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Silver nanoparticles encapsulated in glycogen biopolymer: Morphology, optical and antimicrobial properties

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ABSTRACT

The glycogen biopolymer from the bovine liver has been used as stabilization agent for the growth of silver nanoparticles. The samples with various contents of silver were prepared by two different procedures that include fast (using microwave radiation) and slow (conventional) heating of the reaction mixtures. The TEM images of the two nanocomposites showed the presence of nanoparticles with average diameter of 9.7 and 10.4 nm, respectively. The results also revealed that the optical properties of the obtained nanocomposite samples strongly depend on the method of preparation. The samples prepared using microwave radiation exhibited narrower surface plasmon resonance peaks, while the silver nanoparticles induced quenching of the photoluminescence of glycogen in all of the tested samples. Antimicrobial activity tests were carried out against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* pathogens and showed that the microbial growth was gradually reduced as the concentration of the silver increased. Also, after 2 h of exposure to the nanocomposites the number of cells was significantly reduced (>99%) for all the strains tested.

1. Introduction

Research on integration of polysaccharide biopolymers and inorganic nanoparticles into hybrid systems is witnessing a dramatic activity in current bio-nanoscience. Characteristic macromolecular and supramolecular properties of these biopolymers make them good controlled environments for growth of metallic and semiconductor nanocrystals. Due to a large number of OH groups, polysaccharide chains complexate well with metallic ions in solution, while supramolecular nanostructures formed by inter- and intra-chain hydrogen bonding act as template for nanoparticle growth (Raveendran, Fu, & Wallen, 2003). To date, starch is the most extensively used biopolymer for the stabilization of the growth of metallic (Božanić et al., 2007; Djoković et al., 2009; Raveendran et al., 2003; Sarma & Chattopadhyay, 2004) and semiconductor (Božanić et al., 2007, 2009; Radhakrishnan, Georges, Nair, Luyt, & Djokovic, 2007; Rodriguez et al., 2008; Vigneshwaran, Kumar, Kathe, Varadarajan, & Prasad, 2006) nanoparticles. The other polysaccharide biopolymers such as chitosan (Murugadoss & Chattopadhyay, 2008; Sun et al., 2008; Wei & Qian, 2008), alginate (Pal, Esumi, & Pal, 2005; Brayner, Vaulay, Fievet, & Coradin, 2007), gum Arabic (Kattumuri et al., 2007) or cellulose (Pirkkalainen et al., 2008) also proved to be good stabilization agents. It is also important to mention that nanoparticles synthesized within the biopolymer are biocompatible and hydrophilic, which could be important for their application in biology and medicine. On the other hand, the number of studies in which polysaccharide biopolymers of animal origin were used in preparation of inorganic nanoparticles is limited. In the present study we prepared silver nanoparticles in the presence of a glycogen biopolymer.

Glycogen, the main carbohydrate-storage in animals and microorganisms, is a polysaccharide that consists of highly branched $(1\rightarrow4)(1\rightarrow6)$-linked α-D-glucoses (Manners, 1991). It is produced primarily by the liver and the muscles, but can also be made by glycogenesis within the brain, thymus and skin (Brown, 2004). Glycogen has a high molecular weight (10^6–10^9) and its molecules are packed into spherical granules (β-particles), 20–40 nm in size, which often group together into much larger α-particles. The highly branched, actually fractal (Melendez, Melendez-Hevia, & Canela, 1999), structure of the glycogen enables a very easy pathway of synthesis and degradation as well as simplicity in the regulation mechanism. However, we believe that this dendrimeric structure is also an ideal environment for the controlled synthesis of metallic nanoparticles. The preparation of metal–glycogen hybrid nanostructures could be important from a practical point of view, due to their possible application as probe materials for glucan–biomolecule interactions. Li and co-workers (Xiang, Xu, Liu, Li, & Li, 2009) found, after mixing previously prepared gold nanoparticles and glycogen, that interactions between glycogen and biomacromolecules can alter the aggregation status of

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gold nanoparticles, which produced intensity changes in plasmon resonance light-scattering.

Antimicrobial activity is an important property of silver nanoparticles and/or silver–polymer nanocomposites. A number of studies emerged lately on this subject (Dror-Ehre, Mamane, Belenkova, Markovich, & Adin, 2009; Jain et al., 2009; Kvitek et al., 2008; Lok et al., 2007; Morones et al., 2005; Panacek et al., 2006; Sharma, Yngard, & Lin, 2009), reporting on the influence of different factors, such as the nanoparticle concentration, size and capping agents involved on the inhibition of the antimicrobial growth. It is believed that the antimicrobial activity of silver nanoparticles originates from the formation of Ag^+ active species (Lok et al., 2007), since they exhibit strong affinity towards sulphur and phosphor containing functional groups from the membrane-bound enzymes (McDonnell & Russell, 1999). The accumulation of the nanoparticles in the cells might also be a source of bactericidal activity (Morones et al., 2005). Given that glycogen is a biocompatible polymer of animal origin and that, to our best knowledge, it was not used so far as a stabilization agent for the growth of silver nanoparticles, we considered that it would be interesting from a fundamental as well as from a practical point of view to investigate the antimicrobial effects of this material. The present study is divided into two parts. In the first part, the structure and the optical properties of the silver–glycogen nanocomposites prepared by two different synthetic procedures were investigated. In the second part the antimicrobial activity of the nanocomposite films was tested against the *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* pathogens.

2. Experimental

2.1. Materials and the preparation of the nanocomposites

2.1.1. Materials

Glycogen from bovine liver, ammonium hydroxide (NH_4OH), D-glucose and silver nitrate ($AgNO_3$) were purchased from Sigma–Aldrich and used as received. High purity water (specific resistance $\sim 10^{18}\ \Omega$ m) was used in all synthetic procedures.

2.1.2. Synthesis of Ag–glycogen nanocomposites

Two different green chemical procedures for the preparation of the nanocomposites were employed:

1. In the first procedure, a modified Tollens method for the preparation of silver nanoparticles was used (Panacek et al., 2006). The silver nanoparticles were synthesized by reduction of $[Ag(NH_3)_2]^+$ ions using D-glucose as a reducing agent in the aqueous glycogen solution and under microwave irradiation. In a typical procedure, a 1 mL of a 0.1 M solution of $AgNO_3$ and 2.5 mL of 0.1 M solution of D-glucose were added to 50 mL of a 0.5% aqueous solution of glycogen. The mixture was stirred for 60 min at room temperature and purged with argon. After that, NH_4OH was added to 5 mL of the obtained solution until pH value reached 8.5. Finally, the mixture was treated in a conventional microwave for 30 s at 440 W. The nanocomposite films were obtained after evaporation of water.
2. The second procedure was a "green" synthetic method reported by Raveendran et al. (2003) for the preparation of starch capped silver nanoparticles. In this method Ag–glycogen nanocomposites were prepared by conventional heating on a hot plate. The same initial mixtures as in the previous procedure were purged with argon, sealed, heated to 50 °C and maintained at this temperature for 24 h. Different samples were prepared by changing the initial amount of silver while the nanocomposite films were obtained after solvent evaporation.

Table 1
List of Ag–glycogen nanocomposite samples and the initial amount of chemicals used in their preparation.

Label	n_m ($AgNO_3$) [mM][a]	n_m (D-Glucose) [mM][b]	Ag [wt.%][c]	Treatment
MW-1	0.5	1.25	2	Microwave
MW-2	1.0	2.50	4	Microwave
MW3	2.0	5.00	8	Microwave
HP-1	0.5	1.25	2	Hot plate
HP-2	1.0	2.50	4	Hot plate
HP3	2.0	5.00	8	Hot plate

[a] Silver concentration.
[b] Concentration of D-glucose.
[c] Silver weight fraction in the nanocomposite.

The preparations conditions and the denotations of the samples obtained using these two procedures are given in Table 1. Throughout the discussion the samples prepared by microwave radiation and conventional heating will be referred to as MW and HP samples, respectively.

3. Methods

3.1. Characterization of the nanocomposites

The morphology and dispersion of the Ag nanoparticles in the glycogen matrix were investigated by transmission electron microscopy (Philips CM100 instrument) at a 80 kV operating voltage. A water dispersion of the nanocomposite was deposited on carbon coated (MW3 sample) and formvar coated (HP3 sample) copper grids using a fine pipette. The samples were left to dry in air before they were transferred to the TEM chamber. The distribution of particle sizes was determined by measuring the diameters of the equivalent circular area of 125 (MW3 sample) or 172 (HP3 sample) observed particles. The obtained histograms were fitted by log-normal distribution with parameters D_{LN} (mean) and σ_{LN} (standard deviation).

The surface morphology of the pure glycogen powder and silver–glycogen nanocomposite films (MW3 and HP3 samples) was investigated by scanning electron microscopy (SEM) using a JEOL JSM 6460LV instrument. The images were obtained using both secondary electron and back-scattered electron (BSE) imaging modes. Due to the poor conductivity of the materials, the samples were coated with carbon prior to SEM investigations. Their composition was checked with EDX measurements done with an X-ray microanalysis unit (Oxford Instruments) attached on SEM.

The X-ray diffraction (XRD) measurements of the MW3 and HP3 nanocomposite samples were performed on a Philips PW3710 X-ray diffractometer (Cu radiation, $\lambda = 0.154$ nm).

The UV–vis absorption measurements of the Ag–glycogen solutions were carried out on a Thermo Evolution 600 spectrophotometer.

FTIR spectroscopic analyses of the pure glycogen and the Ag–glycogen nanocomposite films were carried out at room temperature using a Nicolet 380 spectrophotometer in the spectral range of 4000–400 cm^{-1}, with a resolution of 4 cm^{-1}. The datasets were averaged over 200 scans.

The photoluminescence spectra of the pure glycogen and the Ag–glycogen nanocomposite films were recorded using a Perkin–Elmer LS45 fluorescence spectrophotometer.

3.2. Indicator strains and culture conditions

The microorganisms used in this study were Gram-positive bacteria *S. aureus* ATCC 25922, Gram-negative bacteria *E. i* ATCC 25923, and fungus *C. albicans* ATCC 24433. The growth of microorganisms