

THE ELECTROPHORETIC PROPERTIES OF *S*-CARBOXYMETHYLKERATEINES AND *S*-SULPHOKERATEINES FROM WOOL

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Summary

The most suitable conditions for the moving-boundary electrophoresis of *S*-carboxymethylkerateines were determined. The use of urea or sodium dodecyl sulphate to assist the extraction of soluble proteins from wool with alkaline thioglycollate did not alter the electrophoretic behaviour of the extracted proteins.

S-sulphokerateines, prepared from wool by a new method due to Swan (1957), exhibited similar behaviour to the carboxymethylkerateines on electrophoresis and this method of preparation can be adapted to fractional extraction to give one component more homogeneous electrophoretically than the remainder.

Proteins prepared by fractional extraction of wool with 8M urea and sodium bisulphite at pH 5 gave similar results on electrophoretic examination to those obtained by fractional extraction with thioglycollate at pH 11.

The effects of protein interactions, including aggregation, on the electrophoresis diagrams of soluble wool proteins are discussed.

I. INTRODUCTION

Keratin derivatives which are water soluble and stabilized against re-oxidation can be readily prepared from wool by alkaline reduction of the disulphide bonds and substitution of the thiol groups so produced (Goddard and Michaelis 1935; Gillespie 1956). Reagents such as thioglycollic acid, mercaptoethanol, and cysteine give two thiol groups per cystine residue. Sodium bisulphite (or sulphite), which has been widely used in the presence of concentrated solutions of urea, guanidine, and anionic detergents to prepare soluble keratins, converts one of the cystine sulphur atoms to $-SSO_3^-$, the other to $-SH$. Such an unsymmetrical fission must complicate the examination of the soluble proteins for heterogeneity by conventional means. Swan (1957) has shown in this Laboratory that the disulphide bonds of wool can be split specifically and symmetrically by means of sodium sulphite in the presence of cupric ions whereby water-soluble *S*-sulphokerateines may be obtained. In this paper the *S*-sulphokerateines have been examined by moving-boundary electrophoresis and compared with the *S*-carboxymethylkerateines prepared from wool by the alkaline thioglycollate reduction method of Gillespie and Lennox (1953, 1955*a*) and some modifications of it.

II. EXPERIMENTAL

(a) Preparation of *S*-Carboxymethylkerateines

The kerateines were prepared from Merino 64's solvent-scoured wool top by the method of Gillespie and Lennox (1953) using a single extraction at 50°C for

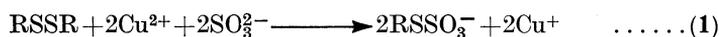
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20 min with 0.1M sodium thioglycollate at pH 12.3 whereby approximately 60 per cent. of the wool dissolved. A liquor to wool ratio of 100 ml of thioglycollate solution to 1 g wool was used. After extraction the pH was lowered to approximately 8.5 and the -SH groups allowed to react with sodium iodoacetate, the pH being kept constant by the addition of alkali. When the nitroprusside reaction was negative the solution was dialysed against tap-water for 48 hr and concentrated by per-vaporation; alternatively the proteins could be first precipitated by half saturation with ammonium sulphate and the precipitates dialysed to remove ammonium sulphate. Several modifications of the extraction procedure were examined. In the presence of 0.5 per cent. sodium dodecyl sulphate or 8M urea, sodium thioglycollate at pH 10.5-11.0 dissolved the same amount of wool as at the higher pH and temperature.

The action of sodium dodecyl sulphate and urea in assisting the solution of the wool proteins is probably due to the fact that both these reagents are excellent disaggregating agents for the isolated proteins (O'Donnell and Woods 1956a), and it has also been found that the disulphide bonds of proteins are more readily reduced in the presence of either urea or sodium dodecyl sulphate (Lindley 1955; Markus and Karush 1957).

(b) *Preparation of S-Sulphokerateines*

The *S*-sulphokerateines were prepared by the method described by Swan (1957) who used the following reactions of cupric ions in the presence of sulphite for the conversion of the disulphide and thiol groups of proteins to $-\text{SSO}_3^-$:



With this method in the presence of 8M urea, 70 per cent. of wool could be dissolved overnight and up to 85 per cent. with longer extraction times. If the urea is omitted the extraction process is slower but the same amount of protein dissolves after about 8 days. The protein solubilities found by this method were in substantial agreement with the values given by Swan (1957).

For electrophoresis the protein was prepared by a fractional extraction process at 2°C. The reagent was 0.04M in cupric ammonium hydroxide, prepared by the addition of conc. NH_4OH to CuSO_4 , 0.1M in Na_2SO_3 , and was adjusted to approximately pH 10 with conc. NH_4OH . A liquor to wool ratio of 100 : 1 was used. Forty per cent. of the wool had dissolved after 18 hr, the solution was filtered and the residue extracted with fresh reagent for 48 hr. A further 25 per cent. of protein went into solution. Triammonium citrate was then added to the extracts (more than sufficient to complex with all the copper present), the pH lowered to about 7 by the addition of conc. HCl and the protein precipitated by the addition of ammonium sulphate to approximately two-thirds saturation. Acid precipitation recovered only 75 per cent. of the soluble proteins; ammonium sulphate precipitation was therefore preferred. The protein precipitates were dissolved in 0.1M potassium bicarbonate or 0.05M borax, more ammonium citrate was added, and the solutions dialysed against running tap-water. If all the copper was not removed as evidenced

by a purple tint on making the protein solutions slightly alkaline, disodium ethylenediaminetetra-acetate was added and the solution redialysed.

Fractional extractions of wool were also made with urea bisulphite at pH 5.0. Under these conditions only one of the disulphide sulphur atoms is converted to $-\text{SSO}_3^-$, the other remaining as $-\text{SH}$. Reaction (2) can then be used to convert this $-\text{SH}$ group to $-\text{SSO}_3^-$ after extraction. The conditions of extraction were similar to those used by Woods (1952) and Friend and O'Donnell (1953). 50 g wool was extracted with 300 ml of 8M urea and 0.04M NaHSO_3 at pH 5.0 for 48 hr at 50°C; 15 per cent. of the protein dissolved and a further 25 per cent. was extracted in 72 hr after the addition of fresh reagent. These extracts were converted to *S*-sulphokerateines by adjusting the pH to 10 with conc. ammonium hydroxide, adding cuprammonium hydroxide and more Na_2SO_3 . After allowing a few minutes for the reaction to be completed, the solution was treated as above to recover the proteins. A further 25 per cent. of soluble protein was recovered from the residue by extraction with the cuprammonium sulphite at pH 10 for 24 hr at room temperature.

(c) *Cystine Analysis on Extracted Proteins*

Determinations of residual disulphide on the extracted proteins were done by Dr. S. J. Leach in this Laboratory. The intact proteins were titrated with mercuric chloride amperometrically at the dropping-mercury electrode in the presence of 0.2M sulphite and 8M urea at pH 9.2. All the *S*-carboxymethylkerateine preparations were free of disulphide, while of four *S*-sulphokerateine preparations one contained approximately 0.5 per cent. disulphide (expressed as cystine), the others contained none. The above method using the intact protein must be used in the case of the sulphokerateines since the $-\text{SSO}_3^-$ group is known to revert to $-\text{SH}$ on acid hydrolysis.

(d) *Electrophoresis*

Electrophoresis of the wool proteins was carried out at 1°C in a standard moving-boundary Tiselius electrophoresis apparatus (LKB, Sweden), using the inclined-slit schlieren optical system for observation of the boundaries. The proteins were dialysed at 2°C for 48 hr against two changes of buffer before electrophoresis. The protein concentration was measured refractometrically after dialysis and adjusted to approximately 1.2 per cent. for electrophoresis. Mobilities were calculated using the conductivity of the protein solution.

III. RESULTS

(a) *S*-Carboxymethylkerateines

Figure 1 shows the ascending and descending patterns at various pH values of the combined unfractionated *S*-carboxymethylkerateines from wool, prepared by urea-thioglycollate (pH 11.0) extraction. The descending pattern is complex at pH values of 8.6 and 9.6, and as the pH is raised the proportion of components changes. In sodium carbonate buffer at pH 11 the areas under the peaks were different to those obtained with glycine buffer at this pH. With piperidine hydrochloride at pH 11 a slow-moving boundary, not evident in the other buffers, occurred. The most suitable buffer for electrophoresis was glycine-sodium hydroxide at pH 11

where there was the least difference between the mobilities of the ascending and descending peaks and the patterns from the two sides of the electrophoresis tube more nearly corresponded.

Figure 2 gives the patterns at pH 11 of the *S*-carboxymethylkerateines extracted with alkaline thioglycollate (pH 10.9) containing sodium dodecyl sulphate,

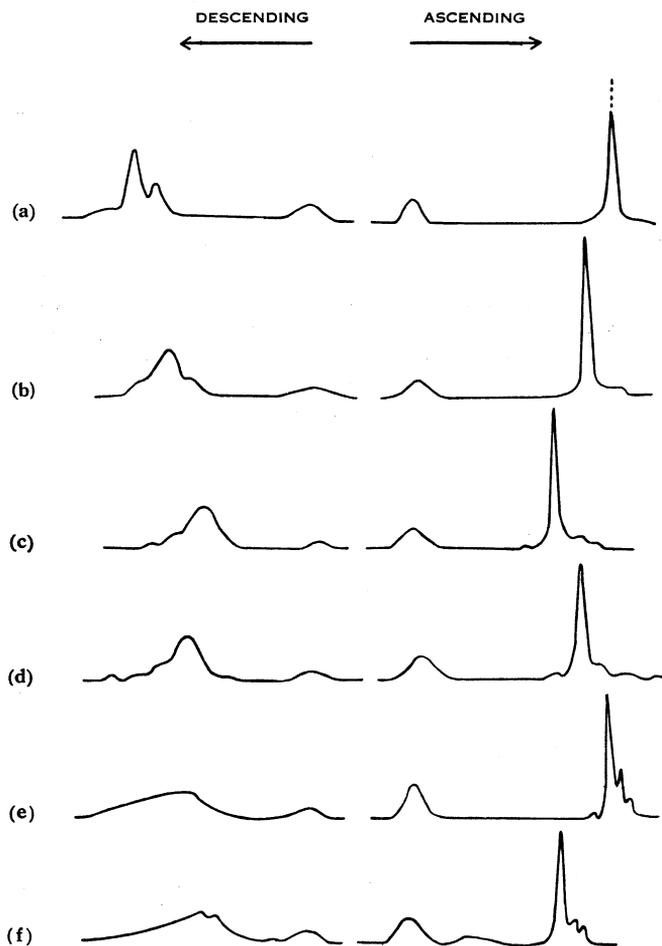


Fig. 1.—Electrophoresis in buffers of ionic strength 0.1 of *S*-carboxymethylkerateines from wool prepared by extraction at pH 11.0 with alkaline thioglycollate-8M urea. (a) Veronal, pH 8.6; (b) glycine, pH 9.6; (c) glycine pH 10.5; (d) glycine, pH 11.0; (e) sodium carbonate, pH 11.0; (f) piperidine hydrochloride, pH 10.9.

and by the Gillespie and Lennox (1953) alkaline thioglycollate procedure. The ascending patterns are almost identical with one another and with the previous urea-thioglycollate preparation (Fig. 1(d)). The splitting of the main peak on the descending side for the detergent alkaline thioglycollate method (Fig. 2(b)) was, however, not a reproducible property and has occurred with carboxymethylkerateines pre-

pared by all three procedures. There is evidence that slight variations in the substitution of the protein $-SH$ groups with iodoacetate may be important in determining anomalies in the pattern. In the preparation of the main component, *S*-carboxy-

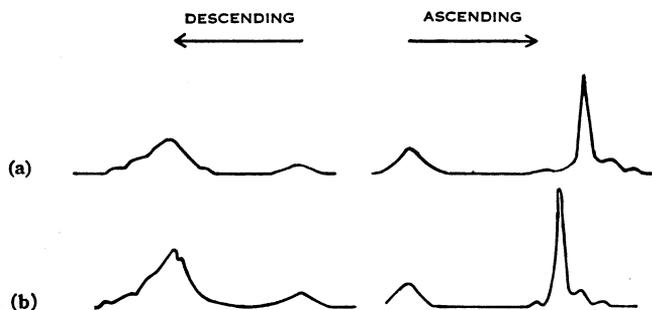


Fig. 2.—Electrophoresis in glycine buffer of ionic strength 0.1 and pH 11.0 of *S*-carboxymethylkerateines (a) prepared by alkaline thioglycollate extraction at pH 12.3, and (b) prepared by alkaline thioglycollate extraction at pH 11.0 in the presence of sodium dodecyl sulphate.

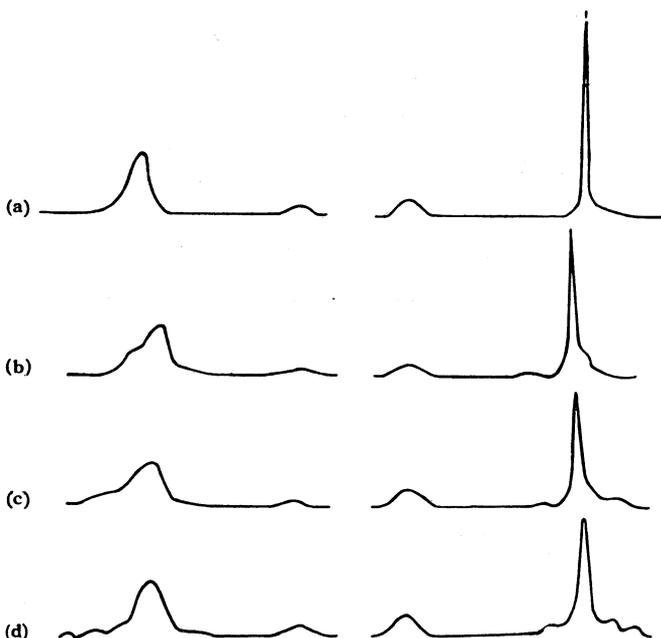


Fig. 3.—Electrophoresis in buffers of ionic strength 0.1 of *S*-sulphokerateines from wool. The proteins comprise the first 40 per cent. of extractable material. (a) Veronal, pH 8.6; (b) glycine, pH 9.5; (c) glycine, pH 10.6; (d) glycine, pH 11.1.

methylkerateine 2 (SCMK2), by the fractional extraction procedure of Gillespie and Lennox (1955a) it has been observed that variations in the conditions for substitution with iodoacetate and subsequent dialysis sometimes led to double peaks

on the descending boundary. For example, when dialysis was carried out at pH 8 to remove the excess iodoacetate and dialysable products, a single peak was obtained on electrophoresis at pH 11, whereas lowering the pH to 6 after substitution followed by dialysis gave a double peak. Further, a preparation of SCMCK2, which showed about 90 per cent. of the material as a single descending peak on electrophoresis of a 1.2 per cent. solution, gave a double peak on electrophoresis after it had been fractionated by the ammonium sulphate procedure of Gillespie (1957). At a protein concentration of 0.4 per cent., however, electrophoresis gave a single symmetrical peak in both limbs of the electrophoresis tube for the ammonium sulphate-fractionated protein.

(b) *S-Sulphokerateines*

Figures 3 and 4 show the electrophoretic patterns at four pH values of the *S*-sulphokerateines prepared by fractional extraction of wool with the cuprammonium sulphite reagent at pH 10. The first extract was obtained by extraction for 24 hr at 2°C and the second by a further extraction for 48 hr. The patterns for the first extract are similar to those observed for the *S*-carboxymethylkerateines and the best resolution of components occurs on electrophoresis at pH 11. The second extract is slightly more homogeneous and appears to correspond to the kerateine 2 of Gillespie and Lennox (1955*a*). In the combined extracts of the *S*-sulphokerateines it appears in the same position in the electrophoretic diagram as the kerateine 2 in the combined kerateine extracts, or the SCMCK2 in the combined carboxymethyl proteins. For this reason it is labelled *S*-sulphokerateine 2 (SSK2) although there is some evidence that the protein fractionated from the early extracts of wool may not be the same as the SCMCK2 of later extracts (Gillespie 1958). The electrophoretic pattern at low ionic strength (0.02) at pH 9.6 shows the descending pattern to be very spread out (Fig. 4(*e*)).

Figure 5 gives the electrophoretic patterns at pH 11 of the proteins obtained by the fractional extraction of wool with urea bisulphite at pH 5 and subsequent conversion to the *S*-sulphokerateines. The first extract represented 15 per cent. of the wool, the second 20 per cent., and the third extract, which was obtained by treating the residue with cuprammonium sulphite at pH 10, about 20 per cent.

IV. DISCUSSION

It is clear from Figures 1, 3, and 4 that the best resolution of the wool proteins is obtained on electrophoresis at pH 11 in 0.1 ionic strength glycine buffer. Fewer components are observed at lower pH values. At pH 11 glycine is at the upper limit of its alkaline buffering range but it was the most suitable buffer tried at this pH since it gave ascending and descending patterns which more nearly corresponded. The small fast-moving peak in the descending pattern, which moves faster than any peak on the ascending side, is possibly a false boundary of the type described by Svensson (1946) or Woods (1958). The electrophoretic patterns of the carboxymethylkerateines are similar to those obtained by Gillespie and Lennox (1953, 1955*a*) for the kerateines. Four components were resolved with possibly additional minor components. Conversion to the *S*-carboxymethyl derivatives is advantageous in that it

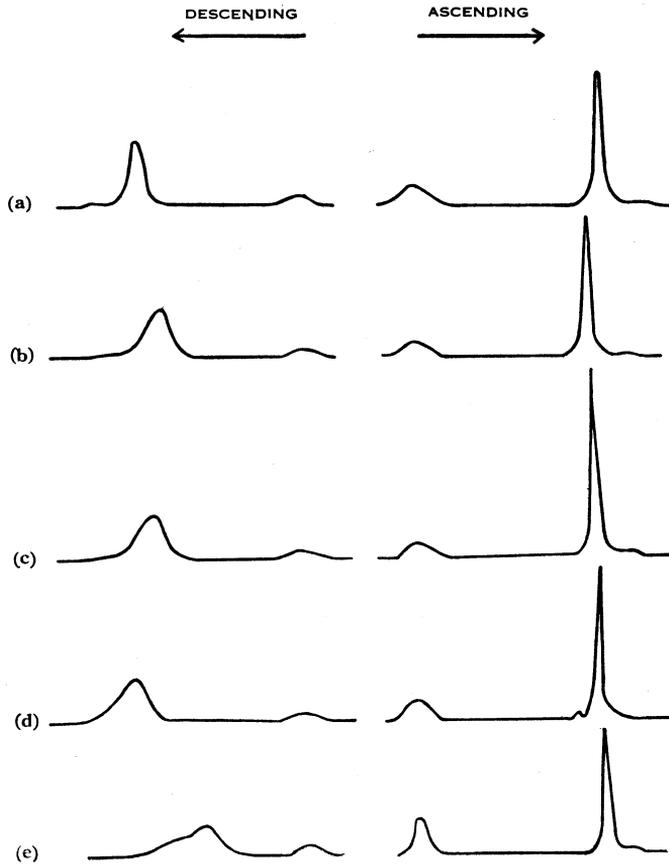


Fig. 4.—Electrophoresis in buffers of ionic strength 0.1 of *S*-sulphokerateines from wool. The protein represents the fraction labelled *S*-sulphokerateine 2, and comprises 20 per cent. of material from the second extraction of the wool. (a) Veronal, pH 8.6; (b) glycine, pH 9.6; (c) glycine, pH 10.5; (d) glycine, pH 11.1; (e) glycine, pH 9.6, ionic strength 0.02.

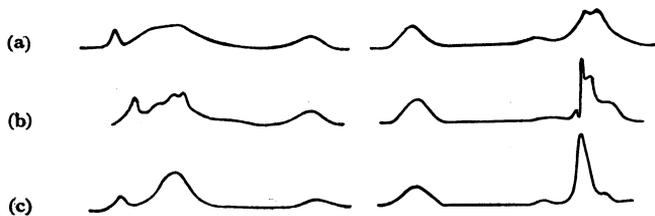


Fig. 5.—Electrophoresis in glycine buffer of ionic strength 0.1 and pH 11.0 of *S*-sulphokerateines prepared by urea-sodium bisulphite extraction at pH 5.0, followed by reaction with cuprammonium sulphite at pH 10.0. (a) First 15 per cent. of the protein extracted at pH 5.0 by 8M urea-sodium bisulphite; (b) the next 20 per cent. of the protein extracted at pH 5.0 by 8M urea-sodium bisulphite; (c) the next 20 per cent. of the protein extracted at pH 10.0 by cuprammonium sulphite.

eliminates some of the problems associated with the electrophoresis of -SH proteins. It is not then necessary to incorporate thioglycolic acid in the buffers and the descending patterns are free from some of the anomalous effects which occurred with the kerateines.

The use of sodium dodecyl sulphate or 8M urea with alkaline thioglycollate for extraction of the protein does not alter the electrophoretic patterns of the isolated protein and has the advantage that the whole extraction can be carried out between 10.5 and 11.0 at room temperature whereas in the method of Gillespie and Lennox (1953, 1955*a*) a temperature of 50°C is used and an initial pH of >12 for the final extraction. However, prolonged dialysis is necessary to remove sodium dodecyl sulphate.

TABLE 1
MOBILITIES OF COMPONENTS EXTRACTED FROM WOOL
Electrophoreses carried out in glycine-NaOH buffer, ionic strength 0.1, pH 11.0

Method of Extraction	Descending Mobility* $\times 10^5$ ($\text{cm}^2 \text{sec}^{-1} \text{volt}^{-1}$)			
Alkaline thioglycollate-8M urea (pH 11.0)	<i>7.0</i>	8.7	10.2	11.3
Alkaline thioglycollate-0.5 per cent. sodium dodecyl sulphate (pH 11.0)	7.0	8.6	10.3	11.6
Alkaline thioglycollate (pH 12.3)	6.3	8.2	9.5	
Cuprammonium sulphite-8M urea (pH 10.0)	6.7	8.7	9.8	
Cuprammonium sulphite (pH 10.0)	7.4	8.9	10.5	11.4
Sodium bisulphite-8M urea (pH 5.0)	7.0	8.4	10.6	14.6

*Main component italicized.

The cuprammonium sulphite method of Swan (1957) gives similar results to the thioglycollate method and can be adapted for the fractional extraction of components. Although a complete quantitative analysis of the electrophoretic patterns was not carried out it was observed that if the electrophoretic patterns of the *S*-carboxymethylkerateines and *S*-sulphokerateines were compared for the same percentage of wool extracted, then the area under the main peak was approximately the same percentage of the total area in each case. The descending mobilities at pH 11 of the components (Table 1) are very similar for all methods of extraction, the differences between the methods being no greater than variability between preparations using the same method of extraction. The mobility of the isolated SCMK2 was $7.2 \times 10^{-5} \text{ cm}^2 \text{sec}^{-1} \text{volt}^{-1}$ (unpublished data), while that of SSK2 was $7.0 \times 10^{-5} \text{ cm}^2 \text{sec}^{-1} \text{volt}^{-1}$ indicating that the main component obtained by two different extraction methods is similar as regards electrical charge. The isolated SSK2 showed a very diffuse descending boundary at low ionic strength (Fig. 4(e)) and this is probably a similar phenomenon to that observed by Gillespie (1956) for SCMK2 and other keratin derivatives at low ionic strength. The main disadvantage in the preparation of the *S*-sulphokerateines is the difficulty of removing the excess copper from the extracted protein and often dialysis for several days in the presence

of disodium ethylenediaminetetra-acetate was necessary for complete removal. There was no evidence of any decomposition during electrophoresis of the *S*-sulphokerateines at pH 11 although Swan (1957) reports a small amount of decomposition of the protein $-\text{SSO}_3^-$ group at this pH.

Examination of proteins extracted fractionally at pH 5.0 with urea bisulphite and subsequent stabilization of the $-\text{SH}$ group by conversion to $-\text{SSO}_3^-$ shows that the proteins extracted exhibit similar electrophoretic behaviour to those extracted at higher pH values with other reducing agents. For small amounts of protein extracted the electrophoretic patterns are very similar to the first alkaline thioglycollate extracts obtained by Gillespie and Lennox (1955*a*). It appears that the order in which soluble proteins are extracted from wool is independent of the reagents used to reduce the disulphide bond or the pH of the reduction. Similar considerations would be expected to apply to the isolation of proteins from oxidized wool by the method of Alexander (Alexander and Hudson 1954). The electrophoresis experiments on *S*-sulphokerateines prepared from urea bisulphite extracts of wool are also of interest in that they show that the first extracts are more heterogeneous electrophoretically than the later extracts, whereas Friend and O'Donnell (1953) found that the osmotic molecular weights of urea bisulphite extracts were the same irrespective of whether 20 or 70 per cent. of the wool dissolved.

Interpretation of the Electrophoretic Patterns of Wool Proteins

In this and other work from this Laboratory concerning the electrophoresis of wool proteins there are many unexplained features of the electrophoresis diagrams. For example, descending and ascending patterns of the *S*-carboxymethyl proteins given in Figure 1 are not enantiographic. At low ionic strength the descending pattern of *S*-sulphokerateine 2 (Fig. 4(e)) is extremely spread out and does not correspond with the ascending pattern. Buffer ion effects certainly play some part in determining the type of patterns shown in Figure 1 where at pH 11 different patterns are obtained if carbonate or piperidine buffers are used instead of glycine. In the situation where thioglycollic acid is incorporated in the electrophoresis buffer to keep the protein in the reduced state (Gillespie and Lennox 1955*a*, 1955*b*), thioglycollate-protein interaction is a complicating factor and would be difficult to disentangle from protein-protein and other buffer ion-protein interactions. The thioglycollate-protein interaction is avoided, however, by stabilizing the proteins by substitution of the $-\text{SH}$ group. With the more homogeneous kerateine derivatives prepared by Gillespie (1956), some peculiar descending electrophoretic patterns have been observed. The "twinning" of the main descending peak on the electrophoresis of the combined extracts and of the more homogeneous SCMK2 (reported in this work) in 0.1 ionic strength glycine buffer at pH 11 also needs some explanation. In view of the fact that physical measurements have shown that soluble wool proteins exist in solution in an aggregated state (O'Donnell and Woods 1956*a*, 1956*b*; Harrap and Woods 1958) the effect of reversible aggregation on the electrophoretic patterns is discussed below.

In order to fully interpret the electrophoretic patterns of a monomer in equilibrium with a series of polymers it is necessary to know, firstly, the relation

between the electrophoretic mobility of a monomer and its polymers and, secondly, the time taken for equilibrium to be established compared to the time of electrophoresis since this determines the type of pattern.

There is no general answer to the first question. The relation between the electrophoretic mobility and net charge of solid spherical non-conducting spheres has been given by Henry (1931) and the effects of ionic strength of the medium and size and shape of the particle have been clearly set out by Abramson, Moyer, and Gorin (1942). From these relations it is possible to calculate the effect of linear aggregation on the electrophoretic mobility if it is assumed that the net charge and volume are increased n -fold when n monomer units aggregate linearly, and that for linear aggregation of spherical particles the polymer is assumed to be a cylinder. The results of such calculations for the range of sizes in which most proteins fall show that dimerization leads to little change in mobility and resolution would not be expected in a mixture of monomer and dimer. The calculated mobility increases slightly on further aggregation. No predictions can be made for the more likely case of non-linear aggregation since spheres and cylinders are the only models that can be treated by the above method. Recently, however, the electrophoresis of polyelectrolytes has been treated theoretically by Hermans and Fujita (1955) and Overbeek and Stigter (1956). At sufficiently high salt concentration polyelectrolyte molecules behave as free-drained on electrophoresis and the mobility should be independent of molecular weight and ionic strength. At lower ionic strengths polyelectrolytes becomes less free-drained; however, the effect of molecular weight on mobility is not very pronounced. Independence of mobility on molecular weight has been observed experimentally for poly-4-vinyl-*N-n*-butyl pyridinium bromide (Fitzgerald and Fuoss 1954) and polyphosphates (Strauss, Woodside, and Wineman 1957). In some proteins which dissociate, e.g. haemocyanin (Svedberg 1937) and ground-nut globulins (Johnson, Shooter, and Rideal 1950; Johnson and Naismith 1953), the rates of migration were shown to be independent of the degree of association. In studies on α -keratose (O'Donnell and Woods 1956b) prepared from wool it was found that resolution was obtained in the ultracentrifuge the results suggesting that much reversible aggregation occurred in solution, whereas on electrophoresis only a single peak was observed. These results are explained if these proteins behave on electrophoresis as free-drained or almost free-drained.

For cases where a polymer migrates faster than the monomer then normal resolution will take place if the equilibrium is established slowly compared to the time of electrophoresis. For times of equilibrium which are fast or comparable to the time of electrophoresis then a readjustment of equilibrium takes place during electrophoresis. A theoretical treatment of the effects of reversible polymerization during electrophoresis or ultracentrifugation has been given by Gilbert (1955) for the case where equilibrium is instantaneous. Where dimerization occurs a single peak is obtained. For a higher degree of polymerization a partly resolved boundary indicating two components will be formed. The situation is more complicated where a group of complexes each of different degree of polymerization is present, but the same principles are applicable and additional incompletely resolved boundaries would be expected.

Possibly the patterns obtained with kerateine derivatives at low ionic strength and high protein concentration could be explained by the presence of a monomer and its aggregates in equilibrium (Gillespie and Lennox 1955*b*; Gillespie 1956) if the assumption is made that the polymers migrate faster than the monomers. The latter assumption is doubtful. The complex boundaries formed by β_1 -lactoglobulin on electrophoresis have been attributed to polymerization (Tombs 1957). In the case of this protein, phase rule solubility tests show it to be homogeneous whereas the purest wool protein prepared is heterogeneous by this test (Gillespie 1957). Hence it seems safer to conclude that the anomalous electrophoretic patterns arise from the heterogeneity of the materials although buffer ion interactions are not excluded as a contributing factor. Caution is needed in the interpretation of electrophoretic patterns of mixtures of components which interact since Colvin and Briggs (1952) have shown that the presence of reproducible shoulders or inflexion points in the schlieren diagrams is not always due to the presence of additional distinct components.

V. ACKNOWLEDGMENTS

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VI. REFERENCES

- ABRAMSON, H. A., MOYER, L. S., and GORIN, U. H. (1942).—"Electrophoresis of Proteins." (Reinhold: New York.)
- ALEXANDER, P., and HUDSON, R. F. (1954).—"Wool: Its Chemistry and Physics." (Chapman and Hall: London.)
- COLVIN, J. R., and BRIGGS, D. R. (1952).—*J. Phys. Chem.* **56**: 717.
- FITZGERALD, E. B., and FUOSS, R. M. (1954).—*J. Polymer Sci.* **14**: 329.
- FRIEND, A. J., and O'DONNELL, I. J. (1953).—*Aust. J. Biol. Sci.* **6**: 630.
- GILBERT, G. A. (1955).—*Disc. Faraday Soc.* **20**: 68.
- GILLESPIE, J. M. (1956).—Proc. Int. Wool. Text. Res. Conf. Aust. 1955. Vol. B. p. B-35.
- GILLESPIE, J. M. (1957).—*Aust. J. Biol. Sci.* **10**: 105.
- GILLESPIE, J. M. (1958).—*Biochem. Biophys. Acta* **27**: 225.
- GILLESPIE, J. M., and LENNOX, F. G. (1953).—*Biochem. Biophys. Acta* **12**: 481.
- GILLESPIE, J. M., and LENNOX, F. G. (1955*a*).—*Aust. J. Biol. Sci.* **8**: 97.
- GILLESPIE, J. M., and LENNOX, F. G. (1955*b*).—*Aust. J. Biol. Sci.* **8**: 378.
- GODDARD, D. R., and MICHAELIS, L. (1935).—*J. Biol. Chem.* **112**: 361.
- HARRAP, B. S., and WOODS, E. F. (1958).—*Aust. J. Chem.* **11**: 581.
- HENRY, D. C. (1931).—*Proc. Roy. Soc. Lond.* **A135**: 106.
- HERMANS, J. J., and FUJITA, H. (1955).—*Proc. Acad. Sci. Amst.* **B58**: 182.
- JOHNSON, P., and NAISMITH, W. E. F. (1953).—*Disc. Faraday Soc.* **13**: 98.
- JOHNSON, P., SHOOTER, E. M., and RIDEAL, E. (1950).—*Biochem. Biophys. Acta* **5**: 376.
- LINDLEY, H. (1955).—*J. Amer. Chem. Soc.* **77**: 4927.
- MARKUS, G., and KARUSH, F. (1957).—*J. Amer. Chem. Soc.* **79**: 134.
- O'DONNELL, I. J., and WOODS, E. F. (1956*a*).—*J. Polymer Sci.* **21**: 397.
- O'DONNELL, I. J., and WOODS, E. F. (1956*b*).—Proc. Int. Wool. Text. Res. Conf. Aust. 1955. Vol. B. p. B-71.

- OVERBEEK, J. H. G., and STIGTER, D. (1956).—*Rec. Trav. Chim.* **75**: 543.
- STRAUSS, M. P., WOODSIDE, D., and WINEMAN, P. (1957).—*J. Phys. Chem.* **61**: 1353.
- SVEDBERG, T. (1937).—*Chem. Rev.* **20**: 81.
- SVENSSON, H. (1946).—*Ark. Kemi. Min. Geol. A* **22**: 10.
- SWAN, J. M. (1957).—*Nature* **180**: 643.
- TOMBS, M. P. (1957).—*Biochem. J.* **67**: 517.
- WOODS, E. F. (1952).—*Aust. J. Sci. Res. A* **5**: 555.
- WOODS, E. F. (1958).—*J. Phys. Chem.* **62**: 308.

