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FORMATION KINETICS OF CHITOSAN SULFATE BOMBYX MORI IN THE PRESENCE OF SULPHATING AGENTS

Abstract: *The regularities of sulfation of chitosan Bombyx mori were studied, the molecular weight characteristics, the kinetics of the reaction of formation of chitosan sulfate and its physicochemical properties were determined. Also, the medico-biological properties of chitosan sulfate were analyzed.*
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Introduction

It is known that water-soluble derivatives of chitosan, in particular, chitosan sulfate, are widely used in medicine, since they have hypolipidemic properties in human atherosclerosis [1-3]. The method of obtaining sulfated chitosan isolated from crab chitin was described in [4-6]. It should be noted that sulfating agents such as a mixture of dioxide and sulfur trioxide, sulfuric anhydride with pyridine or dimethylformamide, and chlorosulfonic acid are usually used to sulfate chitosan obtained from shell-containing sources.

Objects and methods of research

Sulfation of chitosan *Bombyx mori* was carried out according to the methods [7-8] developed for chitosan *Bombyx mori*, using sulfuric acid and chlorosulfonic acid (CSA) as a sulfating agent, which was obtained and had a high reactivity [7]. It was

found that the most effective chemical conversion of chitosan into chitosan sulfate occurs at SDA 75 and 95%, temperature 50°C and reaction duration 180 min.

The study of the kinetic regularities of the chemical conversion of chitosan *Bombyx mori* to chitosan sulfate was carried out by varying the duration and temperature of the reaction, as well as the ratio of sulfating agents of sulfuric acid and chlorosulfonic acid with chitosan in the range from 1:2 to 1:10. Chitosan characterized by SDA ≈ 91%, P ≈ 98%, and M_η ≈ 104,000 was chosen as the main object.

Results and their discussion

In order to obtain chitosan sulfate, sulfuric and chlorosulfonic acids were selected as sulfating agents. The main objective of the research was to study the dependence of the ratio of chitosan and sulfating

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reagents of sulfuric acid and chlorosulfonic acid on the formation of chitosan sulfate. To obtain chitosan sulfate with the participation of sulfuric acid, the ratio of acid to chitosan was varied in the presence of

isopropyl alcohol (IPA). We believe that the reaction of formation of chitosan sulfate in the presence of sulfuric acid occurs as follows:
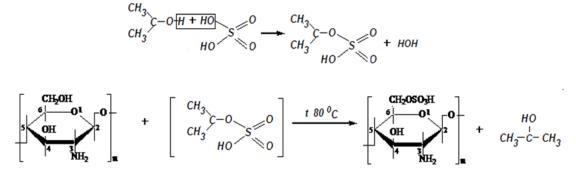

Figure. 1. Formation reaction of chitosan sulfate with sulfuric acid in the presence of IPA

Experiments have shown that as the reaction time (t) increases, the sulfur content (S, %) in the composition of chitosan sulfate increases, which leads to an increase in the degree of substitution (DS) (the degree of substitution of chitosan, depending on the number of functional groups, is up to 3) of sulfo groups in the chain. Moreover, an increase in the amount of S occurs noticeably intensively up to 3

hours, after which the reaction continues with a slight decrease in the rate, and the increase in the value of sulfur and substitution rate (SR) decreases slightly. At the same time, the yield of the final product changes with approximately the same regularity. The reaction of the formation of chitosan sulfate was carried out at 20^0C and a duration of 3 hours. The data obtained are presented in Table 1.

Table 1. Sulfation of chitosan with sulfuric acid by varying the ratio (chitosan:H_2SO_4) at a temperature of 20^0C and a duration of 3 h

№	Ratio chitosan: H_2SO_4	S%	N%	SR	Result, %	S*%	MM, кDa
1	1:3	3.75	5.6	0.22	54.3	61	73.6
2	1:4	6.50	4.5	0.41	38.5	75	62.0
3	**1:6**	**11.5**	**3.4**	**0.85**	**34.0**	**90**	**52.0**
4	1:8	9.31	4.0	0.64	25.4	85	49.4
5	1:9	9.46	3.9	0.66	18.9	86	38.7
6	1:10	8.90	4.2	0.61	20.5	83	35.5

S*-solubility

From the results obtained, it can be seen that with an increase in the ratio of sulfuric acid in comparison with the initial chitosan, a regular increase in the sulfur content and the degree of substitution of sulfo groups occurs, which indicates an increase in the proportion of sulfate groups in the composition of chitosan sulfate. It was found that with an increase in the ratio of the components of chitosan:H2SO4 more than 1:6, there is no significant change in the degree of substitution in the formation of chitosan. However, it is a fact that with an increase in the proportion of sulfuric acid, it is impossible to obtain low molecular weight fractions of chitosan sulfate, as a result of

which a sharp decrease in the yield of final products is found. The results obtained indicate that the most optimal ratio of components is 1:6, at which there is an increase in the degree of substitution and the average yield of the final product. The study of the synthesis time during the formation of chitosan sulfate is an urgent task. This stipulates the control of the sulfur content and the degree of substitution of the obtained samples. In this regard, the synthesis was carried out under constant conditions (the ratio of the components is 1: 6 at a temperature of 20^0C) and for 0.5-5 hours. The data obtained are presented in Table 2.

Table 2. Effect of the synthesis time on the formation of chitosan sulfate
(component ratio 1:6 at a temperature of 20^0C)

№	Synthesis time	S, %	N, %	SR	Reaction rate, $*10^{-4}$ mol/l.s.	Result, %	S*, %	MM, кDa
1	0,5	6.9	4.4	0.44	6.60	38.6	71	86.3
2	1	8.6	4.3	0.56	4.20	35.6	76	71.0
3	2	8.9	4.2	0.60	2.30	33.5	79	60.9
4	**3**	**11.5**	**3.4**	**0.85**	**2.10**	**34.0**	**90**	**52.0**
5	4	9.0	4.0	0.61	1.14	30.1	74	46.5
6	5	9.3	3.9	0.64	0.94	31.3	76	34.0

S*-solubility

The obtained result shows that with an increase in the duration of the synthesis, there is a natural increase in the sulfur content and the degree of substitution of sulfo groups. A decrease in the content of elemental nitrogen was found, which indicates an increase in the proportion of sulfate groups in the composition of chitosan sulfate. It was also revealed that with an increase in the duration of more than 3 hours, the solubility of the obtained samples does not increase, which indicates the loss of low-molecular water-soluble fractions of the final products during the precipitation of the reaction systems. Also, with an increase in the duration, the yield and molecular weight decrease due to the destruction of chitosan sulfate macromolecules. At the same time, the rate of the reaction of formation of chitosan sulfate (v) calculated by the formula

$$\upsilon = \Delta C / t \, V,$$

where ΔC – the change in the concentration of sulfuric acid during the reaction of the formation of chitosan sulfate; V – reaction mixture volume

It was found that with an increase in the synthesis time, a decrease in the reaction rate from 6.6×10^{-4} to 0.94×10^{-4} is observed, which indicates a decrease in the number of free sulfuric acid molecules with a decrease in the reaction rate. To determine the rate constant, the dependence of the logarithm of the rate on the synthesis time was established. The tangent of the slope was used to determine the value of the reaction rate constant, equal to $K=4.8\times10^{-5}$. The data obtained are shown in Figure 2.
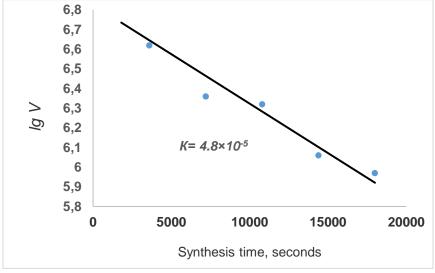

Figure 3. Dependence of the logarithm of the speed on the synthesis time

In the synthesis of chitosan sulfate, the synthesis temperature plays an important role. To study the effect of temperature on the formation of chitosan sulfate, synthesis was carried out under constant synthesis conditions with temperature control in the range of $20-70^0$C. The data obtained are presented in Table 3.

Table 3. Effect of the synthesis temperature on the formation of chitosan sulfate
(ratio of the components of chitosan:H₂SO₄ 1:6 and duration 3 h)

№	T ᵒC	S, %	N, %	SR	Result, %	S*, %	MM, кDa
1	20	11.5	3.40	0.85	34.0	90	52
2	30	11.8	3.36	0.88	32.8	88	49
3	40	12.5	3.24	0.96	31.7	91	38
4	**50**	**12.8**	**3.10**	**1.00**	**30.9**	**92**	**32**
5	60	10.4	3.70	0.74	28.5	63	29
6	70	9.23	3.92	0.63	24.3	73	26

S*-solubility

The results obtained show that with an increase in the synthesis temperature, the degree of substitution and the sulfur content in chitosan sulfate increase. Also, under the influence of high temperatures (60-70⁰C), there is a decrease in the molecular weight and the yield of final products, which indicates a loss of weight at a temperature of chitosan sulfate above 50⁰C. Based on the data obtained, we assume that the ratio of the components of chitosan:H₂SO₄ 1:6 and the duration of 3 hours at a synthesis temperature of 50⁰C are the optimal conditions for the formation of chitosan sulfate. The proposed synthesis conditions make it possible to obtain more water-soluble chitosan sulfate samples.

Also, in order to obtain chitosan sulfate, chlorosulfonic acid was used, the effect of synthesis conditions on the formation of chitosan sulfate was studied. The influence of the ratio of the components, the time and temperature of synthesis on the molecular weight characteristics of chitosan sulfate has been studied.

The use of the above reaction conditions leads to sulfation of NH₂-groups (in position C-2, i.e., N-substitution occurs) and OH-groups (in positions C-6, O-substitution) of the chitosan elementary unit according to the reaction. The reaction of formation of chitosan sulfate in the presence of chitosan sulfate-dimethyl sulfoxide can proceed as follows:
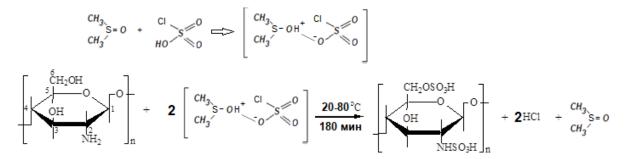

Figure 3. Formation reaction of chitosan sulfate with chlorosulfonic acid in the presence of dimethyl sulfoxide

The reaction of chitosan sulfate formation was carried out at room temperature for 180 minutes in chlorosulfonic acid with dimethyl sulfoxide. The data obtained are presented in Table 4.

Table 4. Sulfation of chitosan with chlorosulfonic acid with varying the ratio of components
(chitosan: chitosan sulfate) at a temperature of 20⁰C, 3 h

№	Ratio (chitosan:chitosan sulfate)	S, %	N, %	SR	Result, %	S*, %	MM, кDa
1	1:2	11.9	4.30	0.90	63.9	78	68.0
2	1:3	12.5	4.08	0.96	73.1	85	55.3
3	1:4	13.4	3.90	1.07	78.4	89	46.4

4	**1:6**	**14.6**	**3.76**	**1.22**	**86.3**	**92**	**35.9**
5	1:8	13.8	3.89	1.12	79.0	90	24.5
6	1:10	14.0	3.83	1.14	82.5	95	20.2

S*-solubility

The results obtained show that with an increase in the ratio of chlorosulfonic acid in the reaction systems, there is a regular increase in the degree of substitution and the yield of final products, and in this regard, the proportion of elemental nitrogen in the composition of chitosan sulfate decreases. A significant decrease in molecular weight was also found in comparison with another sulfating agent (H_2SO_4), which confirms the destruction of chitosan sulfate macromolecules. The solubility of the obtained samples increases depending on the degree of substitution of sulfo groups. It was found that the highest degree of substitution was established when the ratio of the components of chitosan:chlorosulfonic acid was 1:6.

To study the kinetics of the formation of chitosan sulfate, synthesis was carried out at room temperature 20^0C in the ratio of components 1:6. By varying the synthesis time in the range of 0.5-5 hours, the values of the degree of substitution of sulfo groups and the reaction rate of the formation of chitosan sulfate were determined depending on the duration of the synthesis. The data obtained are presented in Table 5.
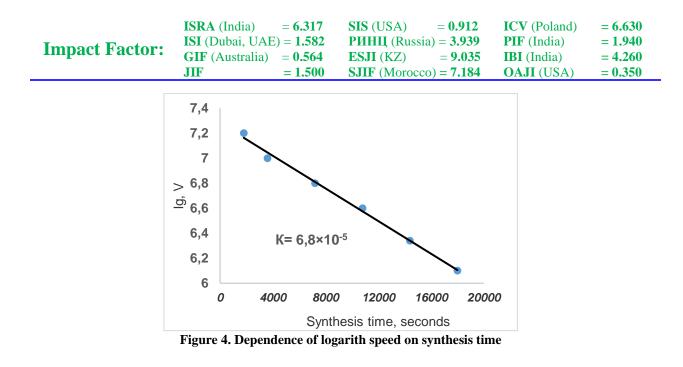
Table 5. Influence of the synthesis time during the formation of chitosan sulfate
(component ratio 1:6 at a temperature of 20^0C)

№	Time, h	S, %	N, %	SR	Reaction rate, *10^{-4} mol/l.s.	Result, %	S*, %	MM, кDa
1	0.5	8.76	5.30	0.56	8.46	66.31	68.0	68.4
2	1	9.76	4.38	0.68	5.14	7.,6	76.0	54.0
3	2	10.8	4.20	0.78	2.95	77.3	86.0	44.7
4	**3**	**14.6**	**3.76**	**1.22**	**3.07**	**86.3**	**92.0**	**35.9**
5	4	14.1	3.90	1.15	2.17	81.2	90.0	26.9
6	5	13.8	4.0	1.12	1.79	79.0	89.0	19.0

S*-solubility

It was found that with an increase in the duration of the synthesis, there is a regular increase in the degree of substitution and the content of sulfur, as well as a decrease in the molecular weight from 68.4 kDa to 19 kDa, which is a confirmation of the destruction of macromolecules under prolonged exposure to chlorosulfonic acid. Based on the results obtained, we assume that the synthesis time of 3 hours is the most optimal for the formation of chitosan sulfate. The results of determining the reaction rate indicate that with an increase in the synthesis time, a decrease in the reaction rate from 8.46×10-4 to 1.79×10-4 is observed, which is due to a decrease in the number of free chlorosulfonic acid molecules. To determine the rate constant, the dependence of the logarithm of the rate on the synthesis time was established. The tangent of the slope was used to determine the value of the reaction rate constant equal to K=6.8×10-5. The data obtained are shown in Figure 5.

$$K = 6{,}8 \times 10^{-5}$$

Figure 4. Dependence of logarith speed on synthesis time

In order to study the dependence of the properties of chitosan sulfate on the synthesis temperature, the synthesis was carried out in the ratio of the components of chitosan:chlorosulfonic acid 1:6 for 3 hours at a temperature of $20\text{-}70^0\text{C}$. The data obtained are presented in Table 6.

Table 6. Effect of the synthesis temperature on the formation of chitosan sulfate

№	T ℃	S, %	N, %	С3	Result, %	S*, %	ММ, кDa
1	20	14.6	3.76	1.22	86.3	92.0	35.9
2	30	14.8	3.46	1.24	83.0	90.0	29.1
3	40	15.6	3.30	1.37	78.2	94.0	24.5
4	**50**	**15.7**	**3.20**	**1.38**	**76.5**	**95.0**	**22.0**
5	60	14.2	3.87	1.17	68.4	87.0	17.4
6	70	14.4	3.70	1.20	65.2	88.0	15.0

S*-solubility

It was found that with an increase in temperature, not only the formation of final products occurs, but the opposite process is also observed, in which the degree of substitution of chitosan sulfate decreases. The results obtained confirm that with an increase in temperature more than 50^0C, there is a decrease in the degree of substitution of the sulfo group and the sulfur content. This can be due to an increase in the rate of reverse reaction under the influence of high temperatures. It was found that at a temperature of 50^0C, there is an increase in the degree of substitution and sulfur content. However, it should be noted that with an increase in the synthesis temperature, a decrease in the yield of final products from 86.3% to 65.2%, respectively, is observed. With this in mind, to study the physicochemical properties of the obtained samples, sample No.1, obtained at room temperature, was selected.

The IR-spectrum of chitosan sulfate samples has characteristic absorption bands due to the presence of the sulfonic ($R\text{–}SO_3H$) group and stretching vibrations of the sulfate groups S=O at 863-1150 см$^{-1}$. With the formation of chitosan sulfate in the presence of the sulfating agent chlorosulfonic acid in the region at 863, 1015, 1149 см$^{-1}$, a clear maximum appears, characterizing the stretching vibrations of sulfate groups (C-O-S bonds). There was a shift in the absorption band of amide II from 1580 см$^{-1}$ to 1520 см$^{-1}$ for chitosan to a lower wavelength region for chitosan sulfate, which may indicate sulphation of chitosan at the amine group at C-2 of the chitosan elementary unit [9-10].

In the spectrum of chitosan sulfate obtained on the basis of sulfating agents of oleum and sulfuric acid, a shift of the absorption band is also observed, which is characteristic of the amino groups of the initial chitosan, and also in the obtained spectrum, a decrease in the intensity of the remaining absorption bands is observed in comparison with chitosan sulfate obtained on the basis of the sulfating agent chlorosulfonic acid.

Such shifts and changes in the intensity of the wavenumbers indicate the occurrence of the sulfation reaction of chitosan. The intensity of the absorption band in the region of 863, 1015, 1149 см$^{-1}$ confirms that under the action of the sulfating agent chlorosulfonic acid, a more intense substitution occurs (Figure 5).
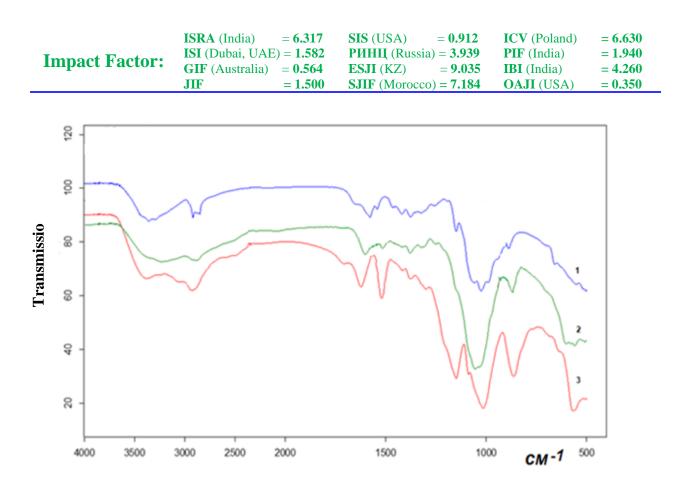
Figure 5. IR-spectra of chitosan (1) and sulfated chitosan: chitosan sulfate with sulfuric acid (2), chlorosulfonic acid (3)

In the IR-spectrum of chitosan sulfate in the presence of the sulfating agent chlorosulfonic acid in the range of 863, 1015, 1149, 1520 см$^{-1}$, a clear maximum appears, characterizing the stretching vibrations of sulfate (C-O-S bonds) and C2- $NHSO_3H$ groups for chitosan sulfate. Also in the spectrum of chitosan sulfate obtained in the presence of sulfuric acid, absorption bands are observed at 863 см$^{-1}$, which may indicate the substitution of C6-OH groups of chitosan.

The conductometric titration of chitosan sulfate was carried out: chitosan-1, chitosan sulfate-2 and chitosan sulfate-3, obtained at different reaction temperatures. From the comparative conductometric titration data shown in Figure 6 it can be seen that the titration curves are characterized by broken areas, and curves 2, 3 for chitosan sulfate are shifted to the region of large Gsm values as compared to curve 1 for chitosan. Comparison of the titration curves of chitosan and chitosan sulfate showed that the titration of sulfo groups occurs on the segment AB and corresponds to the difference in titrant volumes $\Delta V_S \approx |V_A - V_B|$, and the titration of amino groups takes place in the segment BC at the difference $\Delta V_N \approx |V_C - V_B|$. Using these results, we calculated the degree of substitution of sulfo groups and their distribution between hydroxyl and amino groups in sulfated chitosan samples using the formulas:

$$\omega_S \approx km_S \Delta V_S / m_{chitosan\ sulfate} \quad and$$
$$\omega_N \approx km_N \Delta V_N / m_{chitosan\ sulfate}$$

where k - titrant normality; m_S and m_N – molecular weight of sulfur (S) and nitrogen (N), respectively.
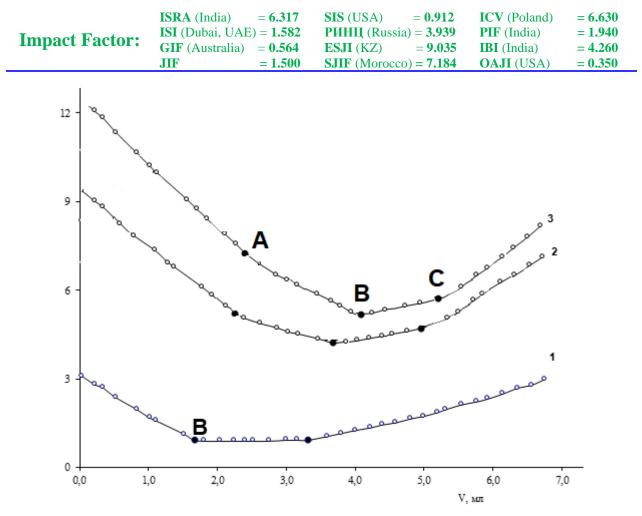
Figure 6. Curves of conductometric titration of sample solutions:
1-Chitosan; 2-Chitosan sulfate-1; 3-Chitosan sulfate-2.

Evaluation of the distribution of sulfo groups over hydroxyl and amino groups showed that the content of SO_3H groups for all samples of chitosan sulfate obtained by varying the reaction conditions is 1.2 times higher for OH groups. This confirms the assumption that the sulfation of chitosan is carried out not only at the C_2-NH_2 groups and at the C_6-OH-groups.

Conclusion

Thus, the reaction rate and rate constant during the formation of chitosan sulfate have been determined, and the effect of temperature and duration of synthesis has been studied. It was found that for the formation of chitosan sulfate, the optimal synthesis conditions are the duration of 3 hours, the temperature of 25^0C, and the ratio of components 1:6.

The results obtained confirm that when chlorosulfonic acid is used in the formation of chitosan sulfate, the value of the rate constant increases from $K=4,8\times10^{-5}$ to $6,8\times10^{-5}$, which contributes to an increase in the rate of substitution of sulfogroup and the entire kinetic process compared to other sulfating systems.

Based on the experimental results of the kinetics of sulfation of chitosan *Bombyx mori*, theoretical calculations were performed using model concepts of the chemical transformation reaction.

The obtained results of conductometric titration confirm that the sulfation of chitosan is carried out not only at the C_2-NH_2 groups, but also occurs at the C_6-OH groups.

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