THE ANALYSIS OF FORCE-AREA CURVES OF PROTEINS

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(Received for publication: March 6, 1952)

ABSTRACT

A method of analysis of the force-area curves obtained upon compression of monomolecular films has been presented; this method permits one to distinguish between two closely related species of protein molecules. Data obtained from the force-area curves are: 1. the surface pressure at which the curve becomes linear: 2. the slope of the linear portion of the curve; 3. the surface pressure at which the curve deviates from linearity in the direction of greater compressibility; 4. the specific area, obtained by extrapolating the linear portion of the curve to zero pressure. In addition the presence or absence of a solid fiber upon complete collapse of the film is recorded. The possible physico-chemical significance of these data are discussed; the fact that all these five data differ significantly before and after treatment of the protein with ultraviolet radiation shows that each of them has some definite relation to the physico-chemical properties of the film molecules.

The study of the surface chemistry of proteins was initiated by the French botanist, Devaux (1), and the Dutch paediatrician, Gorter (2); this latter worker and his collaborators first employed the Langmuir-type film balance to measure the one-dimensional 'force' or 'pressure' exerted by protein monomolecular films against a barrier floating on the surface of an aqueous phase. The data obtained upon compression of the film may be represented graphically in a plot of 'force' (dynes per cm., therefore dimensionally a tension) against specific area (sq. meters of film per mg. protein, or sq. Angstrom units per molecule, where the molecular weight of the protein has been well established.)

A typical force-area curve for egg albumin at the air/water interface is shown in Figure I. Several compressions and decompressions of this film are shown to illustrate the phenomenon of elastic hysteresis of protein monolayers (Langmuir and Waugh 3). The upper curve obtained upon decreasing the area of the film, shows three characteristic regions:

1. Aided by grants from the National Research Council and the Defense Research Board.

2. Summer Fellow of the National Research Council.

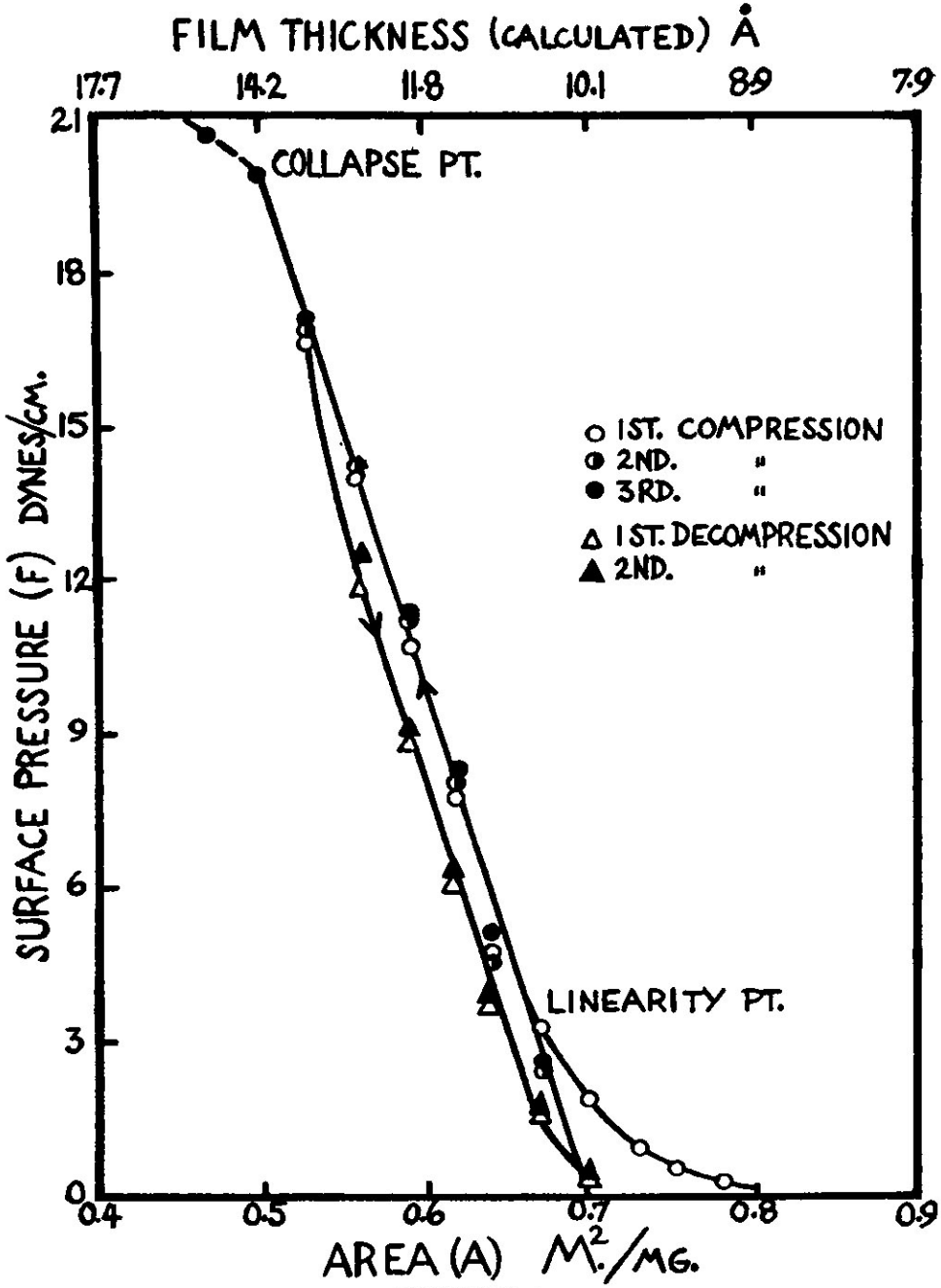

FIGURE 1

Compression and decompression curve for egg albumin. Extrapolated area at zero pressure is 0.7 m²/mg. Cenco 'hydrophil' balance used for surface pressure measurements. Film spread on distilled water buffered at pH 4.8.

1. At low pressure (under four dynes), there is a zone of relatively high compressibility, where decreases in film area lead to but small increases in surface pressure.

2. At about four dynes pressure, the curve becomes linear and remains so up to approximately twenty dynes. The linear portion of the curve represents the zone of lowest compressibility of the film; the pressure at which the curve becomes linear we have designated the 'linearity point'. Bull (9) has stressed his belief that these curves are only "approximately" *linear*; in using the term linear, we mean only that in this region of the force-area curve, a straight line best fits the experimental points (e.g. Figure 3). However, one is tempted to point out that the curves chosen by Bull to illustrate various of his articles (see 9) are as linear as any curves to be found in the scientific literature, including that describing the behaviour of ideal gases.

3. At approximately twenty dynes, the curve deviates from linearity in the direction of greater compressibility; this is the point at which the film begins to collapse. In the case of protein films, the collapse is not so spectacular as that of fatty acid films, but is quite gradual, as various restricted regions of the film 'buckle' separately. The collapse pressure might well have appeared somewhat lower had more sensitive methods of measurement (e.g. electrical or optical) been employed.

As the film is further compressed (not illustrated), more and more areas of the film collapse; these soon become visible as tiny striations running across the trough, parallel to the plane of compression. Upon complete compression, the monolayer is further crumpled up into a solid thread, or fiber, (Devaux effect, 4) resulting from the cramming together of the individual striations mentioned above; this fiber may easily be picked up from the surface of the water and is sturdy enough to survive considerable manipulation. The use of these fibers in the study of the biological properties of proteins has been described. (5, 6.)

The behaviour of the film at very low pressure (below one dyne) where its properties have been described as those of a two-dimensional gas (Guastalla, 7) was not studied, due to lack of the necessary instrumentation. From data obtained in this region, it is possible to calculate the molecular weight of various proteins (7).

In our work on protein monolayers, we record the following data:

1. *The specific area at zero pressure*, obtained by extrapolating the linear portion of the curve to the abscissa. This is a standard procedure in protein surface chemistry, introduced by Gorter and Grendel (8), permitting the comparison of one film with another, but is of no theoretical significance (Bull, 9).

2. *The linearity point*; it seems probable that the lower the pressure at which the curve becomes linear the greater the attractive forces that exist between the film molecules; this point may also be partially determined by the molecular weight or the molecular dimensions of the unfolded protein.

3. *The slope of the linear portion of the curve*; this is inversely related to the compressibility of the film. High compressibility (low slope) indicates that the film molecules are easily deformable (Adam 10). For the sake of convenience, all curves are considered to have a positive slope. The measurement of slope seems to have been introduced by Stallberg (11).

4. *The collapse point*; the collapse of the film is presumably due to the formation of inter-molecular linkages at higher film pressures. Here also, then, the lower the collapse pressure the greater the attractive intermolecular forces.

5. *The ability of the monolayer, upon complete collapse, to form a visible fiber.* The theoretical significance of this type of fiber formation is obscure; insulin, a protein of molecular weight close to that of egg albumin, does not form such a fiber (Wrinch 12). Nevertheless, in the experience of the senior author, the ability of a protein to form a fiber is related to its molecular weight, those of high M.W. forming such

fibers, but often losing their ability to do so upon a treatment so mild that their film-forming capacity is unimpaired.

Other film data, which are sometimes recorded, are the 'gel point' of Hughes and Rideal (13) and the 'coefficient of compressibility' of Bull (1).

The method of analysis outlined above has proven to yield extremely useful results in evaluating the action of physical and chemical agents on protein monolayers and on solutions of proteins. As an example, let us consider the effect of ultraviolet irradiation on a monolayer of protein. Gorter (15) has reported that UV will decrease at a uniform rate the area of such a film, due to destruction of the protein. Without an examination of the force-area curve of such an irradiated film, one might imagine that one is simply observing the elimination of the fragmented protein from the film, leaving the intact molecules still adsorbed, unchanged, at the interface. This, however, is not the case, as the residual film, after irradiation, is strikingly different in properties from the unirradiated control film.

This is illustrated in Figure 2, which shows the force-area curve of a protein film following a period of irradiation and in Figure 3, which represents that of an un-irradiated control. Table I summarizes the properties of these films with respect to the criteria discussed above; the two films presented were selected as typical.

TABLE I

Properties of irradiated and control monolayers

	Unirradiated control	Irradiated experimental
Extrapolated area (m^2/mg)	0.86	0.36
Linearity point (dynes/cm)	5.0	9.5
Slope $\frac{(\text{dynes. cm}^{-1})}{(\text{m}^2.\text{mg}^{-1})}$	72	112
Collapse point (dynes/cm)	20	34.5
Fiber formation	+++	—

The residual film after irradiation differs from the control in that its area is smaller, it has a much higher linearity point, it is of very low compressibility, it has a high collapse point,

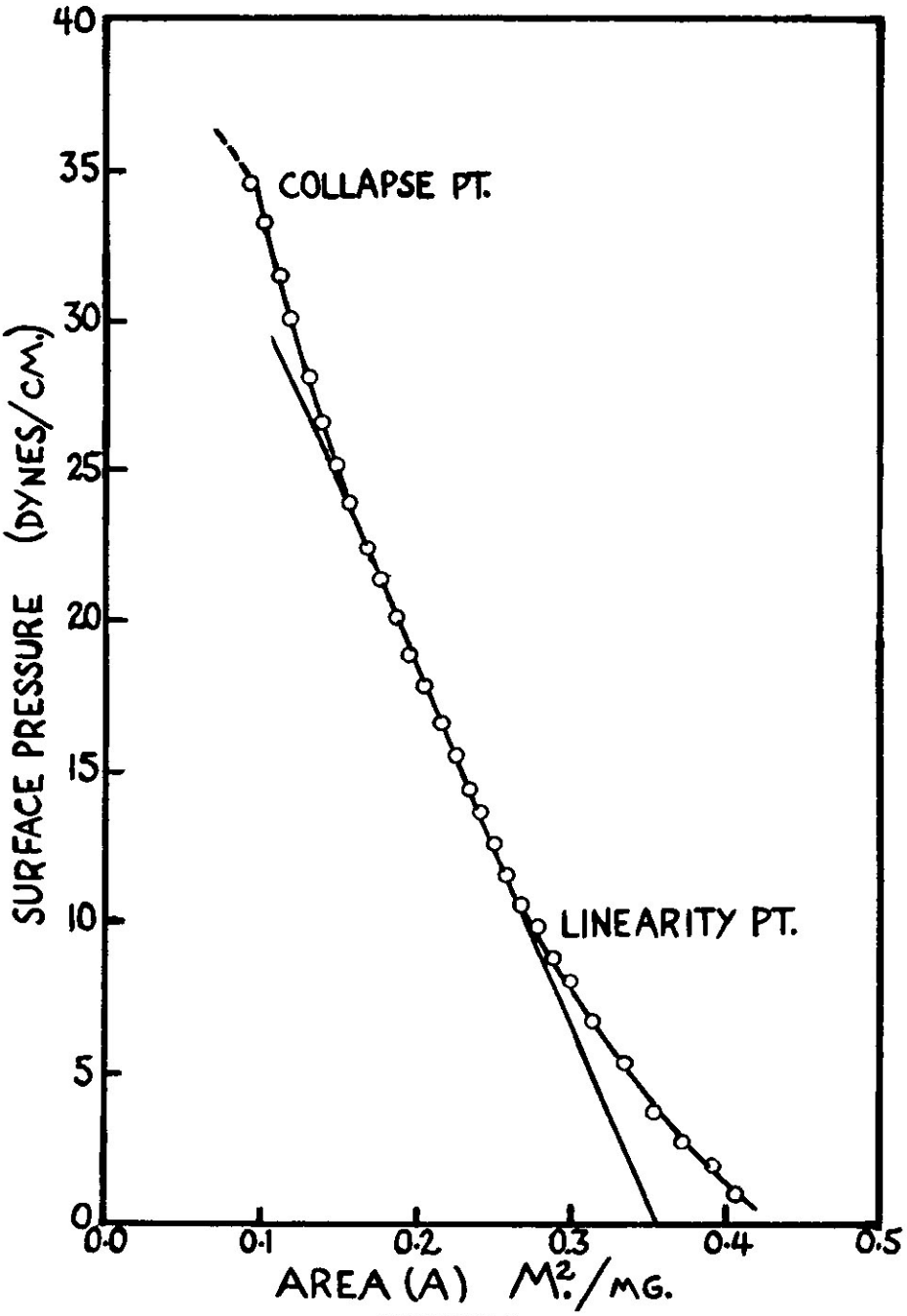

FIGURE 2

Force-area curve for an irradiated film. Note the inflection (in the direction of decreased compressibility) at approx. 23 dynes.

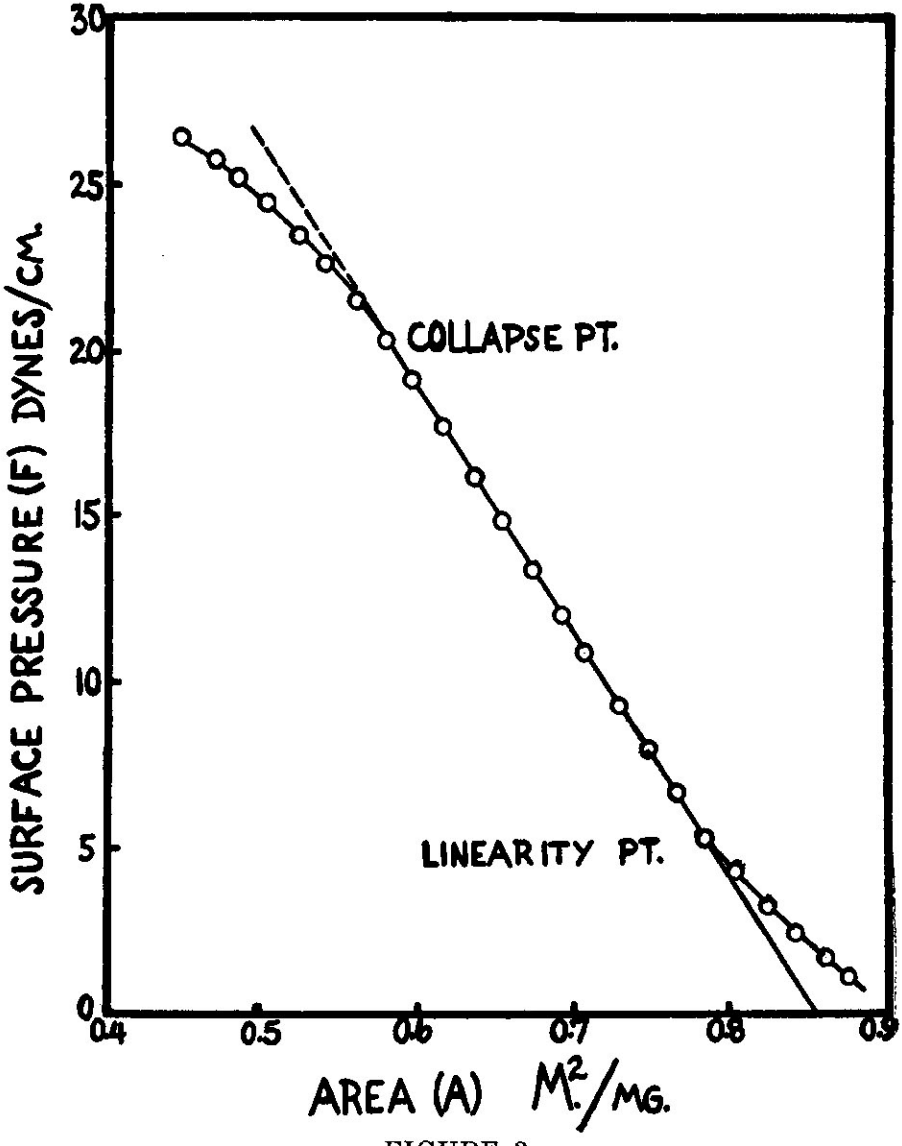

FIGURE 3
Force-area curve for an unirradiated control film

and it cannot form a fiber upon complete collapse. This last point is a genuine difference and does not follow from the fact that much of the protein has been lost from the interface due to the action of the UV. We may tentatively conclude on the basis of this analysis that the molecules of the irradiated film differ from those of the unirradiated film in that they are of lower molecular weight, are more rigid and less deformable, and in that the attractive forces between them are much less.

Two papers, describing application of this method to the study of the effect of UV on protein monolayers and on dilute solutions of protein, are in preparation (16, 17); exact experimental details and statistical treatment of data will be presented there. It is hoped that other workers may find this method useful in the analysis of the action of various physicochemical agents on protein, since it is effective even at protein concentrations of the order of 0.01%.

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