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Determination of the Molecular Mass of Silk Fibroin Using the Method of Spectrophotometry

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ABSTRACT: The molecular mass of silk fibroin was determined by osmometry, ultracentrifugation, SDS-PAGE methods.One of the most convenient ways to determine the molecular masses of high-molecular compounds is the viscometry method. However, this method is not widely used to determine the molecular mass of fibroin. This is because the coefficients of the Kuhn–Mark–Houwink equation are necessary to determine the molecular mass of fibroin in this way, and there is almost no reliable literature on these coefficients for fibroin. We propose a spectrophotometry method to determine the molecular mass of silk fibroin. Experiments to determine the molecular mass revealed 275 nm wavelength absorbance of aqueous solutions of silk fibroin at different concentrations. A graph of absorbance and concentration dependence was drawn. Using the tangent value of the angle determined for the concentration axis of the straight line obtained in the graph, it was determined that the molecular mass of silk fibroin was 411,7kDa.

Keywords: Silk fibroin, UV spectrophotometry, Molecular mass, Dialysis.

INTRODUCTION

Fibroin is a fibrillar protein that forms the polymer basis of natural silk. The macromolecule of silk fibroin is characterized by a conformational variety, that is, it consists of ɑ-spiral (amorphous) and β–structural (crystalline) parts, in which the polymer chain is located consecutively. The sequence of amino acid groups in the crystal parts of the fibroin macromolecules is structured in the following order [1].

$$-(Gly-Ala)_2-Ser-Gly-Ala-Ala-Gly-[Ser-Gly-(Ala-Gly)_2]_3-Tyr-$$

The composition of amino acids in the amorphous part is determined in the following

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order.

– Gly – Val – Gly – Ala – Tyr – Gly – Ala –

The fiber in the silk of the silkworm (Bombyx mori) consists of three chains: a heavy chain, a light chain, and a glycoprotein P-25. Light chain(26 kDa) and heavy chain (390 kDa(heavy chain can be up to 500 kDa)) are available in a 1:1 ratio, connected by a disulfide chemical bond. Glycoprotein (25 kDa), called P-25, is not associated with covalent bonds with heavy and light chain proteins[2]. The structure and weight of the molecule of silk fibroin are also associated with the feeding conditions of the silkworm, the region in which silk is grown.

In the literature, Holmes and Smith have reported that the average molecular mass for fibroin by ultracentrifugation and diffusion methods is 60–150 kDa (average 84 kDa) [3]. Coleman and Howitt measured the osmotic pressure and found that the molecular mass of silk fibroin was 30 kDa [4].

The molecular mass determined by the SDS-PAGE method in a 9,3 M LiBr solution of silk fibroin is up to 250 kDa [5]. When silk fibroin was studied by the SDS-PAGE method in CaCl2-ethanol solution, its molecular mass was found to be 300 kDa [6].

To determine the molecular mass of silk fibroin, it is necessary to dissolve it. In solutions, the molecules of high-molecular compounds occur in different spatial states. In concentrated solutions, macromolecules intertwine to form a twisted shape. In a dilute solution, the shape of the molecules changes to a linear state [7].

One of the most convenient ways to determine the molecular masses of high-molecular compounds is viscometry. However, this method is not widely used to determine the molecular mass of fibroin. This is because the coefficients required to solve the Kuhn–Mark–Houwinkequation to determine the molecular mass of fibroin in this method are required, and the exact literature on these coefficients for fibroin is almost non-existent.

We focused our research on developing a method for determining the molecular mass of fibroin. In our study, we used a spectrophotometry method to determine the molecular mass of fibroin.

EXPERIMENTAL SECTION

Materials

Fibrous waste of silk (Cleaned of additives.Khorezmipagi LLC, Urgench,Uzbekistan), Sodium carbonate (purity 99,9%), Calcium chloride and ethyl alcohol(98%) were purchased from Fortek company (Uzbekistan).

Instrumentation

Bidistilled water is obtained from the “GFL 2104 Double distillation water still” device

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(Germany). The experiments used UV-1800 Shimadzu spectrometer, high-speed microcentrifuge (D2012 PLUS-DLAB Scientific Co-"Fortek" Company), Ultrafiltration Discs,10kDa NMW (Merck KGaA, Darmstadt, Germany).

Procedure

Determination of the measured absorbance of aqueous solutions of silk fibroin

To determine the molecular mass of silk fibroin by spectrophotometry, fibroin is first converted to a soluble state. A solution of $CaCl_2$:H_2O:C_2H_5OH in a ratio of 1:2:8 mol was used as a precipitator[8]. The prepared fibroin saline solutions were dialyzed using a 10 kDa membrane to remove ions and alcohol. Dialysis was performed until the ions in the solution were completely removed. Different concentrations of fibroin solution obtained by dialysis: $5\cdot10^{-5}$, $7\cdot10^{-5}$, $1\cdot10^{-4}$, $2\cdot10^{-4}$, $3\cdot10^{-4}$, $3{,}6\cdot10^{-4}$, $4\cdot10^{-4}$, $4{,}3\cdot10^{-4}$, $4{,}6\cdot10^{-4}$, $5\cdot10^{-4}$, $6\cdot10^{-4}$, $7\cdot10^{-4}$ $8\cdot10^{-4}$, $8{,}6\cdot10^{-4}$, Aqueous solutions with concentrations of $5\cdot10^{-3}$ and 10^{-2} g/cm^3 were prepared.

UV-1800 Shimadzu spectrophotometer obtained UV spectra of prepared fibroin solutions of different concentrations and determined the absorbance [9]. Solutions of silk fibroin showed the maximum absorption wavelength of UV spectra in the region of 275 nm. (Figure 1).

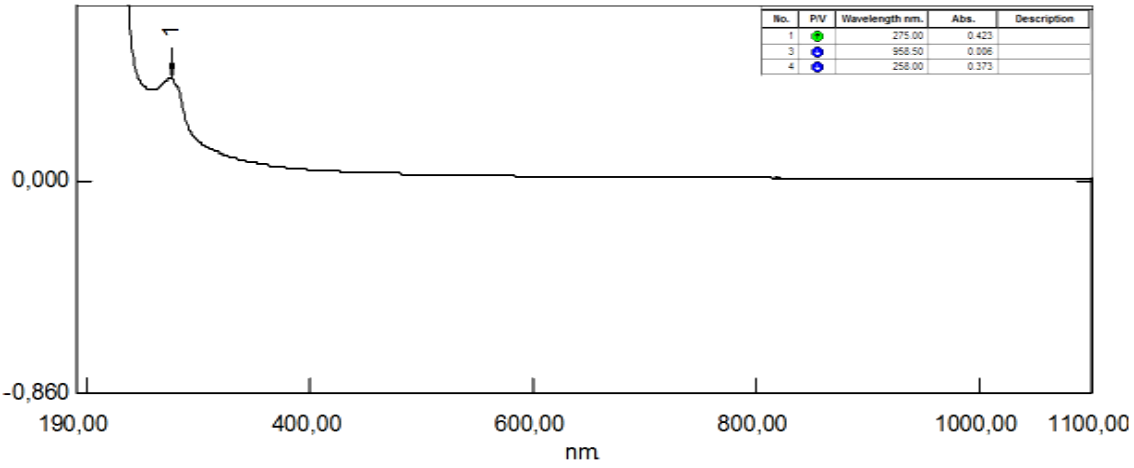

Figure 1. UV spectrum of silk fibroin

$5\cdot10^{-5}$, $7\cdot10^{-5}$, $1\cdot10^{-4}$, $2\cdot10^{-4}$, $3\cdot10^{-4}$, $3{,}6\cdot10^{-4}$, $4\cdot10^{-4}$, $4{,}3\cdot10^{-4}$, $4{,}6\cdot10^{-4}$, $5\cdot10^{-4}$, $6\cdot10^{-4}$, $7\cdot10^{-4}$, $8\cdot10^{-4}$, $8{,}6\cdot10^{-4}$, $5\cdot10^{-3}$ and 10^{-2} g/cm^3 the absorbanceof fibroin solutions with a concentration of were determined.

RESULTS AND DISCUSSION

Drawing a calibration graph of the "concentration-absorbance" relationship for an aqueous solution of silk fibroin

Based on the obtained results, a calibration graph of the "concentration-absorbance" relationship was drawn (Figure 2). This post calibration graph was intended to be used in

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subsequent studies to determine the concentrations of aqueous solutions of fibroin.

The spectrophotometry method was chosen to determine the molecular mass of fibroin obtained from silk fiber waste, and the calculations were performed according to the basic formula of Beer-Lambert–Bouguerlaw $A = \varepsilon \cdot C \cdot l$.

The optical density, according to the Beer-Lambert–Bouguer law, depends on the molar absorption coefficient of the solution of the test substance, the concentration of the solution and the thickness of the absorption layer.

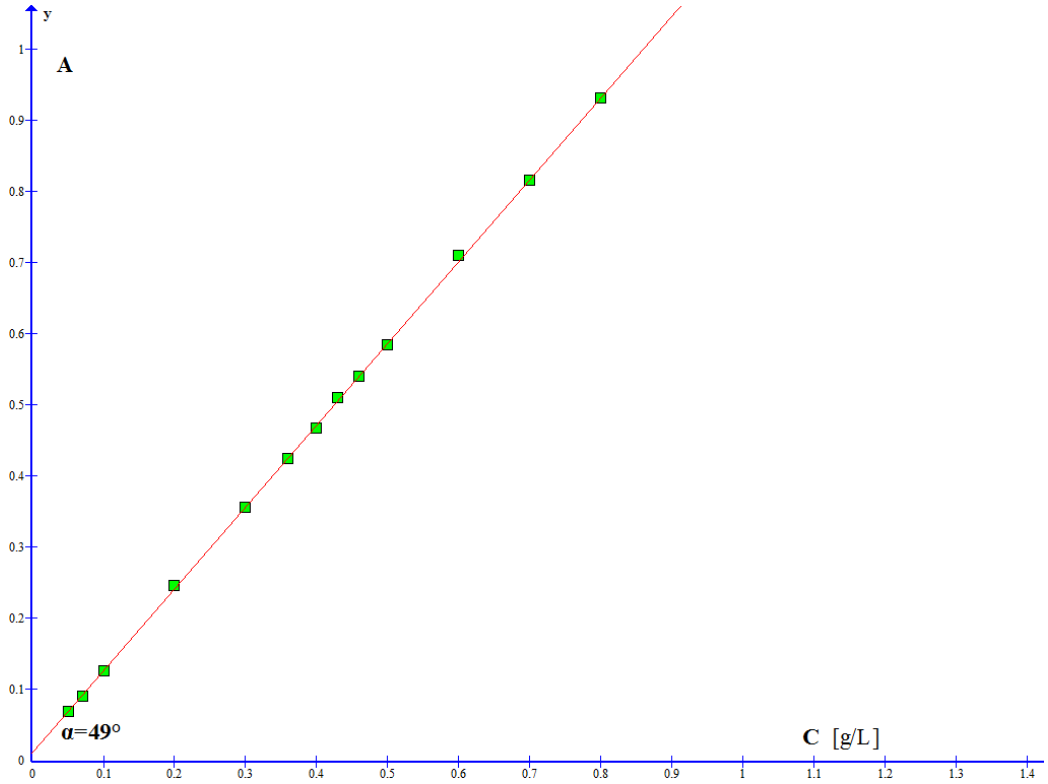

Figure 2. The graph of dependence of absorbance on the concentration of silk fibroin solution in aqueous solution

The molar absorption coefficient of the substance varies from 1 to 10^5. As a result of the calculations, it is known that the molar absorption coefficient is 10^5, the minimum value of optical density is 10^{-2}, and the thickness of the conductive layer is 1 cm for most analytical measurements. Accordingly, the sensitivity of spectrophotometry measurements is 10^{-7}M [10]. The studied absorbance values and concentrations of fibroin solutions derived from silk fiber waste in our studies fully meet the above requirements.

Determination of the molecular mass of silk fibroin using the method of UV spectrophotometry

Subsequent calculations were performed to derive the molecular mass equation of fibroin.

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The equation for determining the molecular mass is given as follows.

In this case, we take into account that the molar concentration C in the formula $A = \varepsilon \cdot C \cdot l$ is expressed as follows:

$$C = \frac{n}{V} \cdot 1000 \quad (1)$$

where: n- is the molar amount of solute; V-solution volume. Knowing that the molar amount of solute is $n = \frac{m}{M}$, the molar concentration formula is given by:

$$C = \frac{m}{M \cdot V} \cdot 1000 \text{ or } C = \frac{C^{/}}{M} \cdot 1000 \quad (2)$$

where we consider that $C^{/}$=m/V, and obtain the following expression: $C^{/}$- concentration of fibroin solution, in g/cm^3 units;

$$C = \frac{C^{/}}{M} \cdot 1000 \quad (3)$$

If we put expression (3) in the equation of Beer-Lambert–Bouguer law, we get the following expression.

$$A = \frac{\varepsilon \cdot C^{/} \cdot 1000 \cdot l}{M} \quad (4)$$

We use the resulting equation (4) to determine the molecular masses of high molecular weight compounds:

$$M = \frac{\varepsilon \cdot C^{/} \cdot 1000 \cdot l}{A} \quad (5)$$

where: molecular mass of M-fibroin-g/mol; Concentration of $C^{/}$ -fibroin in g/cm^3 units.

To determine the molecular mass of fibroin using equation (5), solutions of different concentrations of the sample were prepared. In this case, fibroin: $5 \cdot 10^{-5}$, $7 \cdot 10^{-5}$, $1 \cdot 10^{-4}$, $2 \cdot 10^{-4}$, $3 \cdot 10^{-4}$, $3{,}6 \cdot 10^{-4}$, $4 \cdot 10^{-4}$, $4{,}3 \cdot 10^{-4}$, $4{,}6 \cdot 10^{-4}$, $5 \cdot 10^{-4}$, $6 \cdot 10^{-4}$, $7 \cdot 10^{-4}$, $8 \cdot 10^{-4}$, $8{,}6 \cdot 10^{-4}$, $5 \cdot 10^{-3}$ and 10^{-2} g/cm^3 concentrated aqueous solutions were prepared and research work was carried out to determine the molecular masses of the samples. Based on the data obtained from the experiment, a graph of the “molecular mass-concentration” relationship between the molecular masses and concentrations of solutions of silk fibroin was created (Figure 3).:

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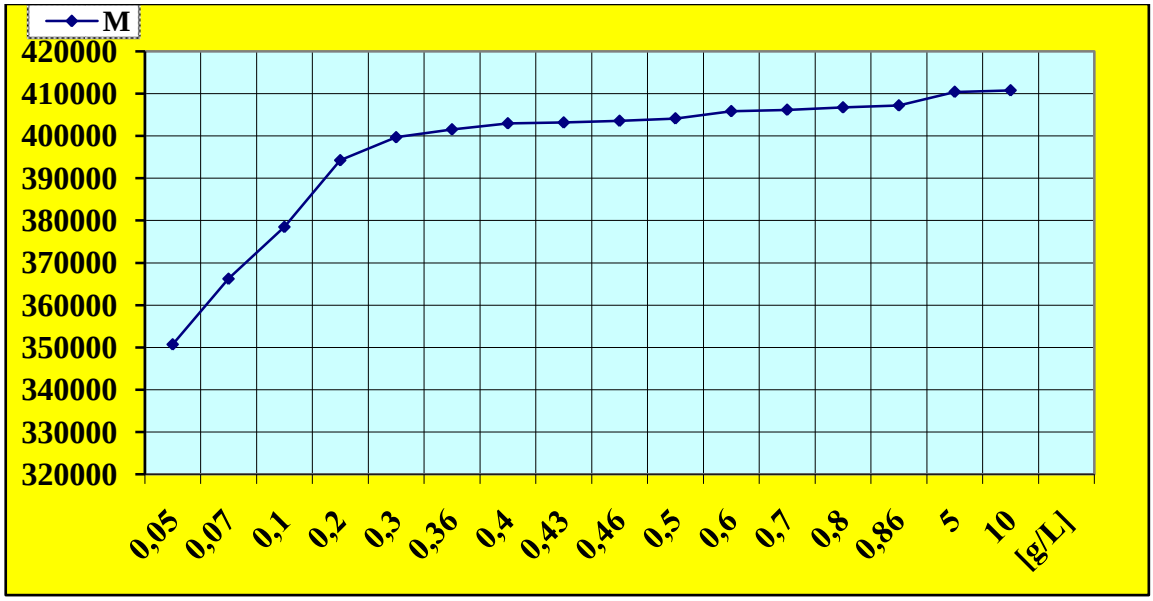

Figure 3. The molecular mass-concentration dependence graph

From the graph, it can be seen that the molecular masses of fibroin increase as the solution concentration increases. In this case, initially, the relationship between the solution concentration and the molecular mass in highly dilute solutions of the samples is directly proportional to each other, and this relationship is observed up to a concentration of $3{,}6\cdot10^{-4}$ g/cm^3 of the sample solution. In a solution of fibroin with a concentration of $4\cdot10^{-4}$ g/cm^3, a proportional relationship between molecular mass and concentration is not observed, and the graph shows that the line in this area approaches a straight line.

In subsequent studies, the relationship between $\frac{A}{l}$ and C$^{/}$ was studied to draw a definitive conclusion about the molecular mass of silk fibroin [11], and based on these calculations, an "$\frac{A}{l}$-C$^{/}$" bond graph was drawn. From the experiments, the thickness of the l-beam layer was obtained as 1 cm, the correlation graph of "$\frac{A}{l}$-C$^{/}$" corresponds to the graph of dependence of "A-C$^{/}$". The angle between the line of the graph and the concentration axis was found to be 49˚.

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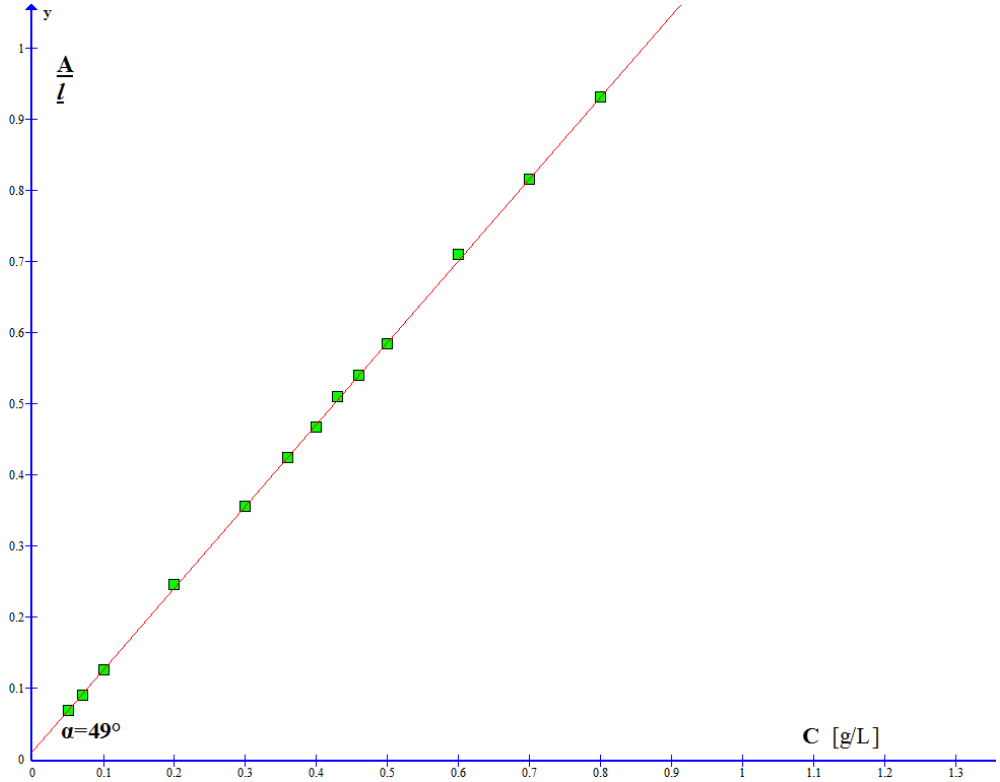

Figure 4. "A/l- C/" dependence graph

The tangent tgα of the angle value determined from the graph in figure 4 was determined. Using the value of tgα, the molecular mass of silk fibroin is found from the following equation:

$$M = \frac{\varepsilon}{tg\alpha} \quad (5)$$

According to the calculations in the study, the molecular mass of silk fibroin was found to be 411721 g/mol (411,7kDa) using equation (5).

Subsequent studies have focused on the effect of temperature on the molecular mass determined value by spectrophotometry method. Experiments were performed to determine the molecular mass of solutions at different temperatures, and the molecular mass of the samples at different temperatures was determined. In this case, the molecular mass of fibroin determined by spectrophotometry methods of solutions at temperatures of 20, 30, 40, 50, 60˚C remains unchanged (411,7kDa). No changes were observed in the results obtained in the molecular mass of fibroin, no significant effect of temperature was observed in the determination of molecular mass by spectrophotometry.

CONCLUSION

Thus, using the proposed spectrophotometry method to determine the molecular mass of silk fibroin allows the simultaneous determination of the solution concentration and the molecular

mass of fibroin. Spectrophotometry does not require the preparation of dilute solutions to determine the molecular mass of fibroin, the method is recommended for use in solutions with a concentration of $4 \cdot 10^{-4}$ g/cm^3. Temperature changes do not significantly affect the detection method. The method is superior to other methods of determining the molecular mass of silk fibroin in terms of speed, convenience, and accuracy.

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