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RESEARCH ARTICLE

Obtaining chitosan sulfate nanoparticles in an aqueous medium and their colloidal protection with polysaccharides

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Abstract

Objectives. To develop a method to obtain a hydrosol of the salt of chitosan with sulfuric acid—chitosanium sulfate (ChS) hydrosol—and to study the effect of various water-soluble polysaccharides on its stability over time, as well as its resistance to indifferent and non-indifferent electrolytes.

Methods. κ-Carrageenan, sodium alginate (SA), and xanthan were used as polymers which perform the function of colloidal protection for ChS nanoparticles. Capillary viscometry was used to study the viscosity of polymer solutions, their molecular weight, and their adsorption on ChS. The stability of the sols over time and their resistance to indifferent and non-indifferent electrolytes were evaluated photometrically. The hydrosol particle size was determined by means of dynamic light scattering.

Results. On the surface of ChS, κ-carrageenan is adsorbed most strongly over a wide range of concentrations. The graphs of the dependencies of the relative change in the turbidity of sols with the addition of various polysaccharides on their weight concentration at a sol lifetime of 2 days have the shape of curves with a maximum. Sols with the addition of 0.0125% SA and κ-carrageenan in the range of 0.04% have the greatest stability over time. According to dynamic light scattering data, the average particle size of freshly prepared sols with the addition of the polymers to ensure their greatest stability over time are 10.8 nm and 14.6 nm, respectively. For freshly prepared sols without polysaccharides, this size is 24.8 nm. The hydrosol coagulation threshold with an indifferent electrolyte (NaCl) is 9.3 times higher than that with a non-indifferent electrolyte (Na_2SO_4). κ-Carrageenan and SA protect the hydrosol from coagulation with an indifferent electrolyte (NaCl) at all their used amounts. At the same polymer concentrations, no protection from coagulation with a non-indifferent electrolyte (Na_2SO_4) was observed.

Conclusions. A method was developed to obtain ChS hydrosol with a positive particle charge. The stability of ChS sols over time was studied both without and with the addition of SA, κ-carrageenan, and xanthan. Sol coagulation thresholds with indifferent and non-indifferent electrolytes, as well as the protective numbers for κ-carrageenan and SA against the coagulation of hydrosols with these electrolytes, were established. The mechanism of stability of sols at certain concentrations of water-soluble polysaccharides was explained using data on the adsorption of these polysaccharides on the surface of chitosan treated with a solution of sulfuric acid. Based on the results of the work, it can be concluded that SA and κ-carrageenan can be used for the efficient stabilization of ChS hydrosols over time and for the colloidal protection of ChS from coagulation with sodium chloride.

Keywords

colloid chemistry, nanoparticles, nanotechnology, sols, dispersed systems, polymers, carbohydrates, polysaccharides, chitosan, sodium alginate, carrageenans, xanthan, stability, coagulation

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НАУЧНАЯ СТАТЬЯ

Получение хитозан-сульфатных наночастиц в водной среде и их коллоидная защита полисахаридами

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Аннотация

Цели. Разработать методику получения гидрозоля сернокислой соли хитозана — сульфата хитозания (СХ), исследовать влияние различных водорастворимых полисахаридов на его устойчивость во времени и при добавлении индифферентного и неиндифферентного электролитов.

Методы. В качестве полимеров, выполняющих функцию коллоидной защиты наночастиц СХ, были использованы κ-каррагинан, альгинат натрия (АН) и ксантан. Для определения вязкости растворов полимеров, их молекулярной массы и для исследования их адсорбции на СХ использовался метод капиллярной вискозиметрии. Оценка устойчивости золей во времени и при добавлении индифферентного и неиндифферентного электролитов проводилась фотометрически. Размер частиц гидрозоля определялся методом динамического светорассеяния.

Результаты. На поверхности СХ в широком диапазоне концентраций сильнее всего адсорбируется κ-каррагинан. Графики зависимости относительного изменения мутности золей с добавками различных полисахаридов от их массовой концентрации при времени жизни золей 2 суток имеют вид кривых с максимумом. Наибольшей устойчивостью во времени обладают золи с добавками 0.0125% АН и κ-каррагинана в диапазоне 0.04%. По данным динамического светорассеяния средний размер частиц свежеприготовленных золей с добавками полимеров, обеспечивших их наибольшую устойчивость во времени, составил соответственно 10.8 нм и 14.6 нм, тогда как для свежеприготовленных золей без полисахаридов — 24.8 нм. Порог коагуляции гидрозоля индифферентным электролитом (NaCl) в 9.3 раза выше порога коагуляции гидрозоля неиндифферентным электролитом (Na_2SO_4). κ-Каррагинан и АН защищают гидрозоль от коагуляции индифферентным электролитом (NaCl) при всех их использованных количествах. В то же время, при тех же концентрациях полимера защиты от коагуляции неиндифферентным электролитом (Na_2SO_4) не наблюдалось.

Выводы. Разработана методика получения гидрозоля СХ с положительным зарядом частиц. Исследована устойчивость золей СХ во времени как без добавок, так и с добавками АН, κ-каррагинана и ксантана во времени. Определены пороги коагуляции золей индифферентным и неиндифферентным электролитами, а также защитные числа от коагуляции гидрозоля этими электролитами для κ-каррагинана и АН. Для объяснения механизма устойчивости золей при определенных концентрациях водорастворимых полисахаридов использованы полученные данные по адсорбции этих полисахаридов на поверхности хитозана, обработанного раствором серной кислоты. По результатам работы можно сделать вывод, что АН и κ-каррагинан могут использоваться как эффективные стабилизаторы гидрозолей СХ во времени и для его коллоидной защиты от коагуляции хлоридом натрия.

Ключевые слова

коллоидная химия, наночастицы, нанотехнология, золи, дисперсные системы, полимеры, углеводы, полисахариды, хитозан, альгинат натрия, каррагинаны, ксантан, устойчивость, коагуляция

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INTRODUCTION

In medical and pharmaceutical nanotechnology, there is currently great interest in the polyaminosaccharide chitosan and its various derivatives. Chitosan and its derivatives are promising polymers for use in various fields of modern medicine. It can be used in the development of new drugs and drug delivery vehicles, wound dressings, suture materials, bone and dental implants, and embolization materials [1–20].

Chitosan is obtained by alkaline deacetylation of chitin: the second most abundant polysaccharide in nature after cellulose. The main natural sources of chitin and, accordingly, chitosan, are the outer integuments of arthropods, primarily crustaceans, as well as fungal cell walls. Due to features of the method for producing chitosan, unreacted chitin units always remain in it (Fig. 1a). Therefore, chitosan is characterized by the fraction of units containing amino groups, or by the degree of deacetylation (DD).

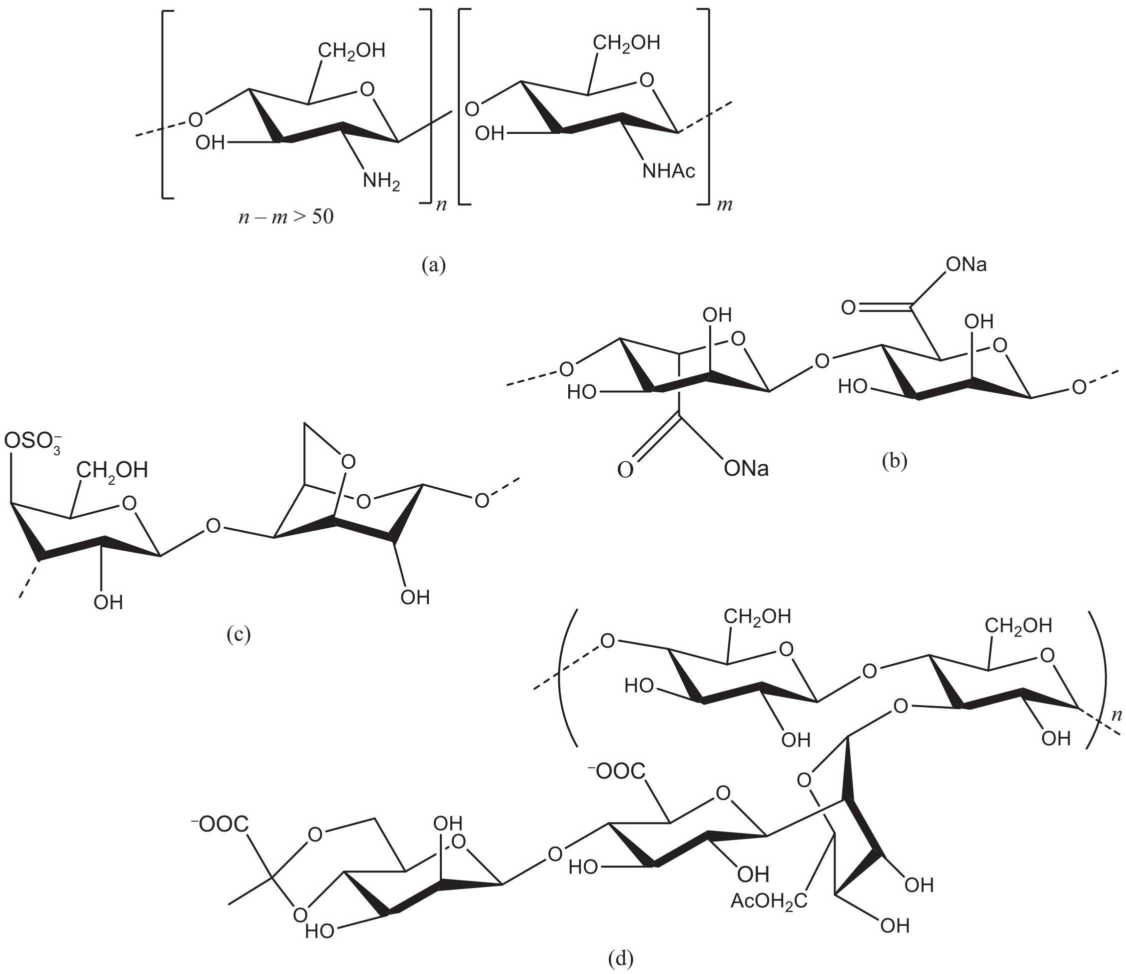

Fig. 1. Formulas of the polysaccharides used:
(a) chitosan,
(b) sodium alginate (SA),
(c) κ-carrageenan, and
(d) xanthan (xanthan gum)

An important advantage of micro- and nanoparticles of chitosan and its derivatives is that they can be prepared and loaded in an aqueous medium without using organic solvents. Chitosan is insoluble in water and organic solvents. Since chitosan is a weak base, in an acidic medium, its groups are protonated. At DD > 70%, it is soluble in an aqueous medium with pH < 6.5.

Salts of chitosan with various acids, e.g., its sulfate salt, chitosanium sulfate (ChS), are of interest for pharmacology and medicine. ChS is insoluble in water because of the cross-linking of chitosan macromolecules by sulfate anions by the interaction with their protonated amino groups (Fig. 2). This makes it possible to use ChS as a basis for nano- and microparticles. Since they possess biological activity themselves, they can also serve as delivery vehicles for other drugs [4, 21–25]. ChS nanoparticles can possibly also find application as fillers in polymer composite materials for bone and dental implants, as is the case with the use of chitosan [18].

In order to study the biological activity of nanoparticles and the possibilities of pharmaceutical use of chitosan and its derivatives, methods for their production and stabilization need to be developed. Furthermore, their stability under physiological conditions and during storage of drugs also need to be studied. The advantage of using biopolymers, including polysaccharides, in stabilizing dispersed systems is their biodegradability, environmental safety, availability, and, in many cases, biocompatibility [4].

The use of polysaccharides for the production and stabilization of various nanoparticles was considered previously (see, e.g., [26–29]). ChS sols were also studied [21–23], but without their stabilization by polymers.

In our study, ChS sols were stabilized by the anionic polysaccharides sodium alginate, κ-carrageenan, and xanthan (xanthan gum). Figures 1b–1d present their structural formulas.

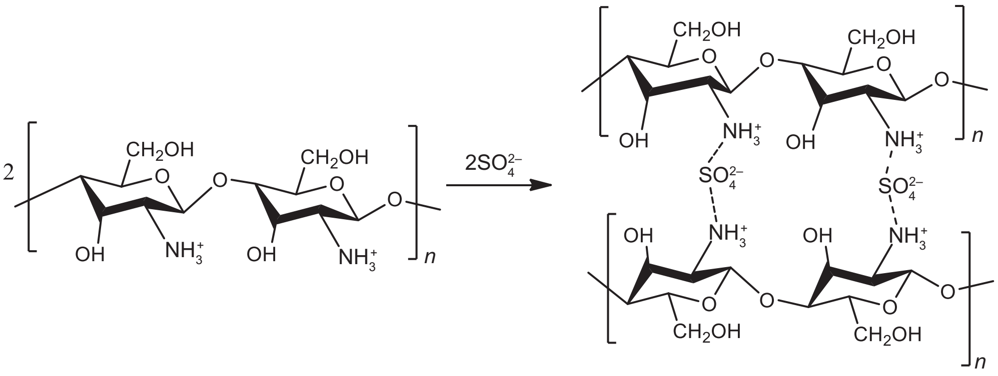

Fig. 2. Cross-linking of protonated chitosan (chitosanium polycations) with sulfate anions

EXPERIMENTAL

The main reagents were chitosan (*Slavyanskii Food Processing Plant*, Russia), sodium alginate (*Reaktivtorg*, Russia), BLK-1120 κ-carrageenan (*Shanghai Brilliant Gum*, China), and Kelzan xanthan (xanthan gum) (*CP Kelco*, Denmark). The other reagents (all of reagent grade, *Reaktivtorg*, Russia) were: sodium sulfate, sodium chloride, hydrochloric acid, sulfuric acid, sodium hydroxide, acetic acid, and sodium acetate.

The DD of chitosan was calculated based on experimental data of its titration with alkali according to the published procedure [30]. The molecular weights of chitosan, SA, κ-carrageenan, and xanthan were determined by capillary viscometry method with a VPZh-2 viscometer (*Ekros*, Russia) with a capillary diameter of 0.73 mm at 25°C using the Mark–Houwink equation.

In order to study the adsorption of SA, κ-carrageenan, and xanthan from their aqueous solutions on the surface of the ChS, a chitosan sample was first treated with a 2% sulfuric acid solution, filtered off, and dried at a temperature of about 70°C. Weighed specimens of the samples obtained were used to measure the viscosity of polysaccharide solutions of different concentrations after reaching adsorption equilibrium. In order to construct calibration graphs, solutions of polysaccharides were premixed with acidified water obtained after keeping the same specimens of a chitosan sample treated with a solution of sulfuric acid in distilled water. The pH of the medium (pH was 2.5–2.7) and ionic strength on the viscosity of the polysaccharide were taken into account. These determined by the presence of H^+ ions due to the dissociation of the surface of chitosan treated with a solution of sulfuric acid. The adsorption of water-soluble polysaccharide on chitosan treated with a solution of sulfuric acid (g adsorbate/g adsorbent) was found from the formula

$$A_{g/g} = \frac{\left(C_{in} - C_{eq}\right)V}{m}, \tag{1}$$

wherein C_{in} and C_{eq} are the viscometrically determined weight concentrations of water-soluble polysaccharides in the initial solution and upon reaching adsorption equilibrium, respectively; V is the volume of the system; and m is the weight of the sample of chitosan treated with a solution of sulfuric acid.

In order to obtain a ChS sol, chitosan was first converted into a soluble salt form by reacting it with hydrochloric acid. The ChS sol was produced by the metathesis reaction between the obtained chitosanium chloride and sodium sulfate:

$$2[\mathrm{Chit}_n \cdot n\mathrm{H}^+]\mathrm{Cl}_n + n\mathrm{Na_2SO_4} = \\ = [\mathrm{Chit}_{2n} \cdot 2n\mathrm{H}^+](\mathrm{SO_4})_n + 2n\mathrm{NaCl}, \tag{2}$$

wherein Chit is the deacetylated chitosan unit.

In order to obtain the sol, the reactants were taken in stoichiometric quantities (in the ratio ChS/Na_2SO_4 = 2/1).

In order to dissolve chitosan in a solution of hydrochloric acid, 0.41 g of chitosan and 0.093 g of hydrochloric acid were taken per 50 mL of water. This weight of chitosan, taking into account the DD, corresponds to an amount of 2 mmol (0.32 g) of deacetylated monomer units which enter into the reaction. Accordingly, the concentration of these units was 40 mM. The reaction mixture was heated in a water bath until the chitosan was completely dissolved. In order to obtain a sol, 50 mL of a 20 mM Na_2SO_4 solution (0.142 g Na_2SO_4) was added by drops into the cooled resulting solution of chitosan chloride with constant stirring using a submersible mixer. This sequence of mixing the solutions of reagents is necessary, in order to ensure a positive charge of the sol particles. The theoretical weight concentration of the obtained sol is 4.2 g/L.

To each of the obtained 100-mL portion of the initial sol, 25 mL of each of the solutions of polysaccharides were added so that the range of obtained polysaccharide concentrations in the sol was 0.0125–0.06 wt %. In order to maintain its concentration, 25 mL of distilled water was added to one of the portions of the sol instead of a polysaccharide solution. After this, all sols were treated with ultrasound for 3 min. The pH values of the sols were about 2.5–2.7. The sols were prepared at a temperature of 25°C.

The studied ChS sols are colorless; therefore, only light scattering is observed for them, and absorption is virtually absent. The optical densities D of all studied sols were determined (wavelength 430 nm, cell thickness 1 cm) using a KFK-3KM single-beam spectrophotometer (*Uyniko-Sis*, Russia) (spectral wavelength range 325–1000 nm). The turbidity for freshly prepared sols and sols at certain time intervals was calculated from the optical density as $2.3D$, cm^{-1}. The error in measuring the optical density (turbidity) of the sols was 4.4%.

As a measure of the stability of sols at a certain time, the relative change in turbidity, τ_{rel}, as a percentage of the initial turbidity τ_0 was taken:

$$\tau_{\text{rel}} = \frac{100\left|\tau - \tau_0\right|}{\tau_0}. \tag{3}$$

A sol can be considered more stable, if its turbidity changes (in percentage) to a lesser extent in the given time, when compared with the turbidity of the sol immediately after synthesis.

In order to determine the coagulation threshold with electrolytes and to study the colloidal protection of sols with polymers, NaCl (3 M) was used as an indifferent electrolyte, while Na_2SO_4 (50 mM) was used as a non-indifferent electrolyte. The coagulation threshold was found from the turbidity of freshly prepared sols with the addition of gradually increasing volumes of polysaccharide solutions. The volume of the sol portion and the volume increment were 10 and 0.05 mL, respectively, for NaCl and 5 and 0.1 mL, respectively, for Na_2SO_4.

The sol coagulation threshold with electrolytes (mol/L) was calculated using the formula (4):

$$C_{\text{coag}} = \frac{c_{\text{e0}}\, V_{\text{e}}}{V_{\text{sol}}}, \tag{4}$$

wherein c_{e0} is the concentration of the electrolyte in the portion of its solution before addition to sol, V_{e} is the volume of the electrolyte solution in which coagulation occurs, and V_{sol} is the volume of the sol in the test tube.

The colloidal protection from each electrolyte by water-soluble polysaccharides was studied by means of the turbidity of portions of sols with the addition of gradually increasing volumes of polysaccharide solutions (in increments of 0.05 mL to protect the sol from NaCl and 0.1 mL to protect it from Na_2SO_4), and the subsequent addition of a portion of electrolyte the volume of which corresponds to a certain coagulation threshold.

The protective numbers (in weight percent) for water-soluble polysaccharides were calculated by the formula (5):

$$C_{\text{prot}} = \frac{c_{\text{p0}}\, V_{\text{p}}}{V_{\text{sol}}}, \tag{5}$$

wherein c_{p0} is the concentration of the polysaccharide in the added portion, wt %; V_{p} is the volume of the polysaccharide solution at which coagulation occurs; and V_{sol} is the volume of the sol in the test tube.

The size distribution of particles of sols was determined by dynamic light scattering using a Delsa Nano C A53878 automatic particle analyzer (*Beckman Coulter*, USA) in the measurement range from 0.6 nm to 30 µm.

RESULTS AND DISCUSSION

The potentiometric titration of chitosan converted into a soluble protonated form by reacting it with hydrochloric acid was performed according to the published procedure [30]. The result showed that the DD of chitosan is 77.28%. In the work of Wang *et al.* [31], the parameters of the Mark–Houwink equation for chitosan were shown to be dependent on its DD. The graphs of the dependencies of the constants k and α on DD were presented. According to the data of Wang *et al.* [31], the values of these constants at DD = 77.28% were found and used, in order to calculate the molecular weight of chitosan using the Mark–Houwink equation using capillary viscometry data.

Table 1 presents the results of determining the molecular weights of chitosan and water-soluble polysaccharides, the conditions for their viscometric determination, the corresponding values of the constants of the Mark–Houwink equation, and the determined values of the intrinsic viscosity of their solutions. According to the DD value, the macromolecule of the chitosan used contains 4968 deacetylated monomer units.

Figure 3 presents the adsorption isotherms (g/g) of water-soluble polysaccharides from their aqueous solutions on the surface of chitosan treated with a solution of sulfuric acid. The isotherms were obtained by processing capillary viscometry data, as shown in Fig. 3. At weight concentrations below 0.05%, the relative viscosity of solutions of the polysaccharides under study before adsorption is close to unity. After achieving adsorption equilibrium, it is unity. Therefore, at such low weight concentrations, the amount of polysaccharides remaining in the solution after reaching adsorption equilibrium for all three polysaccharides is insignificant for the use of viscometry data. In this case, it can also be assumed that almost all of their amount is adsorbed. At a concentration of 0.05% and higher, according to capillary viscometry data, differences in adsorption between different water-soluble polysaccharides are

Table 1. Values of molecular weights, intrinsic viscosity, parameters of the Mark–Hauwick equation, determination conditions, and references to relevant sources for chitosan and water-soluble polysaccharides

Polysaccharide	$k \cdot 10^5$, mL/g	α	Conditions	Reference	Intrinsic viscosity [μ], mL/g	Molecular weight *M*, kDa
Chitosan	50	1.02	0.2 M CH_3COOH/0.1 M CH_3COONa, $T = 30°C$	[31]	660	1001.3
SA	73	0.92	0.1 M NaCl, $T = 25°C$	[32]	300	103.5
κ-Carrageenan	10	0.9	Distilled water, $T = 25°C$	[33]	32	1308.7
Xanthan	2.49	1.2454	0.01 M NaCl, $T = 25°C$	[34]	785	771.4

already clearly visible. As can be seen, on the surface of chitosan treated with a solution of sulfuric acid, xanthan is least adsorbed throughout the concentration range studied here (Fig. 3, curve *3*). Up to a concentration of about 0.15%, κ-carrageenan is adsorbed best. The adsorption isotherm can be attributed to the Langmuir type (Fig. 3, curve *2*). SA is generally adsorbed worse than κ-carrageenan. However, at concentrations above 0.15%, its adsorption already begins to exceed that of κ-carrageenan. It is obvious that the adsorption of κ-carrageenan is facilitated not only by the electrostatic attraction of its polyanions to the positively charged surface, but also by the specific binding of sulfate groups of κ-carrageenan to the protonated amino groups of chitosan, as occurs in the case of SO_4^{2-} anions (Fig. 2).

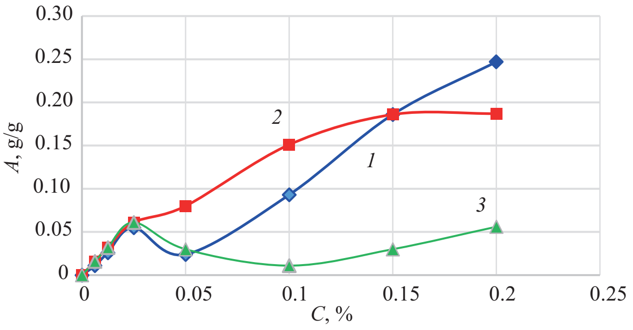

Fig. 3. Adsorption of polysaccharides on the surface of chitosan treated with a solution of sulfuric acid (containing ChS on the surface):
(*1*) SA,
(*2*) κ-carrageenan, and
(*3*) xanthan

Reaction (2) between chitosanium chloride and sodium sulfate gives insoluble ChS. It was shown [22] that the factor which most affects the rate of formation and characteristics of ChS is the molar ratio of the components of the mixture SO_4^{2-} and NH_3^+. With a decrease in the fraction of sulfate ions in the initial solution, the size of the resulting particles decreases. Their ξ-potential increases, helping to increase their stability.

The micelle of the ChS hydrosol, stabilized with chitosan chloride, with the positively charged surface can be represented by the general formula:

$$\{m\mathrm{Chit}_{2n}(\mathrm{SO}_4)_n\ k[\mathrm{Chit}_n\ n\mathrm{H}^+](k-x)n\mathrm{Cl}^-\}xn\mathrm{Cl}^-,$$

wherein Chit is the deacetylated chitosan unit (nondeacetylated fragments of chitosan are not taken into account).

The potential-determining groups are protonated groups of chitosan (chitosanium polycations), and the counterions are chloride anions.

For each of the water-soluble polysaccharides, graphs were constructed of the time dependencies of the turbidity of sols containing different weight concentrations of polysaccharides. Figure 4 presents examples of such graphs for the sol without the addition of a polysaccharide and for sols with polysaccharides at weight concentrations of 0.125 and 0.25%.

These graphs show that over time the turbidity in some cases can not only increase, but also decrease. According to Rayleigh's equation, $\tau \sim \nu V_p^2$, where ν is the number concentration of sol particles and V_p is the average particle volume. As a result of coagulation over time, the increase in the turbidity of the sol with an increase in the volume (size) of particles appears together with a decrease in turbidity and a decrease in the number concentration of the sol. On the one hand, the coagulation helps to reduce the number concentration of particles, thus decreasing the optical density (turbidity). On the other hand, the larger the particle aggregates, the more they scatter light, which contributes to an increase in optical density (turbidity). Thus, depending on the predominance of the former or latter trend, the turbidity either increases or decreases. For example, although the largest aggregates of particles scatter light more strongly, they are deposited to the bottom of the vessel, thereby leaving the bulk of the sol. Consequently, they are absent from the sol placed in the cell for measuring optical density. Thus, because of the ambiguity of assessing the stability of sols using the absolute values of optical density (turbidity), the stability of sols over time was characterized using not the absolute values of turbidity, but the relative change in percentage according

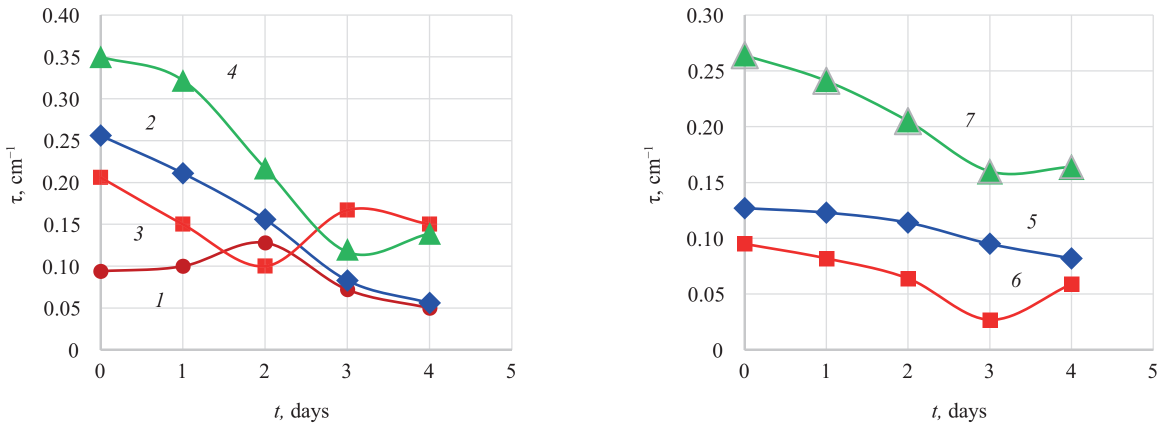

Fig. 4. Time dependencies of the turbidity of ChS sols
(*1*) without the addition of polysaccharides
and with the addition of (*2*) 0.025% SA,
(*3*) 0.025% κ-carrageenan,
(*4*) 0.025% xanthan,
(*5*) 0.0125% SA,
(*6*) 0.0125% κ-carrageenan, and
(*7*) 0.0125% xanthan

to formula (2). The more the turbidity of the sol changes, the less stable it can be considered. Figure 5 illustrates the relative change in the turbidity of sols after 2 days. The relative change in the turbidity of the sol without the addition of polymers for this time is 33%. Figure 5 shows that, at very low weight concentrations of polymers (less than 0.02%), the stability of the sols increases in comparison with the initial sol. The addition of each of all the three polysaccharides to a concentration of 0.0125% decreases the relative change in turbidity. These points of decrease in the relative change in turbidity at polysaccharide concentrations of 0.0125% can be conditionally considered points of minimum. However, a more accurate determination of the presence of a minimum and its value requires several more points in the concentration range less than 0.0125% to be examined. At concentrations above 0.0125%, the relative changes in turbidity for xanthan and SA increase, pass through a maximum, and decrease. In this case, the greatest decrease is observed for κ-carrageenan at its concentration of 0.04%. In this case, the turbidity changes only by 4.9%, which is close to the error in measuring the optical density (turbidity) of the sol.

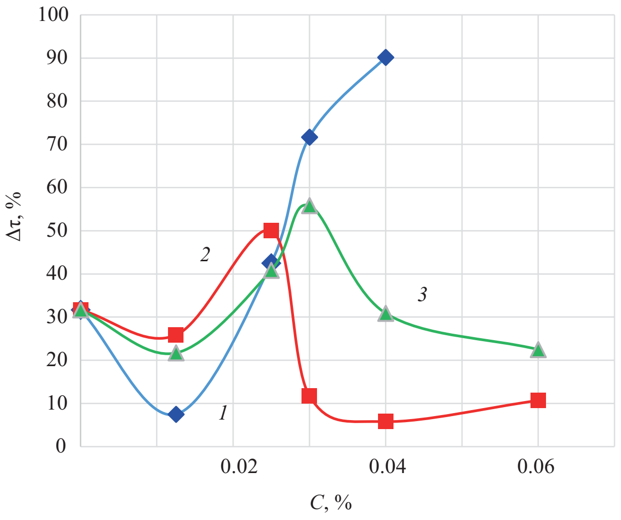

Fig. 5. Relative change in the turbidity of sols after two days in the presence of various weight concentrations of stabilizing polymers:
(*1*) SA,
(*2*) κ-carrageenan, and
(*3*) xanthan

Thus, polymers in certain weight concentration ranges contribute to a smaller change in the turbidity of the sol and, accordingly, to an increase in its aggregative stability. In this case, the curves of the relative change in turbidity over time for sols with the addition of different polymers have a similar shape to the curves with portions of decrease in relative change in turbidity and its maxima. At very low concentrations, the addition of polymer contributes to the stability of the sol. With increasing concentration, the stability of sol decreases, passing through a minimum (which is also a maximum of the relative change in turbidity over time), and then increases again. The type of dependence of the relative change in turbidity on the concentration of polysaccharides shown in Fig. 5 can be explained by the relationship between the amount of adsorbed polysaccharide and its ability to induce particle flocculation. All the polysaccharides studied are polyelectrolytes which carry a charge opposite to the charge of the particle surface. Therefore, they contribute to a decrease in the thickness of the electric double layer of micelles and, accordingly, to a decrease in the stability of the sol. At very low concentrations of water-soluble polysaccharides, the relative change in turbidity decreases. This indicates a stabilization of particles by polysaccharides at their given concentrations. In this case, the contribution of the stabilization of sols by polysaccharides exceeds their contribution to their destabilization. This is due to a decrease in the thickness of the electric double layer of micelles. The decrease in the relative change in turbidity with the subsequent increase can be explained as follows: due to the low degree of filling the surface of particles with polysaccharides, the macromolecules adsorbed by some of their ends on one particle can be adsorbed by other ends on the surface of another particle, connecting them by a bridging mechanism. Owing to this, the tendency of polysaccharides to cause flocculation of particles begins to increase, compensating for their stabilizing ability and then prevailing over it. Thus, after a certain decrease, the increase in the concentration of the polysaccharide enhances flocculation, and the relative change in turbidity increases. The value for the sol without the addition of polysaccharides begins to be exceeded, and thus the first maximum is reached. Then the relative change in turbidity decreases. This can be associated with the high degree of filling of the particle surface with adsorbed macromolecules. The surface of the particle is saturated with polymer macromolecules which begin to counteract the coagulation of particles. As a result, a stabilizing effect gradually begins to appear, reducing flocculation. Further, with increasing polysaccharide concentration, the relative change in turbidity gradually increases again. When the adsorption of polysaccharides on the surface is sufficiently high, their destabilizing effect on the sol due to a decrease in the thickness of the electric double layer of micelles begins to increase.

Initially, SA promotes flocculation less than the other two polysaccharides. This can be due to the fact that it has a significantly lower molecular weight (Table 1) and, consequently, a smaller macromolecule size. As a result, its ability to link sol particles with each other by a bridging mechanism is lower. This can explain the more pronounced decrease in stability at a concentration of 0.0125% in the case of the addition of SA.

The maxima on the curves in Fig. 5 for κ-carrageenan and xanthan are observed at their concentrations of 0.025 and 0.03%, respectively. This can be explained by the predominance of flocculation over the stabilizing effect of polymers at these concentrations. Moreover, in the case of κ-carrageenan, which has a higher molecular weight than xanthan (Table 1), this maximum occurs at a lower concentration. The degree of stabilization of the sol is greatest in the case of the addition of κ-carrageenan, which, as suggested by Fig. 3, in this concentration range, is adsorbed on the surface of ChS more strongly than the other two polymers and, moreover, has the highest molecular weight (Table 1). Consequently, in the concentration range where the stabilization begins to prevail over the flocculation, κ-carrageenan was found to be the most efficient stabilizer. This is due to its stronger adsorption resulting from the presence of specific interactions of its sulfate groups with the protonated amino groups of chitosan. For this reason, the adsorption of κ-carrageenan on the surface of sol particles creates a more voluminous and stronger structural-mechanical barrier. Therefore, in this concentration range, xanthan stabilizes sols over time much worse than κ-carrageenan. SA, which is adsorbed on ChS better than xanthan, exhibits an even lower stabilizing ability. This can be explained by the lower molecular weight of SA and, therefore, the less pronounced manifestation of the structural-mechanical stability factor. As can be seen from Fig. 5, the curve for SA obviously approaches a maximum only at concentrations above 0.04%. However, precipitation occurs at higher concentrations of the added SA (Fig. 5, curve *1*). As Fig. 3 shows, the adsorption of SA at this concentration is close to the adsorption of xanthan, but significantly lower than the adsorption of κ-carrageenan. This can be explained by the fact that the structural-mechanical barrier for SA is obviously less pronounced because of its lower molecular weight.

At higher concentrations, the stabilizing ability of water-soluble polysaccharides decreases again due to the increasing contribution of neutralization of the positive charge of the particle surface by polysaccharide polyions. At sufficiently high concentrations, polysaccharide anions cause rapid coagulation of positively charged ChS particles; something which is not considered in this work. Thus, although the water-soluble polysaccharides used in this study can cause coagulation of sol particles, there are certain concentration ranges in which they exhibit a stabilizing ability owing to the structural-mechanical stability factor. A mechanically strong protective shell is created around the ChS particle by the adsorption of polysaccharide macromolecules. This protective shell also carries negative charges, promoting the repulsion between polyanions adsorbed on different particles when these particles collide. Thus, despite the decrease in the electrostatic stability factor when polysaccharides are added to the sol, in the case of collision between two ChS particles with polyions adsorbed on them, the polyions can interfere with the coagulation for these reasons.

By means of dynamic light scattering with a Delsa Nano C particle size analyzer (*Beckman Coulter*, USA), the particle sizes measured in the sols demonstrated the highest stability over time and the sol without the addition of polymer. Table 2 presents the results for freshly prepared sols (time elapsed since preparation was about 1 h). In the histograms of the particle number size distribution for all sols, there is one peak with a fraction of 65–75%. The other peaks in the submicron and micron ranges are negligible (Fig. 6).

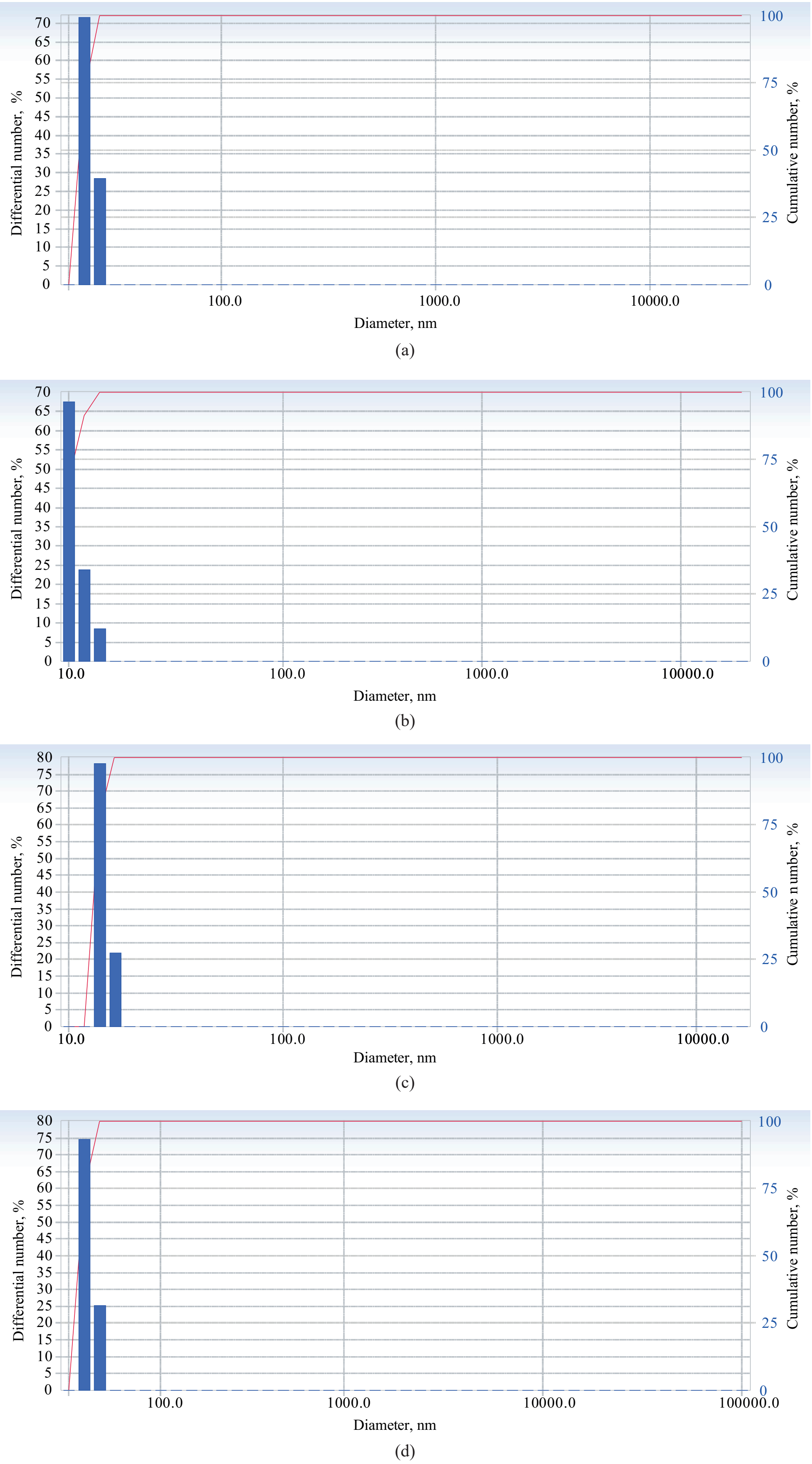

Fig. 6. Histograms of the particle number size distributions for ChS sols (a) without the addition of polysaccharide and with the addition of (b) 0.0125% SA, (b) 0.04% κ-carrageenan, and (d) 0.0125% xanthan

As can be seen, in the case of the addition of solutions of SA and κ-carrageenan to the sols to the indicated concentration, the average particle size decreases, while it increases in the case of addition of a xanthan solution. The decrease in the particle size in the sol can be related to the stabilizing effect of added polysaccharides at these concentrations. At the same time, the observed slight increase in the turbidity of freshly prepared sols in the case of the addition of κ-carrageenan and SA (in accordance with Fig. 4) can be explained by an increase in the number concentration of particles which are more protected from coagulation when polysaccharides are added.

At a concentration of 0.0125%, the smallest average particle size (smaller than the particle size without the addition of polysaccharides) is observed for SA, whereas that for xanthan is noticeably larger. This is consistent with the above considerations since at this concentration, SA contributes less to flocculation. Despite the fact that when xanthan is added, the particles become almost twice as large, sols with the addition of xanthan retain their particle size longer than without it (Fig. 5). This can be explained by the fact that the tendency for xanthan to stabilize particles over time begins to prevail over the tendency to promote flocculation. At a κ-carrageenan concentration of 0.04%, the particle size is smaller than that without the addition of polysaccharides. It is close to that with the addition of 0.0125% SA.

Figures 7 and 8 show the graphs of the dependencies of the turbidity of solutions on the amount of added electrolytes. Sodium chloride is an indifferent electrolyte in relation to ChS. Therefore, it does not affect the charge and electrical potential of the particle surface, but only compresses the electric double layer, promoting concentration coagulation. Coagulation begins when 0.1 mL of 3M sodium chloride solution is added to 5 mL of sol.

Sodium sulfate is a non-indifferent electrolyte in relation to ChS, since it can bind potential-determining chitosanium polycations, thereby changing the surface charge. When Na_2SO_4 is started to be added, the surface charge decreases because of the interaction of sulfate anions with potential-determining chitosanium polycations. When 1.75 mL of 0.5 M sodium sulfate is added to 10 mL of sol, the turbidity sharply increases, indicating that the coagulation threshold has been reached. In region II in Fig. 8, the sol is rapidly coagulated. When 3 mL of the electrolyte is added, the turbidity decreases and a new region of sol stability appears (Fig. 8, region III). This can be explained by the attainment of the equivalence point. This is the point where the electrolyte recharges the surface of the micelle, making it negatively

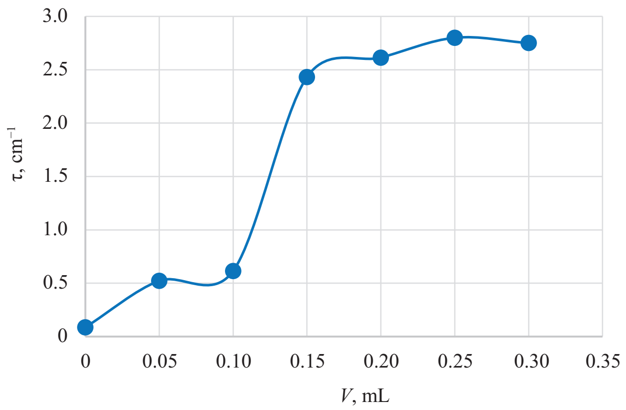

Fig. 7. Dependence of the turbidity of the ChS sol on the volume of the added NaCl solution

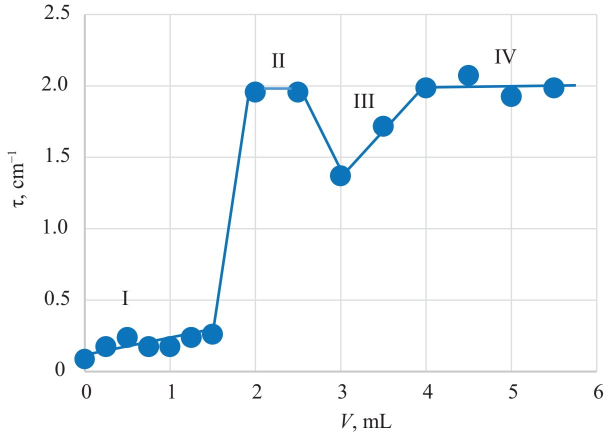

Fig. 8. Dependence of the turbidity of the ChS sol on the volume of the added Na_2SO_4 solution

Table 2. Average particle sizes of sols with the addition of various polysaccharides

Sol composition	Average particle size, nm	Coefficient of variation, %
Without addition of water-soluble polymers	25	17
With addition of 0.0125% SA	11	23
With addition of 0.04% κ-carrageenan	15	30
With addition of 0.0125% xanthan	55	8

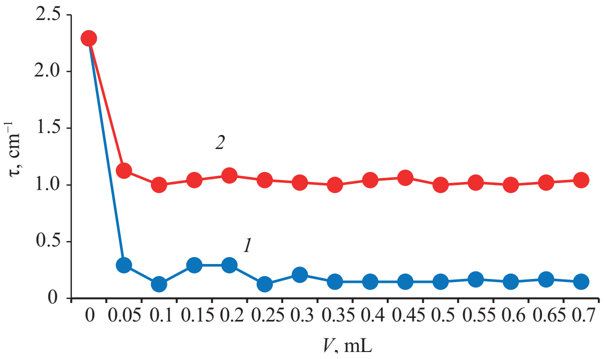

Fig. 9. Dependence of the turbidity of the ChS sol on the volume of added solution of (*1*) SA and (*2*) κ-carrageenan in the presence of NaCl sufficient for coagulation

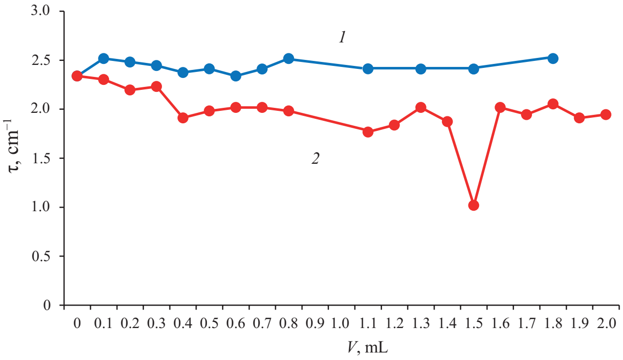

Fig. 10. Dependence of the turbidity of the ChS sol on the volume of added solution of (*1*) SA and (*2*) κ-carrageenan in the presence of Na_2SO_4 sufficient for coagulation

charged, thereby exerting a stabilizing effect. Then, when sodium sulfate is added, the turbidity of the sol begins to increase again and a new region of coagulation appears (Fig. 8, region IV). This is due to a decrease in the thickness of the electric double layer with an increase in the concentration of counterions which are now sodium ions. This type of dependence of turbidity on the volume of added electrolyte is typical of the case where a sol is supplemented with a non-indifferent electrolyte carrying non-indifferent ions charged opposite to the potential-determining ions. This proves that the surface of the resulting sols is positively charged.

For colloidal protection of the ChS sol from coagulation with electrolytes, SA and κ-carrageenan were taken as polymers which showed themselves to be the most optimal stabilizers.

These polysaccharides protect the sol from coagulation under the action of sodium chloride (Fig. 9), although inefficient against the action of sodium sulfate (Fig. 10). The decrease in turbidity by approximately half at one of the points in curve *2* (Fig. 10) is likely to be caused by the fact that κ-carrageenan, the molecules of which have a greater electrical charge, recharges the surface when taken in the given amount. However, this stabilizing effect is insufficient to prevent coagulation.

The sol coagulation thresholds with electrolytes were calculated using formula (4), while the protective numbers for polysaccharides were calculated using formula (5) (Table 3). The coagulation threshold with an indifferent electrolyte is 9.3 times higher than that with a non-indifferent electrolyte. The protective number against the coagulation with an indifferent electrolyte is 4.8 times lower for SA than for κ-carrageenan. The protective numbers for both of these polysaccharides are significantly lower than the concentrations at which, in accordance with Fig. 5, the relative changes in the turbidity of the sols over time (their maximum stability over time) are minimum. Therefore, at these concentrations of SA and κ-carrageenan, their efficient protection against the coagulation with sodium chloride is also ensured.

CONCLUSIONS

A method was developed to obtain ChS hydrosol with a positive particle charge. It was shown that the addition of polysaccharides to certain concentrations improves the stability of sols. The mechanisms of the stabilization of sols by polysaccharides are explained using experimental capillary viscometry data on the adsorption of these polysaccharides on the surface of ChS. In this case, in the widest concentration range, κ-carrageenan

Table 3. Coagulation thresholds of ChS sols with sodium chloride and sodium sulfate, and the protective numbers of polysaccharides against the coagulation of ChS sols with these electrolytes

Electrolyte causing coagulation	Coagulation threshold, mM	Stabilizer	Protective number, %
NaCl	73	SA	$6.1 \cdot 10^{-5}$
NaCl		κ-Carrageenan	$2.9 \cdot 10^{-4}$
Na_2SO_4	7.88	SA	Does not protect
Na_2SO_4		κ-Carrageenan	Does not protect

is adsorbed most strongly, whereas xanthan is adsorbed least of all. The stronger adsorption of κ-carrageenan can be attributed to the specific binding of sulfate groups to the ChS surface.

Based on experimental data on the relative change in turbidity over time, which has complex behavior with extrema, the polysaccharides studied herein predominantly either stabilize or destabilize the sol according to the degree of filling the surface of the particles with polysaccharide macromolecules, depending on their concentration. The stabilization of sols by polysaccharides is ensured by the structural-mechanical stability factor.

The most stable over time are the sols with the addition of 0.0125% SA and κ-carrageenan in the range of 0.04%. In the former case, this can be explained by the lower molecular weight of SA, which is less conducive to the flocculation of sol particles at lower polysaccharide concentrations. In the latter case, at a higher polysaccharide concentration, this may be a consequence of better adsorption of κ-carrageenan, as well as its higher molecular weight. This makes a greater contribution to the structural-mechanical stability factor.

κ-Carrageenan and SA protect the sol against coagulation with an indifferent electrolyte (NaCl) at all amounts of polysaccharides used. At the same time, at the same amounts of polysaccharides, no protection against the coagulation by a non-indifferent electrolyte (Na_2SO_4) was observed. The protective number against the coagulation with sodium chloride is lower for SA than for κ-carrageenan.

Based on the results of the work, it can be concluded that SA and κ-carrageenan can be used as efficient stabilizers of ChS hydrosols over time and to protect them against the flocculation with sodium chloride.

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Authors' contributions

V.S. Erasov—idea of the study, acquisition and processing of experimental data, analysis of results, and writing the text of the article.

Yu.O. Maltseva—obtaining and processing experimental data.

The authors declare no conflict of interest.

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