# THE MOLECULAR WEIGHT AT THE AIR-WATER INTERFACE OF SOME KERATIN DERIVATIVES EXTRACTED FROM WOOL

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[*Manuscript received July* 2, 1954]

*Summary*

Using the monolayer technique, number-average molecular weights have been determined for a series of extracts of wool prepared by successive treatments with alkaline sodium thioglycollate. The molecular weights of these extracts have been discussed in relation to their electrophoretic patterns. The change in the number-average molecular weight in the successive extracts has been correlated with the presence of certain electrophoretic components. The possibility of extraction of lipoidal or other non-protein material from the cortical cell walls is discussed. A reversible dissociation of the major electrophoretic component at high pH was observed.

## I. INTRODUCTION

The development, by Guastalla (1939, 1951), of methods for measuring the surface pressure of amphipathic molecules spread at the air-water interface at very low surface concentrations has made it possible to determine the molecular weight of large molecules.

Subsequent work by Bull (1950) has shown that the surface pressure is thermodynamically equivalent to a two-dimensional osmotic pressure, and that the variation of surface pressure ($\pi$) with area ($A$) may be represented by an equation

$$\pi\,(A-A_0) = n\,R\,T = NW/M\;.\;RT, \quad .. \qquad .. \quad (\mathbf{1})$$

where $A_0$ is a factor representing the "co-area" of the film molecules, $N$ is Avogadro's number, $M$ the molecular weight of the film molecules, $W$ the weight of material spread at the interface, and $R$ and $T$ the gas constant and absolute temperature respectively. Since $W/A$ is the surface concentration, the analogy between equation (**1**) and the familiar three-dimensional osmotic pressure-concentration equation is apparent. Thus, by measuring the variation of surface pressure with surface concentration, the molecular weight of the spread molecules may be determined.

However, it has not always been realized that equation (**1**), in common with its three-dimensional analogy, holds only at low surface concentrations where interaction between the film molecules is negligible; otherwise higher-power terms in $A$ must be introduced into equation (**1**). To illustrate this point, let us consider a typical protein molecule which is thought to spread in the $\beta$ configuration (Bull 1947). From X-ray data, the mean side-chain length

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is of the order of 10 Å for most proteins in this configuration and the unit repeat distance per residue along the polypeptide chain is about 3·5 Å. Thus, if the protein molecule is oriented at the interface with one of its side-chains parallel to the water surface the area occupied by that residue will be about 35 Å$^2$. If any other protein molecule approaches within that area interaction will occur. Taking the mean residue weight of a typical protein as 110, this area corresponds to

$$\frac{35 \times 6{\cdot}06}{110} = 1{\cdot}9 \text{ m}^2/\text{mg}.$$

Hence, if the protein film is compressed to areas below this figure intermolecular interaction is possible. Several earlier workers have reported measurements at areas as low as 1 m$^2$/mg: the molecular weights calculated from these observations must therefore be treated with reserve.

This paper reports measurements of the molecular weights of some of the wool protein extracts prepared and described by Gillespie and Lennox (1955). All measurements were made at areas greater than 2 m$^2$/mg where it is likely that intermolecular interaction is very small and that equation (**1**) holds.

## II. Experimental

### (*a*) *Surface Balance*

The surface balance used was basically of the conventional Adam-Langmuir horizontal-float type, but incorporating the modifications suggested by Few and Schulman (1953) (q.v.), together with the following new features:

(i) The light aluminium framework which follows the movement of the float was supported by two agate knife-edges bearing on two agate flats.

(ii) A movable brass bob mounted on top of this framework was used to vary the sensitivity of the balance.

(iii) By means of an externally controlled rider mechanism, similar to that on a conventional beam balance, the sensitivity of the balance could be checked during a series of measurements without disturbing the temperature equilibrium inside the balance case.

At the setting of maximum sensitivity of the balance, surface pressure changes of about 0·3 millidyne/cm could be readily estimated. For maximum reproducibility it was found essential to stand the balance on a vibration-free pier and to protect it from draughts.

### (*b*) *Spreading Solutions*

The protein solutions were spread using an "Agla" micrometer syringe which could be manipulated from outside the balance case (Cheeseman 1952). The surface concentration was varied by keeping the surface area constant and adding successive volumes of the protein solution. Even at the highest surface concentration the pressures involved were so low that there was no likelihood of the formation of the so-called *B*-films of Joly (1939).

Stock solutions of the wool protein were prepared by dissolving the freeze-dried extracts, supplied by Gillespie and Lennox (1955), in phosphate buffer of ionic strength 0·05 which was also 0·001M with respect to thioglycollic acid.

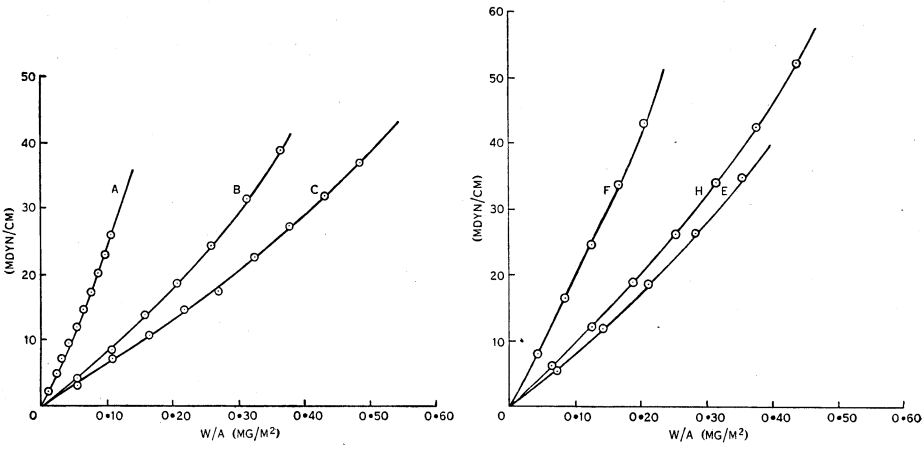


Fig. 1.—Surface pressure: surface concentration curves for alkaline thioglycollate extracts of wool, spread from undialysed solutions. Subsolution pH 6·4.

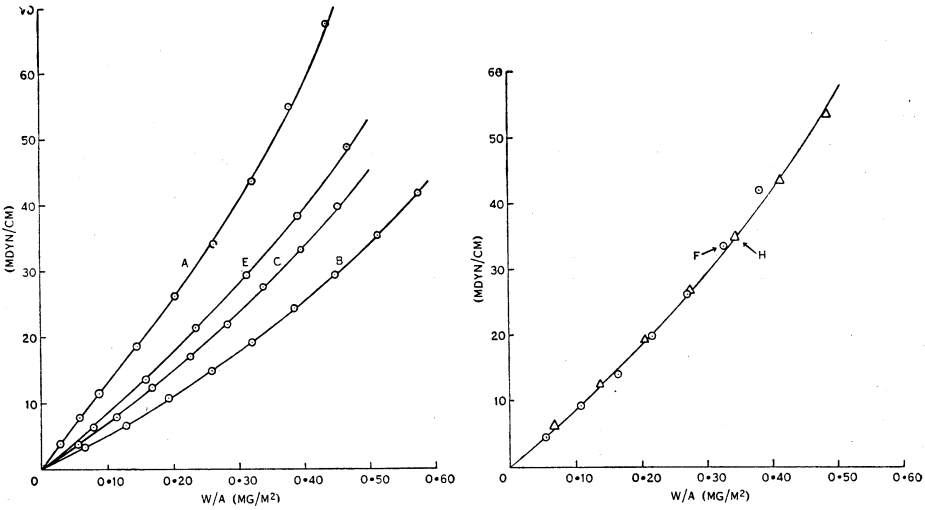


Fig. 2.—Surface pressure: surface concentration curves for alkaline thioglycollate extracts of wool, spread from exhaustively dialysed solutions. Subsolution pH 6·4.

The concentrations of these stock solutions were determined by Kjeldahl nitrogen estimations and solutions for spreading prepared by quantitative dilution with the same buffer to give *c.* 0·01 per cent. solutions.

The protein was spread on a subphase consisting of 0·9M KCl to which phosphate buffer salts were added to give the required pH and ionic strength $\Gamma/2 = 1$. On this subphase complete spreading (checked as suggested by Bull

1947) occurred spontaneously, without the addition of any extraneous materials, such as alcohols, to assist spreading.

### (c) *Materials*

All water used was doubly distilled, the second time from alkaline permanganate in an all-glass still. Buffer salts and sodium chloride were of analytical reagent quality and where possible were ignited at 500°C to remove surface-active impurities. All solutions were allowed to stand for 2 hr before use to enable surface-active impurities to accumulate at the surface. The solution to be used was then siphoned off from below the surface.

TABLE 1

MOLECULAR WEIGHT OF KERATIN EXTRACTS CALCULATED FROM FIGURES 1 AND 2

| Extract | pH of Extraction | Molecular Weight ($\times 10^{-3}$) | |
|---|---|---|---|
| | | Before Dialysis* | After Dialysis† |
| *A* | 10·5 | 11 | 22·9±1 |
| *B* | 10·5 | 32 | 46·8±1 |
| *C* | 10·5 | 38 | 37·7±1 |
| *E* | 10·5 | 32 | 29·6±1 |
| *F* | 12·3 | 12·5 | 32·5±1 |
| *H* | 12·3 | 31 | 32·7±0·6 |

*Since these values are for purposes of comparison only they have been simply calculated from plots of *FA v. F.*

† These values have been calculated by the method of least squares.

## III. RESULTS AND DISCUSSION

Curves of $\pi$ *v.* $W/A$ for the extracts *A*, *B*, *C*, *E*, *F*, and *H* are shown in Figures 1 and 2. These extracts were prepared by Gillespie and Lennox (1955) by successive extractions of wool with alkaline thioglycollate.

The freeze-dried extracts were dissolved in phosphate-thioglycollate buffer of pH 10·4, ionic strength $\Gamma/2 = 0{\cdot}05$, and spread on a subphase of pH 6·4. Figure 1 gives the results using spreading solutions prepared from undialysed stock solutions. The results in Figure 2 were obtained using spreading solutions prepared from stock solutions, 25 ml of which had been previously exhaustively dialysed through "Visking" sausage casing against eight successive 200-ml changes of the same phosphate-thioglycollate buffer over a period of 4 days. The molecular weights obtained from these curves are shown in Table 1.

Considering first extracts *A-E* (extracted at pH 10·5), the molecular weights* in Table 1 show that extract *A*, which was found by Gillespie and Lennox (1955) to comprise several electrophoretic components, contains a con-

* The molecular weights obtained by this technique are number-average values, similar to those obtained from osmometry.

siderable quantity of low-molecular-weight material. Some of this is dialysable, indicating a molecular weight of less than 10,000. Extract *B*, which is also electrophoretically polydisperse, contains some dialysable material, together with a very high molecular weight component. Extracts *C* and *E*, on the other hand, show no significant change in molecular weight on dialysis and their electrophoretic patterns show that components 1 and 4 have virtually disappeared. It therefore seems reasonable to identify these two components with the low-molecular-weight dialysable material in extracts *A* and *B*. Indeed, Gillespie and Lennox (1955) have noted that the relative proportions of the subcomponents comprising component 1 vary with the preliminary equilibrium dialysis time prior to electrophoresis. It is possible that component 1 or 4 may contain non-protein material, since Gillespie and Lennox (1955) observed very little effusion of nitrogenous material on dialysis of extracts *A* and *B*. The discrepancy between this and the large change in number-average molecular weight on dialysis may arise from the presence of low-molecular-weight, but highly surface-active, lipoidal material in the fibre extract. This hypothesis is supported by the electrophoresis diagram for the supernatant liquid obtained after precipitating the protein in extract *B* with acid; this shows a broad, flat band, uncharacteristic of protein solutions.

Table 1 also shows a decrease in the number-average molecular weight from extracts *B-E*, indicating a decrease in the proportion of high-molecular-weight material. The decrease in the range of molecular weights present on passing from *B* to *E* is paralleled by the enhancement of electrophoretic homogeneity, resulting in the emergence of component 2 as virtually a single electrophoretic peak in extract *E*. It is likely therefore that this component has a molecular weight in the vicinity of 30,000 at pH 6·4, whilst component 3, which persists to extract *D* in the electrophoretic diagrams, is probably of high molecular weight and thereby responsible for the high number-average molecular weights observed for extracts *B* and *C*.

Turning to extracts *F* and *H*, prepared by raising the pH to 12·3, Table 1 shows a large increase in the molecular weight of the *F* extract on dialysis, suggesting that raising the pH results in the extraction of a further quantity of low-molecular-weight, dialysable material. The molecular weight of the dialysed extract is, however, not significantly different from that for extract *E* and the electrophoretic diagrams show that the two extracts contain the same electrophoretic component. We must therefore look for the origin of the low-molecular-weight material in the undialysed sample. A possible explanation is suggested by the rate of extraction of the major electrophoretic component 2. Gillespie and Lennox (1955) have found that very small amounts are extracted at pH 10·5 with each successive treatment, the process seeming to be one of diffusion. On raising the pH to 12·3, however, a much larger quantity is extracted, suggesting that there has been a marked change in the barrier to diffusion. It seems likely that the low-molecular-weight material in the undialysed extract *F* may be the breakdown products of this diffusion barrier. For this extract also Gillespie and Lennox (1955) observed very little dialysable nitro-

genous material. Thus the large change in molecular weight on dialysis seems again to be due to non-protein material. It is known that medulla cells of hair contain considerable quantities of phospholipid (Stoves 1946). If the cortical cell walls contain similar material, possibly in the form of a lipoprotein complex, one would expect the low-molecular-weight lipoidal products from the breakdown of these walls to have a profound effect on the number-average molecular weight of the protein released from within the cells. It is unlikely that the low-molecular-weight material could be a breakdown product of the major component itself as a result of the increased pH, since extract *H*, which has been

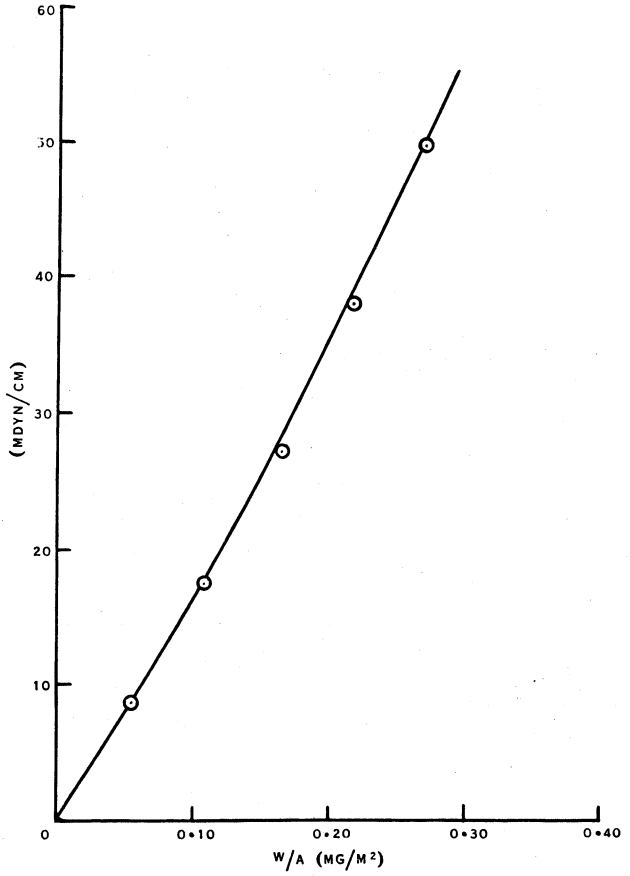


Fig. 3.—Surface pressure: surface concentration curve for extract *F*, spread from exhaustively dialysed solution on pH 9·4 subsolution.

subjected to the same conditions, shows no significant difference between the molecular weights of the dialysed and undialysed samples. Moreover, the molecular weight of extract *H* is not signficantly different from those of dialysed extracts *E* and *F*. It appears that in these three solutions we are dealing with a molecule which, either by itself or in combination, is a major component

of the wool fibre. The electrophoretic diagram of extract *H* shows, in addition to the major component, a faster-moving spike; since no significant change in molecular weight occurs, this is apparently due to a change in the molecular configuration of the protein.

A few preliminary experiments have also been made to determine the effect of pH of both the spreading solution and the subphase on the molecular weight. First, extract *F* was dialysed against phosphate-thioglycollate buffers of pH 8·1, 10·4, and 11·5, and the resulting solutions spread on a pH 6·4 subphase. There was no significant difference between the $\pi$ *v.* *W/A* curves for these three solutions and hence between the molecular weights calculated from them (32,000, 30,000, and 31,000 respectively). The pH 10·4 solution was also spread on subphases of pH 2·1 and 9·4. On the former a molecular weight of 29,000 was estimated, again an insignificant change, but on the pH 9·4 subphase the $\pi$ *v.* *W/A* curve shown in Figure 3 was obtained, leading to a molecular weight of 16,000. This suggests a dissociation of the major electrophoretic component as the pH is raised. It appears that the state of aggregation of the *spread* molecule is a function of the pH of the subphase rather than of the solution from which it is spread. Therefore, if the states of aggregation in both the surface phase and in the bulk phase are the same at the same pH, we must conclude that the dissociation of the molecule is reversible since a solution of high pH spread on a subphase of neutral pH gives a surface film of "dimer."

Friend and O'Donnell (1953), from osmotic pressure measurements, obtained a value of 15,000 for the molecular weight of an extract of wool by urea-bisulphite at pH 8. It is tempting to speculate whether the same major component is being dealt with in each case. If we postulate a "dimeric" reduced molecule of molecular weight *c.* 30,000 at neutral pH, the two halves being held together by hydrogen bonds, then we should expect the cleavage of these bonds either by concentrated urea solution or by increasing the pH of the solution.

## IV. Acknowledgments

The author wishes to thank Mr. W. B. Hall for making a statistical analysis of the results presented in this paper, and Mr. E. W. Walton for constructing the sensitive surface balance used.

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