \left(\frac{z \sigma_2}{A_1 - \sigma_1} \right) + 1 \qquad 13$$

Substitute equation 13 into equation 4 and solve for π, while retaining the activity coefficient ϕ_2

$$\pi = \frac{K T}{\sigma_2} \ln \left\{ \left[\frac{z \sigma_2}{A_1 - \sigma_2} + 1 \right] \frac{1}{\phi_2} \right\} \qquad 15$$

For ideal dilute solution $\phi_2 = 1$.

2. Equation of State Derived From The Statistical Mechanical Partition Function of a Two-Dimensional Ideal Gas [4a, 6]

In a two-dimensional surface system, at very low surface concentration where the surfactant is considered to be in a " gaseous " state, the partition function for the surface molecules may be determined by statistical mechanics and used to calculate a " surface pressure ".

A two-dimensional perfect gas which satisfies corrected Boltzman statistics is assumed to be constrained in a rectangular area A = x y, with coordinate x and y, and it possesses two degrees of translational freedom. The energy levels of a monatomic two-dimensional perfect gas are given by

$$E_{tr} = \frac{h^2}{8m} \left(\frac{l_x^2}{x^2} + \frac{l_y^2}{y^2} \right) \qquad 16$$

where h = Plancks' constant

m = mass per molecule

l_x, l_y = integral quantum numbers

E_{tr}=individual energy level for translation.

The total molecular energy is

$$E = E_{tr} + E_{int}$$

where E_{int} is the internal energy, includes rotational, vibrational and electrical energies. The molecular partition function q is defined

as a summation over all the quantum states[6].

$$q = \sum e^{-E_i / K T} \qquad 17$$

The total molecular partition function is then

$$q_{total} = q_{tr} \times q_{int}$$

For a monatomic gas, $q_{int} = 1$. This relation can also be used for a polyatomic gas, if the molecular motion is assumed to be translational only. From equations 16 and 17

$$\begin{aligned} q_{tr} &= \sum e^{-E_{tr} / K T} \\ &= \sum_{l_x=1}^{\infty} \sum_{l_y=1}^{\infty} e^{\frac{-h^2}{8 m K T}\left(\frac{l_x^2}{x^2} + \frac{l_y^2}{y^2}\right)} \\ &= \sum_{l_x, l_y} \left(e^{-h^2 l_x^2 / 8mKTx^2} \right)\left(e^{-h^2 l_y^2 / 8mKTy^2} \right) \\ &= \left(\sum_{l_x} e^{-h^2 l_x^2 / 8mKTx^2} \right) \left(\sum_{l_y} e^{-h^2 l_y^2 / 8mKTy^2} \right) \\ &= q_x \; q_y \end{aligned}$$

If $h^2 / 8mKTx^2 \ll 1$, as is typical for a gas. we can replace the sum by an integral

$$q_x = \int_{l_x=0}^{\infty} \left(e^{-h^2 l_x^2 / 8mKTx^2} \right) d\, l_x = (2\pi mKT/h^2)^{1/2} \cdot x$$

$$q_y = (2\pi mKT/h^2)^{1/2} \cdot y$$

$$q_{tr} = (2\pi mKT/h^2)\, xy = (2\pi mKT/h^2)\, A$$

If N is the number of molecules on the surface, the total partition function for a gas, Q is given by[6]

$$Q = \frac{1}{N!} \; q_{tr}^{N}$$

The Helmholtz free energy in term of Q is

$$F = - KT \ln Q$$

For an equilibrium system containing a surface phase, any variation from equilibrium is described by :

$$d F = - S\, dT - \pi_s \; dA + \mu\, dN_m \qquad 18$$

where S = entropy

π_s = surface pressure

μ = Chemical potential

N_m = moles of any spesies in surface phase

A = monolayer area

At constant T and N_m, π_s is

$$\pi_s = -\left(\frac{\partial F}{\partial A}\right)_{T,\, N_m} = K\,T\left(\frac{\partial \ln Q}{\partial A}\right)_{T,\, N_m} \qquad 19$$

where $Q = \frac{1}{N!}\, q_{tr}^{\,N} = \frac{1}{N!}\left(2\pi mKTA/\, h^2\right)^N$

Using the Stirling formula for large N, $N! = \frac{(N)^N}{(e)^N}$, so that

$$\ln Q = -N \ln N + N + N \ln q_{tr}$$

$$\ln Q = N \ln \left(\frac{2\pi m_s KT}{h^2}\,\frac{Ae}{N}\right) \qquad 20$$

From experimental surface pressure π_s, the molar partition function can be calculated at a given surface area A, and the calculated and the experimental values of π_s can be compared.

If $B = \frac{2\pi m\, K\, T\, e}{h^2}$, equation 20 becomes

$$\ln Q = N \ln \left(\frac{A\,B}{N}\right)$$

or $$Q = \left(\frac{A\,B}{N}\right)^N$$

If the factor " z " is used to represent the degree of dissociation of the surface molecule, similar to the usage of Fowkes, equation 19 becomes

$$\pi_s = \left[\frac{d \ln \left(\frac{A\,B}{N}\right)^N}{d\,A}\right]_{T, N_m} z\,K\,T \qquad 21$$

If $N/A = \delta$ = surface concentration in molecules per cm, the integrated equation 21 reduces to

$$\Pi_s = z\delta K T \qquad 22$$

Bibliography

Chapter One

1. Guastalla, J., Compt. Rend., 206, 993, (1938).

1b. Guastalla, J., Compt. Rend., 208, 1078, (1939).

2. Davies, J. T. and Rideal, E. K., " Interfacial Phenomena ", p.230, Academic Press, N. Y., (1963).

3. Benhamon, N., J. Chim. Phys., 48, 5, (1951).

4. Rosano, H. L., J. Colloid Science, 10, 362, (1955).

5. Romeo, D. and Rosano, H. L., J. Colloid and Interface Science, 33, 84, (1970).

6. Christodoulou, A. P., Ph. D. Dissertation, The City University of New York, N. Y., N. Y., (1968).

7a. Bull, H. B., J. Am. Chem. Soc., 67, 4, (1945).

b. Bull, H. B., J. Am. Chem. Soc., 67, 8, (1945).

c. Bull, H. B., J. Am. Chem. Soc., 68, 745, (1946).

d. Bull, H. B., J. Biol. Chem., 185, 27, (1950).

e. Bull, H. B., Advanced in Protein Chemistry, 3, 95, (1947).

8. Allan, A. J. G. and Alexander, A. E., Trans. Faraday Soc., 8, 863, (1954).

9. Harrap, B. S., J. Colloid Science, 10, 351, (1955).

10. Fredericq, E., Biochim. et. Biophys. Acta, 9, 601,(1952).

11. Fowkes, F. M., J. Phys. Chem. 66, 385, (1962).

12. Guastalla, J., J. Chim. Phys., 48, (1951).

13. Rosano, H. L. and Karg, G., J. Phys. Chem., 63, 1692, (1959).

14. Scatchard, G., Batchalder, A. E. and Brown, A., J. Am. Chem. Soc., 68, 2320, (1946).

15. Sinsheimer, R. L., J. Mol. Biol., 1, 37, (1959).

16. Dandliker, W. B., J. Am. Chem. Soc., 76, 6036, (1954).

17. Schulman, S., Arch. Biochem. Biophys., 44, 230, (1943).

18. Klainer, S. M. and Kegeles, G., J. Phys. Chem., 59, 952, (1955); Klainer, S. M. and Kegeles, G., Arch. Biochem. Biophys., 63, 247, (1956).

19. Noelken, M. and Reibstein, M., Arch. Biochem. Biophys., 123, 397, (1968).

20. Payens, T. A. J. and Van Markwijk, B. W., Biochim. Biophys. Acta, 71, 517, (1963).

21. Sullivan, R. A., Fitzpatricle, M. M. and Stanten, E. K., Arch. Biochem. Biophys., 55, 455, (1955).

22. Kalen, et al. J. Diary Science, 48, 884, (1965).

23. Peterson, R. F. and Kopfler, F. C., Biochim. Biophys. Res. Comm. 22, 388, (1966).

24. Ribadeau Dumas, B., Eur. J. Biochem., 25 (3), 505, (1972).

25. Muramatsu, M. and Sobotka, H., J. Phys. Chem., 66, 1918, (1962).

26. Schulman, J. H. and Hughes, A. H., Proc. Roy. Soc., A 138, 430, (1932).

Chapter Two

1. Davies, J. T., Proc. Roy. Soc., A 224, 208,(1951).

2. Ter Minassian-Sagara, L., Proc. Second Intl. Cong. Surface Activity, London, 1956, 1, 36, (1957),Butterworths, London.

3. Lucassen-Reynders, E. H., J. Phys. Chem., 70, 1777, (1966).

4. Fowkes, F. M., J. Phys. Chem., 66, 385, (1962),

5. Bull, H. B., J. Biol. Chem., 185, 27, (1950).

6. Motomura, K., J. Colloid Science, 19. 425, (1964).

7. Davidson, N., " Statistical Mechanics ", Mc. Graw-Hill, N. Y., p. 248, (1962).

8. Hill, T. L., " An Introduction to Statistical Thermodynamics ", Addison-Wesley, Reading, Mass., 78, (1960).

9. Christodoulou, A. P., Ph. D. Thesis, The City University of New York, N. Y., N. Y., 1968.

10. Rosano, H. L., J. Colloid Science, 10, 362, (1955).

11. Rosano, H. L., Christodoulou, A. P. and Chen, S. H., Berichte von VI. Intl. Kongress für Grenzflächenactive Stoffe, Carl Hauser verlag, München, 1973, p.21.

Chapter Three

1. Hughes, A. H. and Rideal, E. K., Proc. Roy. Soc., A 137, 62, (1932).

2. Hughes, A. H., Schulman, J. H. and Rideal, E. K., Nature, 129, 21, (1932).

3. Bull, H. B., J. Am. Chem. Soc., 67, 4, (1945).

4. Harrap, B. S., J. Colloid Science, 10, 351,(1955).

5. Glazer, J. and Dogan, M. Z., Trans. Faraday Soc., 49, 448, (1953).

6. Trurnit, H. J., J. Colloid Science, 15, 1, (1960).

7. Evan, M. T. A., Mitchell, J., Mussellwhite, P. R. and Irons, L., " Surface Chemistry in Biological System ", Edited by Martin Blank, p. 1.

8. Dervichian, D. G., Nature, 144, 629, (1939).

9. Crisp, D. J., J. Colloid Science, 1, 161, (1946).

10. Ställberg, S. and Teorell, T., Trans. Faraday Soc., 35, 1413, (1939)

11. Mitchell, J. Irons, L. and Palmer, G. J., Biochim. Biophys. Acta, 200, 138, (1970).

12. Minones Trillo, J., Iribarnegaray Jado, E., Garcia Fernandez, S. and Sanz Pedrero, S., Kolloid-Z. u. Z. Polymer, 250.318,(1972); 250, 325, (1972).

13. Gaines, G. L., Jr., " Insoluble Monolayers at Liquid-Gas Interfaces ", Interscience Publishers, N. Y., 1966. Chapter 3 .

14. Christodoulou, A. P. and Rosano, H. L., Advances in Chemistry Series, 84, 210,(1968).

15. Schulman, J. H. and Hughes, A. H., Proc. Roy. Soc., A 138, 430, (1932).

16. Davies, J. T. and Rideal, E. K., J. Colloid Science Suppl., 1, (1954).

Chapter Four

1. Boussinesq, J., Ann. Chim. Phys. Ser., 8, 29, 349, (1913).

2. Harkins, W. D. and Kirkwood, J. G., J. Chem. Phys., 6, 53, (1938).

3. Joly, M., Kolloid Z., 89, 26, (1939).

4. Wilson, R. E. and Ries, E. D., "Colloid Symposium Monograph ", Univ. of Wisconsin Press, Madison, Wisconsin, 1953. pp.145-173.

5. Joly, M., Kolloid-Z. 162, 35, (1952).

6. Harkin, W. D. and Myers, R. J., Narure, 140, 465, (1937).

7. Tschoegl, N. W., Kolloid-Z., 181, 91, (1962).

8. Ellis, S. C., Lanham, A. F. and Pankhurst, K. G. A., J. Sci. Intr., 32, 70, (1955).

9. Jarvis, N. L., J. Phys. Chem., 70, 3027, (1966).

10. Nutting, G. C. and Harkins, W. D., J. Am. Chem. Soc., 62, 3155, (1940).

11. Biswas, B. and Haydon, D. A., Proc. Roy. Soc., A 271, 296,(1963).

12. Van Voorst Vader, F., Erkens, Th. F. and Van Den Tempel, M., Trans. Faraday Soc., 60, 1170, (1964).

13. Tachibana, T. and Inokuchi, K. , J. Colloid Sci., 8, 341, (1953).

14. Criddle, D. W., " Rheology" Vol. 3, Frederick R. Eirich, ed., Acad. Press, N. Y., (1960), p. 429.

15. Joly, M., "Surface and Colloid Science ", Vol. 5, Egon Matijevic, ed; Wiley-Interscience, (1972), p. 1 .

16. Schulman, J. H. and Teorell, T., Trans. Faraday Soc.,34, 1337, (1938).

17. Pak, C. Y. and Gershfeld, N. L., Nature, 214, 888, (1967).

18. Crisp, D. J., Trans. Faraday Soc., 42, 619, (1946).

19. Panaiotov, I., Private Communication.

20. Panaiotov, I. and Ter-Minassian-Sagara, L., Unpublished work.

21. Abribat, M., Rosano, H. L. and Vaillet, G., C. R. Acad. Sci., Paris, 238, 1219, (1954).

22. Flory, P. J., " Principles of Polymer Chemistry", Cornell University Press, 1953.

23. Huggins, M. L., Annal of the N. Y. Acad. Sci., XLIII, 1, (1942).

24. Flory, P. J., J. Chem. Phys., 13, 453, (1945).

25. Singer, S. J., J. Chem. Phys., 16, 872,(1948).

26. Frisch, H. L. and Simha, R., J. Chem. Phys., 27, 702, (1957).

27. Silberberg, A., J. Phys. Chem., 66, 1872, (1962).

28. Blank, M., J. Colloid Science, 29, 205, (1969).

29. Rosano, H. L., J. Colloid Interface Sci.,23, 73, (1967).

30. Gibbs, J. W., "Collected Works " Vol. 1, Longmans Green & Co. Ltd., London, (1928), p. 300 .

31. Gaines, G. L., " Interscience Monographs on Physical Chemistry", Interscience Publishers, N. Y., 1966, Chapter 4.

32. Davies, J. T. and Rideal, E. K., " Interfacial Phenomena ", Acad. Press, N. Y., 1963, Chapter 5.

33. Adam, N. K., " The Physics and Chemistry of Surface ", Dover Publications Inc., N. Y., 1968, Chapter 2.

34. Adamson, A. N., "Physical Chemistry of Surface ", 2nd. ed., Interscience Publishers, N. Y., 1967, pp. 123-136.

35. Karel, M., J. Food Sci., 38, 756, (1973).

36. Colacicco, G., Buckelow, A. R. Jr. and Scarpelli, E. M., J. Colloid Interface Sci., 46, 1, (1974).

37. Blank, M. and Lee, B., J. Colloid Interface Sci., 36, 1, (1971).

38. Boyd, J. V., Mitchell, J. R., Irons, L., Musselwhite, P. R. and Sherman, P., J. Colloid Interface Sci., 45, 478, (1973).

39. Joly, M., Recent Progr. Surface Sci., 18, 555, (1963).

Chapter Five

1. Karel, M., J. Food Sci., 38, 756, (1973).

2. Korn, E. D., J. Biol. Chem. 215, 1, 15, (1955).

3. Gordon, R. S., Jr. and Cherkes, A., Proc. Soc. Exp. Biol. Med.,97, 150, (1958).

4. White, J. E. and Engel, F. L., Proc. Soc. Exp. Biol. Med.,99, 375, (1958).

5. Eley, D. D. and Hedge, D. G., Disc. Faraday Soc., 21, 221, (1956).

6. Eley, D. D. and Hedge. D. G., J. Colloid Sci., 11, 445, (1956).

7. Eley, D. D. and Hedge, D. G., J. Colloid Sci., 12, 419, (1957).

8. Colacicco, G., Lipid, 5, 636, (1970).

9. Colacicco, G., Annals of the New York Acad. Sci., 195, 224, (1972).

10. Boyer, P. D., Ballou, G. A. and Luck, J. M., J. Biol. Chem.,162, 199, (1946).

11. Ballou, G. A., Boyer, P. D. and Luck, J. M., J. Biol. Chem., 159, 111,(1945).

12. Putnam, F. W. and Neurath, H., J. Am. Chem. Soc., 66, 692, (1944).

13. Putnam, F. W. and Neurath, H., J. Biol. Chem., 159, 195, (1945).

14. Neurath, H. and Putnam, F. W., J. Biol. Chem., 160, 397, (1945).

15. Pankhurst, K. G. A., "Surface Chemistry", Butterworths, 1949, p. 109.

16. Elkes, J.J., Frazer, A. C., Schulman, J. H. and Stewart, H. G., Proc. Roy. Soc., A 184, 102, (1945).

17. Schulman, J. H. and Hughes, A. H., Biochem. J., 29,1236, 1243, (1935).

18. Schulman, J. H. and Rideal, E. K., Proc. Roy. Soc., B 122, 29, 46, (1937).

19. Doty, P. and Schulman, J. H., Disc. Faraday Soc., 6, 21, (1949).

20. Matalon, R. and Schulman, J. H., Disc. Faraday Soc., 6, 27, (1949).

21. Chatelain, P., Berliner, C., Ruysschaert, J. M. and Jaffe, J., J. Colloid Interface Sci., 51, 239, (1975).

Introduction

1. Same as references 3,4,5,6, chapter 1.

Appendices

1. Brown, J. R., Low, T., Bakerns, P., Sepulveda, P., Parker, K. and Blakeney, E., (Spon: R. J. William), Fed. Proc. 30,1241, (1971).

2. Mercier, J. C., Grosclaude, F. and Ribadeau Dumas, B., Milckwissenschaft, 27 (7), 402, (1972).

3. Block, Richard J., " The Amino Acid Composition of Proteins and Foods ",second edition, Charles C. Thomas, Publisher, Springfield, P.300 (1945).

4a. Christodoulou, A. P., Ph. D. Dissertation, The City University of New York, New York, N. Y., (1968).

5. Fowkes, F. M., J. Phys. Chem.,65, 355, (1961).

Fowkes, F. M., J. Phys. Chem., 66, 385, (1962).

6. Davison, N., " Statistical Mechanics " Mc. Graw-Hill, N. Y.,P248, (1962).

Hill, T. L., "An Introduction to Statistical Thermodynamics ", Addison-Wesley, Reading, Mass., P.74, (1960).