transition breadth at $\theta = {}^1/_2$ in good approximation, but not for $F_{AT} = 0.9$. One can see a marked effect of base-pair distribution on the transition breadth.

The shift in the melting temperature expressed in eq 31 is illustrated in Figure 6 as a function of sequence probability. For a copolymer with a random sequence distribution, the actual melting temperature T_m differed from the mean melting temperature $T_m{}^0$ by an experimentally insignificant and negligible amount, as pointed out by Fixman and Zeroka.[13] For a copolymer with a Markoffian sequence distribution with $F_{AT} = 0.5$, $\langle \Delta T_m \rangle$ does not depend on the sequence probability. But, on the other hand, for $F_{AT} = 0.9$, a marked effect on the shift was found, as indicated in Figure 6.

The molecular weight dependence of the transition breadth for T2 DNA assuming a Markoffian distribution ($P_{GC-GC} = 0.2$) and $\sigma = 10^{-6}$ is given by the broken curve in Figure 3. Figure 3 indicates that the theoretical curve obtained by assuming a Markoffian distribution is more satisfactorily in accord with experimental results than that obtained by assuming a random distribution.

We are concerned with other interesting quantities that can be calculated from the partition function. The average number of helical sequence $\langle \bar{N}_{hc} \rangle$ and the average length of a helical sequence $\langle \bar{L}_h \rangle$ are plotted in Figure 7 as a function of the average helical fraction $\langle \theta_h \rangle$. Calculations were carried out for molecules having a base composition corresponding to T2 DNA, by assuming two typical sequence distributions, *i.e.*, random and Markoffian ($P_{GC-GC} = 0.2$). The effect of sequence distribution on these quantities is found to be appreciable but not large.

Lastly, we will again mention that comparison of theroetical and experimental results should be confined to general trends, because the coil size dependence of σ may indicate a considerable effect on the transition of a heterogeneous copolymer, although it seems not to be so drastic as shown by Zimm[4] for homogeneous polynucleotides.

A Study of Films of Poly(γ-methyl L-glutamate) Adsorbed on Water Using Wave Damping and Other Methods

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ABSTRACT: Simultaneous measurements of wave damping coefficient, film pressure, and surface potential difference as a function of area per monomer have given much information concerning the behavior and structure of a monolayer of poly(γ-methyl L-glutamate) (PMG) adsorbed at the air–water interface. At large monomer areas the PMG film is composed of widely separated polypeptide units in the α-helical conformation but, at about 18 Å^2 per monomer, the molecules come into close contact to form a uniform and nearly incompressible film. Upon further compression the film molecules are packed forcibly into their most compact state, where the average area per monomer is about 16.5 Å^2. Up to that point the film is completely reversible on expansion. The sole effect of compressing the film further is one of displacing segments of the polymer chain from the interface while the packing and orientation of the molecules remaining in the surface remain constant. If the external pressure is relieved at any point after molecules are expelled from the interface, the film exhibits hysteresis as it enters a lower energy state. After that state is attained, displaced molecules again enter the surface and, when all of the molecules are back on the surface once more, the film returns to its original state in which the monomer area is about 18 Å^2. Therefore, the gradual near-equilibrium compression and expansion behavior of the film is such as to exclude the possibility of a permanent change occurring in the polymer molecules as a result of compression.

The behavior of synthetic polypeptides when adsorbed at the air–water interface has been studied during the last decade to help elucidate the way the more complex proteins function in aqueous environments. Interesting studies at three laboratories have been carried out in this area on poly(γ-methyl L-glutamate) (PMG) which have led to a divergence of opinions about the form this molecule assumes when it is spread and compressed on aqueous substrates.[1-5] Isemura and Hamaguchi concluded from surface pressure[1] and viscosity[2] measurements that PMG was in the α_{II} conformation proposed by Ambrose and Hanby[6] when adsorbed on water. Malcolm's vigorous and persistent investigations have combined the traditional methods of surface chemistry[3] with spectral and deuterium-exchange studies.[4] He rationalized the results by proposing that: (a) PMG was in the α-helical structure of Pauling and Corey[7] when spread on water, (b) compression beyond a certain point caused the polymer molecules to form a multilayer, and (c) the structure of the film molecules was fixed on the surface of the water and was independent of the degree of compression. In addition, Malcolm[3] suggested that the difference in the spreading solvent used by him (chloroform) and by Isemura and Hamaguchi (pyridine) might account for the discordant results obtained in the two studies. Loeb's subsequent investigation[5] confirmed this suspicion by showing that the relative concentration of pyridine and chloroform in the spreading solvent can influence considerably the properties and structure of PMG films on water.

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(1) T. Isemura and K. Hamaguchi, *Bull. Chem. Soc. Jap.*, **25**, 40 (1952).
(2) T. Isemura and K. Hamaguchi, *ibid.*, **27**, 125 (1954).
(3) B. R. Malcolm, *Polymer*, **7**, 595 (1966).
(4) B. R. Malcolm, *Proc. Roy. Soc., Ser. A*, **305**, 363 (1968).
(5) G. I. Loeb, *J. Colloid Interface Sci.*, **27**, 38 (1968).
(6) E. J. Ambrose and W. E. Hanby, *Nature (London)*, **163**, 483 (1949).
(7) L. Pauley and R. B. Corey, *Proc. Nat. Acad. Sci. U. S.*, **37**, 235 (1951).

It appears that the structure and behavior of PMG films at the air–water interface are not yet firmly established, although the bulk of the evidence suggests that, at least under some conditions the molecules are in the α-helical form, in contradiction to the earlier belief stated by Cheesman and Davies[8] that all proteins and polypeptides when spread on aqueous substrates assume an extended conformation (β form).

The investigation reported herein differs from previous work mainly in two respects: (a) the capillary wave damping method was included in studying film properties and (b) the films were slowly expanded after a slow compression to look for hysteresis effects, a treatment usually found informative in surface chemistry research but neglected in the referenced investigations of PMG films. Recent published research of this laboratory concerning the capillary wave damping effects of adsorbed monolayers of polyorganosiloxanes[9] and polyoxyethylene[10] on water had demonstrated that the wave damping method was much more sensitive to conformational and phase changes occurring in those films than were the more conventional measurements of surface film pressure (F) and surface potential difference (ΔV) *vs.* area per adsorbed monomer (A). Therefore, the additional use of the wave damping method, together with a study of film hysteresis, offered a convenient and powerful way of obtaining more information on the behavior of PMG films spread on water, particularly in regard to detecting any conformational transitions, phase changes, or polymolecular film development.

Experimental Section

Materials Investigated. Poly(γ-methyl L-glutamate) having an average molecular weight of 300,000 (according to the supplier) was obtained from Mann Research Laboratories and used without further purification. Since the sample was not easily soluble in pure chloroform, the desired spreading solvent, standard spreading solutions were prepared by first dissolving about 10 mg of the polymer in about 1 ml of freshly distilled dichloroacetic acid (bp 193.6°, lit. 194°). Enough chloroform (ACS reagent grade) was then added to give a concentration of about 0.5 mg of polymer per milliliter of solution. The water used as the substrate was singly distilled through a solid tin still and, after driving off any chlorine, was equilibrated with the CO_2 in the atmosphere so that the pH stabilized at 5.7.

Apparatus Used. The experimental apparatus used to measure film pressure, wave damping coefficient, and surface potential difference as a function of area per monomer has been described in sufficient detail previously.[10] The equipment was arranged so that all of the above quantities could be measured simultaneously on the same film. A box-shaped Lucite cover protected the water surface from contamination by atmospheric particles.

Surface pressures were measured with a Langmuir–Adam type of film balance modified by using the torsion head of a Cenco "Hydrophil Balance." The steel piano wire used in the torsion head had a sensitivity of 0.114 dyn/cm of film pressure per degree of rotation, and the film pressure could be measured with a precision of about ±0.10 dyn/cm.

The ionizing electrode method using americium-241 as the radiation source was used to measure the surface potential difference (ΔV) as before;[10] however, a slight change was made in this study to make the system more useful. In previous work, the radioactive air electrode was supported by the top of the protective cover over one specific area of the water surface. In this investigation, it was desired to establish the uniformity of the film by moving the air electrode over the water surface. This was done by making the top of the protective cover from two pieces of Lucite which were slightly separated. The electrode could then be moved in this slot to cover the water surface from the float of the film balance to the opposite end of the trough. The reproducibility of the surface potential difference was about ±10%, a much greater spread than had been noticed in the previous study[10] involving polyoxyethylene films on water (about ±1%). Since the same equipment components were used to measure ΔV in both studies, the difference in reproducibility must be attributable to a difference in the nature of the two films.

The system used to generate capillary waves on the water was the same as that used earlier,[10] but in this investigation the amplitudes of the ripples were estimated by a different scheme which was much easier to use. In both systems, the frequency of the waves propagated across the water surface was synchronized with the flashing light from a stroboscope placed beneath the glass trough. The light rays from the stroboscope were sent through the bottom of the trough and were then refracted by each ripple crest which acted as a cylindrical lens. In previous work, the light rays were projected onto a frosted glass screen maintained above the water so the experimenter could see on the screen a series of parallel bright lines corresponding to successive ripple crests.

From the geometrical optics of the system, the amplitude "a" of a capillary wave is given by the approximate equation[9]

$$\text{“}a\text{”} = 0.075\lambda^2\left(\frac{1}{L_1} + \frac{1}{L_2} + \frac{3w}{\lambda L_2}\right)$$

where λ is the wavelength, L_1 is the distance between the light source and the water surface, L_2 is the distance between the water surface and the projected light image, and w is the width of the light image projected on the screen. The wave amplitudes decayed exponentially with distance from the wave source, and the damping coefficient was determined experimentally from the slope of the line obtained when log "a" was plotted against distance from the wave source.

Previously, the focal length of a cylindrical lens equivalent to a wave crest was estimated by moving the glass screen vertically until that light image became brightest and sharpest. Under these circumstances, L_2 became the focal length and w could be considered to be negligible; therefore, the last term in the above equation was neglected in calculating the wave amplitude. However, this method was laborious and slow because frequently it was very difficult to reasonably ascertain just where the light image was brightest and sharpest.

In an effort to make the determination of the wave amplitudes more precise, it was decided to measure the width of the light images rather than to judge the focal distances. This was accomplished by moving the frosted glass screen to one side and projecting the refracted light rays onto a sheet of tracing paper placed on the transparent top of the protective cover. The light images on the paper were found to be fairly well defined and the desired number of lines, usually 11, could be marked off easily with a thin-pointed tracing pen. After the experiment was completed, the distance between the lines, each of which corresponded to a wave, was measured with the use of a small magnifying comparator. The widths of the lines varied from about 6.3 mm for the first wave measured to about 0.7 mm for the last and were measured to the nearest 0.05 mm. Wave amplitudes were calculated from the above equation and were found to be identical with those calculated by the method used previously. However, this new method was adopted because it was much less time consuming.

Methods. Details of the experimental procedure have been described earlier.[10] In general, the polymer was spread to the desired area per monomer by carefully placing small drops of the spreading solution on different areas of the clean water surface with a micrometer-driven hypodermic syringe. It was found that only about 5 min was required for the film to reach equilibrium after spreading at low film pressures. At the end of this period, compression was started by manually moving a barrier slowly across the surface until the area per monomer was reduced in most cases about 0.75 unit. At high surface pressures, the area decrements, or

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(8) D. F. Cheesman and J. T. Davies, *Advan. Protein Chem.*, 9, 439 (1954).

(9) W. D. Garrett and W. A. Zisman, *J. Phys. Chem.*, 74, 1796 (1970).

(10) R. L. Shuler and W. A. Zisman, *ibid.*, 74, 1523 (1970).

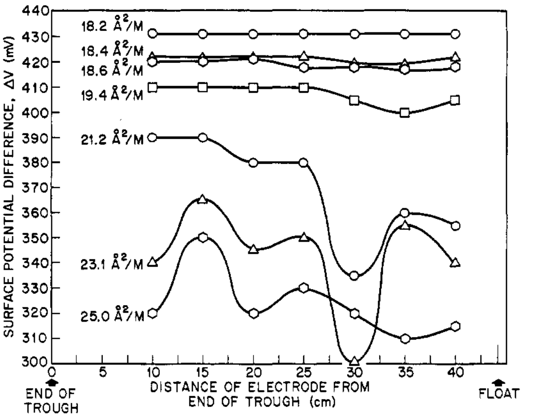

Figure 1. Variation of surface potential difference with distance of air electrode from end of trough at several areas per monomer for PMG films on water at 22° and pH 5.7

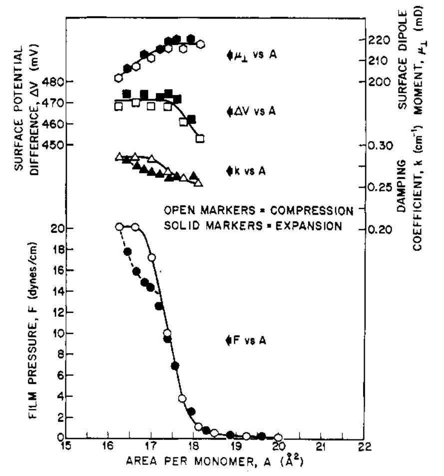

Figure 3. Variation of F, k, ΔV, and $\mu_\perp$ with A for PMG film on distilled water at 22° and pH 5.7.

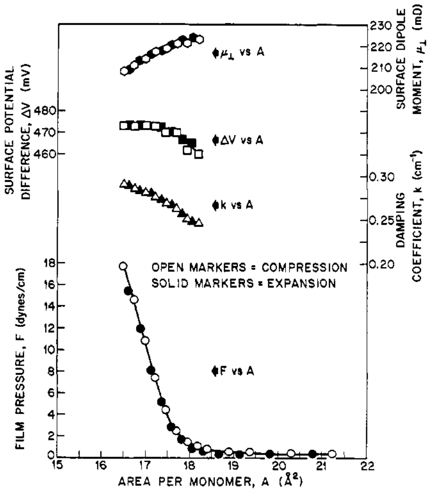

Figure 2. F, k, ΔV, and $\mu_\perp$ as functions of A for PMG films on distilled water at 22° and pH 5.7.

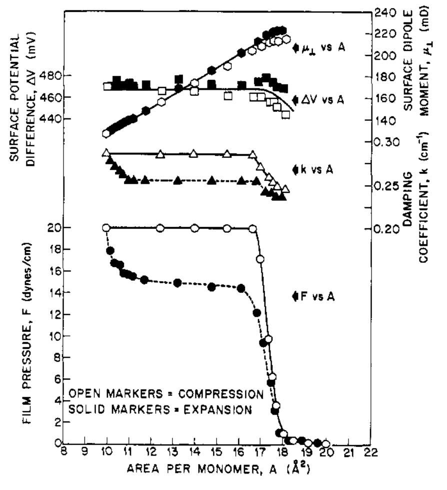

Figure 4. Variation of F, k, ΔV, and $\mu_\perp$ with A for PMG film on distilled water at 22° and pH 5.7.

increments when the film was expanded, were made smaller, being less than 0.15 area-per-monomer unit under some conditions. After each compression or expansion, the film was considered to have reached equilibrium if the surface pressure did not change significantly in a 2-min period; however, the time required for the film to equilibrate depended on the degree of compression, varying from less than 2 min at low film pressures to about 18 min as the film approached the maximum film pressure.

Results and Discussion

Homogeneity of Film. The homogeneity of the film was established by moving the air electrode in a straight line over the water surface and measuring the surface potential difference (ΔV) caused by the adsorbed film at several degrees of compression. Typical results are shown in Figure 1. It may be seen that ΔV fluctuated widely and erratically at high areas per monomer, but the fluctuations became smaller the more the film was compressed. Finally, at a value of A of 18.2 Å or less per monomer, ΔV did not change as the electrode was moved over the water surface, showing that the film covered the surface uniformly at that area. Malcolm[3] had also noted fluctuations in ΔV across the water surface but reported that the film became homogeneous at about 20 Å² per monomer instead of the 18.2 Å² per monomer observed here.

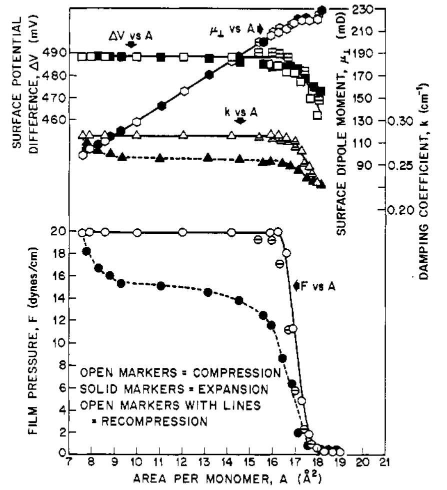

Figure 5. F, k, ΔV, and $\mu_\perp$ as functions of A for PMG film on distilled water at 22° and pH 5.7.

The inhomogeneity of the film also caused a great deal of scatter of the points in the wave amplitude *vs.* distance plots used to determine the wave damping coefficient k. Consequently, it was difficult, if not impossible, to get a reasonably straight line in these plots until the film became fairly uniform. Because of this and because of the aforementioned fluctuations in ΔV, the values of k and ΔV will be shown only at areas per monomer below about 18.2 Å^2.

Film Behavior before Collapse. PMG films were progressively compressed to smaller areas per monomer and then were allowed to expand as shown in Figures 2–5. The first appreciable rise in F came at about the same area per monomer at which the film became uniform, as would be expected by a closing up of the unfilled areas of the film by the application of external pressure. This result means that a monomer unit must have occupied approximately 18.2 Å^2 when the polypeptide was first spread as a uniform film on the water surface. This value is particularly interesting since it is in close agreement with the value of 17.9 Å^2 per monomer calculated from X-ray diffraction measurements of PMG in the solid state on the assumption that the molecules were in the α-helical conformation and packed hexagonally.[11] It is not surprising that the value obtained in this study was somewhat larger than that obtained from X-ray studies, because PMG molecules, even though in close contact in a monolayer, undoubtedly would not be as closely packed at low film pressures as they would be in the solid state. Although other conformations are certainly not ruled out, the excellent agreement between the monomer area deduced from these experiments and that predicted from solid-state studies may be taken as evidence that the PMG molecules were in the rodlike α-helical conformation when spread.

In full accord with that idea are the shape of the F *vs.* A plots of Figures 3–5 and the low compressibility of the film. The rapid rise in F at about 18 Å^2 per monomer and the sharp break in the curve at about 16.5 Å^2 per monomer, hereafter called the collapse point, both denote considerable rigidity in the film of the type to be expected if the film were constituted of stiff α helices. If the film were mobile or liquid-like, F should rise more slowly at first and gradually approach a collapse pressure. The linear part of the F *vs.* A graph is seen to be very steep, and the film compressibility coefficient (calculated from $C = -(1/A)(\mathrm{d}A/\mathrm{d}F)$) in that region was about 0.003 cm/dyn. Thus, the PMG monolayer exhibited a behavor between a liquid condensed and solid film,[12] and, based on the reversibility of the film in that region of the F *vs.* A curve (Figure 2), it could best be described as behaving like a liquid-condensed film.

The graphs show that k and ΔV as well as F increased as the film was compressed below about 18 Å^2 per monomer and both reached a maximum value. The normal component ($\mu_\perp$) of the surface dipole moment (μ) did not change very much until ΔV reached the plateau region, at which point $\mu_\perp$ necessarily decreased almost linearly with A. The small slope of the initial part of the $\mu_\perp$ *vs.* A curve evidently rules out any major reorientation of the polymer molecules and indicates that compression from about 18 Å^2 per monomer to the flat plateau in the curves simply caused the molecules to be fitted somewhat closer together. This would increase the number of molecules per unit area of water surface, causing both k and ΔV to rise as observed. This is a reasonable physical picture, since there is probably some free space between the polymer molecules at low surface pressures caused by the ester side chains projecting from the peptide backbone which could be reduced without disturbing the integrity of the molecule. On compression to less than 18 Å^2 per monomer, one might anticipate, therefore, that the film would be forced into a strained arrangement judging from the low compressibility of the linear part of the F *vs.* A curve. If this explanation is correct, one would expect, in the absence of strong intermolecular bonding, that when the pressure was relieved the molecules would revert quickly to their most favorable state. In accord with that expectation, the film which was compressed to the collapse point and allowed to expand (Figure 2) was found to be completely reversible with respect to all measured quantities.

The film thickness at the collapse point where the film presumably was most dense was calculated by assuming that the film density was 1.31, the same as the solid polymer. For all of the films shown in Figures 2–5, the thickness was estimated to be about 11 Å, which is close to the value found for most proteins. Molecular models of PMG in the α-helical structure were constructed and showed that this was about the thickness to be expected also if the peptide chain were lying flat in the water surface with the ester side chains projecting radially from the backbone.

Film Behavior in the Plateau Region. As shown in Figures 3–5, F, k, and ΔV reached maximum values in the vicinity of 16.5 Å^2 per monomer, at about the same area per monomer where the plateau region began in the F *vs.* A and ΔV *vs.* A curves obtained by Malcolm.[3] On the other hand, Loeb,[5] who did not measure ΔV, found that F leveled off at the lower area of about 15 Å^2 per monomer.

At the commencement of the flat plateaus in the curves (see Figures 3 and 4), the film is believed to be composed of a more or less orderly array of α helices packed to the utmost. If this is an accurate description of the state of affairs, it must be concluded that any further reduction in the surface area must bring about a drastic alteration in the film. The possibility of the film undergoing a significant structural change seems to be eliminated by the constancy of k and ΔV at values of A below the initial collapse value. Malcolm[3] has stated that the constancy of ΔV observed in his experiments is consistent with his view that compression below the collapse point forced molecules out of the interface, and the wave damping results reported here firmly support that idea. It has already been mentioned in an earlier section that the wave damping method has been shown to be a powerful probe for detecting conformational and other changes occurring in polymer films. The remarkable sensitivity of the method was amply demonstrated in our previous work[9,10] because some of the changes taking place in those films which were manifested only vaguely in the F *vs.* A and ΔV *vs.* A curves caused pronounced maxima and minima in the k *vs.* A curve. Although the polymers studied earlier (polyorganosiloxane and polyoxyethylene) undoubtedly had more flexible chains, it is difficult to conceive of any appreciable rearrangement taking place in the PMG films which would not be accompanied by some irregularities in the k *vs.* A curve. Instead, the virtual constancy of F, k, and ΔV strongly suggests that the film may be treated as incompressible with the packing and orientation of the adsorbed molecules remaining constant as the film is compressed past the collapse point. As a consequence of this conclusion, the energy of compressing the film in the plateau region must be used solely to displace molecular segments and then whole molecules from the air–water interface.

However, the experimental results did not give any indication that compression beyond the collapse point caused the formation of a bimolecular layer of helices as proposed by

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[11] C. H. Bamford, A. Elliott, and W. E. Hanby, "Synthetic Polypeptides," Academic Press, New York, N. Y., 1956, p 239.

[12] J. T. Davies and E. K. Rideal, "Interfacial Phenomena," Academic Press, New York, N. Y., 1961, p 265.

Malcolm.[3] If this occurred, some change in film properties should be noted at approximately the halfway point in the plateau region, where bilayer formation would be completed. Malcolm[3] and Loeb[5] did observe a second rise in F, although at different areas of about 10 and 7 Å^2 per monomer, respectively, and Malcolm also noted a second rise in ΔV. The difference in the area at which the second rise in F was observed is understandable since, as already mentioned, the location of the F *vs.* A curves obtained in the two investigations differed. In our experiments, as shown in Figure 5, F and ΔV did not change after reaching a maximum value but remained constant down to about 7.5 Å^2 per monomer. This was as much as a film could be compressed under the experimental conditions employed in this study, since it was found that the polymer would spread only at areas greater than the collapse point. Hence, an experiment had to begin beyond that point, and thus placed a limit on the lowest area per monomer to which the film could be compressed. When some spreading solution was placed on the surface after the film had been compressed into the plateau region, a globule would form on the water, indicating the presence of a highly condensed, solid-like film.

Since there was no indication in this investigation of a change in F, ΔV, or k, once these values became constant, the conclusion is inevitable that a true equilibrium state was not reached experimentally in the other two investigations. To see if the generation of capillary waves on the surface might be implicated, a film was compressed but only F and ΔV measurements were made. As shown in Figure 6, a definite rise in F and a drop in ΔV were observed at about 10 Å^2 per monomer; hence, it appears that, at least under the experimental conditions used in this study, the disturbance of the water surface by the wave generator facilitated the attainment of equilibrium.

Hysteresis Effects. Figure 3 shows that once a film was compressed beyond the collapse point, it became definitely irreversible on expansion with respect to both F and k. These values dropped fairly rapidly after the external pressure was relieved, indicating that compression did not cause any strong intermolecular interactions in the film. Instead, the immediate drop in F and k is in accord with the view proposed earlier that at the collapse point and in the plateau region the film was in a state of stress. Thus, a reduction in pressure would allow the molecules to adopt a less stressed and more stable arrangement, and hence, a decrease in F and k resulted.

In contrast to F and k, the value of ΔV stayed essentially the same when the films were expanded. This is easily explained if ΔV was less sensitive to changes occurring in the films upon decompression than were F and k. This is not surprising, since F has been known to change significantly in other films without concomitant changes in ΔV.[13] However, the present situation may be unique because there is evidence that the surface potential difference arising from PMG films is derived not from permanent dipoles in the molecules themselves but rather from the reorientation of the water dipoles just beneath the surface caused by the presence of the adsorbed organic film. This is a distinct possibility, because α-helical poly(amino acids) have a spiral symmetry and should not have a significant dipole moment vertical to the backbone of the chain due to vectorial cancellation of the contributing dipoles. Furthermore, the polar side chains which might be expected to make a sizeable contribution to ΔV apparently do not do so, since ΔV values for PMG are comparable to those of polyalanine which has no polar side chains.[3] The above idea is in agreement with the hypothesis that in *all* such condensed films ΔV originates almost entirely from the reorientation of the dipoles in the aqueous substrate and that a point may be reached in compression when ΔV becomes constant.[14] Therefore, it is conceivable that expansion could produce small changes in the molecules of a PMG film which would readily change F and k but would not substantially alter the arrangement of those dipoles in the underlying water mainly responsible for the observed ΔV.

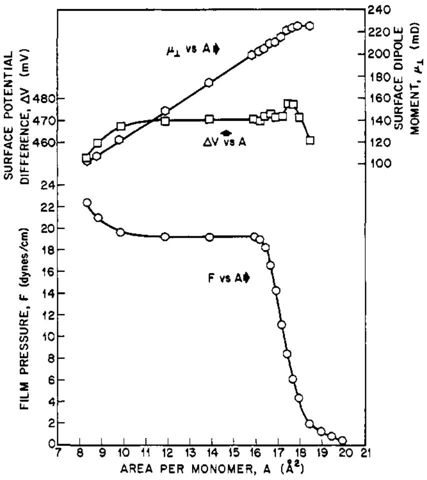

Figure 6. Variation of F, ΔV, and $\mu_\perp$ with A for PMG film on distilled water at 22° and pH 5.7 (no capillary waves were generated on water surface).

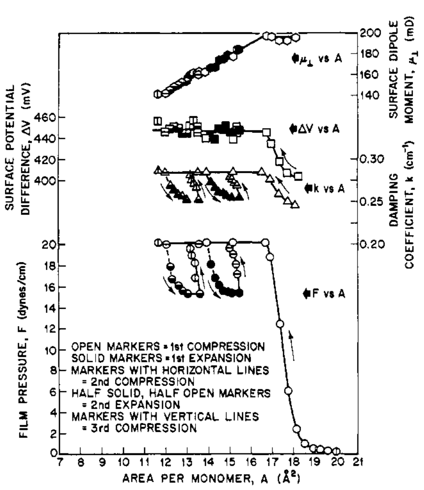

Figure 7. F, k, ΔV, and $\mu_\perp$ as functions of A for PMG film on distilled water at 22° and pH 5.8.

Upon expansion the film apparently reached a lower energy state when the film pressure was about 15 dyn/cm, at which point F and k leveled off to form a plateau which is quite analogous to the one in the compression curve. Inferentially, therefore, we can assume that the molecules which were forced out of the interface by compression reentered the surface along the plateau region in the expansion curve. At the end of the plateau, where all of the polymer molecules are presumably once more in the surface, F and k decrease again and eventually fall on, or close to, the compression curve at about 18

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(13) See, for example, W. D. Harkins, "The Physical Chemistry of Surface Films," Reinhold, Princeton, N. J., 1952, p 128.

(14) J. Goldfarb, S. Gonzalez, and B. A. Pethica, *Bol. Soc. Chilena Quim.*, 12, 24 (1962).

Å^2 per residue. Thus, when there was sufficient room at the interface, the film ultimately returned to its initial state. That this was the case was shown by recompressing one of the films (Figure 5) after it had been expanded to 18.8 Å^2 per monomer. As may be seen in the graph, the recompression values of all measured quantities fell close to the initial compression curve.

Therefore, the compression and expansion curves obtained when F and k are plotted against A form a closed cycle, showing that no permanent change occurred in the film as a result of compression. However, on the basis of the appearance of the steady state in the expansion curve where all experimentally measured quantities were largely independent of film area, it is difficult to avoid the conclusion that the film goes through some kind of intermediate state before reverting to its position of minimum energy, which is presumably found when the whole molecule is lying flat in the surface in the α-helical form. This intermediate state exerts a relatively high surface pressure of about 15 dyn/cm, which may result from the driving force of the displaced molecules to come back to the interface.

Other experiments were performed in which films were recompressed before the film had been expanded to its original state, and typical results are illustrated in Figure 7. This film was compressed to about 14 Å^2 per monomer, expanded until F became constant, and recompressed to about 12 Å^2 per monomer. The film was then expanded and recompressed as before. The observed hysteresis loops in the F *vs.* A and k *vs.* A curves show that, although transient change may be induced in the film by compression, the film does not irreversibly collapse, since the molecules in the surface can easily be recompressed to their most compact state, that is, back to the plateau region in the compression curve.

At this point it might be mentioned that some interesting calculations were made from the F *vs.* A and k *vs.* A plots shown in Figure 7. These calculations were predicated on the assumptions made earlier that all of the PMG molecules were still on the surface at the collapse point and that compression beyond that point displaced an increasing number of molecules from the interface. From these assumptions the number of monomers remaining in the surface at any point along the plateau region can be easily calculated because the molecules left in the surface decrease with the fractional decrease in film area. For example, for the film shown in Figure 7, there were 10.6×10^{17} monomers deposited on the water. Calculations show that at 14.0 and 12.1 Å^2 per monomer, the two areas to which the film was compressed, 8.91×10^{17} and 7.69×10^{17} monomers remained at the interface, respectively. If these molecules expanded back to their most preferred arrangement, *i.e.*, where each monomer occupied about 18 Å^2 each time the pressure was decreased, calculations show that these points would be reached at 15.1 and 13.0 Å^2 per monomer. Figure 7 shows that these are exactly the areas where F and k became constant and where it has been suggested that the displaced molecules came back into the surface.

Films compressed to other points along the plateau region were also found upon decompression to reach a steady state at the areas predicted, assuming the molecules in the surface expanded so that a monomer occupied about 18 Å. An exception was noted, however, for the film compressed to the lowest area per monomer (Figure 5),[15] which casts considerable doubt on the validity of the results obtained in the other films. Despite the inconsistency of the results obtained with the film shown in Figure 5, it is tempting to postulate that if a film is compressed into the plateau region and allowed to expand, the molecules left in the surface first arrange themselves in a way which is characteristic of the film in its most preferred state where a monomer occupies about 18 Å^2 per monomer. It is only after this process is completed that displaced molecules are permitted to reenter the interface to establish a steady state as film expansion proceeds. However, as suggested previously, if a film is compressed only to the collapse point, all of the molecules are still at the interface and a reduction of pressure allows the molecules to return directly and reversibly to their most favored state, as observed.

Conclusions

The experimental data obtained in this investigation have confirmed the basic hypothesis of Malcolm,[3] *viz.*, that PMG is in the α-helical form when spread on water and retains that conformation throughout compression of the film. In addition, our results have allowed some new conclusions to be reached about the way the polymer behaves when appropriately compressed and expanded on water, and to demonstrate freedom from multilayer formation when conditions are changed slowly enough to keep the film in a state as close to equilibrium as possible.

The area occupied by a monomer when the polypeptide film was in its most stable state was found to be in excellent accord with that predicted from X-ray diffraction studies, buttressing the idea that the molecule is in the α-helical conformation. Furthermore, the wave damping data rule out any significant conformational change taking place in the film but instead lend some support to Malcolm's proposal that compression of the film past the collapse point forces molecular segments out of the water surface. However, the F *vs.* A, k *vs.* A, and ΔV *vs.* A plots gave no indication that a bimolecular layer was being formed as compression proceeded along the horizontal part of those graphs, as suggested by Malcolm. That investigator compressed the films continuously and the second rise in F and ΔV observed by him, but absent in this study, might have been caused by the compression rate exceeding the rate at which the film molecules could be displaced from the surface thereby putting the film in a metastable state.

The hysteresis phenomena observed in this investigation can be explained on the basis that the PMG molecule retained its basic conformation at all stages of compression. However, the appearance of a plateau in the expansion F *vs.* A and k *vs.* A curves suggested that the film went through an intermediate state before reverting to its most stable state where all the molecules were in the surface and in the α-helical structure.

(15) It may be worth noting that this film behaved as though it were two polypeptide chains thick, based on the above mathematical scheme. Calculations showed that if each monomer occupied 18 Å^2 when F and k leveled off in the expansion curve, there would be 5.8×10^{17} monomers at the interface or exactly half the number placed on the surface initially.