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ELECTROPHORETIC STUDIES OF SILK

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ABSTRACT

A microelectrophoretic technique was used to determine the characteristic pH-mobility curves for fibroin and for sericin and to ascertain the extent of removal of the latter during degumming by the soap and enzyme processes. The data suggest that sericin contains a much greater proportion of reactive groups than fibroin and indicate the importance of obtaining either uniform or complete removal of sericin during degumming processes.

The isoelectric points are shown to vary slightly with ionic strength. In acetate buffers of 0.02 *M* ionic strength, the isoelectric point of fibroin is 3.6 and that of sericin, 4.3.

Large ions of complex structure, such as phthalate or picrate, appear to have specific effects which are readily detectable by the electrophoretic technique.

Dissolved fibroin adsorbed on glass particles and fibroin regenerated from similar solutions had the same isoelectric points but different pH-mobility curves. Fibroin regenerated after dissolving in solutions of lithium bromide gave the same pH-mobility curve as untreated fibroin.

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I. INTRODUCTION

One of the principal difficulties involved in defining many of the characteristics of silk results from the fact that the naturally occurring fiber consists of at least two proteins, namely, silk fibroin and silk sericin, which have very different properties. Since the latter is generally removed from the fiber by empirical methods, the available data on many of the properties of the fibroin may be of questionable validity, especially since ordinary chemical methods are inadequate for determining the extent of removal of the sericin.

Sericin is a soluble protein which appears to form a coating on the surface of the fibroin. It has been shown by Abramson and others that a number of surfaces, when coated with any one of several proteins, assume electrophoretic properties similar to those of the ad-

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sorbed materials; and it was felt, therefore, that an electrophoretic technique could be employed to advantage in studies of silk. Such an approach cannot only be used for studying the removal of sericin from fibroin but also has the additional advantage of contributing information relative to the amphoteric properties of the constituents of the naturally occurring fiber.

Electrophoretic techniques have been frequently used to investigate the properties of protein surfaces. Excellent discussions and reviews on the general subject have been presented by Abramson [1, 2],² and by Moyer [3].

One characteristic constant of a protein which is determined by its electrokinetic behavior is the isoelectric point. As defined by Abramson [1], the isoelectric state of a surface of a particle is that electrical state in which the sum of the positive and negative charges at the surface over a time average is equal to zero. The isoelectric point then is defined as the reference concentration of hydrogen ion or some other ion (Th^{++++} , for example) at which this condition is found. In other words, although it has become customary to define the isoelectric point in terms of the pH scale, it must be defined in terms which include a complete description of the medium in which no migration takes place. In the electrophoretic technique, the isoelectric point is, therefore, the point at which the electric mobility is zero.

It has been assumed by some investigators that the isoelectric state of a substance depends only on the state of its dissociable acid and basic groups (its combination with hydrogen or hydroxyl ions). Actually, however, it depends not only on the combination with hydrogen or hydroxyl ions but also with other anions or cations which may be in the solution.³ The point at which dissociable groups of the substance combine equally and only with hydrogen and hydroxyl ions is defined as the *isoionic point* [4]. It should be noted that this is identical with the isoelectric point only when the substance does not combine with ions other than hydrogen or hydroxyl. It follows then, that while it may be possible to determine the isoelectric point by several methods (for example, by titration, provided the substance binds *only* H^+ or OH^-), electrophoresis is the only method by which the isoelectric point is determined in every case.

The most satisfactory results with electrophoretic techniques have been obtained with soluble proteins in solution or adsorbed on inert surfaces such as quartz, glass, or paraffin oil. In many cases it has been found that a protein adsorbed on such a surface has electrokinetic properties which are very similar to those obtained for the same protein when studied in the dissolved state or in the form of relatively insoluble particles. It has also been noted by Abramson that the titration and mobility curves are congruent for a number of soluble proteins, and by making certain assumptions, he has predicted the following: In solutions of the same ionic strength, the electric mobility of the same protein at different hydrogen-ion activities should be directly proportional to the number of hydrogen or hydroxyl ions bound by each molecule. In the case of some of the soluble proteins, the isoelectric and isoionic points have been found to be approximately the same.

² Figures in brackets indicate the literature references at the end of this paper.

³ It should be noted that a surface may conceivably adsorb hydrogen or hydroxyl ions other than those bound by the acidic and basic groups.

Considerably more difficulty, however, is encountered in interpreting the results of investigations dealing with insoluble materials, especially those which are more or less crystalline in nature [5]. For example, in some materials of this type, the isoelectric and isoionic points have been found to be far apart. As a result, no attempt is made at this time to relate the data obtained to the specific acidic and basic properties of silk. However, it was found that the electrophoretic technique provided an excellent tool for distinguishing protein surfaces, and it was in this connection that it was used in the present investigation.

II. DESCRIPTION OF APPARATUS, METHODS, AND MATERIALS

An Abramson horizontal microelectrophoresis cell was employed in this investigation. The apparatus and methods were essentially the same as those described by Moyer [6]. The apparatus was checked from time to time by measuring the mobility of human erythrocytes in *M*/15 phosphate buffer at pH 7.4 [7]. A 28 \times ocular and 40 \times water-immersion objective were used for measurements involving soluble proteins adsorbed on glass particles. Particles of ground silk were observed with a 20 \times ocular and 20 \times objective.

In most cases, the mobilities were measured by observing the velocity during migration of the particles at the 0.21 and 0.79 depth levels of the cell, where the effect of the electroosmotic flow of the liquid is eliminated [6]. When the mobilities were very low, the velocities of the particles were measured in successive layers from the top to the bottom of the cell, and the mobility was evaluated by graphical integration of the curve obtained by plotting velocity against depth [1].

The majority of measurements were made on samples of raw and degummed silk which were reduced to a suitable particle size by cutting in a small, laboratory Wiley mill until the particles passed through a 60-mesh screen. A suspension of the particles in water was made and allowed to settle. The supernatant liquid was removed and found to contain particles approximately 1 to 5 microns in diameter.

The mobilities of dissolved silk or sericin were measured by adsorption of the protein on particles of Pyrex glass of 0.1 to 2 microns in diameter. The particles were thoroughly cleaned by washing with cleaning solution, water, concentrated hydrochloric acid, water, 0.001 *N* ammonium hydroxide, and then finally soaked for 3 days in distilled water, during which time the water was changed daily.

The mobilities of soluble materials were always measured in solutions containing a concentration of protein sufficient to insure complete coating of the glass particles. The required concentration was determined by measuring the mobility as a function of concentration of protein at constant pH and determining the point beyond which further increase in protein concentration caused no further change in the mobility of the glass particles.

All mobility measurements were made in a room at approximately 25° C. All values were corrected to 25° C by applying a factor of 2 percent per degree [6].

The pH values of the suspensions were measured with a MacInnes and Belcher type glass electrode and an electron ray meter. The pH values were referred to potassium acid phthalate (0.05 *M*) which was assigned a pH value of 4.01 [8].

The silk used was the product of the silkworm *Bombyx mori*.

III. EXPERIMENTAL

1. ELECTROPHORETIC PROPERTIES OF RAW SILK

Curve 1 in figure 1 shows the mobilities as a function of pH for three different samples of raw silk. The mobilities were measured after overnight immersion of the particles in hydrochloric acid-potassium chloride (pH range of 1.7 to 3.0) or acetic acid-sodium acetate (pH range of 3.0 to 6.5) solutions of ionic strength 0.02. Since

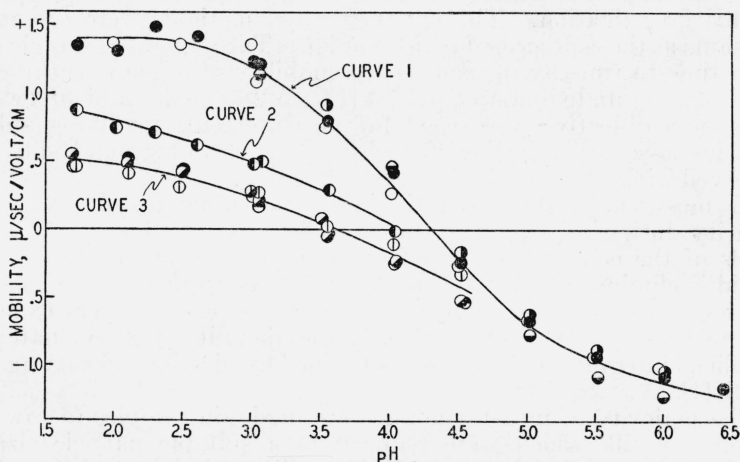


FIGURE 1.—A comparison of the pH-mobility curves for raw and degummed silks.

Curve 1 represents the mobilities for three different samples of raw silk, ●, ○, and for sericin adsorbed on glass particles, ○. Curve 2 is that for silk degummed for 2 hours with soap. Curve 3 represents the results for 1, 2, and 3 cycles of degumming treatment as listed in the text.

sericin is relatively soluble, it was expected that some of the material would dissolve in this time and then completely coat the particles of cut-up silk. That curve 1 actually represents the mobility of sericin adsorbed on silk was demonstrated by the following experiments.

A sample of sericin was obtained by boiling 10 g of natural silk in two 150 ml portions of water for 15 minutes each. One drop of a suspension of Pyrex particles was then added to each of a series of solutions of the same pH but containing varying amounts of sericin. The mobilities of the glass particles were measured after allowing 15 minutes for adsorption. The two curves, shown in figure 2, were obtained by plotting mobility as a function of sericin concentration and indicate that complete coating of the glass particles by sericin occurs in solutions containing as little as 0.0001 percent of sericin. The mobility of sericin adsorbed on glass was then measured as a function of pH. The solutions used contained approximately 0.001 percent of the protein. The results as shown by the open circles in

curve 1, figure 1, indicate that the pH-mobility curve for the adsorbed sericin is identical with that obtained for the sericin on the silk fibers. The data also indicate that the sericin is isoelectric at pH 4.3.

2. ELECTROPHORETIC PROPERTIES OF DEGUMMED SILK

(a) SOAP DEGUMMING

Fifty grams of natural silk was boiled successively in four 2-liter portions of a 1-percent solution of soap for a total time of 2 hours, then extracted with cold alcohol and ether, and finally washed twice with water at 50° C. Curve 2 (fig. 1) is the pH-mobility curve for the degummed sample. After the above degumming treatment, some of

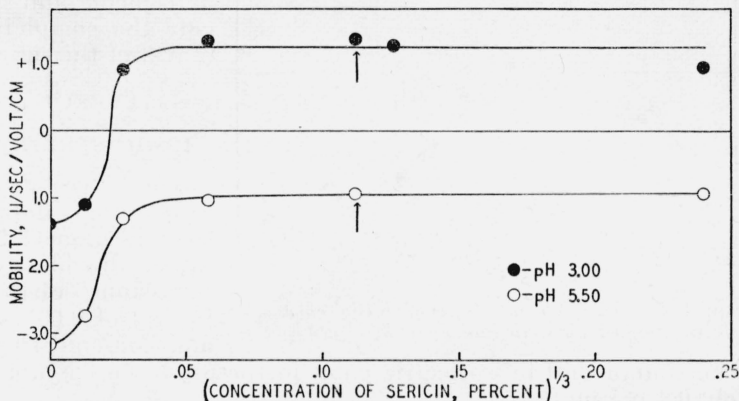


FIGURE 2.—Mobility as a function of concentration of sericin at pH 3.0 and pH 5.5 in 0.02 M ionic strength buffers.

the fibers were shaken with a hydrochloric acid-potassium chloride solution (ionic strength 0.02) of pH 1.77 containing some glass particles. It was found that an amount of sericin sufficient to alter the mobility of the glass particles was still present.

In an attempt to remove all of the sericin from the fibers, 10-g samples of the silk were subjected to successive cycles of the following treatments: a boil-off in 2-liter portions of a 1-percent solution of soap for three successive $\frac{1}{2}$ -hour periods, a 5-minute boil in 2 liters of water, a 5-minute rinse in 2 liters of 0.1 N ammonium hydroxide, a 5-minute rinse in 2 liters of 0.1 N hydrochloric acid, a 1-day wash in running distilled water, a 4-hour extraction with cold alcohol, and finally three successive rinses in distilled water at 50° C.

Curve 3, figure 1, shows results for samples subjected to one, two, and three cycles of the above series of treatments. No measurable change was produced after the first treatment, which appears to indicate that the samples were completely degummed. Curve 3 therefore represents the pH-mobility curve for fibroin and indicates that the material is isoelectric at pH 3.6 in 0.02-ionic-strength acetate buffers.

(b) ENZYME DEGUMMING

A 10-g sample of silk was treated with 1 liter of a solution containing 20 ml of Serizyme at 50° C for a total of about 500 hours. The enzyme solution was changed three times during the first 100

hours of treatment. Removal of sericin, as measured by loss in weight and nitrogen determinations, was rapid during the first 50 hours of treatment. The fibers continued to lose weight during the first 400 hours but appeared to reach a constant value after that time.

Figure 3 shows the results of pH-mobility measurements on the sample in hydrochloric acid-potassium chloride and acetic acid-sodium acetate solutions (ionic strength 0.005).⁴

Similar measurements, made at the same ionic strength on a sample of which had been completely degummed by the soap boil-off method,

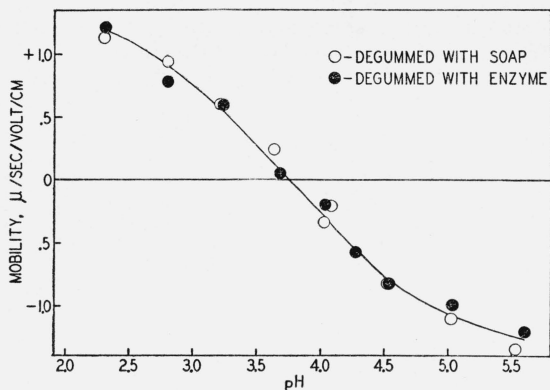


FIGURE 3.—A comparison of pH-mobility curves for silk degummed by soap and enzyme treatments.

The maximum shift in isoelectric point in these solutions is approximately 0.4 pH unit.

Figure 5 represents similar curves at 0.005 and 0.02 ionic strength for dissolved fibroin coated on glass particles. The material was prepared as follows: 0.5 g of degummed fibroin was heated in 5 ml of a 50-percent solution of lithium bromide (sp gr 1.53) on the steam bath for 5 minutes. The solution was dialyzed (specific resistance approximately 30,000 ohms), coated on glass particles, and the mobilities were measured. The curves show that the same relation between mobility and ionic strength holds for the dissolved fibroin as for the solid material.

4. SPECIFIC ION EFFECTS

The isoelectric points (3.5 to 3.9) obtained in the present investigation are higher than that (2.5) previously reported by Harris [9]. The differences appear to be too large to be accounted for by the differences in ionic strengths used in the two investigations. However, it was noted that hydrochloric acid-potassium acid phthalate buffers were used in the earlier investigations, which suggested that the differences might result from specific ion effects.

The effect of different ions in shifting the point of maximum flocculation and other protein characteristics has long been recognized [10, 11]. This is especially true for polyvalent ions [12]. Figure 6 shows the results of pH-mobility measurements in 6 different types of solutions on fibroin degummed by the soap boil-off method. All of the buffers except the phthalate were of ionic strength 0.02. The phthalate solutions varied in ionic strength from 0.05 to 0.10.

⁴ The ionic strength was decreased from 0.02 used previously to 0.005 in order to facilitate measurements by increasing the mobilities.

3. EFFECT OF VARIATION OF IONIC STRENGTH

The curves in figure 4 represent data for soap-degummed fibroin in hydrochloric acid-potassium chloride buffers of 0.005, 0.02, and 0.05 ionic strength.

Curve 2 represents the data obtained in the hydrochloric acid-potassium chloride, acetic acid-sodium acetate, and sulfuric acid-potassium sulfate solutions. In these systems the samples were isoelectric at about pH 3.6, although there may have been slight

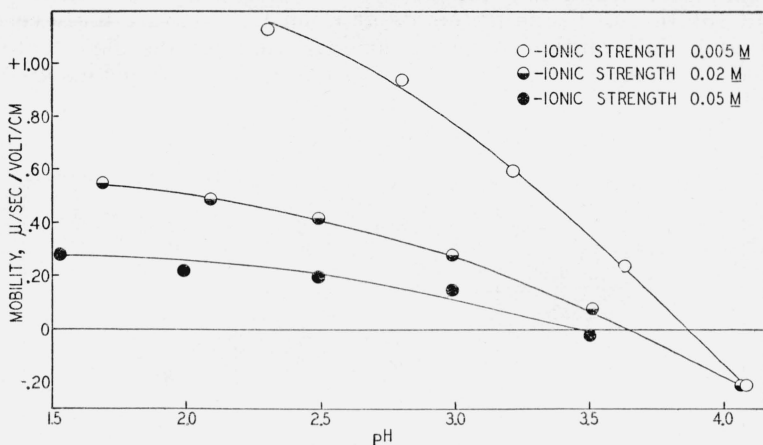


FIGURE 4.—Effect of variation of ionic strength on the pH-mobility curve of degummed silk.

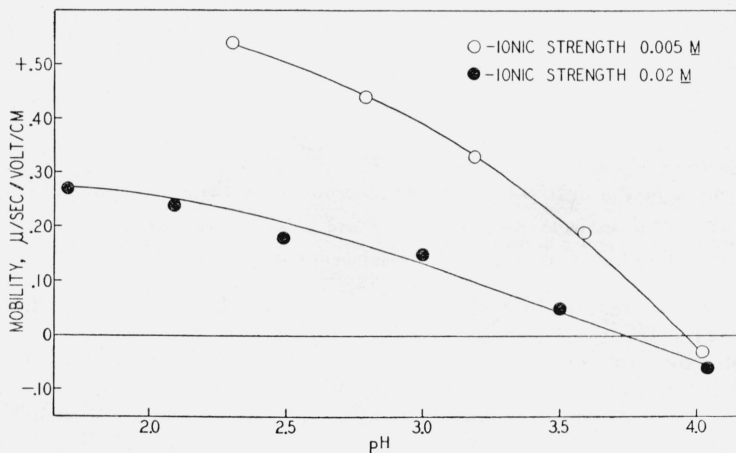


FIGURE 5.—Effect of variation of ionic strength on the pH-mobility curve of dissolved fibroin adsorbed on glass particles.

divergences between the different valence types. The phthalate buffers (curve 3) caused a shift in the isoelectric point of approximately 1 pH unit to pH 2.6, in agreement with the data of Harris.⁵ Oxalic acid causes a smaller shift in the opposite direction (curve 1). In the picrate solutions (curve 4) the sample was so electronegative that it no longer exhibited an isoelectric point in the pH range used.

⁵ The results for phthalate solutions (Clark's buffers) are not directly comparable with those for the other buffer types because of the difference in ionic strengths. However, the data given above for fibroin in solutions of different ionic strengths indicate that only a minor portion of the shift is caused by this factor. This is confirmed by the fact that a fivefold decrease in the ionic strengths of Clark's buffers causes an increase of only about 0.3 pH unit in the isoelectric point of wool [16].

5. EFFECT OF STATE OF AGGREGATION

Silk is readily dissolved by concentrated solutions of certain neutral salts [13, 14, 15]. With some salts, such as lithium bromide, solution is accomplished at room temperature.

A sample of degummed silk was dissolved by shaking with a 50-percent solution of lithium bromide at room temperature for several hours and the solution dialyzed against running distilled water. After about 2 days a precipitate was formed. Microscopic examina-

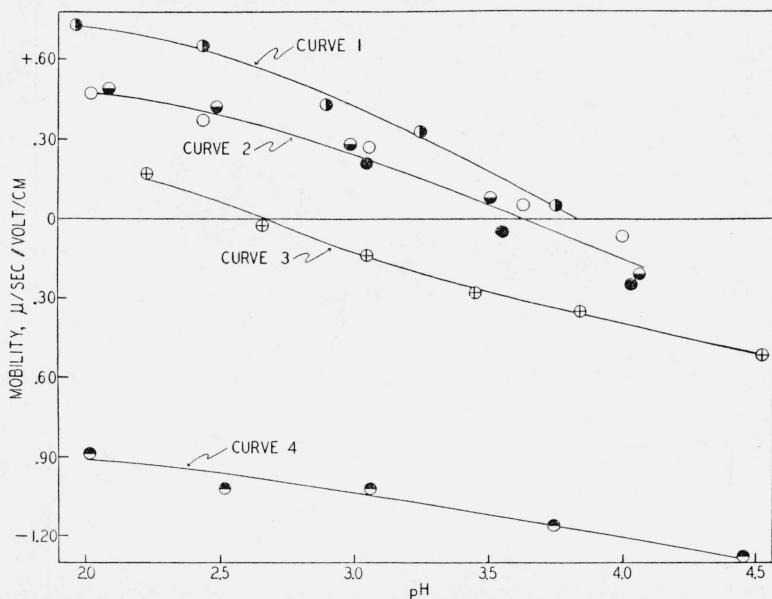


FIGURE 6.—Effect of different ions on the electrophoretic mobility of degummed silk.

Curve 1, oxalic acid-potassium hydroxide solutions. Curve 2, solutions of hydrochloric acid-potassium hydroxide, \ominus ; sulfuric acid-potassium hydroxide, \circ ; and acetic acid-sodium hydroxide, \bullet . Curve 3 Clark's phthalate buffers. Curve 4, picric acid-potassium hydroxide solutions.

tion of the material showed it to be made up of well-defined fibers which were strongly birefringent.

When the fibroin was dissolved by heating in a lithium bromide solution on a steam bath, spontaneous formation of a fibrous material did not occur during subsequent dialysis. On addition of ether, a precipitate which appeared to be somewhat fibrous and of low-order birefringence was obtained.

The pH-mobility curves in solutions of 0.005 *M* ionic strength for both samples of regenerated fibroin as well as for the dissolved materials coated on glass particles are given in figure 7.

IV. DISCUSSION

A comparison of the curve for sericin (curve 1, fig. 1) with that for fibroin (curve 3, fig. 1) shows that the mobility of the former is much greater, which strongly suggests that sericin contains a much greater proportion of the reactive carboxyl and basic amino groups. This has recently been confirmed by chemical analyses [16]. The practical significance is at once obvious. Many of the problems related to silk

and silk processing are in part, at least, concerned with the surface characteristics of the fiber. For example, the presence of a small amount of sericin may have a profound influence on the dyeing properties, and uneven distribution of the residual sericin would probably result in uneven dyeing of the fibers. It is not within the scope of the present investigation, however, to do more than point out some of the implications of these electrophoretic studies. The relationship of the difference in chemical properties between fibroin and sericin to the more practical aspects of silk chemistry is being studied separately [16].

These specific ion effects noted in figure 4 indicate that considerable care must be exercised in choosing buffer systems for protein investigations. These data also emphasize the necessity of including in the definition of the isoelectric point a description of the medium in which no migration takes place.

Sufficient data are not available for the elucidation of the nature of specific ion effects. In general, it appears that the greatest anomalies arise when large ions of complex structure, such as phthalate or picrate, are used. The results suggest a number of possible explanations. For example, the acid of the buffer solution might react with basic groups of the fibroin to form a slightly ionized compound; the anion might react with or be adsorbed by the fiber in such a manner as to allow one of the acid groups of the adsorbed ion to be free; the strength of the acid or basic groups in the fiber might be altered by the proximity of the polar groups of the anion; or the adsorbed anion might provide charged centers which might even permit the adsorption of other ions.

It is well known that picric acid forms compounds with many proteins. Silk undoubtedly also forms picrates as evidenced by the color of the fibers. In the case of colorless ions such as phthalate ions, however, similar reactions are not at once obvious. The electrophoretic technique accordingly may often prove useful as a qualitative indication of such reactions.

Examination of the curves in figure 7 reveals a number of points of interest. The dissolved fibroin, adsorbed on glass particles (curve 2), exhibits lower mobilities than the untreated or regenerated fibroin (curve 1) throughout the pH range tested. The mobilities of the undissolved fibroin were roughly twice those of the dissolved material adsorbed either on glass or quartz particles. These data are in disagreement with earlier data by Harris [9] in which it appeared that the

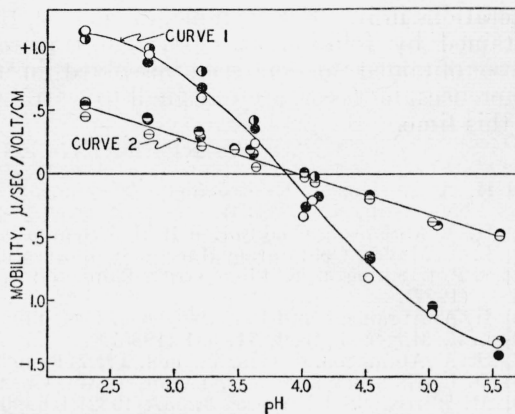


FIGURE 7.—Effect of state of aggregation on the electrophoretic mobility of fibroin.

Curve 1 represents the mobilities of degummed fibroin, ○; fibroin regenerated after solution in hot lithium bromide, ●; and fibroin regenerated after solution in lithium bromide at room temperature, ●. Curve 2 is for dissolved fibroin adsorbed on glass. The solutions were obtained by heating fibroin in solutions of lithium bromide for 5 minutes, ●, and for 2 hours, ●, and by solution of fibroin at room temperature, ○.

mobilities were the same. The present work, however, indicates that the solutions used previously were too concentrated and, as a result, the majority of particles appearing in the solution were those of regenerated fibroin. These, as shown (solid and half-solid circles, curve 1), give a pH-mobility curve which is identical with that of untreated fibroin (open circles, curve 1).

Although only one curve is drawn to represent the points for the solid fibroin and one for the points for the dissolved material, it appears that dissolving fibroin in hot solutions may have produced slight alterations in it. For example, in curve 2, the points for the material obtained by solution at room temperature are consistently below those obtained for material dissolved in the hot solutions. The differences, however, are too small to warrant any further conclusions at this time.

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