NATURAL SILK FIBROIN MICRO- AND NANOPARTICLES WITH POTENTIAL USES IN DRUG DELIVERY SYSTEMS

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În această lucrare este prezentată sinteza şi caracterizarea de micro- şi nanoparticule pe bază de fibroină din mătase naturală de Bombyx mori, cu potenţiale aplicaţii în sisteme de eliberare controlată a principiilor active. S-au obţinut micro- şi nanoparticule pe bază de fibroină prin metoda precipitării în diferiţi solvenţi organici. Particulele astfel obţinute au fost caracterizate prin spectroscopie FTIR-ATR şi analiză SEM. Aceste particule pot fi utilizate în transportul şi eliberarea controlată de principii active a medicamentelor în scop terapeutic şi pentru regenerarea ţesuturilor

The paper reports on the synthesis and characterization of domesticated Bombyx mori silk fibroin (SF) micro- and nanoparticles used for controlled drug delivery systems. SF micro- and nanoparticles were obtained by precipitation in different organic solvents. The fibroin samples were characterized by FTIR-ATR spectroscopy and SEM analysis.

The use of such SF particles may range from the controlled delivery of drugs and protein therapeutics to their use as a platform for the delivery of growth factors for regenerative tissue repair.

Keywords: silk fibroin, nanoparticles, organic solvents, drug delivery

1. Introduction

Drug delivery is a vast research topic studied by many scientists and researchers throughout the world.

Basically, the concept behind drug delivery is to provide more constant concentrations in the organism, and to bring the compound with pharmaceutical activity directly to the site of need in order to enhance the effectiveness of action.

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One way to bring the active substance to the site of action is to modify their bio-distribution by entrapping them in particulate drug carriers such as microspheres, micro and nanocapsules, or liposomes. The need for encapsulation lies in the instability of many drugs, and in some cases it can improve the bioavailability of the therapeutic compounds.

Micro- and nanoparticles have attached great research interest in the field of drug delivery due to their ability to deliver many kinds of drugs to targeted areas of the body for sustained periods of time [1]. Also, nanoparticles and nanoformulations have already been applied as drug delivery systems with great success; and nanoparticulate drug delivery systems have still greater potential for many applications, including anti-tumour therapy, gene therapy, and AIDS therapy, radiotherapy, in the delivery of proteins, antibiotics, virostatics, vaccines and as vesicles to pass the blood - brain barrier.

Nanoparticles provide massive advantages regarding drug targeting, delivery and release and, with their additional potential to combine diagnosis and therapy, emerge as one of the major tools in nanomedicine. Nowadays, major effort has been addresses to develop drug-delivery nanospheres for treating various diseases such as cancer, due to the potential for more targeted localization in tumors with active cellular uptake.

Micro- and nanoparticles can be made from a number of synthetic and natural biodegradable polymers. Synthetic polymers used for micro- and nanoparticles preparation include poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA) whilst natural polymers include gelatin, chitosan, collagen, albumin, whey protein and silk fibroin [2-7].

As a fibrous protein, silk fibroin (SF) fibres from *Bombyx mori* have been used as a biomedical suture material as a long time, because of their outstanding mechanical properties, in contrast with the catalytic and recognition functions of globular proteins [8-9].

Silk fibroin (SF) has been explored as a versatile protein biomaterial for the formation of films, three-dimensional scaffolds, electrospun fibres and microspheres due to its superior properties like excellent biocompatibility, favourable oxygen permeability, good mechanical properties, high thermal-stability, slow biodegradation, minimal inflammatory and immunogenic response [10-13].

The domesticated silkworm *Bombyx mori* silk fibroin consist of two proteins: a light chain (~25 kDa) and heavy chain (~370 kDa) which are present in a 1:1 ratio and linked by a single disulfide bond [14]. The molecular structure of silks consists of large regions or domains of hydrophobic amino acids, segregated by relatively short and more hydrophilic regions. For drug delivery, especially protein drugs, silk materials exhibit high encapsulation efficiency and controllable drug release kinetics due to the control of crystalline beta-sheet formation.

Various *in vitro* and *in vivo* studies have demonstrated that silk fibroin is biocompatible and less inflammatory than other common biodegradable polymers such as poly(lactide) and collagen [15-17]. The degradation rate can be modified and corrected by controlling the crystalline state (β-sheet content) of silk during processing, to regulate the release profile of bioactive molecules.

Silk fibroin (SF) protein based micro- and nanoparticles provide new options for drug delivery due to their unique combination of biocompatibility, biodegradability, self-assembly, controllable structure and morphology, their tunable drug loading and release properties, which could be regarded as significant advantages compared with the properties of other synthetic and natural materials [15-18].

Silk fibroin micro- and nanoparticles could be obtained by several methods: emulsion-solvent evaporation/extraction methods, phase separation/coacervation, self-assembly, solvent displacement, rapid expansion of supercritical solution, and spray-drying [19]. Each method has advantages and disadvantages; therefore the selection of the right method is very important in fabricating micro- and nanospheres for drug delivery applications.

In this paper, we present one alternative method for the obtaining silk fibroin (SF) globular micro- and nanoparticles. We have also characterized and compared the morphological aspects of silk fibroin particles. These particles could be further used for encapsulation of active principles in drug delivery systems.

2. Experimental

2.1. Materials

Bombyx mori cocoons were kindly supplied by S.C. SERICAROM S.A. (Bucharest, Romania). Sodium bicarbonate and sodium dodecyl sulphate (SDS) were provided by Alfa Aesar GmbH&Co KG, Germany, lithium bromide (LiBr) was provided by Sigma Aldrich, dialysis tubing cellulose membrane from Sigma Aldrich, acetone (S.C. Reagents COM S.R.L.), ethanol, methanol, isopropanol (Chemical Company) and butanol (Aldrich, 99%).

2.2. Methods

2.2.1. Preparation of silk fibroin solution

Silk fibroin stock solutions were prepared as follows. *Bombyx mori* silkworm cocoons were boiled for 30 min in an aqueous solution of 0.05 % (w/v) $NaHCO_3$ and SDS and then rinsed thoroughly with double distilled water to extract the sericin protein and other impurities. This operation was repeated three times to get the pure silk fibroin. The degummed silk fibroin was dried at 40 °C

and atmospheric pressure. The extracted silk fibroin was then dissolved in a 9.3 M LiBr solution at 60 °C for 5 h. The resulted silk fibroin solution was then dialyzed in double distilled water using a dialysis tubing cellulose membrane (MWCO 12.4 kDa) for 4 days with several changes to remove the residual lithium bromide. The solution was filtered to remove the debris from original cocoons. The final concentration of the silk fibroin aqueous solution was 8 wt. %, which was determined by weighing the remaining solid after drying the solution at 60 °C. This stock solution was stored at 4 °C and diluted with double distilled water to 3 and 5 wt. % prior to use.

2.2.2. Preparation of silk fibroin particles

Silk fibroin micro- and nanoparticles were obtained in water-miscible organic solvents. The method consists in the obtaining of silk fibroin micro- and nanoparticles by precipitation with different organic solvents (acetone, methanol, ethanol, isopropanol and butanol). Briefly, silk fibroin solutions of 3, 5 and 8 wt. % concentration were rapidly introduced in organic solvent at room temperature. SF particles were collected by centrifugation at 12 000 rpm, 15 min and then dried under vacuum at 40 °C for 24 h. Various volume ratios between solvents and silk fibroin solutions were employed: Solvent/SF, 90/10, 70/30 and 50/50.

2.3. Characterization

The FTIR-ATR spectra were taken on a Jasco 4200 spectrometer equipped with a Specac Golden Gate attenuated total reflectance (ATR) accessory, using a resolution of 4 cm^{-1} in the 4000-400 cm^{-1} wavenumber region.

Morphological information including shape and size of the silk fibroin particles was obtained through the scanning electron microscopy (SEM) analysis of the gold-coated samples. The analysis has been performed using a QUANTA INSPECT F SEM device equipped with a field emission gun (FEG) with a resolution of 1.2 nm and with an X-ray energy dispersive spectrometer (EDS).

3. Results and discussions

The results of the FTIR–ATR spectra gave us the specific absorbance wavelengths of the specific bonds which appeared in the silk fibroin particles. Typical peaks of silk fibroin are 1621, 1515 and 1231 cm−1, characteristic for amide I (C=O stretching), amide II (NH deformation and C-N stretching) and amide III (C-N stretching and N-H deformation) [20]. FTIR spectra of silk fibroin particles show slightly shifted peaks as shown in Fig. 1: 1637 cm^{-1} for amide I,

1519 cm^{-1} for amide II and 1235 cm^{-1} for amide III. This shifting of the amides is characteristic for β-sheet structure of regenerated silk fibroin particles.

The fibroin micro- and nanoparticles could be rapidly obtained from the fibroin solution by using protonic and aprotonic organic solvents such as methanol, ethanol, isopropanol, butanol and acetone. The volumes of solvent added to the fibroin solution were different as the data in literature are quite controversial. Some authors suggest that adding a small amount of solvent could lead to the precipitation of fibroin as nanoparticles. Others suggest that not all the solvents lead to the formation of fibroin micro- and nanoparticles, but to a viscous gel solution [21, 22]. Therefore our goal was to investigate the formation of micro- and nanoparticles in various solvents and different volumes (ratio solvent/fibroin solution, 90/10, 70/30 and 50/50, v/v). Morphology of silk fibroin particles obtained from acetone is shown in Fig. 2.

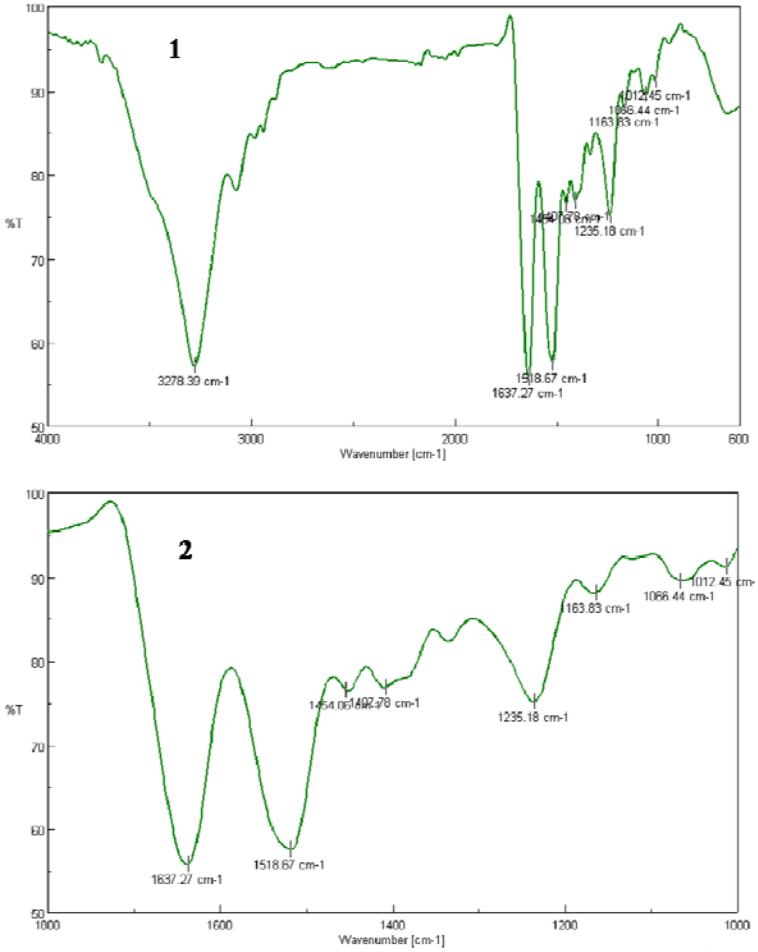

Fig. 1. FTIR-ATR spectra of silk fibroin particles: 1-600–4000 cm^{-1};

2-zoom of the amides region 600–1800 cm^{-1}.

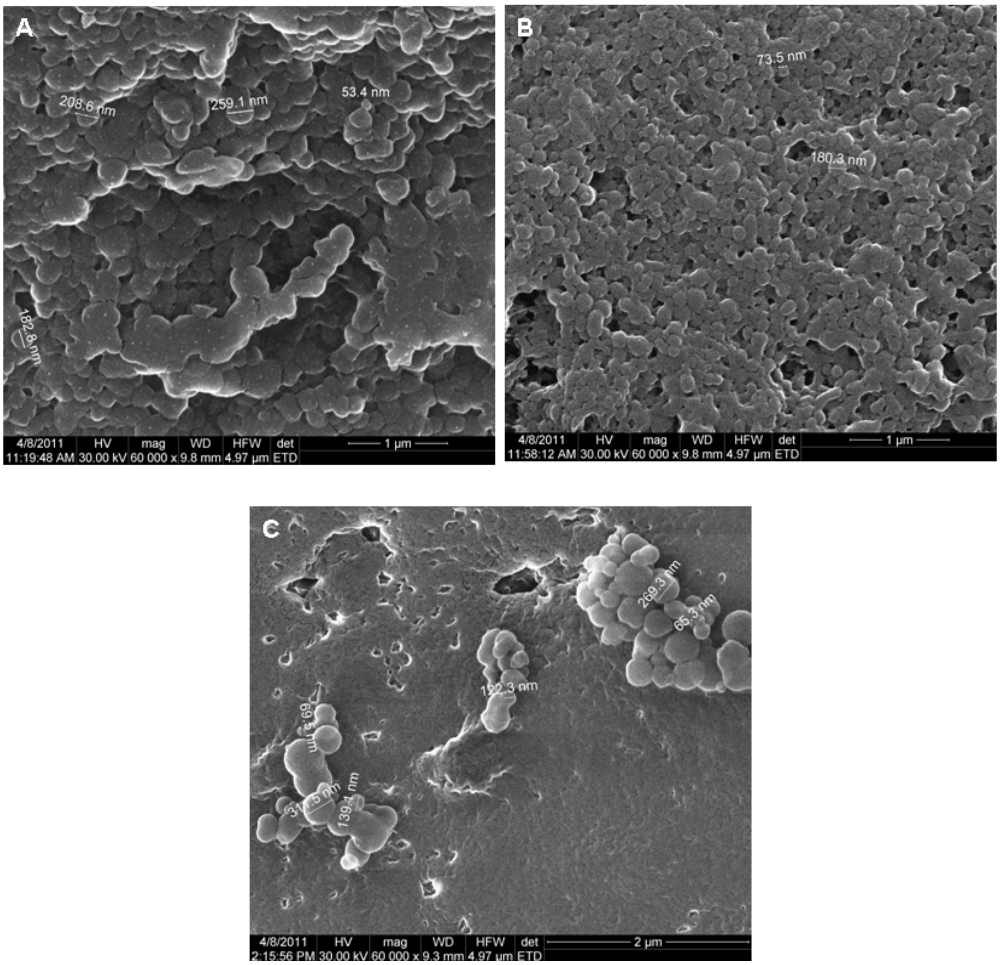

Fig. 2. SEM microphotographs of silk fibroin nanoparticles obtained from acetone/silk fibroin solution, 90/10, v/v: A-3 wt. % fibroin solution; B-5 wt. % fibroin solution; C-8 wt. % fibroin solution.

As we could see from Fig. 2, acetone is able to precipitate silk fibroin solution as fine globular particles with nanometer size diameters (ranging from 50 to 300 nm). As the concentration of silk fibroin solution increases from 3 to 8 wt. % the number of nanoparticles formed is diminished and the diameters are a little bit higher. If the ratio between solvent and silk fibroin solution is changed (70/30 and 50/50, v/v) the precipitation of fibroin solution as nanoparticles is still maintained. But the globular morphology tends to disappear and to obtain irregular shapes of nanometer size that will finally aggregate at 50/50 volumetric

ratio. Similar results were also obtained with isopropanol for the precipitation of silk fibroin solution (Fig. 3).

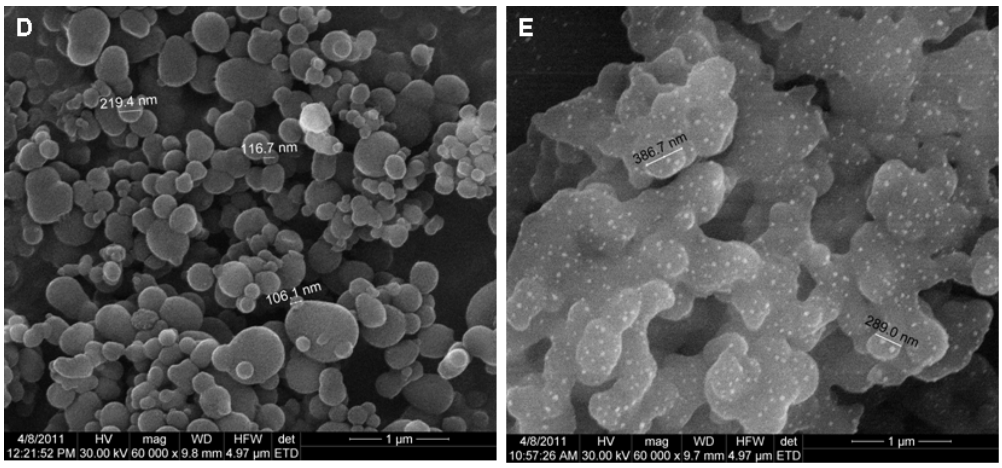

Fig. 3. SEM microphotographs of silk fibroin nanoparticles obtained from isopropanol/silk fibroin solution, 90/10, v/v: D-3 wt. % fibroin solution; E-5 wt. % fibroin solution.

Methanol and ethanol are well-known to induce the transition of silk fibroin conformation from random coil to β-sheet. Nevertheless our findings suggest that these solvents have poor ability to induce the formation of globular nanoparticles (Fig. 4).

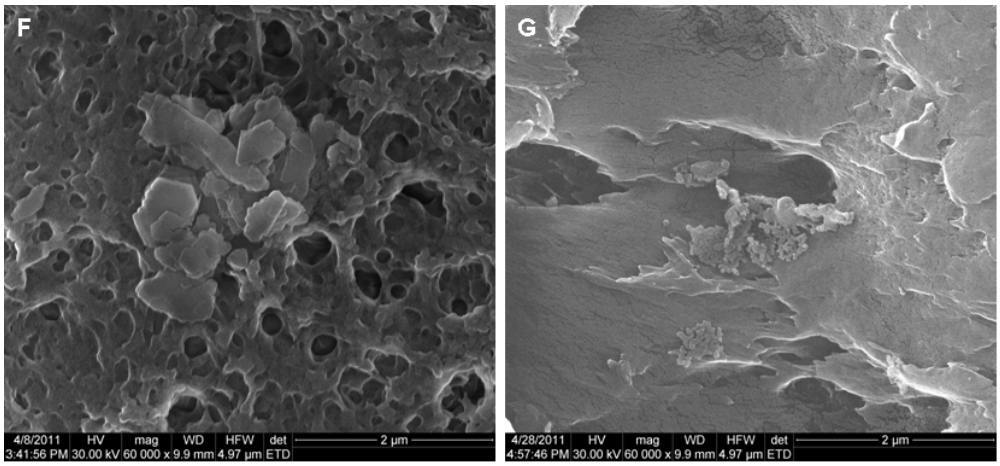

Fig. 4a). SEM microphotographs of silk fibroin nanoparticles obtained from methanol and ethanol: F- methanol/silk fibroin solution 90/10, v/v, 5 wt .% SF solution; G-ethanol/silk fibroin solution 90/10, v/v, 5 wt .% SF solution

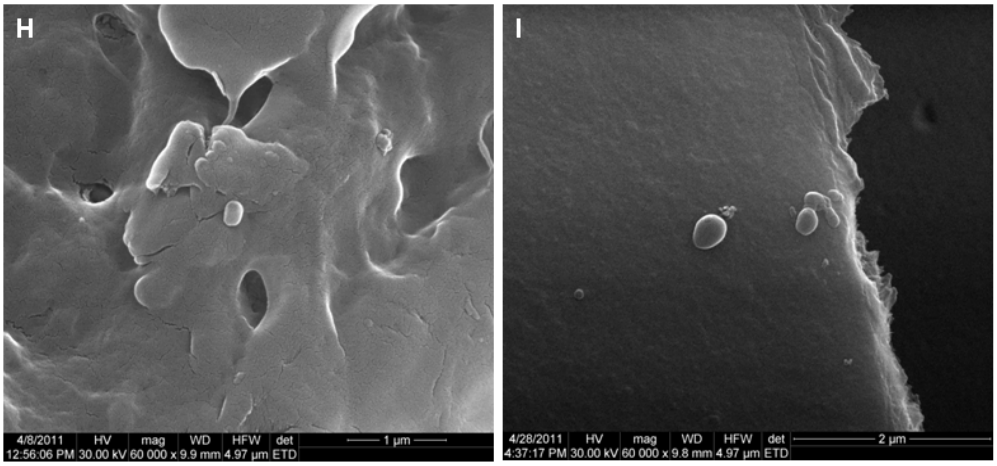

Fig. 4b). SEM microphotographs of silk fibroin nanoparticles obtained from methanol and ethanol: H-methanol/silk fibroin solution 90/10, v/v, 3 wt .% SF solution; I-ethanol/silk fibroin solution 90/10, v/v, 3 wt .% SF solution.

Butanol only induced aqueous solution of silk fibroin agglomerating, but could not lead to the obtaining of fine nanoparticles as acetone or isopropanol.

Acetone and isopropanol proved to be the best solvents to achieve the precipitation of silk fibroin solution as fine globular nanoparticles with various diameters. This is obtained preferentially by adding an excess of solvent volume in silk fibroin solutions with various concentrations. The concentration of fibroin solution seemed to be important as the high tendency towards globular nanoparticles formation is attributed to the lowest concentration (3 wt. %). At higher concentrations the silk fibroin particles agglomerate and went down.

4. Conclusions

Silk micro- and nanoparticles with different sizes (50–300 nm) were obtained from silk fibroin solution by precipitation in various organic solvents. The best results were obtained with acetone and isopropanol. These two solvents have a high capacity of precipitating the silk fibroin solution as fine globular nanoparticles well dispersed and stable in aqueous solution. Methanol and ethanol are not able to induce the nanoparticles formation, but they could be used to change the conformation of regenerated silk fibroin from random and α-helix type to a more organized β-sheet. This method for nanoparticles preparation is easy to be performed, time and energy efficient, and could be useful for silk fibroin-based drug delivery systems. Further studies will be dedicated to more complex physico-chemical analysis and drug loading with model molecules and anticancer drugs.

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