Biocompatibility of Silicon Nanoparticles as a New Material for Diagnostics and Treatment of Common Diseases

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Abstract. In the present day medicine intense work is performed to study the properties of nanoparticles, aimed at their further use in the diagnostics and treatment of widespread diseases, including the oncologic ones. The synthesis of biologically active nanocomposite silicon-based materials gave an impetus to intensive studies of this element. Particularly, it was found that owing to their biological compatibility with living tissues and ability of fast removal from the organism, the silicon nanoparticles (Si-NPs) can be used as photosensitisers in photodynamic therapy of cancer or as matrix for drug delivery systems. Nanoparticles, which are planning to be used in medicine, should be low-toxic and stable structure, exhibit high selectivity of accumulation in tumours, combined with fast removal from the organism. Therefore, it is of primary importance to study the interaction of Si-NPs with the basic proteins of the blood serum – albumin and gamma-globulin. These studies may be performed using the infrared spectroscopy method. Comparison of IR spectra for systems "water + Si-NPs + protein" and "water + Si-NPs" allow the judgement about the presence or absence of aggregates that can arise as a result of interaction of proteins' macromolecules and silicon nanoparticles.

Introduction

Current medicine studies are focused at different nanoparticles' properties, aimed at their further use in the diagnostics and treatment of widespread diseases, including the oncologic ones. Among the substances, that may be used for these purposes, special attention is paid to silicon.

Healthy human body weighing 70 kg normally contains about 1 g of silicon [*Canham,* 2007]. It makes silicon one of the most common minerals in the human body. In the human organism silicon is responsible for providing the safety functions, metabolic processes and deintoxication.

The synthesis of biologically active nanocomposite silicon-based materials gave an impetus to intensive studies of this element. Particularly, it was found that owing to their biological compatibility with living tissues [*Canham,* 1995], and ability of fast removal from the organism the silicon nanoparticles (Si-NPs) can be used as photosensitisers in photodynamic therapy of cancer or as matrix for drug delivery systems [*Tasciotti et al.,* 2008].

Main objective is nanoparticles close delivery to diseased cells. The delivery is commonly performed through the vascular system. Therefore, investigation of interaction between Si-NPs and blood serum proteins — albumin and gamma-globulin is important.

We can study this interaction using the infrared spectroscopy method. Comparison of IR spectra for systems "water + Si-NPs + protein" and "water + Si-NPs" allow the judgment about the presence or absence of aggregates that can arise as a result of interaction of proteins' macromolecules and silicon nanoparticles.

Materials and methods

Proteins

Albumin is globular protein with molecular weight 65kDa.Albumin binds water, cations (such as Ca^{2+}, Na^{+}, and K^{+}), fatty acids, hormones, bilirubin, thyroxine (T4), and pharmaceuticals, including barbiturates. Albumin main function is to regulate the colloidal osmotic pressure of blood and to transport various substances. Albumin molecule size is 5×5×15 nm.

Gamma-globulin is a protein fraction of blood serum containing many antibodies that protect against bacterial and viral infectious diseases. Gamma-globulin molecular weight is 200 kDa, molecule size is 10×10×20 nm.

Both proteins have a huge surface charge and dipole moment. Isoelectric point for albumin is at pH = 4.9 and for gamma-globulin at pH = 6.5.

Silicon nanoparticles

We worked with mesoporous silicon nanoparticles. There are Si-NPs clusters with size range from 100 nm to 1 mkm in water solution. Each cluster consists of nanocrystals with size range from 2 to 10 nm, as seen at TEM images (Figure 1). Also there are separated nanoparticles with size range from 20 to 50 nm. Porous Si-NPs clusters can be used as matrix for drug delivery systems. Single nanoparticles can be used in medicine aimed to their ability to penetrate into the cells.

Figure 2 shows the electron diffraction pattern obtained in the "transmission" geometry. Periodical arrangement of the reflections indicates that the crystalline structure of porous silicon remains unchanged during etching and grinding retains the crystal structure.

IR spectroscopy method

IR-spectroscopy is based on investigation of vibrational and rotational transitions in molecules, it allows to obtain information about substance's chemical composition. Vibrational-rotational spectrum of substance is determined by its molecules' composition. The number and the frequencies of IR-absorption bands in spectrum depend on the number of atoms forming a molecule, their nuclear masses, geometry and symmetry of equilibrium nuclear configuration and intramolecular forces potential field.

Every molecule has its unique vibrational spectrum, which is a set of bands of different frequencies and intensities. Experimentally obtained characteristic frequencies allow, according to the spectrum, to define the presence of different groups and bonds in molecule without additional calculations, thus to determine the molecule's composition.

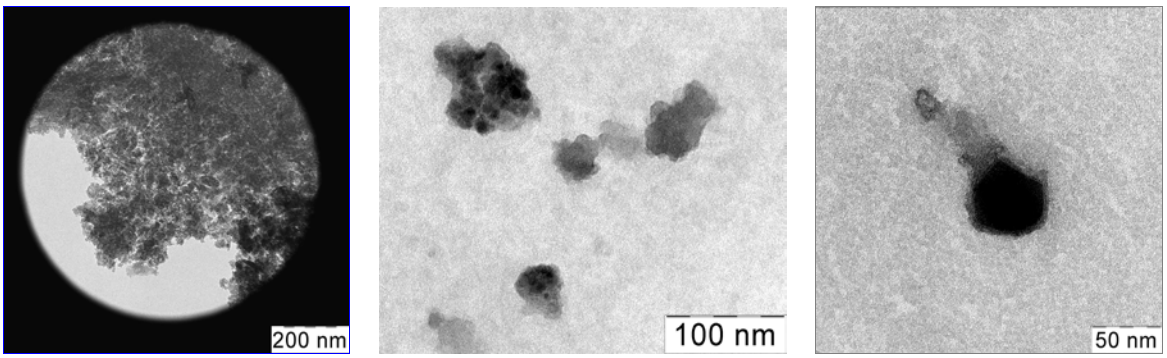

Figure 1. TEM images of Si-NPs.

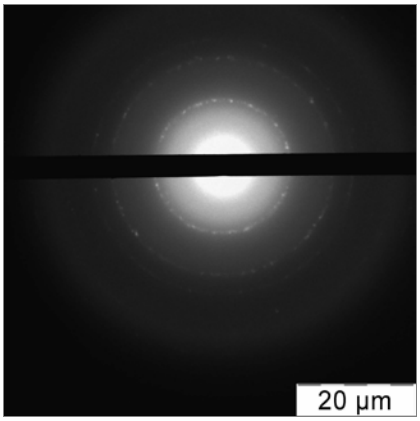

Figure 2. Mesoporous silicon powder diffraction pattern.

In our researches we used IR spectrometer Bruker IFS 66v/S in transmission mode with scanning range from 7500 to 370 cm^{-1} and resolution 0.25 cm^{-1}. The measuring chamber was evacuated to 3 mbar. All spectra were recorded at room temperature.

Main results and discussion

Preparing the samples

Nanoparticles for experimental samples were prepared by grinding the powder of porous silicon in the planetary type mill (Pulverisette-7, FRITSCH, Germany) during 30 minutes according to the technique [*Timoshenko et al., 2006*]. The powder was obtained from the exfoliated films of porous silicon, formed using the electrochemical etching of crystalline silicon (c-Si) plates in the solution HF(48 %):C_2H_5OH=1:1. The etching current density was 60 mA/cm^2. We used c-Si plates with p-type conductivity, (100) surface orientation and the specific resistance 25 mΩ·cm.

We used Bovine Serum Albumin (BSA), fraction V, and Bovine Gamma-globulin, fraction II, III, produced by Sigma Aldrich.

Studying of samples by the FTIR spectroscopy comprises two steps. First, the water solutions of investigated materials were prepared. BSA concentration — 2 g/l, gamma-globulin concentration — 2 g/l, Si-NPs — 1 g/l. IR-spectra in proteins' and Si-NPs water solutions presented strong absorption peaks with 1500 and 3500 cm^{-1} frequencies. These peaks are associated with the OH-groups vibrations in water. To reveal BSA, gamma-globulin and Si-NPs absorption peaks in second step water was desorbed by desiccation the solutions at 38 °C and the atmospheric pressure for an hour.

IR-spectra

For silicon nanoparticles the characteristic IR absorption band is observed at 1050–1100 cm^{-1} due to Si–O stretching vibrations in Si–O–Si) (Figures 3 and 4).

The protein backbone amide groups generate a number of characteristic IR bands: amide-I (1700–1600 cm^{-1}), amide-II (1600–1500 cm^{-1}) и amide-III (1330–1220 cm^{-1}). NH stretching vibrations give rise to a strong band at 3300 cm^{-1} [*Vonhoff et al.,* 2010].

Amide-I band is primarily due to the C=O and C–N stretching vibrations, amide-II band is due to the C–N stretching vibrations and C–N deformation vibrations and amide-III is due to C–N stretching vibrations and N–H rotation overlay additional vibrations of neighboring protein's group

According to the previously obtained data the shifts of the amide groups' absorption bands are indicating the presence or absence of interaction between proteins and other substances , metal salts [*Peng et al.,* 2010] and organic [*Belal,* 2011].

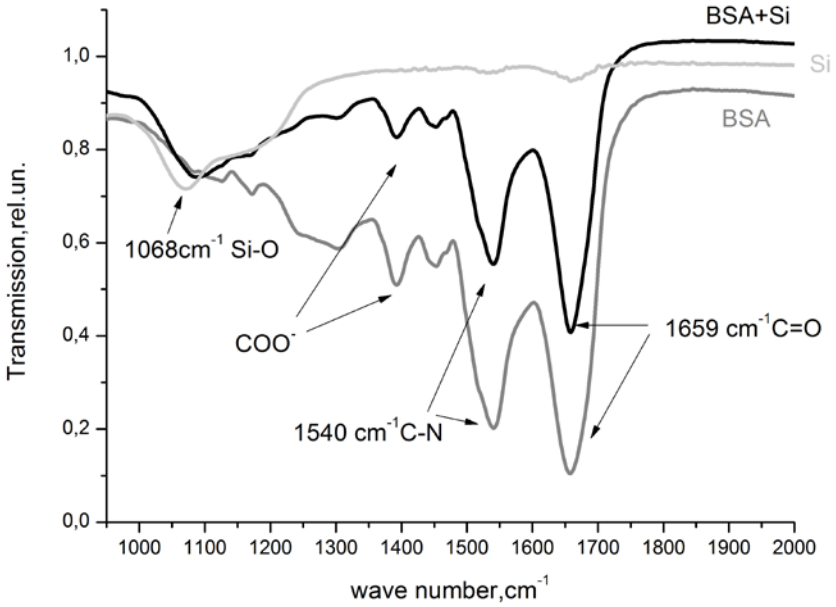

Figure 3. IR transmission spectra of bovine serum albumin (BSA), silicon nanoparticles (Si-NPs) and BSA + Si-NPs mix from 950 to 2000 cm^{-1}.

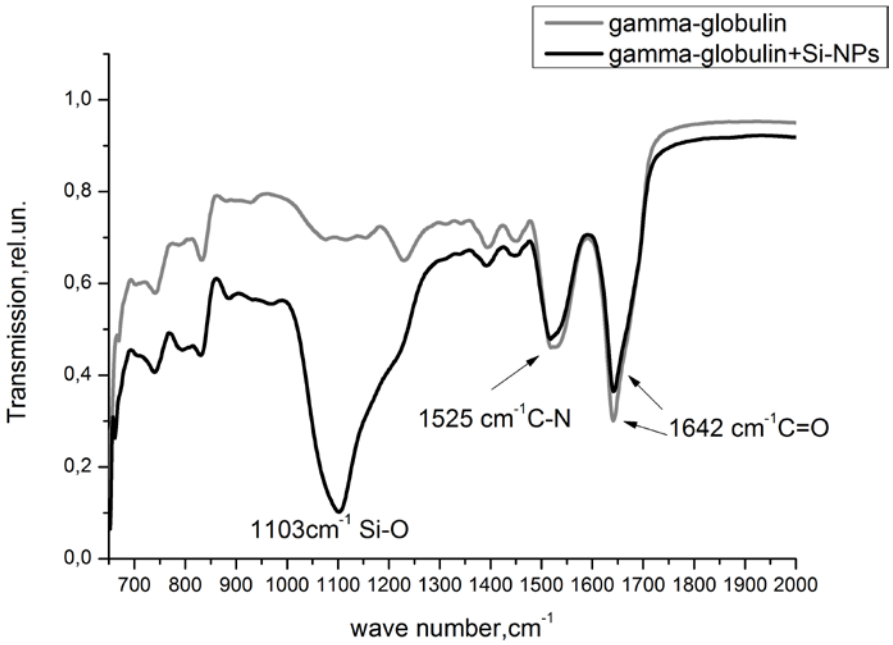

Figure 4. IR transmission spectra of gamma-globulin and gamma-globulin + Si-NPs mix from 950 to 2000 cm^{-1}.

We are looking for amide-I and amide-II bands frequencies shifts to the low frequency region. These shifts are characteristic for the formation of hydrogen bonds between the carbonyl groups of the proteins and the OH groups on the surface of Si-NPs.

As can be seen in Figures 3 and 4, amide-I and amid-II frequencies are the same for pure proteins and for protein + Si-NPs mixes. It means that there are no secondary structural changes that may be caused by interaction of nanoparticles and proteins. Thus, we suppose, that there is no interaction between Si-NPs and BSA or between Si-NPs and gamma-globulin.

Conclusion

Present results show that there is no interaction between protein's molecules and silicon nanoparticles in the investigated systems. This fact may be helpful for the further use of silicon nanoparticles in medicine. It means, than the entry of particles into a blood flow will not be followed by forming the protein + silicon aggregates directly in blood plasma.

Presumably, the amount of hydrogen on the nanoparticles' surface is insufficient to form a stable hydrogen bound C = O • • • • H-Si-O. Also, the absence of interaction can be explained by the mesoporous silicon nanoparticles hydrophilic properties. If necessary, silicon nanoparticles' surface can be modified by some amino acids to change its charge and other physical and chemical properties.

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