STRUCTURAL CHARACTERIZATION OF CHITOSAN COATED SILICON NANOPARTICLES –A FT-IR APPROACH

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În lucrarea de față sunt prezentate rezultatele noastre experimentale privind biofuncționalizarea nanoparticulelor de siliciu (SiNP), utilizând un polymer biocompatibil-chitosanul. Procedura constă în două etape şi a fost dezvoltată pentru a ataşa eficient bioplimerul chitosan de suprafața nanoparticulelor de siliciu. Utilizând triethoxsililbutiraldehida, un compus al siliciului cu grup funcțional carboxil terminal, în locul altor silani cunoscuți a redus timpul necesar pentru a ataşa acoperirea necesară. Analiza spectrelor FT-IR înregistrate pentru nanoparticulele de siliciu acoperite cu filmul biopolimeric a demonstrat prezența la suprafața acestora a grupărilor funcționale –NH2 şi –OH disponibile pentru legarea ulterioară a unor medicamente utilizate în tratamentul cancerului. Filmul polimeric chitosan şi-a menținut structura în stare nativă, pastrându-şi astfel proprietățile de biocompatibilitate.

This paper presents our experimental results on the bio-functionalization of silicon nanoparticles (SiNP) with a polymer, chitosan. The procedure consists of two steps and it was developed in order to bind efficiently and effectively chitosan biopolymer to the silicon nanoparticles surface. Using triethoxsilylbutyraldehyde, a silane compound possessing terminal carboxyl functional group instead of other known silanes, has reduced the time required to attach the desired coating. FT-IR analysis performed on chitosan coated SiNP samples has proved the presence of –NH_2 and –OH functional groups on their surface, available for further attachment of some drugs used in cancer therapy. The attached chitosan biopolymer film has maintained its native structure, therefore preserving its biocompatibility properties.

Keywords: silicon nanoparticles, biofunctionalization, chitosan biopolymer coating, Triethoxsilylbutyraldehyde, structural analysis

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1. Introduction

Silicon nanoparticles display a number of properties of interest for targeted drug delivery [1]. Nanocomposite systems based on silicon are promising platforms for pharmaceutical applications as they present low toxicity. Their ability to decompose into relatively harmless silicic acid by-products present fewer challenges for long-term use than carbon nanotubes or gold nanoparticles which are not metabolized and must be excreted after administration [2, 3]

The surface of Si nanoparticles can be easily modified via convenient chemistry with a wide range of organic or biological molecules (drugs, peptides, antibodies proteins and biocompatible polymers), allowing flexibility in the drug release profiles.

The optical properties of Si nanoparticles provide a useful dimension for *in vivo* sensing or therapeutics. Silicon nanoparticles exhibit fluorescence deriving from Si quantum dot structures which are produced during chemical etching [4], and it can be prepared with unique optical reflectivity spectra. These characteristics allow porous silicon to exhibit a signal that is affected in a predictable way when exposed to environmental changes, presenting possibilities for the development of advanced functional systems that incorporate sensors for diagnostic or therapeutic functions.

In order to prevent rapid degradation after administration and to increase their blood half life, biocompatible polymers coating was performed on silicon nanoparticles. Different type of biocompatible polymers (dextran [5], polylactic acid [6], chitosan [7], hyaluronic acid [8]) was used, biopolymeric film attachment was carried out through organosilane chemistry [9] or physisorption [5].

Chitosan is a biodegradable and biocompatible polymer derived from crustacean shells. Chitosan exhibits several favourable characteristics for drug delivery. Chitosan is soluble in acid solutions (pH<5.5) and can form complexes with anionic macromolecules to yield nanoparticles, microparticles, hydrogels. Most of the positive charges in the chitosan polymer chain would be neutralized at physiological pH (pKa of the side chain amino groups is 6.5), rendering chitosan molecule hydrophobic and less water soluble. This unique property ensures that chitosan based nanocomposites systems formed at low value of pH remain stable at physiological pH. This paper presents our experimental results on the bio-functionalization of silicon nanoparticles using chitosan biopolymer.

Silanization reaction has been employed to modify the surface of silicon nanoparticles [9]. Using a silane compound possessing terminal carboxyl functional group reduced the number of steps needed for functionalization, thus reducing the time required to attach the biopolymeric film [10]. Various techniques have been used to obtain information about morphological and structural characteristics of biopolymer coated silicon nanoparticles [11, 12].

Fourrier Transform Infrared (FT-IR) Spectroscopy was used to identify and characterize the functional groups on the surface of silicon nanoparticles.

2. Experimental

2.1 Materials

Silicon nanoparticles (SiNP) were provided by National Institute for Research and Development in Microtechnologies Bucharest - Nanosic Project coordinator. Si nanoparticles (diameter around 15-30 nm) were prepared by electrochemical etching of single-crystal silicon wafers in ethanolic HF solution. Chitosan (Chi) from crab shells (medium molecular weight, 85% deacetylated), acetic acid, sulfuric acid, hydrogen peroxide were purchased from Sigma-Aldrich provenience. Triethoxsilylbutyraldehyde (TESBA) was purchased from ABCR GmbH & Co. All used reagents were of analytical grade.

2.2. Methods

2.2.1. Surface functionalization of Silicon nanoparticles (SiNP)

Piranha treatment was used as method to generate –OH functional group on pristine silicon nanoparticles surface. Silicon nanoparticles (5 mg) were cleaned in freshly prepared Piranha solution (a mixture of H_2O_2 and H_2SO_4, 3:1 volume ratio) for at least 5 hours at 90°C, rinsed twice with Milli Q water and dried at 100°C in an oven.

The method performed for TESBA and chitosan deposition was adapted by us from the work of H.J. Martin et al. [10]. The procedure consists of two steps and it was developed in order to efficiently and effectively binds chitosan biopolymer to the silicon nanoparticles surface. Reaction steps are schematically presented in figure 1. In the silane deposition step, the dried “piranha” solution treated SiNP were mixed with 2% (v/v) TESBA solution in toluene and allowed to react for 24 h under stirring. Following the 24 h reaction time, SiNP were placed in toluene and sonicated for 30 minutes. To remove any residual toluene, SiNP were rinsed with ethanol and Milli Q water and then dried at 60°C. The second step in the reaction series involved chitosan film attachment to the nanoparticles surface. TESBA functionalized SiNP were mixed with a 2% chitosan solution prepared in 1% acetic acid and allowed to react for 2 h. Following the reaction step chitosan functionalized SiNP were rinsed with Milli Q water. Finally functionalized SiNP were rinsed with a 0.1 M NaCl solution and dried at 50°C in order to obtain a homogenous and stable biopolymer film on nanoparticles surface.

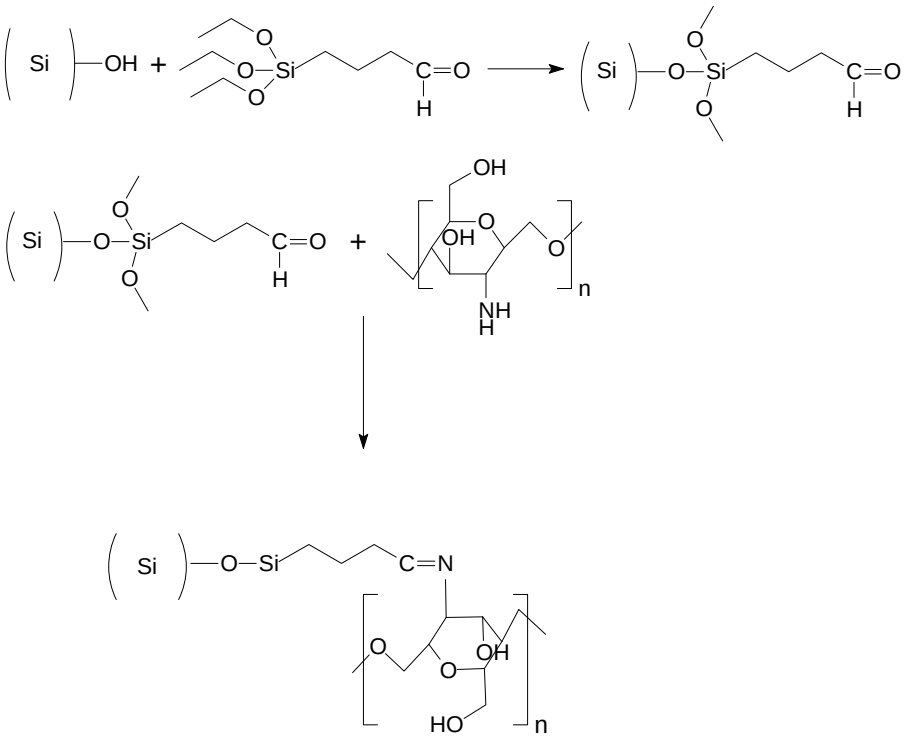

Fig. 1. Reaction steps involved in the chitosan attachment to silicon nanoparticles surface

2.2.2. Structural characterization of SiNP

FT-IR spectroscopy was used to identify and characterize the functional groups on the silicon nanoparticles surface. Infrared spectra were recorded on KBr pellets with a Bruker Tensor 27 infrared spectrometer under dry air at 25 C. Each FT-IR spectrum represents the average of 64 scans at 4 cm^{-1} resolution. Brucker OPUS 6 software was used to record and analyse sample spectra.

3. Results and discussions

Reactions mechanism presented in figure 1 was proposed in order to efficiently attach chitosan biopolymer film on SiNP surface. First reaction step involving silane chemistry was performed to obtain a stable silane monolayer on hydroxylated silicon nanoparticles surface. By using triethoxsilylbutyraldehyde instead of other known silanes has reduced the number of steps needed for functionalization, thus reducing the time required to attach the desired coating. The second reaction step implied a covalent interaction between the carboxyl group present in the used silane molecules and the chitosan polymer amino

functional group and it was done in order to develop a stable and uniform layer of biopolymer on the SiNP surface.

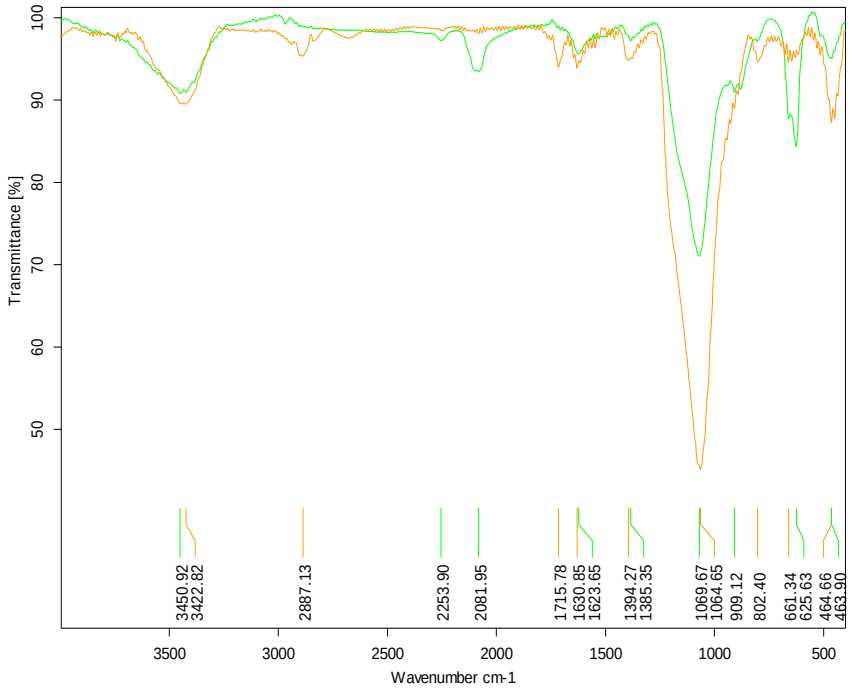

Fig. 2. Recorded spectra of the silicon nanoparticles (A) and TESBA modified silicon nanoparticles (B)

The samples were analyzed before and after each reaction step. For clearness, the spectra recorded on the coated samples were always compared to the spectra obtained from pristine SiNP and pure chitosan powders.

In figure 2 are presented the recorded spectra of oxidized SiNP (curve A) and TESBA functionalized SiNP (curve B). Broad band of medium intensity occurring at 3460 cm^{-1} was related to the hydroxyl deformation vibrations of the silanol group present on SiNP surface. The strong sharp peak occurring around 1069 cm^{-1} can be ascribed to Si-O-Si functional group asymmetric stretching vibration. Two other vibrational modes with the medium intensity peaks occurring around 625 cm^{-1} and 463 cm^{-1} confirm Si-O-Si functional group presence on functionalized SiNPs. Moreover it could be noticed that 1069 cm^{-1} strong peak indicates the presence of intermolecular bonds between SiNPs due to the aggregation phenomena. A slight shifting to the right of the 3400 cm^{-1} peak and an increase in band intensity were observed on the TESBA functionalized SiNP

(figure 2B). This phenomenon was ascribed to the increasing number of Si-OH functional groups on the nanoparticles surface.

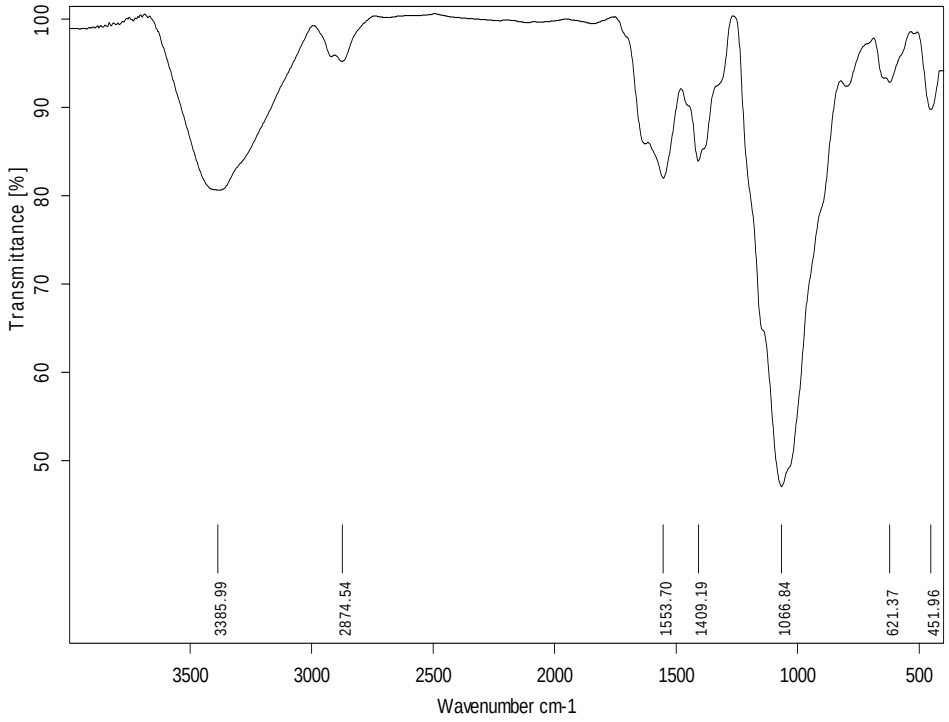

Fig. 3. Recorded spectra of chitosan modified SiNP and pure chitosan (inset) in KBr pellets

A slight shift to the right and an intensity increase of the 1069 cm^{-1} peak denoted a conjugation between Si-OH group present on silicon nanoparticles surface and Si-OH functional group from TESBA molecules during reaction step 1. Carbonyl functional group presence on TESBA functionalized SiNP was confirmed by the stretching vibrations peak occurring around 1716 cm^{-1} on the recorded spectrum.

SiNP spectrum recorded after final reaction step is shown in figure 3. For clearness, spectrum recorded for chitosan coated SiNP was compared to the spectrum recorded for pure medium molecular weight chitosan powder (figure 3-inset). The broad band of medium intensity occurring around 3400 cm^{-1} both in chitosan coated SiNP and in pure chitosan spectra proved –OH functional group existence on silicon nanoparticles surface. Free hydroxyl group presence is also confirmed by the 1400 cm^{-1} medium intensity peak due to the stretching vibrations from –C-O functional group. Primary aliphatic amino group presence

on chitosan coated SiNP was proved by the deformation vibration peak occurring around 1659 cm^{-1}-1553cm^{-1}. The slight shift of the peak toward lower wavenumbers observed in the case of chitosan coated SiNP spectrum was ascribed to the covalent interaction between carboxyl functional group and amino functional group during the last step of functionalization.

4. Conclusions:

The two step method developed to attach chitosan biopolymer film to silicon nanoparticles surface reduced considerably the time required for functionalization.

FT-IR analysis of chitosan coated SiNP proved the presence of – NH_2 and –OH functional groups on their surface, functional groups available for further attachment of some drugs used in cancer treatment. Moreover, it should be noticed that the attached chitosan biopolymer film maintains its native structure, therefore preserving its biocompatibility properties.

Further experiments will be carried out in order to study the possibility of using these nanocomposite systems as drug delivery vehicles.

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R E F E R E N C E S

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