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NONLINEAR OPTICAL CHARACTERISTICS OF ALBUMIN AND COLLAGEN DISPERSIONS WITH SINGLE-WALLED CARBON NANOTUBES

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Abstract. The interaction of laser radiation with aqueous dispersion of only bovine serum albumin (BSA) 25 wt. %, only bovine collagen (BC) 2 wt. %, and 25 wt. % BSA with single-walled carbon nanotubes (SWCNTs) 0.3 wt. % and 2 wt. % BC with 0.3 wt. % SWCNT was studied. The beam was absorbed mainly by nanotubes, that confirmed by the small value of the nonlinear absorption coefficients for aqueous dispersed media with BSA 6 cm·GW^{-1}, as well as dispersion with BK 4 cm·GW^{-1} and the large values of coefficients for these media with addition of SWCNTs, respectively 350 cm·GW^{-1} and 70 cm·GW^{-1}. Determination of nonlinear optical parameters was obtained by the method of fixed sample location. Knowledge of the values of these parameters allowed calculating theoretical curve of Z-scan with open aperture what made possible to compare with the experimental data.

Keywords: nonlinear optics; laser applications; three-dimensional printing; absorption.

1. Introduction

Laser printing of multilayered three-dimensional cellular- and tissue-engineered constructions with a structured internal scaffold is currently a promising task [1]. It is necessary to use bio-inks, which are capable of biodegradation, photo-cross-linking and providing sufficient strength of the formed structure for formation of constructions for bioengineering of human organs and tissues. Proteins that perform many functions are the most numerous organic substances in the human body, have high biocompatibility and biodegradability [2, 3] and are almost transparent in the visible range [4] are well suited for printing cellular and tissue-engineered constructions in solution of the practical challenges of laser surgery. Water dispersed media, containing only bovine serum albumin (BSA) 25 wt. %, only bovine collagen (BC) 2 wt. %, 25 wt. % BSA with single-walled carbon nanotubes (SWCNTs) 0.3 wt. % and 2 wt. % BC with SWCNTs 0.3 wt. % have desired properties. The printed samples on the basis of bio-inks with such a composition are able to ensure, during implantation, the germination of blood vessels through itself during the process of self-biodegradation [5]. Moreover, there is no hemolysis (the value of the hemolysis level is less than 0.5%), when blood contacts with such tissue-engineered constructions, which makes

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it possible to use them for the restoration of damaged heart tissues heart and blood vessels. The tensile strength and hardness of such multilayered three-dimensional constructions in the presence of a structured scaffold from SWCNT surpasses similar characteristics of porous human bone tissue [6].

In this paper, the main attention is paid to the investigation of the interaction of pulsed nanosecond laser radiation. Moreover, the intensity of laser radiation varies greatly so that the effect of light fluence on the formation of three-dimensional cellular and tissue-engineered constructions can be studied in detail. It is known that the effect of high-intensity laser radiation leads to the noticeable manifestation of nonlinear effects, when a certain threshold intensity is exceeded [7]. As a result, the energy, absorbed by the substance, can sharply increase, and this increase has a nonlinear character. Therefore, the study of nonlinear optical characteristics of proposed dispersion media was carried out to determine the optimal parameters of laser radiation for the formation of three-dimensional cellular and tissue-engineered structures.

At present, work is being carried out to create new photo-cross-linkable, biodegradable polymers for increasing the number of materials that are currently available for laser printing using laser sterolyolography (SLA) technology, which is very limited for choice [8, 9]. Another common method is digital light processing (DLP) [10]. Both of these techniques make it possible to fabricate tissue-engineered constructions from cell-saturated bio-inks, but differ in time, which is required for the formation of multilayered three-dimensional constructions [11]. In turn, the use of visible light instead of UV light reduces the potential risk of cells' DNA damage [12]. Therefore, in this paper, we studied the effect of laser radiation with a wavelength of 532 nm on the previously proposed in this paper composition for bio-ink.

The possibility of tissue-engineered structures formation by methods of laser printing make easier conducted procedures in laser surgery and simultaneously it improves the quality of these operations. That is why, it makes promising the development of this direction. This is achieved through the possibility of making implants of any anatomical shape, based on bio-inks. Using a laser can reduce the probability of infection due to a lack of the working surface contact with surgical instruments [13]. However, in comparison with the non-biological seal, it is necessary to solve a number of problems related to the sensitivity of cells and the choice of the correct layer design [14].

2. Materials

Aqueous dispersed media of BSA and BC proteins and similar dispersions with SWCNTs were investigated in this paper. The BSA and BC proteins were weighed in the form of a powder on an analytical weighing-machine AND HR-100A. After that, this amount of powder was mix with distilled water in proportions of 25 wt. % by weight and 2 wt. % by weight, respectively. The resulting dispersion was stirred on a magnetic stirrer for 30 minutes. SWCNTs were also mixed in the necessary concentration with distilled water and placed in "Sonicators Q700" homogenizer for 40 minutes. The power of the homogenizer was controlled by the program and was set to 60 – 65 W. The processing time of the dispersion with nanotubes was 30 minutes, the temperature did not exceed 70 °C. After reaching a homogeneous state, the dispersions were intermixed. The obtained dispersion of proteins with SWCNTs was processed by ultrasound for 30 minutes in the "Sapphire" ultrasonic bath. Before the experiment, the solution was additionally stirred on a magnetic stirrer for 10 minutes.

3. Experiments and methods for determining the nonlinear optical parameters of samples

In the course of the experiments, a nanosecond Nd: YAG laser was used, which generated radiation with a wavelength of 532 nm. The focal length of the lenses was 10 cm. The experimental apparatus used was described in detail in [15]. Based on the measured dependence of the normalized weakening coefficient K_{norm} from the input energy U_0 by the method of fixed sample location (direct nonlinear transmission), nonlinear optical characteristics of the prepared samples of proteins with SWCNTs such as nonlinear absorption coefficient, limiting threshold and maximum of weakening coefficient were calculated. In addition, this scheme allows one to make measurements of Z-scan with open aperture method. This method makes it possible to determine the nonlinear properties of the materials without changing the energy of the laser beam by varying its width, which leads to an increase in the light fluence of the beam [16, 17].

Unlike the Z-scan, during the direct nonlinear transmission experiment, the sample was placed in the focus of the lens and did not move during the study. The use of this method makes it possible to determine the linear α and non-linear β absorption coefficients and the threshold intensity I_c, without taking into account the change of the beam radius depending on the position of the sample in the mathematical model. Thus, there is a decrease in the number of variable parameters, which have an influence on the results of the experiment. As the result, it allows simplifying calculations that simultaneously increase accuracy of obtained results.

Dependence of the normalized weakening coefficient on the input energy of the laser beam was found by experiments with a fixed sample location:

$$K_{norm}(U_0) = \frac{K_{nonlin}(U_0)}{K_{lin}}, \tag{1}$$

where the value of the nonlinear weakening coefficient K_{nonlin} is calculated as

$$K_{nonlin}(U_0) = \frac{U_0}{U}. \tag{2}$$

The determination of the sample's nonlinear optical parameters from the known dependence of the transmitted energy U on the incident U_0 is described in detail in [18]. The value of the linear weakening coefficient K_{lin} is determined from the experimental data, obtained by the method of a fixed sample location with a laser radiation intensity not exceeding the threshold value. Knowing the nonlinear optical parameters of the sample, a theoretical Z-scan with open aperture curve can be calculated by the method described in [7]. The dependence of the light fluence on the distance from the center of the beam was determined by a technique that is described in detail in [15].

4. Results

The normalized weakening coefficient was increase with growth of input energy, but in aqueous dispersion of proteins without SWCNTs, this increasing was inconsiderable in comparison with dispersions containing SWCNTs. Fig. 1 shows experimental data, obtained by direct nonlinear transmission and theoretical curves.

The addition of nanotubes to the dispersion leads to a sharp growth of the nonlinear optical effects. The nonlinear absorption coefficient β was 6 $cm\cdot GW^{-1}$ and 4 $cm\cdot GW^{-1}$, respectively, for the dispersions of BSA and BC, however, the same dispersions with SWCNTs showed much larger values of the nonlinear absorption coefficient (350 $cm\cdot GW^{-1}$ for BSA with SWCNTs and 70 $cm\cdot GW^{-1}$ for BC with SWCNTs). It should be noted that the linear absorption coefficient α also increased with the addition of SWCNTs, but the increase was small and had little effect on the value of the linear weakening coefficient K_{lin}. For dispersions of BSA and BC, it was 1.92 cm^{-1} and 2.16 cm^{-1}, respectively, and 2.7 cm^{-1} and 2.91 cm^{-1} for the same dispersions with SWCNTs.

The limiting threshold for BSA and BC dispersions was 0.2 MW/cm^2 and 0.3 MW/cm^2, and for the same dispersions with SWCNTs, it was 1.8 MW/cm^2 and 0.9 MW/cm^2, respectively.

Knowledge of the values of these parameters allowed calculating theoretical curve of Z-scan with open aperture what made possible to compare with the experimental data (Fig. 2). Thus, the calculations, conducted with the help of the threshold model, was in good agreement with the values of the normalized weakening coefficient obtained experimentally. It was found that the behavior of the Z-scan with open aperture curve can be predicted, if the values of linear α and nonlinear β absorption coefficients, threshold intensity I_c, and the waist radius w_0, created by the lens, are determined.

The graphs show that the addition of SWCNTs to aqueous BSA and BC dispersions leads to a significant increase in the normalized weakening coefficient, i.e. to a sharp increase in absorption of laser radiation by the dispersion.

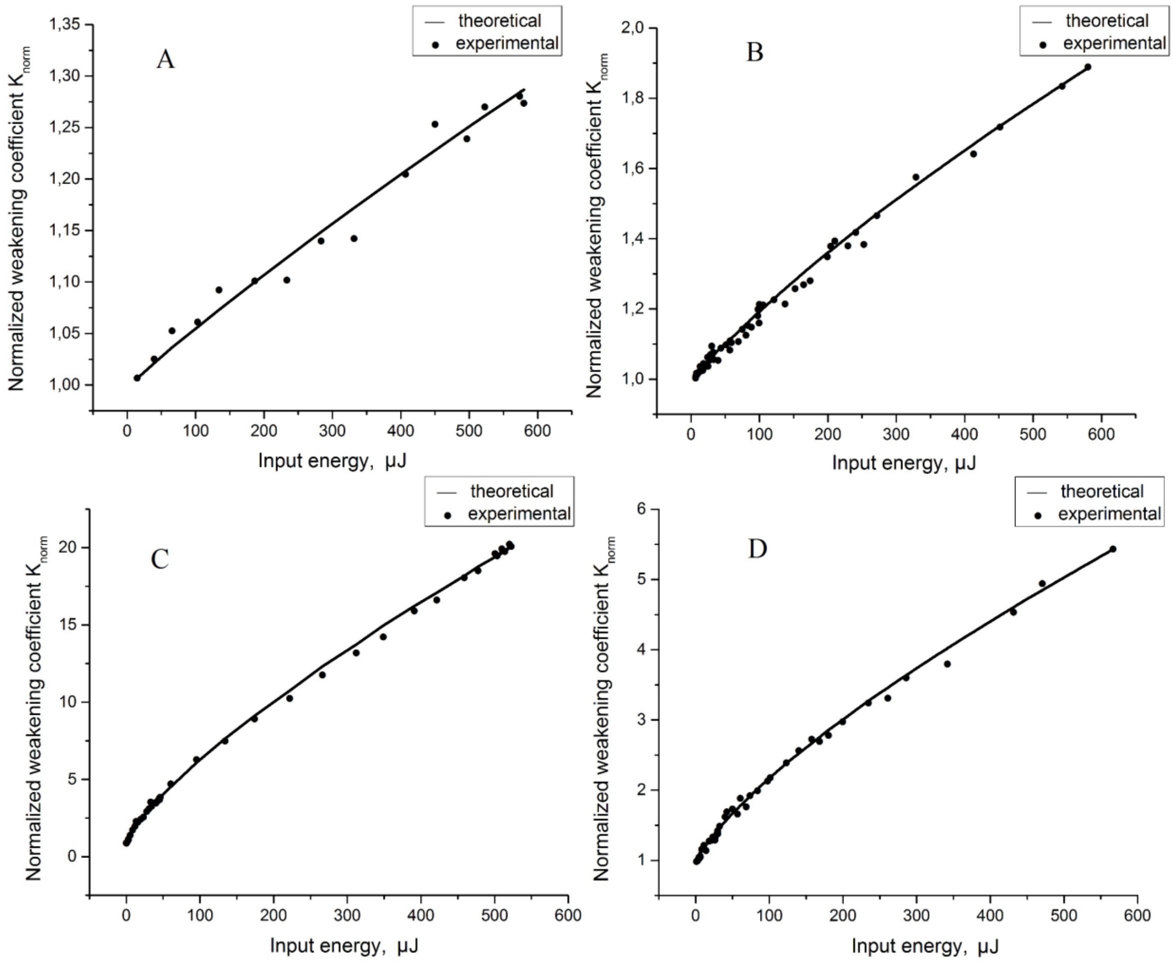

Fig. 1. Dependence of the normalized weakening coefficient on the input energy of the beam for aqueous solutions: (A) BSA (25 wt. %), (B) BC (2 wt. %), (C) BSA (25 wt. %) with SWCNT (0.3 wt. %), (D) BC (2 wt. %) with SWCNT (0.3 wt. %).

Figure 3 shows the dependence of the light fluence distribution on the distance from the center of the beam. In aqueous dispersions of BCs without SWCNTs, the waist radius was equal to 22 μm, and in the same samples with SWCNT was 23 μm. The calculated curves show that the greatest amount of energy is absorbed by the central region of the beam and the absorption is greatly reduced, by approaching its edges. The light fluence distribution, which is shown in Fig. 3, is calculated for the position of the sample in the focus of the lens. Thus, these graphs show the minimum size of the laser spot.

5. Conclusion

The addition of SWCNTs to the aqueous dispersion of albumin and collagen proteins results in an insignificant increase in the linear absorption coefficient, which characterizes the passage of laser radiation at low laser radiation power and a sharp decrease of transmittance at high degrees. However, as the light fluence increases, a single pulse energy decreases sharply, which is characterized by a nonlinear absorption coefficient. This suggests that SWCNTs not only help create biodegradable forests, but also significantly increase the thermal effect of laser radiation.

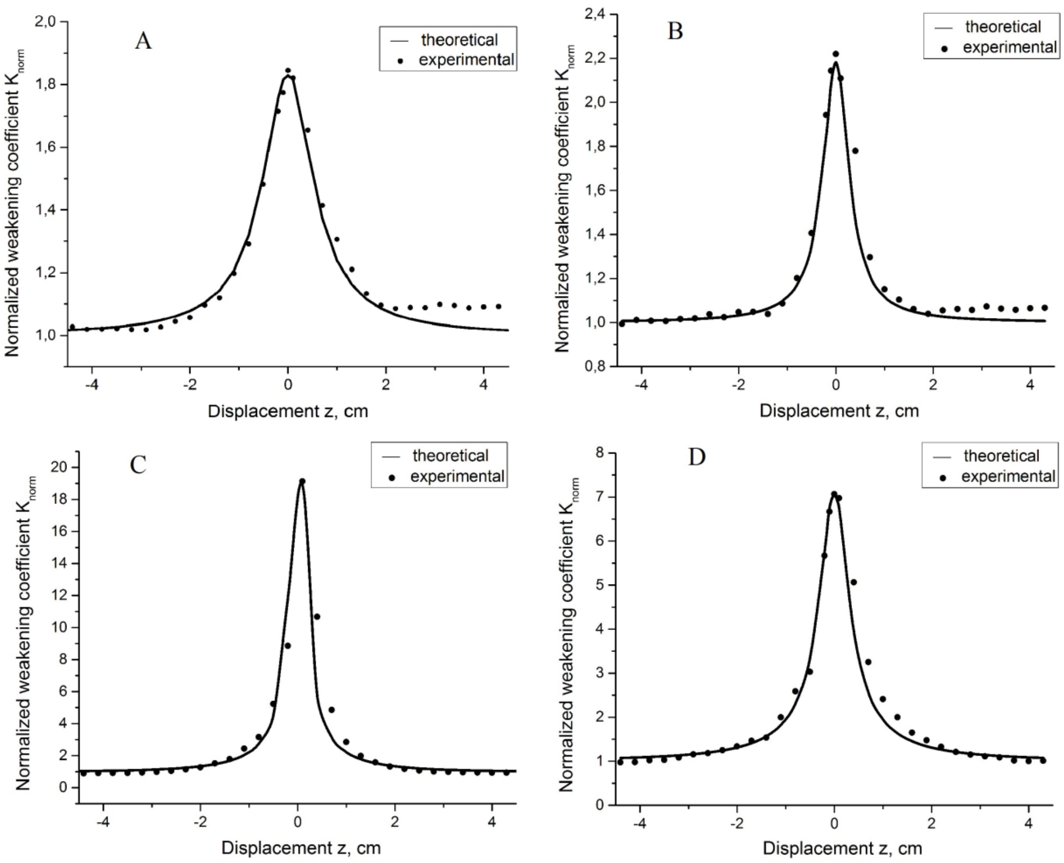

Fig. 2. Dependence of the normalized weakening coefficient on the position of the sample relative to the focus of the lens for aqueous solutions: (A) BSA (25 wt. %), (B) BC (2 wt. %), (C) BSA (25 wt. %) with SWCNT (0.3 wt. %), (D) BC (2 wt. %) with SWCNT (0.3 wt. %).

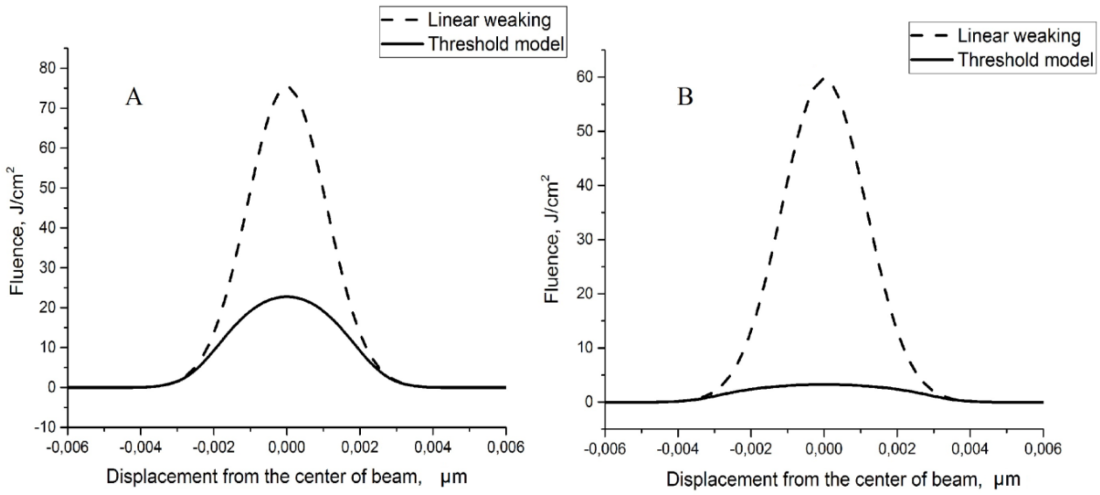

Fig. 3. Dependence of the light fluence distribution on the distance from the center of the beam for dispersions: (A) BC (2 wt. %), (B) BC (2 wt. %) with SWCNT (0.3 wt. %).

The use of nanosecond laser pulses with light fluence above the threshold values makes it possible to reduce the thermal effect on proteins, since the most radiation is absorbed by nanotubes during the formation of the scaffold. Thus, laser printing of multilayered three-dimensional cellular and tissue-engineered constructions with a structured internal nanocarbon scaffold with molecules of proteins such as albumin and collagen can be made. Subsequently, these constructions can be used for the implantation in the damaged area of the cardio-vascular system.

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