Physicochemical properties of graft copolymers of collagen and fibroin with polyacrylic acid

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Abstract. Certain physicochemical properties of the synthesized graft copolymers of raw skin collagen and natural silk fibroin with polyacrylic acid have been determined. The dependence of the solubility, density, and thermal properties of copolymers on the ratio of components and synthesis conditions has been established.

1. Introduction
A graft copolymerization is of particular importance as a method for the synthesis of composite high-molecular substances and materials among the various methods of joint polymerization [1, 2]. Establishing the regularities of obtaining, physicochemical and mechanical properties of grafted copolymers of natural polymers with functionally active monomers, taking into account the specific properties of polymer compositions, is an urgent problem in the chemistry and technology of polymers. Studies related to the regularities and mechanism of the synthesis of graft copolymers and the production of reinforced films based on collagen and polyacrylic acid have been carried out in the past [3].
The present work is devoted to the determination of interdependence of solubility, density and thermal properties of copolymers on the ratio of components and synthesis conditions, processes occurring during heating of copolymers.
The composition for flame retardant treatment of cellulosic textile materials is proposed. The composition comprises an acrylic emulsion as a film former and a binder, collagen as a binder and flame retardant component, boric acid and potassium persulfate for chemically binding the components to a textile material [4]. A technological scheme for applying the composition to the material has been developed. The processes that occur when the temperature was applied to
the treated material were studied and graft copolymerization of acrylic monomers with collagen and cellulose was carried out [5]. Based on the results of IR and PMR spectroscopic studies of collagen, cellulose, initiator, monomers and graft copolymer, a mechanism for interaction of the components was proposed. In the interaction of potassium persulfate with collagen and cellulose, radical active centers are formed, through which the grafted polymer chains are initiated and grown [6-8]. Active polymerization centers are formed in the nitrogen atom of the peptide group of collagen and in the oxygen atom of the hydroxyl group of cellulose. The obtained material was tested for fire resistance: the material was not ignited when exposed to combustion for 10 s; the mass loss was 4-8% [9, 10].

2. Materials and Methods

The object of research is collagen obtained from animal skin waste, natural silk, acrylic acid (AA), potassium persulfate (PC). The synthesis of the grafted collagen copolymer was carried out in a three-necked flask equipped with a stirrer, thermometer and reflux condenser in a nitrogen flow [11]. Collagen solution, acrylic acid (AA), and PC were loaded into a flask and stirred in a thermostat. Fibroin of natural silk was freed from sericin by decoction in a soap-soda solution. The synthesis of the graft copolymer of fibroin was carried out in ampoules at a constant temperature [12]. The calculated amount of fibroin fibers preliminarily kept in a solution of PC, AA, and solvent was loaded into an ampoule, purged with an inert gas, sealed, and placed in a thermostat. Upon reaching a certain time, the flask or ampoule was removed from the thermostat, cooled to a room temperature, and a reaction mixture was poured into a glass with ethanol [13]. Residual monomer and homopolymer dissolve in ethanol, but the copolymer does not dissolve. The resulting copolymer was washed several times with ethanol and dried to constant weight in a desiccator [14].

A density of collagen and polyacrylic acid (PAA) copolymers has been determined by pycnometric method, thermal properties were investigated by differential thermal analysis (DTA) in the range 0-900 °C. A copolymer of fibroin with PAA was studied by differential scanning calorimetry (DSC). Thermoanalytical studies of copolymers were carried out on a Netzsch Simultaneous Analyzer 409 PG (Germany) instrument, with a K-type thermocouple (Low RG Silver) and aluminum crucibles. All measurements were carried out in an inert nitrogen atmosphere with a nitrogen flow rate of 50 ml/min. The temperature range of measurements was 0-560 °C, the heating rate was 10K/min. The amount of sample per measurement is 20-30 mg. The measuring system was calibrated with a standard set of substances KNO₃, In, Bi, Sn, Zn, and CsCl [15].
3. Results and Discussion

A collagen copolymer with acrylic monomers is a yellowish plastic mass that swells in water and organic solvents. At a conversion rate of up to 20-25%, the resulting product is soluble in water and dimethylformamide, insoluble in ethanol, dioxane, acetone, hydrocarbons. The density of the product is influenced by both the composition of the copolymer and the conditions for its production (see Table 1).

According to the table, with an increase in the share of PAA and PC concentration, a density of the copolymer decreases. This is due to two factors: an emergence of a three-dimensional structure at high PAA contents and an increase in the frequency of intermolecular bonds; an increase in the proportion of polymer with a lower density.

Table 1. An interdependence of density of grafted collagen copolymers on composition and required conditions for its preparation

| Collagen composition | PC concentration during synthesis, mol \cdot \text{L}^{-1} \cdot \text{10}^{-2} | Synthesis temperature °C | Density, g \cdot \text{ml}^{-1} |
|----------------------|---------------------------------|---------------------------|-----------------------------|
| Collagen PAA         |                                 |                           |                             |
| 88 12                | 0.38                            | 60°C                      | 1.138                       |
| 74 26                | 1.12                            | 60°C                      | 1.125                       |
| 65 35                | 1.87                            | 60°C                      | 1.117                       |
| 57 43                | 1.12                            | 60°C                      | 1.112                       |
| 74 26                | 1.12                            | 50°C                      | 1.128                       |
| 74 26                | 1.12                            | 70°C                      | 1.123                       |

A compaction of the copolymer indirectly indicates the formation of a network structure with a sufficient degree of conversion and a sufficient content of synthetic polymer. Differential thermal curves of collagen, a copolymer of collagen with PAA, were taken. The nature of the curves of thermogravimetry (TG), DTA and differential thermogravimetry (DTG) is determined mainly by the collagen component.

The loss of the collagen mass sample and its copolymer with AA begins at temperatures above 100 °C. Intensive loss of mass of collagen begins at a temperature of 280 °C, and its copolymer at a temperature of 230 °C. A small weight loss (up to 8%), occurring with insignificant heat absorption, in the range of 100-230 °C (with the highest rate at 172 °C for collagen and at 137 °C for collagen-PAA copolymer) corresponds to the volatilization of bound water. Decomposition of protein macromolecules of collagen and grafted PAA chains for the copolymer. Weight loss in the range 284-425 °C (with the highest rate at 337 °C) is accompanied with a slight endothermic effect, which indicates the melting and decomposition of the sample, volatilization of low-molecular substances. Moreover, a 50% loss in the mass of collagen and its copolymer with PAA is observed at 340-350 °C.
Two exothermic maxima can be seen on the DTA curves, at 500 and 660 °C for collagen and at 505 and 820 °C for its copolymer with PAA. Probably in this temperature range, the processes of crosslinking and oxidation of the polymer residue takes place. Moreover, for the copolymer in a temperature region of 550-800 °C, the TG curve remains horizontal, i.e. no weight loss occurs in this interval. The presence of PAA chains leads to a decrease in the onset of the temperature of intense weight loss, but the network structure of the copolymer helps to reduce weight loss at higher temperatures. The DTA curves of collagen and its copolymer with PAA do not have definite maxima or minima from the baseline, which allows determining the temperatures of phase transitions.

When fibroin is heated in the range from 100 to 340 °C, a uniform decrease in the mass of the sample occurs with a slight endothermic effect (Fig. 1). In this temperature range, about 30% of the mass is lost as a result of decomposition with a release of low molecular weight products. In the range from 340 to 500 °C, weight loss is accompanied by an exothermic effect. During this interval, the processes of cyclization and cross-linking occur as a result of the interaction of functional groups of fibroin with the release of water, ammonia and other low molecular weight products.

The heat effect of the process is $\Delta H = 1167$ J/g. The total weight loss up to a temperature of 500 °C is 60%. No maxima or minima corresponding to phase transitions were found on the DSC curve. Apparently, when heated, fibroin decomposes in an amorphous state and no melting has been observed.

When comparing two data sets of DSC analysis of a copolymer of fibroin with PAA, some differences and similarities can be observed (Fig. 2). The copolymer of fibroin and PAA loses its mass from the start of heating; by 1600°C the mass of the sample decreases by 7%. The initial weight loss is
accompanied by an endothermic effect with a minimum at 56.5°C. This is apparently caused by the volatilization of the solvent bound to the copolymer. The TG curve of the copolymer changes more evenly than the same curve of fibroin. At 200°C, the copolymer has about 10% weight loss, while at this temperature, the fibroin weight loss is more than 15%.

Figure 2. DSC analysis of a copolymer of natural silk fibroin and PAA

At 250°C, the weight loss of the copolymer is 13%, and the weight loss of fibroin is 20%. At a temperature of 300°C, the mass of the copolymer and fibroin decreases approximately the same - by 30%. With a further increase in temperature, fibroin loses mass more intensively, by 500°C, the loss of fibroin mass is 60%, and the loss of copolymer mass is 50%.

The exothermic peak of the copolymer of fibroin and PAA is much less intense than the same peak of fibroin. Moreover, for the copolymer, the maximum of this peak is observed at a lower temperature. The DSC curve of the copolymer showed a wide deviation from the baseline towards the endothermic process at 260-279°C. Apparently, in this temperature range, partial melting of the copolymer occurs due to PAA presence.

The results obtained show that the number of functional groups in fibroin decreases after participation in graft copolymerization. Accordingly, the total weight loss decreases to 250°C. The copolymer has a network structure, therefore, cyclization and cross-linking of macromolecules occurs less intensively, thus, with a lower thermal effect.

4. Conclusions

The physicochemical properties of grafted copolymers of collagen and fibroin of natural silk with PAA depend on the composition, structure and preparation conditions. With an increase in the degree of grafting and, accordingly, the
frequency of crosslinked networks, the density and thermal stability of the copolymer increases. Collagen melts at 172 °C, collagen-PAA copolymer at a higher temperature. When fibroin is heated, phase transitions are not observed, they decompose without melting. An insignificant endothermic process is observed in the fibroin-PAA copolymer, which indicates partial melting of the sample. At higher temperatures, an intense weight loss of copolymers occurs, accompanied by an exothermic peak of the DSC curve, as a result of crosslinking and cyclization reactions with the release of low molecular weight products. The results obtained confirm the network structure of grafted copolymers of natural fibers with functionally active monomers, and will be used for prediction of properties of textile materials based on grafted copolymers.

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