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SYNTHESIS AND THERMAL PROPERTIES OF COLLAGEN COMPLEXES WITH COPPER ION

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Abstract. Coordination complexes of collagen with copper (II) sulfate have been synthesized at various initial ratios of components for dyeing textile materials. The complex compound is formed by mixing dialyzed collagen solution and copper sulfate solution. With an increase in the amount of salt, the yield of the complex compound increases. Thermal properties of coordination complexes of collagen with copper (II) sulfate were studied using thermogravimetry and differential scanning calorimetry. The temperatures of the stage-by-stage decomposition of the complexes and the magnitudes of the thermal effects of dehydration of crystallization water have been determined.

INTRODUCTION

In recent decades, collagen biomaterials have received special attention due to their excellent properties, such as low immunogenicity, biodegradability, biocompatibility, hydrophilicity, ease of processing, etc. [1]. However, collagen also has some unsatisfactory physical and chemical properties (mechanical strength, thermal stability, enzyme resistance, etc.). Therefore, collagen modification is necessary in the process of obtaining various materials. Metal-containing polymer complexes are ubiquitous in biological systems, and their unique structures provide a variety of biological properties [2]. Combining multiple components or hierarchies from a natural source using a simple method can act as a precursor in the design and application of collagen materials. A biosystem of organometallic compounds based on a collagen fibrous matrix with a chromium (III) complex has been obtained [3]. The resulting complex preserves the internal conformation of collagen and the morphology of its fibers in the skin, showed improved water resistance and air permeability. Revealing the relationship between the structure and the combined properties of the complex contributes to the progress of intelligent protein materials.

Metal ions affect the thermal stability of proteins, increasing or decreasing their resistance to unfolding [4]. The interaction between metal ions and peptides has generated interest due to the important role of metal ions in many biological processes and potential applications in various fields. Conformational changes in peptides depend on the nature of binding to metal ions [5]. Copper participates in several biological processes, forming complexes with protein substances [6]. The coordination complex of copper-bioactive scaffolds was used as a carrier of two anticancer agents [7]. Collagen membranes, in combination with complexes of gold and nucleic acids, serve as materials for corneal repair, can quickly regenerate the cornea, and effectively inhibit scar formation [8]. The formation of complexes of proteins with metal ions promotes their crystallization, and advances in macromolecular crystallization have rapidly multiplied in recent years with the advent of practical, easy-to-use screening kits and the use of laboratory robotics [9]. Complex compounds of heteroatom-containing polymers, including proteins, are of great importance in various fields, especially in medicine. Collagen materials are also used in the textile industry. It is known to form collagen fibers [10], use it in tissue engineering [11] as a framework for nanoparticles [12]. Porous carbon fibers with diameters ranging from 30 to 50 μm were obtained by wet spinning collagen materials [13]. Collagen solution is also used for sizing [14] and fire retardant treatment of cotton yarn before weaving [15], as part of a composition to impart dimensional stability [16] and fire resistance [17] to the material. Not enough attention is paid to the possibility of using collagen metal complexes as dyes for textile materials. Time-stable complexes of collagen with metal ions are a kind of colored substances. The fixation of complex dyes on the fibers of textile material, especially synthetic material, is achieved after short-term curing at elevated temperatures. Therefore, thermal stability of collagen complexes is of great importance. The aim of this work is to synthesize and determine the thermal properties of complexes of copper (II) sulfate with collagen for use as a dye for textile material.

MATERIALS AND METHODS

Getting collagen solution. The wool-free waste of raw skin of cattle was cut into pieces 3-4 mm in size and placed in a container. A 2% sodium hydroxide solution was poured into the container at a weight ratio skin : solution of 1.2 : 2. Pieces of the skin swell in an alkali solution for 12-24 hours. Then the solution was mixed until a homogeneous mass was formed, heating, if necessary, to a temperature of 50°C. The solution was sieved through a sieve with mesh sizes of 0.05-0.1 mm. Acetic acid was added to the sieved solution to neutralize to pH = 7 ± 0.2. In order to remove ions and low-molecular substances from the solution, the collagen solution was dialyzed using a dialysis bag made of horse intestine. The time and completeness of dialysis was controlled by the specific conductivity of the solution. In the studies, a collagen solution was used after 24 hours of dialysis, the conductivity of this solution was 46·10-4 ohm-1·cm-1, pH = 6.8, and the mass fraction of collagen was 6%.

Synthesis of $CuSO4$ complexes with dialyzed collagen. For the synthesis of the complexes, a 14.4% solution of

$CuSO4$ (density 1.16 g / ml) and a 6% solution of collagen (density 1.02 g / ml) were used. Solutions at different mass ratios were placed in a conical flask. During the day, the unreacted part precipitates, which was separated by filtration. Upon evaporation of the filtrate (an aqueous solution of a complex compound), crystals of the $CuSO4$ complex with collagen are formed. The complex salts were dried at a temperature of 60 ° C to constant weight.

The thermal properties of coordination complexes of collagen with copper (II) sulfate were studied using thermogravimetric (TG) and differential scanning calorimetry (DSC) methods. Samples were studied using 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 0-400 oC, the heating rate was 10 K / 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, $CsCl$.

Results and discussion

The synthesis of coordination complexes of collagen with $Cu2+$ ions was carried out at various ratios of dialyzed collagen and copper (II) sulfate. When determining the number of amino acid units of collagen in moles, the average molecular weight of one unit is calculated as the sum of the products of the molecular weight of one unit by its mass fraction. Table 1 shows the dependence of the yield of the complex compound on the ratio of the components.

TABLE 1. Initial ratios of components and the yield of a complex compound

Kollagen			$CuSO_4$			Complexes	
solution, г	G	mol	solution, g	g	mol	g	%
2	0.12	0.0015	8	1.152	0.0072	1.5227	119.7
4	0.24	0.0030	6	0.864	0.0054	1.2399	112.3
6	0.36	0.0045	4	0.576	0.0036	0.9106	97.3
8	0.48	0.006	2	0.288	0.0018	0.6669	86.6
9	0.54	0.0068	1	0.144	0.0009	0.5167	75.5

As can be seen from the data in Table 1, with an increase in the mass fraction of $CuSO4$, the yield of the complex increases. Moreover, with the mass ratio of solutions $O4$: collagen = 8: 2 and 6: 4, the mass of the complex is greater than the sum of the masses of salt and collagen. This indicates that some of the water is bound into a complex through a coordination bond. The presence of water of crystallization strongly affects the thermal properties of complex compounds.
Further, the thermal properties of the synthesized coordination complexes of dialyzed collagen with $CuSO4$ were investigated. The results of thermogravimetric analysis of collagen : $CuSO4$ complexes synthesized at different initial ratios of components confirmed the presence of water of crystallization in their composition, but in different amounts (table 2).

TABLE 2. Results of thermogravimetric analysis of coordination complexes of collagen with $CuSO4$

Temperature, ºC	Loss of mass, %					
	Kollagen	$CuSO_4 \cdot 5H_2O$	Complex synthesized at a molar ratio of collagen : $CuSO_4$			
			0,15:0,72	0,30:0,54	0,45:0,36	0,60:0,18
60	0,3	0	0	0	0	0
70	0,8	0	0	0	0	0
80	2,3	0,6	0,2	0,3	1,1	0
90	2,8	0,4	1,2	1,3	6,6	1,8
100	3,6	2,5	3,1	3,3	10,8	3,5

110	4,1	5,9	9,2	9,9	11,1	4,0
120	4,9	9,3	14,1	14,9	11,3	4,1
130	5,0	14,1	14,1	19,9	11,3	4,2
140	5,0	21,9	22,4	24,8	11,9	4,7
150	5,0	23,0	23,9	24,9	12,4	5,9
160	5,0	25,1	27,9	27,2	12,5	6,3
170	5,3	24,1	27,9	27,2	12,5	6,3
180	5,7	24,1	27,9	27,2	12,5	6,3
190	5,9	27,8	27,9	27,2	12,6	6,2
200	6,1	28,1	28,0	27,2	12,8	6,4
210	7,2	28,2	28,1	28,9	12,8	6,4
220	7,3	28,7	28,3	30,5	12,7	6,4
230	7,6	29,0	28,8	30,6	14,1	6,6
240	7,8	29,1	29,3	30,8	17,8	6,6
250	8,0	32,1	32,5	32,1	15,5	6,4
260	8,1	33,1	32,8	32,2	15,5	7,2
270	8,1	33,3	33,1	32,3	15,6	7,2
280	8,2	34,7	34,0	32,6	15,8	7,3
290	8,9	35,2	35,1	33,2	15,8	7,3
300	9,3	35,7	35,8	34,5	15,9	7,3

As can be seen from the data in Table 2, the loss of collagen mass begins at 70°C, but in general it turned out to be thermally stable up to 300°C. Up to 300°C, the loss of collagen mass is less than 10%, most likely due to moisture and low molecular weight condensation products by functional groups. There is no need to talk about a significant decomposition of collagen up to this temperature.

Thermal decomposition of copper sulfate begins above 100°C, here it can be argued that weight loss occurs due to dehydration of condensation water. Moreover, dehydration occurs gradually, the complete dehydration of five water molecules is completed at 300°C with a decrease in 36% of the mass of copper sulfate.

The results of thermogravimetric analysis of the coordination complexes synthesized with a molar excess of

$CuSO4$ resemble the results of the analysis of copper sulfate. The total weight loss up to 300oC is 34-36%, which confirms the presence of coordinated water in the complex. With a slight excess of collagen, the total weight loss of the complex is 16%, and with a significant excess of collagen, the weight loss is even less - 7%. More accurate information on the thermal properties of the objects under study is obtained from the DSC curves.

Figure 1 shows the DSC curve of copper sulfate. The DSC curve of pure copper sulfate (copper (II) sulfate pentahydrate) clearly shows the stages of dehydration $CuSO4 \cdot 5H2O$ - pentahydrate (119.6oC), trihydrate (125.1oC), monohydrate (137.5oC), anhydrous form (258.4oC).

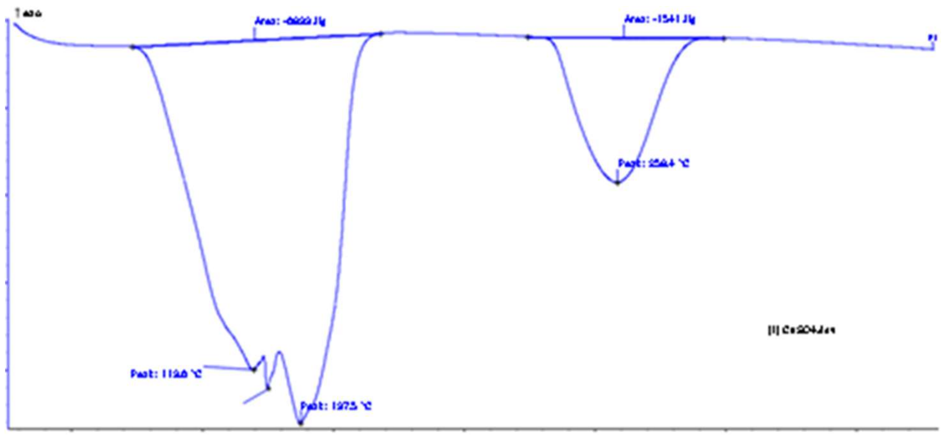

FIGURE 1. DSC curve $CuSO4 \cdot 5H2O$

Up to a temperature of 150°C, four molecules of crystallization water are split off with the absorption of 6839 J of heat per 1 g of crystalline hydrate. The cleavage of the fifth crystal water molecule occurs at a higher temperature (260°C) with the absorption of 1541 J of heat per 1 g of crystalline hydrate. In general, the heat of dehydration of copper sulfate is 8.38 kJ/g or 2095 kJ/mol of crystalline hydrate.

Judging by the DSC curve of collagen, when heated to 400°C, processes with an insignificant endothermic effect occur (Figure 2). These are the processes of melting and partial decomposition of the polymer with the release of low molecular weight substances.

The DSC curves of the coordination complexes of collagen with metal ions confirm the previously stated assumption that water molecules are also involved in the formation of the complex as a donor. The DSC curve of the coordination complex synthesized at the initial molar ratio of collagen: $CuSO4$ = 0.0015: 0.0072 clearly shows three endothermic minima - 108.2oC, 131.5oC, 259.1oC (Figure 3). The total heat of dehydration of this complex is 7.33 kJ/g, which is 12.5% less than the heat of dehydration of copper sulfate.

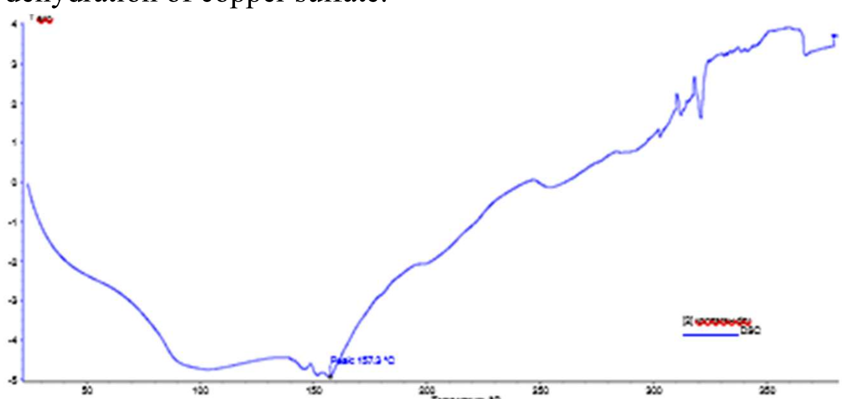

FIGURE 2. DSC curve of collagen.

With a decrease in the amount of salt in the complex, the number of endothermic peaks and the total heat of dehydration decrease, and the peaks shift to the low-temperature region. The DSC curve of the coordination complex synthesized at the initial molar ratio of collagen: $CuSO4$ = 0.0030: 0.0054 has three peaks (116.7oC, 136.7oC, 279.2oC) with a total warm dehydration of 6.22 kJ / g, which is 25.8% less than the heat of dehydration of copper vitriol (Figure 4).

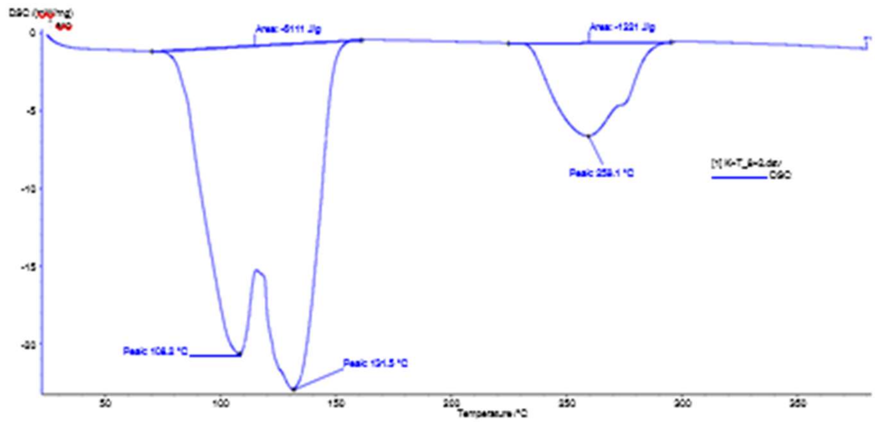

FIGURE 3. DSC curve of the coordination complex synthesized at the initial molar ratio collagen: $CuSO4$ =

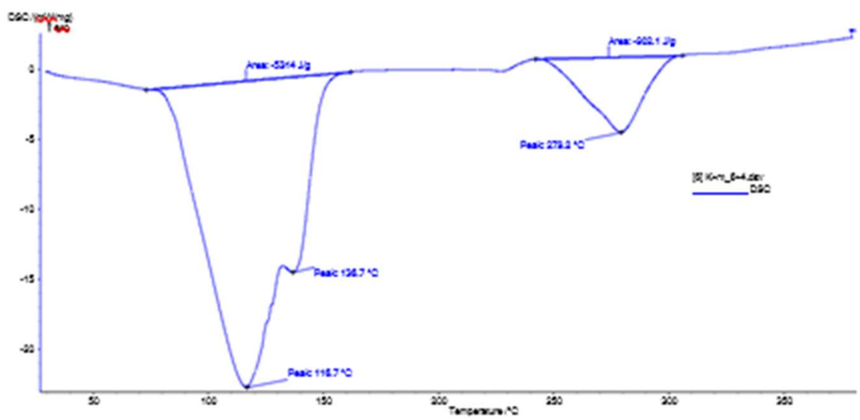

FIGURE 4. DSC curve of the coordination complex synthesized at the initial molar ratio collagen: $CuSO4$ =
0.0030 : 0.0054

The DSC curve of the coordination complex synthesized at the initial molar ratio collagen: $CuSO4$ = 0.0045: 0.0036 has two peaks (109.6°C, 266.8°C) with a total warm dehydration of 5.87 kJ / g, which is 30.0% less than the heat of dehydration of copper sulfate (Figure 5).

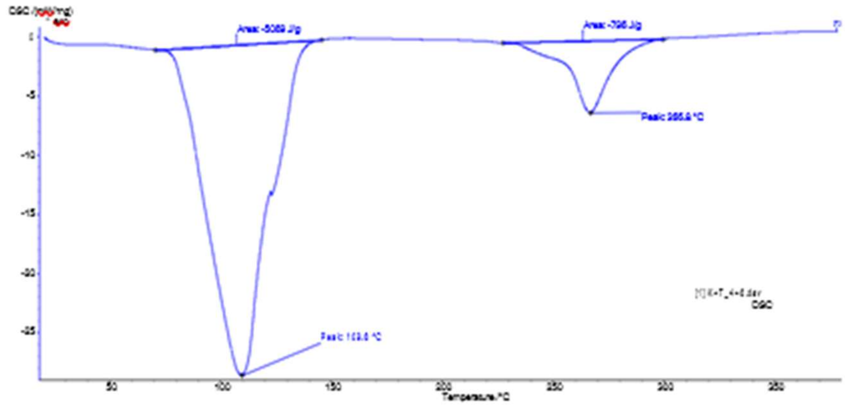

FIGURE 5. DSC curve of the coordination complex synthesized at the initial molar ratio collagen: $CuSO4$ =0.0045 : 0.0036

The DSC curve of the coordination complex synthesized at the initial molar ratio collagen: $CuSO4$ = 0.0060: 0.0018 has two peaks (103.1oC, 277.3oC) with a total warm dehydration of 4.20 kJ/g, which is 49.9% less than the heat of dehydration of copper sulfate (Figure 6).

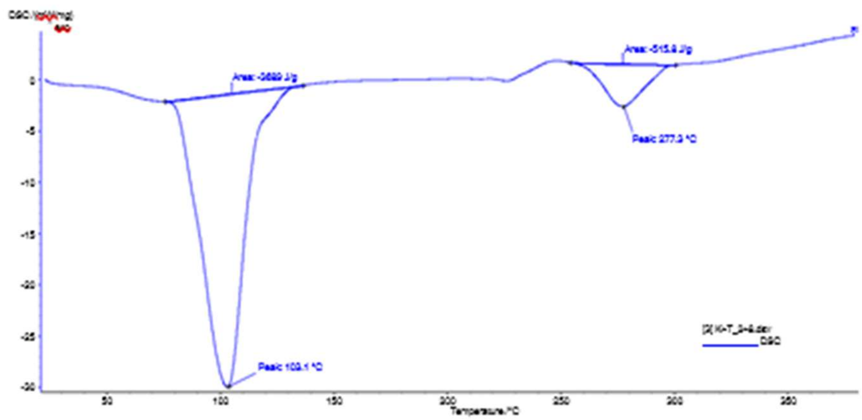

FIGURE 6. DSC curve of the coordination complex synthesized at the initial molar ratio collagen: $CuSO4$ =0.0060 : 0.0018

The DSC thermograms of the obtained complexes with different component ratios clearly show the shift of endothermic peaks to the low-temperature region with an increase in the amount of collagen. A simultaneous decrease in the heat of dehydration indicates a decrease in the coordinatedly bound water molecules.

CONCLUSIONS

When mixing a solution of copper sulfate and a collagen solution purified from electrolytes, a collagen complex compound is formed: $CuSO4$. The yield of the complex compound decreases with an increase in the amount of collagen in the initial mixture. With an increase in the amount of collagen in the initial mixture, the amount of water molecules coordinated as ligands decreases, the number of endothermic peaks, and the total energy of dehydration decrease. Each endothermic peak in the DSC curve corresponds to the dehydration of one water molecule. The overall thermal effect of dehydration of complex compounds in the temperature range from 103 to 277 ° C decreases from 7.33 to 4.20 kJ / g with an increase in the amount of collagen.

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