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Computer simulation of chitosan solvation in water and solutions with reduced active acidity

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Abstract. *The molecular properties of fragments of molecules of natural chitosan and with protons attached to nitrogen have been studied using computer chemistry. The block of periodic boundary conditions in the HyperChem application was used to simulate the dissolution of chitosan in water and a solution with an acidity of less than 7 (protonated chitosan). The possibility of formation of mesh gels in an acidic environment has been revealed. The theoretical results obtained were confirmed by the study of chitosan solutions in whey. It was found that when chitosan is added to the subsurface serum, viscous solutions are formed and the value of active acidity increases, which opens up the possibility of using chitosan and whey for various types of food products.*

Keywords: chitosan, molecular properties, periodic boundary conditions, whey, active acidity

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Компьтерное моделирование сольватации хитозана в воде и растворах с пониженой активной кислотностью

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Аннотация. *С использованием компьютерной химии изучены молекулярные свойства фрагментов молекул натурального хитозана и с присоединенными к азоту протонами. С помощью блока периодических граничных условий в приложении HyperChem выполнено моделирование растворение хитозана в воде и растворе с кислотностью меньше 7 (протонированный хитозан). Выявлена возможность образования сетчатых гелей в кислой среде. Полученные теоретические результаты подтверждены при исследовании растворов хитозана в молочной сыворотке. Установлено, что при добавлении хитозана в подсырную сыворотку образуются вязкие растворы и увеличивается значение активной кислотности, что открывает возможность использования хитозана и молочной сыворотки для различных видов пищевых продуктов.*

Ключевые слова: хитозан, молекулярные свойства, периодические граничные условия, молочная сыворотка, активная кислотность

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Introduction. Chitosan is a natural product and is a promising raw material for obtaining sorbents. Chitosan is classified as an aminopolysaccharide, the monomers of which are linked by a β glycosidic bond (Fig. 1). Chitosan is usually obtained from the shell of crustaceans, although other sources (fungi, insects, mollusks, etc.) can also be used [1, 2, 3].

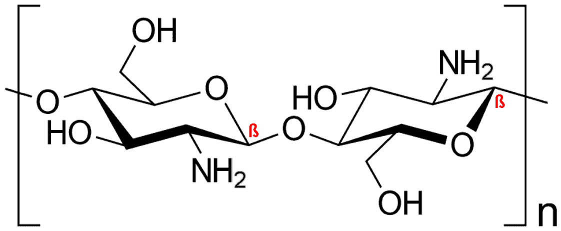


Figure 1 – Repeating fragment of two monomers of the chitosan molecule

Chitosan is obtained from chitin by deacetylation. The main molecular properties of chitosan depend on the degree of deacetylation, which is in the range of 60–100%. The base dissociation constant (pK_{b}) in the region of the amino group has a value of 6.5, which promotes protonation of the nitrogen atom in neutral and acidic environments [1, 2, 3].

There is information that chitosan in solutions is capable of forming cross-linked polymer networks, which characterize the gel-forming properties of this chemical compound. One of the main advantages of chitosan is also its environmental friendliness, safety and harmlessness.

In medicine, chitosan is widely used for the production of artificial skin and surgical sutures, for the treatment of ulcers, skin burns and wounds, as well as for the production of anti-varicose and anti-sclerotic drugs [4, 5, 6, 7].

Chitosan is widely used as a functional additive as a gelling agent and emulsifier in food formulations, it has a preventive effect by regulating intestinal peristalsis, lowers blood pressure, removes toxins and cholesterol from the body, restores lymphatic cells, facilitating the elimination of cancerous tumors. In medicine, chitosan is widely used for the production of artificial leather and surgical sutures, for the treatment of ulcers, skin burns and wounds, as well as for the production of anti-varicose and anti-sclerotic drugs [8, 9, 10, 11, 12, 13].

Materials and research methods. The aim of the study was a theoretical assessment of the molecular and gelling properties of chitosan and practical confirmation of the dissolution of the studied polysaccharide in whey.

To achieve this goal, the following tasks were to be solved:

- perform computer modeling of fragments of the initial and protonated molecules chitosan;

- to study the molecular properties of a fragment of the biologically active additive chitosan;
- using the HyperChem periodic boundary conditions block, analyze the possibility of forming network gels;
- to study the solubility of chitosan in a solution of milk whey.

In the experimental studies, food grade chitosan manufactured according to TU 9289-001-44162258-98 and milk (cheese) whey with pH = 4.51 GOST 34352-2017 were used. Computer modeling and evaluation of the molecular and gelling properties of chitosan were carried out in the HyperChem v. 8.0 application [14, 15].

Research results and their discussion. Using computer chemistry, we will create structural models of sixteen monomers of chitosan molecule fragments and perform geometric optimization. In the optimized structures, we will study the surface of the electrostatic potential and the molecular properties of chitosan molecule fragments. Since it is planned to conduct studies of the gel-forming properties using a block of periodic boundary conditions (Periodical boundary condition), calculations in which are carried out by molecular-mechanical methods, we use the force field amber2. In order for the calculated data of the studied variants to be correctly compared, we use the same method of using the force field (amber2) in all calculations.

There is information in literary sources that chitosan attaches a proton in the region of nitrogen atoms and forms gels in solutions with active acidity (pH) below 7. Let us analyze the magnitude of the charges on the nitrogen atoms (marked in purple) of the chitosan molecule fragment (Fig. 2).

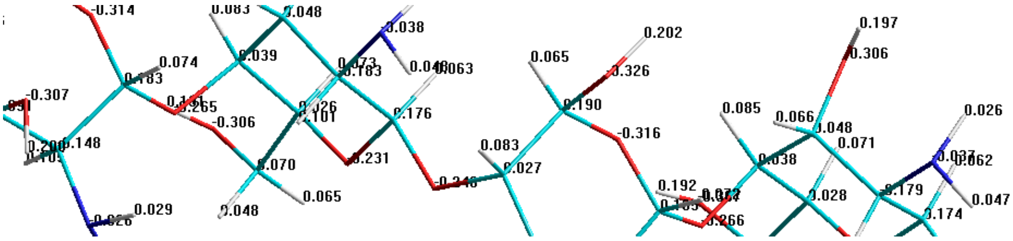
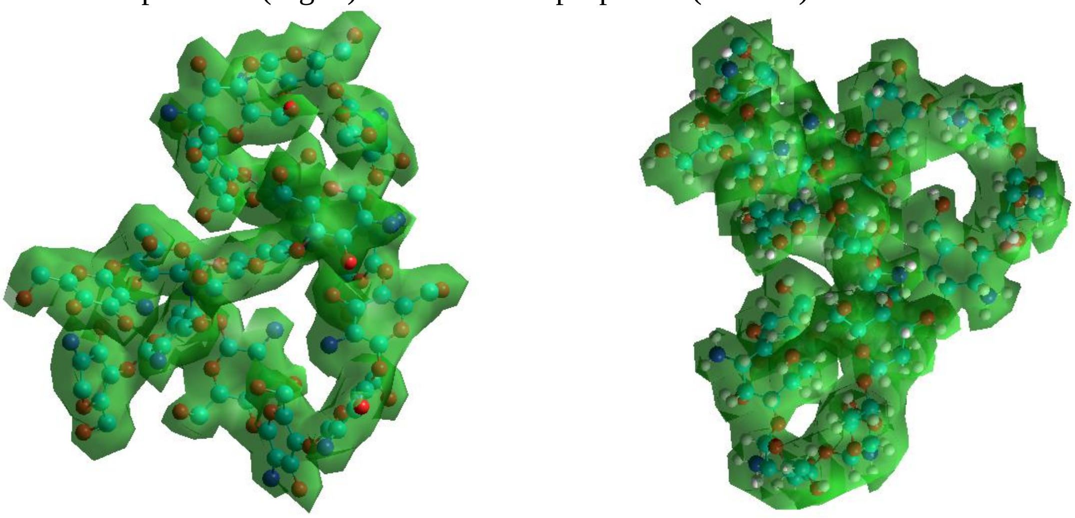


Figure 2 – Distribution of charges in atoms of a fragment of a chitosan molecule

When examining a fragment of 16 monomers, it was found that the charge of nitrogen atoms is in the range of -0.026 – -0.038 C. The data obtained indicate the possible addition of a proton to the nitrogen atom. Guided by literature data, we will create models of fragments of natural chitosan molecules with protonated nitrogen atoms and study the surface of the electrostatic potential (Fig. 3) and molecular properties (Table 1).



a b

Figure 3 – Distribution of molecular electrostatic potential on the surface natural (a) and with protonated nitrogen chitosan

Molecular electrostatic potential (MEP) is one of the characteristics of the reactivity index of the studied fragment of the molecule. MEP primarily characterizes the point energy of interaction between charges. Visualization of MEP (Fig. 3) shows that the fragment of the molecule with protonated nitrogen and natural chitosan differ little from each other. In both fragments, the surface of the electrostatic potential is not electrically neutral, but has a positive charge (green color of the surface), therefore, both fragments are hydrophilic. At the same time, using molecular-mechanical calculations, the molecular properties of the fragments of the chitosan molecule were studied (Table 1).

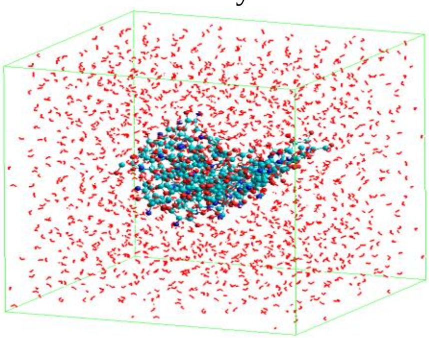
Table 1 – Molecular properties of fragments of the chitosan molecule

Properties	Fragment of a chitosan molecule (16 monomers)	
	natural	with attached protons
Potential energy, kcal/mol	46,44	268.91
Root Mean Square Gradient (RMS Gradient), kcal/(Å×mol)	0,04767	0.04992
Dipole moment, Debye	0	0

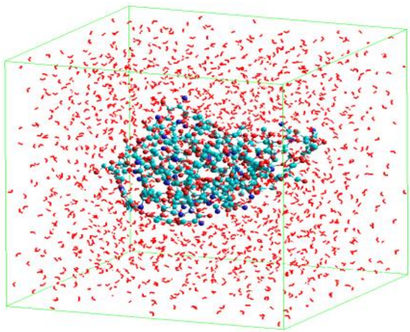
Potential (stored) energy (PE) of a molecule is characterized primarily by the arrangement of atoms and ensuring the stability of the structure confirmation. Molecular-mechanical calculations do not allow to effectively optimizing the geometry of molecules. In this case, it is important to note that the difference in PE between protonated and natural chitosan is significant (Table 1) and is equal to 222.47 kcal/mol (268.91 - 46.44), which is due to a change in the structure and the addition of protons. The root-mean-square gradient characterizes the stability of the molecular structure. Both fragments under study have a stable structure, since the RMS gradient of the fragments is close to zero (0.04767 and 0.04992). The dipole moment in both fragments is zero, which indicates a good balance of positive and negative charges in the studied fragments of chitosan molecules.

Since the main interest is in the behavior of chitosan in aqueous systems and the possible formation of gels, the most efficient way to create an aqueous environment around the molecules is to use the periodic boundary conditions block in the HyperChem application. We solvate three molecules of natural and protonated chitosans.

Chitosan in a cube of periodic boundary conditions (Fig. 4) and carry out geometric optimization of the system.



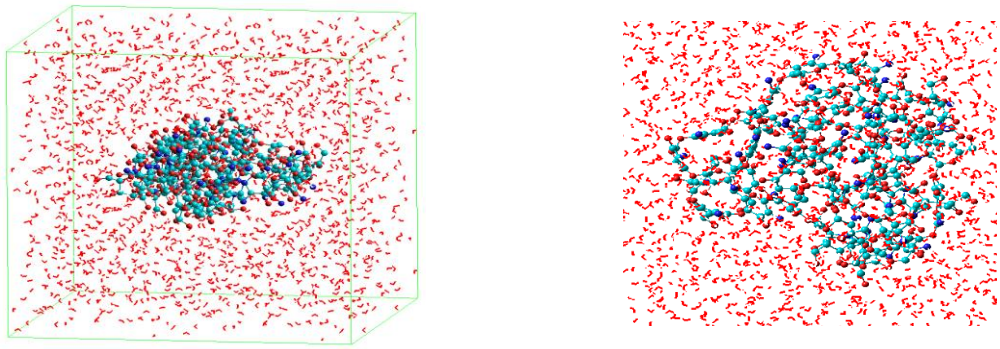
a



b

Figure 4 – Simulations of solvation in water of natural chitosan before (a) and after (b) geometric optimization

We will perform a similar simulation with three chitosan molecules with protons attached to nitrogen (Fig. 5).



a b

Figure 5 – Simulations of solvation in water of chitosan with protons attached to nitrogen before (a) and after (b) geometric optimization

The simulation results show that fragments of natural chitosan molecules (Fig. 4) change their configuration slightly, they only swell slightly, but do not form a gel. Chitosan molecules with protons attached to nitrogen after geometric optimization fill almost the entire volume of the periodic box, forming a mesh gel. After geometric optimization, the molecular properties (Table 2) of two molecular systems after solvation were determined. It should be borne in mind that the calculated indices of molecular properties in periodic boundary conditions relate only to the molecular system entered into the box of periodic boundary conditions. Interaction with the aqueous environment is taken into account, but is not summed up with the indices for the solvated molecules.

Table 2 – Molecular properties of fragments of the chitosan molecule after solvation in an aqueous medium

Properties	-solvated fragments of the chitosan molecule (3 fragments)	
	natural	with attached protons
Potential energy, kcal/mol	-14446,80	-11094,70
Root mean square gradient (RMS gradient), kcal/(Å×mol)	0.09837	0.09999
Dipole moment, Debye	619.1	56.6

The obtained data (Table 2) indicate that both systems (fragments of natural and protonated chitosan) are geometrically optimized quite well, since fragments of natural chitosan have a potential energy of -14446.8 kcal/mol, with attached protons – -11094.7 kcal/mol. The lower (compared to attached protons) energy of fragments of natural chitosan is obviously due to the dense packing of molecular fragments. The root-mean-square gradient for both systems (0.09837 and 0.09999) has low values, which indicates a high efficiency of the performed geometric optimization procedure. The dipole moments of both systems (for natural chitosan 619.1; with attached protons 56.6 Debye) differ from each other due to the formation of network gels and more effective charge balancing for chitosan with attached protons.

The conducted studies confirm the ability of chitosan to dissolve and form gels in the presence of organic acids. Milk whey, which contains lactic, acetic, formic, citric, propionic, butyric and other volatile fatty acids necessary for protonation of chitosan nitrogen, can be used as an effective solvent for chitosan. The composition, nutritional and biological value of milk whey are ambiguous and depend on the type of manufactured products and the raw materials used. In addition to organic acids, milk whey contains milk fat, soluble nitrogen-containing compounds, minerals, vitamins, lactose and other oligosaccharides, vitamins and enzymes [16, 17].

The chemical composition of whey determines its high nutritional value. Including whey in the diet regulates the functions of the gastrointestinal tract, improves blood circulation, normalizes blood pressure, and has an antioxidant effect on free radicals and a therapeutic effect on the condition of the skin [18, 19].

Whey is a secondary raw material of the dairy industry; it is widely used in the production of beverages, in pharmaceuticals, as part of personal hygiene products and medicine. At the same time, whey remains a significant by-product. About 42% of its amount is used to produce low-value products, including fertilizers and animal feed, or is simply disposed of. Processing and reuse of whey remains an important problem in the dairy industry [20]. Often, in the food industry, the main obstacle (primarily in the meat industry) remains the value of active acidity, which usually fluctuates in the range of 4.2 - 5.5 [21].

The use of whey to expand the range of special food products is promising. For this purpose, it is possible to use milk or water in various dishes with whey. Such a replacement gives a more delicate and airy taste to baked goods preserves the juiciness of meat and fish when baking, adding to marinades fulfills the set technological goals and enriches the products with components with proven beneficial properties. Whey has found application as one of the main ingredients in the production of protein shakes and sports nutrition.

Active acidity determines the prospects for the use of whey. The problem of active acidity can be solved and the valuable biologically active components of whey can be used using chitosan. The theoretical studies have confirmed the possibility of attaching a proton to the nitrogen of chitosan, which will affect changes in active acidity. The solubility of chitosan depends on the degree of its deacetylation, so the results of studies of the same brand may differ slightly. The effect of the quantitative content of chitosan in cheese whey on changes in the active acidity of the solution was studied (Table 3). A mixture of chitosan (the degree of deacetylation according to technical documentation is not less than 75%) and cheese whey was subjected to heat treatment for 10 minutes at 80 °C in order to completely dissolve chitosan , then the solution was cooled to room temperature and the active acidity was measured.

Table 3 – Dynamics of changes in active acidity of cheese whey depending on the concentration of chitosan

Concentration of chitosan in cheese whey, %			
0	2	4	6
Active acidity (pH)			
4.51	6.02	6.11	6.28

After the cooling process is complete to room temperature (18 - 20 °C viscous translucent solutions were formed. Table 3 shows the dynamics of the increase in active acidity from 4.51 to 6.28 at a chitosan concentration in the solution of 6%. The formation of viscous solutions is most likely due to the formation of hydrogen bonds with unshared electrons along the entire chitosan molecule.

The results obtained indicate that aminopolysaccharide Chitosan, like proteins, is capable of participating in gel formation during food production.

Conclusion. Modeling of fragments of the chitosan molecule, natural and with protons attached to nitrogen, was performed.

1. When studying the molecular properties of the investigated fragments of molecules, it was found that natural chitosan has a more stable structure since its potential energy is lower (compared to protonated chitosan 268.91 kcal/mol) and is equal to 46.44 kcal/mol.

2. Using periodic boundary conditions in the HyperChem application, the formation of chitosan cross-linked gels in acidic conditions was confirmed.

3. It has been established that dissolving chitosan in whey increases the value of active acidity of the solution; adding 2% chitosan helps to increase pH by 1.51 (6.02-4.51), which opens up the possibility of using whey in the production of a wide range of food products.

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