Chitosan succinate and food-grade chitosan for the creation of tissue-engineering structures by the laser method

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Abstract. Laser radiation makes it possible to form a composite material with laser radiation, which is intended for use as cellular- and tissue-engineering structures. It is important to obtain a uniform distribution of components throughout the volume of such material. Intermolecular interactions between chitosan and single-walled carbon nanotubes (SWCNTs) affect the structure of the composite. The results of IR spectroscopy and nonlinear optical studies have shown that the effect of laser radiation has different effects on the two types of chitosan, which was clearly demonstrated by SEM studies. Upon detailed examination, agglomerates in the composite material are clearly visible. Chitosan succinate less prevents the formation of agglomerates from SWCNTs, as a result of which a high agglomeration of nanotubes occurs among themselves in the composite material. The corresponding formations are distinguishable in the SEM images.

1. Introduction
Chitosan is used to increase the biocompatibility of various materials. Chitosan coatings can be applied after the surface is polished to smoothness, oxidized in air, and immersed in a chitosan solution [1]. The degree of deacetylation has little effect on the surface properties. Differences in molecular weight, spatial structure of chains, surface charge, distribution and order of radicals are important. In the present work, we carried out a comparative study of two types of chitosan with different spatial structure of the chains. Aminosugar chitosan is an amino polysaccharide, the simplest derivative of chitin. Chitosan is a polydisperse polymer of D-glucosamine, which consists of acetamide groups (5-15%) and groups connected to amino acids and peptides (1%). The source of chitosan production and the production technology determines its molecular weight, which varies from 300 to 1000 kDa. Food-grade chitosan (FCTS) is insoluble at neutral and basic pH and dissolves upon acidification when the free amino groups are protonated. Due to its biocompatibility with human tissues, low cytotoxicity, the ability to enhance regenerative processes during wound healing, as well as the rapid rate of biodegradability, chitosan is of particular interest for medicine. In addition, due to the presence of various functional groups, chitosan provides the possibility of the formation of bonds between amino sugar and various substances, which makes it easy to create a variety of chemical compounds based on it.

Chitosan succinate (SCTS) is a pearlescent cream-colored powder obtained from crab chitosan by deacetylation and interaction with succinic anhydride (residual moisture - 4.79%, pH of aqueous
solution - 7.7). Due to the deacetylation procedure, SCTS molecules acquire a positive charge, thus, a suspension based on SCTS actively binds to negatively charged biomolecules. In addition, chitosan has high biodegradability and biocompatibility. FCTS is a white powder (the content of the main substance is 88.4%, the mass fraction of moisture is 3.13%, the mass fraction of minerals is 0.53%), obtained from high-molecular chitosan by chemical hydrolysis. SCTS has a high hydrophilicity, therefore, it dissolves better in water and a coarse-grained structure does not form, as is the case with FCTS. In general, chitosan is obtained from chitin in deacetylation and depolymerization reactions during alkaline hydrolysis or from the shell of crustaceans using the hydrolytic enzymes chitinase and deacetylase.

2. Preparation of dispersed media before the experiment
First, aqueous dispersed media (dispersions) were prepared. For this, an aqueous paste of single-walled carbon nanotubes (SWCNT) was used to which distilled water was added to achieve a concentration of 0.001 wt. %. Preliminary mixing was carried out for 30 minutes on a magnetic stirrer, followed by stirring with an ultrasonic submersible homogenizer for an hour at a power of 40 W. Subsequently, SCTS or FCTS is added in the amount of 2 mass. % with constant stirring. In Figure 1 shows the form of dispersed media for both types of chitosan.

![Figure 1](image_url)

**Figure 1.** Aqueous dispersions of carbon nanotubes with two types of chitosan.

3. Optical measurement
Nonlinear optical characteristics were studied using a neodymium pulsed laser with a wavelength of 532 nm and a pulse duration of 16 ns. The procedure for determining the values of these characteristics from experimental data is described in [2, 3]. Cuvette thickness of 2 mm selected according to Rayleigh length [4]. This technique allows not only to identify materials with a threshold effect [5], but also to determine the most effective of them [6]. To obtain correct values, when carrying out an experiment by the Z-scan method, it is necessary that the focused laser beam has a Gaussian shape:

\[ w(z) = w_0 \sqrt{1 + \left( \frac{\lambda z}{\pi w_0^2} \right)^2}, \]

where, \( w_0 \) is the waist radius, \( w(z) \) is the radius at an arbitrary point of the optical axis \( Z \), \( z \) is the position of the sample relative to the lens focus, \( \lambda \) is the laser wavelength. To check the
correspondence of the used laser beam to a Gaussian beam, a study of the beam radius depending on the distance from the lens focus was carried out. For this, the CCD camera was placed on a motorized ruler and, similarly to the Z-scan experiment, moved relative to the lens focus.

Nonlinear optical parameters of the medium can be found with using the radiation transfer equation for the case of a threshold character of interaction with the material [5]. The absorption coefficient $\mu(I)$ in this case depends on the intensity of the incident radiation, and this dependence manifests itself only after exceeding a certain threshold intensity $I_{th}$ [7]:

$$\mu(I) = \alpha + \beta(I - I_{th})\eta(I - I_{th}),$$

where $\alpha$ and $\beta$ are linear and nonlinear absorption coefficients; $\eta(\cdot)$ is the Heaviside step function.

3.1. Beam profile measurement

As a result, experimental values of the beam radius at different points were obtained, which correlate well with the theoretical values obtained in the course of studying the materials (Figure 2). Figure 3 shows the registered profile of the laser beam corresponding to the sample position at $z = -4$.

![Figure 2](image2.png)

**Figure 2.** Dependence of the beam radius on the position of the CCD camera relative to the lens focus.

![Figure 3](image3.png)

**Figure 3.** Images of the CCD camera for the beam at the point $Z = -4$ cm: a - 2D image, b - 3D image.

3.2. Z-scan experiments of chitosan

Studies of the nonlinear optical properties of dispersions have been carried out. In the case of SCTS with SWCNTs, the nonlinear absorption coefficient was $561 \text{ cm/GW}$ with a threshold intensity of
2.7 MW/cm², for FCTS with SWCNTs 902 cm/GW and 5.5 MW/cm² (Figure 4 and Table 1). This difference is explained by the large size of the formed agglomerates and, accordingly, the smaller number of fractions in the dispersion in the case of SCTS; in addition, the strong interaction of the SWCNT surface with FCTS can lead to a slight decrease in heat transfer to the liquid and, accordingly, the processes, that associated with local heating of the liquid, appear at higher values of intensity [7].

4. Formation and study of the composite

4.1. Composite fabrication

The formation of a composite material from a dispersion droplet was performed using femtosecond laser pulses. The beam diameter was selected according to the droplet size. The duration of laser irradiation was chosen to ensure complete drying of the composite, since even small amounts of moisture make it difficult to measure IR spectra. Unlike Z-scan, the action of laser radiation on the liquid is carried out from above, which is achieved by changing the path of the beam using a prism. Figure 5 shows a typical view of the material formed using such method.

**Figure 4.** Normalized transmittance of samples depending on the position relative to the focus of the lens.

**Table 1.** Z-scan data with open aperture.

| Symbol | Dispersion composition | Data type | Nonlinear absorption coefficient ($\beta_{\text{eff}}$), cm/GW | Threshold intensity ($I_{\text{th}}$), GW/cm² |
|--------|------------------------|------------|------------------------------------------------------|------------------------------------------|
| ■      | FCTS (2%) SWCNTs (0.001%) | Experimental data | – | – |
| ———   | FCTS (2%) SWCNTs (0.001%) | Theoretical data | 902 | 0.0055 |
| ●      | SCTS (2%) SWCNTs (0.001%) | Experimental data | – | – |
| ———   | SCTS (2%) SWCNTs (0.001%) | Theoretical data | 561 | 0.0027 |
4.2. IR spectroscopy

The main difference in the structure of the two types of chitosan used was determined by the differences in the amide bands (1630, 1540 cm\(^{-1}\)) in the IR spectrum (Figure 6). Bending vibrations of N-H FCTS at 1562 cm\(^{-1}\) shift to 1513 cm\(^{-1}\) and their intensities become relatively strong. This may be due to the electrostatic attraction between the cationic groups in FCTS and the carboxyl groups of SWCNTs. The spectrum of SCTS with SWCNTs shows no displacements, only an increase in the peaks was observed. In this case, the anionic nature of succinic acid leads to some electrostatic repulsion between such chitosan and the negatively charged surface of SWCNTs.

![Figure 6. Study of the structure of the composite material after drying with a laser: a - IR spectrum of FCTS; b - IR spectrum of SCTS. Dotted lines mark spectra with SWCNTs, and solid lines without nanotubes.](image)

4.3. SEM studies

Agglomerates are indistinguishable on the surface of the composite material with FCTS, while in the case of SCTS, convex rounded structures are clearly distinguishable (Figure 7). This shows the effect of the chemical composition of chitosan on sample uniformity. In this case, SWCNTs are distributed fairly evenly, but some SWCNT bundles are randomly oriented.

![Figure 7. SEM images of samples after laser drying: (a) FCTS with SWCNT; b - SCTS with SWCNT](image)
5. Conclusion
Exposure to femtosecond laser pulses makes it possible to create a composite of chitosan and carbon nanotubes. The main influence on the internal structure of the composite is exerted by the spatial structure of the chain and the surface charge. SCTS exhibited greater surface roughness due to the formation of agglomerates. This enhances the proliferation and adhesion of cells on the surface of such a composite, which in turn leads to an increase in cell survival, and subsequently to accelerate the formation of a continuous layer of cells. The different interactions of the two types of chitosan with carbon nanotubes were confirmed by the study of nonlinear optical effects and IR spectroscopy. Subsequently, the study of the biocompatibility of such a material can be performed using microfluidic systems. The presence of a threshold intensity is associated with the formation of gas bubbles near carbon nanotubes, as well as with the activation of their interaction with chitosan.

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