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Abstract. In this paper, a new method for the synthesis of the sulfated starch-casein complex is proposed. The resulting new complex was characterized by FTIR and UV-spectroscopy. It has been shown by FTIR spectroscopy that both nitrogen atoms and the carboxyl group of amino acid residues are protonated in casein. The sulfated starch-casein complex obtained in this work may have biological activity, as well as its analogues isolated from plants, fungi, and microorganisms, as well as synthesized in laboratory conditions.

1. Introduction

Polysaccharides are vital biomacromolecules consisting of homo- or heteromonosaccharides and uronic acids linked by glycosidic bonds [1-3]. They are found in various parts of plants, animals, fungi, bacteria and algae and play an important role in numerous physiological processes [4]. Over the past decades, bioactive polysaccharides have been actively studied as therapeutic agents against many chronic diseases due to their biodegradability, non-toxicity and biocompatibility [5]. Studies have shown that polysaccharides have a wide range of pharmacological properties, such as antioxidant, antitumor, antimicrobial, lipid-lowering, antidiabetic and hepatoprotective [6–8]. Polysaccharides have been well studied as new products in the fields of cosmetics, food, medicine, petrochemicals, and paper production [3,9,10]. In particular, in the medical industry, polysaccharides are mainly used as pharmaceuticals and medical biomaterials (hypoglycemic, anti-osteoarthritis, and antitumor drugs) to reduce the effect of the corresponding metabolic syndromes [9,11].

Sulfated polysaccharides play various roles in biology, acting as structural components of tissue for signaling agents in physiological processes. Their composition and structure, as well as their physicochemical, biomechanical, and biological properties, are of great interest for basic research and for the development of new products in the pharmaceutical, medical, and food industries [12].

Proteins due to the presence of many NH-groups are surface active and play an important role in the formation and stabilization of emulsions in the presence of polysaccharides, interacting with them through electrostatic forces. Since polysaccharides are often hydrophilic substances and remain in the aqueous phase, they help to control such characteristics as thickening, gelation, and also act as stabilizing agents. The formation and deformation of polysaccharide-protein complexes and their solubility depend on various factors, such as the charge and nature of biopolymers, pH, ionic strength, and ambient temperature [13, 14].

Non-covalent interactions between polysaccharides and proteins in the emulsion composition play a major role in changing the interfacial behavior and stability of food colloids. The driving forces behind these non-covalent interactions are electrostatic interactions, hydrophobic interactions, H-bonds, and Van der Waals interactions [14-17].

Polysaccharide-protein complexes have the following properties: immunomodulating, antioxidant, and they also inhibit aging and reduce blood glucose [18]. Thus, the development of new methods for the synthesis of polysaccharide-protein complexes becomes relevant.

The aim of this work was to develop a new method for the synthesis of sulfated starch-casein complex.

2. Experimental part

The work used hydrolyzed casein (Sigma). Sulfated starch was obtained according to the procedure [19] by sulfating starch with a sulfamic acid-urea complex. To obtain the sulfated starch-casein complex (SSCC), sulfated starch with a sulfur content of 14.3 wt.% was taken.

The casein-containing complex of sulfated starch was obtained from its sodium salt by the ion exchange method using the KU-2-8 ion exchange resin according to the previously described method [20]. Ion exchange was carried out in a dynamic mode. Preliminarily, the KU-2-8 ion-exchange resin located in the Na^+ form was converted to the H^+ form. To this end, an aqueous 2M HCl solution was passed through a layer of KU-2-8 resin in a Na^+ form placed in a vertical glass column with a diameter of 15–20 mm and a capacity of 50 ml to equal concentrations of the hydrochloric acid solution entering and leaving the burette. After washing the cation exchange resin with distilled water until a neutral reaction of the washing water, casein was sorbed on it. To this end, a 1M casein solution was passed through cation exchange resin, washed with distilled water until there was no casein in the wash water. Then, an ion exchange of the sodium cation in the sodium salt of sulfated starch was carried out for the protonated form of casein sorbed by cation exchange resin. To this end, a solution of sulfated starch (2.0 g) purified by dialysis of the sodium salt of sodium sulfate starch was passed through a layer of prepared cation exchanger. After passing through the column 25 ml of a salt solution of sulfated starch, the resin in the column was washed with distilled water (3 times 25 ml). After passing a second volume of water, the presence of casein was determined using a qualitative reaction. Then the washing liquids were collected and evaporated to dryness under the vacuum of a water-jet pump on a rotary evaporator at a temperature of not more than 40°C. The solid residue obtained after drying in vacuum (sulfated starch-casein complex) was analyzed by FTIR and UV-spectroscopy.

The FTIR spectra of initial starch and sulfated starch were recorded using a Shimadzu IR Tracer-100 spectrometer (Japan) within the wavelength range of 400–4000cm-1. The spectral information was analysed using the OPUS program (version 5.0). Solid samples for analysis were prepared in the form of pills in a KBr matrix (2 mg sample/1000 mg KBr).

The UV–vis spectra were measured with a Leki SS2109-UV scanning spectrophotometer (Leki Instruments, Finland) using 1 cm quartz cells. Cell thermostating (± 0.1 K) was performed with a Haake K15 thermostat connected to a Haake DC10 controller. The absorbance of solutions was measured over the 220–450 nm wavelength range. All measurements were performed at 298 ±0.1 K.

3. Results and discussion

The process of modifying sulfated starch with casein included the following steps:

1. KU-2-8 cation exchanger, manufactured in industry in the Na form, was converted into the H form:

$$R–SO_3Na + H^+ \rightarrow R – SO_3H + Na^+,$$

where R is the matrix of the resin KU-2-8.

2. Sorption of casein on the H-form of cation exchanger KU-2-8:

$$R–SO_3H + \text{Casein} \rightarrow R–SO_3–[\text{Casein H}]^+.$$

3. Exchange of sodium cations in sulfated starch for the cation of protonated casein sorbed on cation exchange resin was carried out:

$$R–SO_3–[Casein\ H]^+ + Starch–OSO_3–Na^+ \rightarrow R–SO_3–Na^+ + Starch–OSO_3–[Casein\ H]^+.$$

Ion exchange was carried out in a dynamic mode.
The obtained sulfated starch-casein complex was analyzed by FTIR spectroscopy (figure 1).

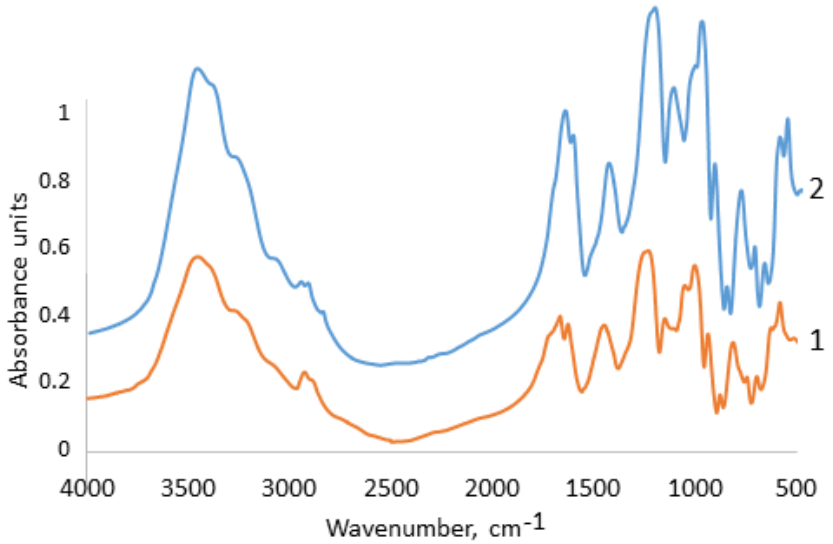

Figure 1. FTIR-spectra of the sulfated starch (1) and sulfated starch-casein complex (2).

According to FTIR spectroscopy, there is also a significant change in the nature of the spectrum in the region of 1658-1514 cm^{-1}, in which there are absorption bands corresponding to stretching vibrations of the C=N, C=C bonds of the caseine and the ionized carboxyl group. The spectral region 3450–2911 cm^{-1} corresponds to stretching vibrations of N–H, O–H, and CH bonds [21-23].

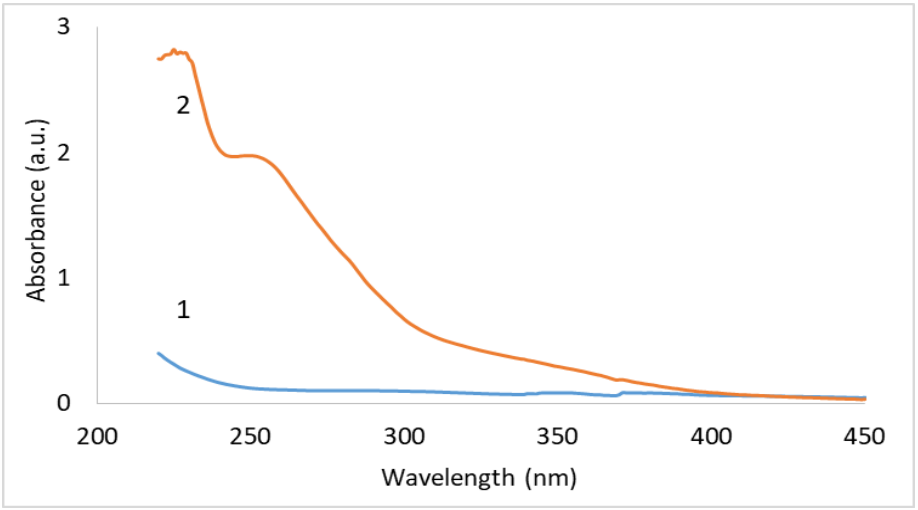

Figure 2. UV-spectra of the sulfated starch (1) and sulfated starch-casein complex (2).

The FTIR spectrum of the casein-containing starch sulfate derivative has a weak absorption band in the region of 3091 cm^{-1}, which corresponds to stretching vibrations of NH_3^+. The absorption bands in the region of 1658–1514 cm^{-1} can be attributed to the absorption bands of COO^- and NH_3^+; in addition, a weak absorption band is observed in the region of 1737 cm^{-1}, which should correspond to stretching C=O vibrations of the undissociated carboxyl group. In the spectrum of the casein-containing starch sulfate derivative, a broad band is observed in the region of 3450-2910 cm^{-1}, in which superimposition of absorption bands occurs, corresponding to stretching vibrations of N–H bonds in NH_3^+, NH_2, and O–H bonds. As in the spectrum of the sodium salt of sulfated starch, there is a high-intensity absorption band in the region of about 1240–1220 cm^{-1}, corresponding to stretching vibrations of the O=S=O bonds [19]. It should be noted that, in accordance with the FTIR spectrum of the casein-containing derivative of sulfated starch, both the carboxyl group and the amino group of amino acids in the protein structure undergo protonation.

Initial sulfated starch and obtained sulfated starch-casein complex was analyzed by UV-spectroscopy (figure 2).

It is shown in figure 2, the UV spectrum of the sulfated starch-casein complex is significantly different from the spectrum of the original sulfated starch. In the UV spectrum of the sulfated starch-casein complex, intense peaks are observed in the range 220–230 nm and 245–260 nm, which correspond to the peaks of the protonated amino acids that make up casein [24,25].

Based on the analysis, a conditional scheme of a complex of sulfated starch-casein is proposed. The scheme of the sulfated starch-casein complex is shown in figure 3.

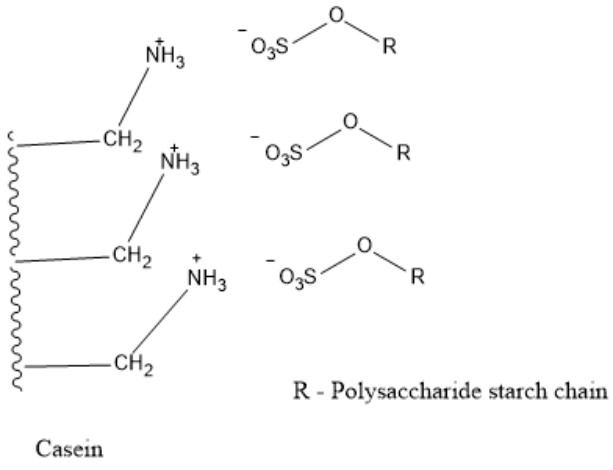

Figure 3. Scheme of the sulfated starch-casein complex.

4. Conclusions

For the first time, the sulfated starch-casein complex was synthesized by ion exchange method.

The presence of casein in the sulfated starch-casein complex was proved by FTIR and UV spectroscopy. So, in the FTIR spectrum of the sulfated starch-casein complex, there are absorption bands in the regions of 1658-1514 cm^{-1} and 3450-2910 cm^{-1}, corresponding to the vibrations of the NH group. Moreover, absorption bands in the region of 1658–1514 cm^{-1} can be attributed to the absorption bands of COO^- and NH_3^+; in addition, a weak absorption band is observed in the region of 1737 cm^{-1}, which should correspond to stretching C=O vibrations of the undissociated carboxyl group.

The introduction of casein into the structure of sulfated starch has also been proved by UV spectroscopy. In the UV spectra of the sulfated starch-casein complex, in contrast to the initial sulfated starch, intense peaks appear in the wavelength regions of 220-230 and 245-260 nm.

Acknowledgments

The reported work was funded by RFBR and the government of Krasnoyarsk region according to the research project № 18-43-242003. The study was carried out using equipment of the Krasnoyarsk

Regional Center of Research Equipment, Federal Research Center "Krasnoyarsk Science Center SB RAS". The authors are grateful to I.V. Korolkova for obtained FTIR-spectra.

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