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Synthesis, characterization and antimicrobial activity of dextran sulphate stabilized silver nanoparticles

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ABSTRACT

Dextran sulphate stabilized silver nanoparticles (AgNPs - DS) were synthesized from aqueous solution of silver nitrate ($AgNO_3$) and dextran sulphate sodium salt (DS). The characterization of AgNPs - DS was performed by ultraviolet–visible spectroscopy (UV-VIS), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and antimicrobial activity. The formation of AgNPs - DS was monitored by colour changes of the reaction mixture from yellowish to brown and by measuring the surface plasmon resonance absorption peak in UV-VIS spectra at 420 nm. The SEM analysis was used for size and shape determination of AgNPs - DS. The presence of elemental silver and its crystalline structure in AgNPs - DS were confirmed by EDX and XRD analyses. The possible functional groups of DS responsible for the reduction and stabilization of AgNPs were determinated by FTIR spectroscopy. The AgNPs - DS showed strong antibacterial activity against *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Bacillus luteus in haus strain*, *Bacillus subtilis* ATTC 6633, *Listeria monocytogenes* ATCC 15313, *Escherichia coli* ATTC 25922, *Pseudomonas aeruginosa* ATTC 27853, *Klebsiella pneumoniae* ATTC 700603, *Proteus vulgaris* ATTC 8427, and antifungal activity against *Candida albicans* ATTC 2091.

1. Introduction

Different methods for the synthesis and characterization of nanoparticles of silver (AgNPs) that are based on the reduction of silver ions by chemical, electrochemical, or photochemical methods are described in the literature [1]. From all these methods, the chemical reduction is most frequently used and in order to avoid the use of hazardous substances green synthetic methods, which involve the application of non-toxic and biodegradable agents such as polysaccharides and their derivatives for the reduction and stabilization of AgNPs, are intensively developed [2–12,28,29]. Methods for the synthesis and characterization of AgNPs with the natural polysaccharides or their derivatives such as dextran, pullulan, starch, carboxymethyl dextran (CMD), carboxymethyl cellulose (CMC), chitosan, heparin, hyaluronic acid, biocomposites AgNPs chitosan/gelatin are described in the literature. The characterization of these particles was performed by using various techniques like UV-VIS and FTIR spectroscopy, EDX, XRD; size and morphology were examined by using scanning electron microscopy (SEM) and transmission electron microscopy (TEM); and the antimicrobial activity of the synthesized compounds was tested. However, there are still open questions about interactions between AgNPs and reducing and stabilizing agents. According to one opinion, they are the result of silver ions coordination with carboxyl group [6,11], and another explanation is in terms of the physical-steric interactions [8]. Dextran derivates, such as carboxymethyl dextran or dextran sulphate (DS, Fig. 1), contain one or more either carboxyl or sulpho-groups, therefore allowing conditions for forming complex compounds of different composition and coordination with copper(II) ions [13]. CMD was already used as reducing and stabilizing agent for the preparation of AgNPS - CMD [12]. Formation of AgNPs - CMD was confirmed by the maximum surface plasmon resonance peak at 420 nm in the UV-VIS spectra. SEM analysis showed that the dominant nanoparticles were spherical in form, their size was 10–60 nm, but they were spotted

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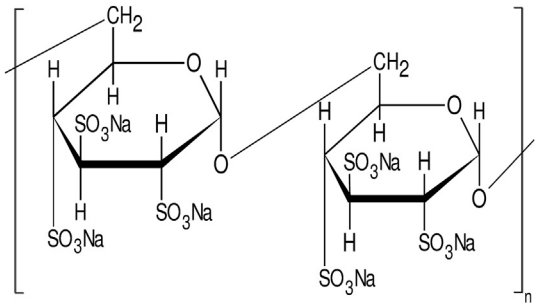

Fig. 1. Molecular structure of dextran sulphate sodium salt (DS).

and aggregated in undefined shapes at large scale. The crystalline structures of AgNPs - CMD were found to be face centered cubic type. Analysis of FTIR spectra indicated a strong interaction between Ag and two oxygen atoms of the −COOH group, i.e. complex formation of CMD and Ag ions from AgNPs surface. Synthesized AgNPs - CMD showed good antibacterial and antifungal activity. Having in mind the similarity of structure between CMD and DS we expected that DS can be used for the synthesis of AgNPs, on which to the best of authors knowledge there are no data in the literature. In this paper the method of synthesizing AgNPs – DS and their characterization by UV-VIS, FTIR, SEM, XRD, and EDX techniques is described. The antimicrobial and antifungal activity was also investigated.

2. Experimental section

2.1. Materials

All reagents in this paper were analytical grade and were used as received without further purification. $AgNO_3$ was used as the silver precursor, and it was obtained from Merck (Darmstadt, Germany). All the other chemicals; NaOH, the reducing agent, and DS (dextran sulphate, Mw ≈ 500000 g/mol, 17% S) were obtained from Sigma – Aldrich (Sweden). All aqueous solutions were prepared with doubly distilled water.

2.2. Synthesis procedure

A solution containing ligand (0.002 M) DS in 100 mL of H_2O, was added dropwise in a solution containing of $AgNO_3$ (100 mL, 0.001 M), was added to the reactor under constant stirring for 4 hours at a temperature of 100 °C, pH was adjusted to 7.5 by the addition of NaOH (0.4 mL, 0.001 M) in reaction mixture. The viscous white-colored solutions turned yellow, which confirmed the formation of AgNPs-DS. The reaction mixture was then cooled to room temperature, and the AgNPs-DS complex was precipitated by the addition of 96% ethanol. After standing overnight, the ethanol was decanted, and precipitate was redissolved by redistilled water. Finally, the complex was precipitated again from the solution by 96% ethanol and after decantation dried for 3 hours at 105 °C in a vacuum oven.

2.3. Characterization methods and instruments

The prepared AgNPs - DS complexes were characterized by UV-VIS spectroscopy, SEM, EDX, XRD, FTIR spectroscopy and used antimicrobial activity testing.

2.3.1. UV-VIS spectroscopy

Absorption spectra have been recorded on Varian Cary-100 Conc. UV-VIS spectrophotometer in the wavelength range of 200–900 nm. The computer processing of the recorded UV-VIS spectra has been performed by software application Cary UV-Conc. (Copyright©, Varian 1999) under Windows XP OS platform.

2.3.2. Scanning electron microscopy (SEM)

For size and shape analysis of the synthesized AgNPs - DS complexes JEOL JSM 5300 scanning electron microscope was used and obtained SEM images were transformed into a PC format for further morphological analysis. The samples for SEM analysis were prepared by room temperature overnight air drying of thin layer of AgNPs - DS suspension onto metallic sample carrier. Dried samples were then covered with a thin film (about 10 nm thick) of gold by cathode sputtering in JPC JEOL-1100 apparatus. After preparation, SEM images of AgNPs - DS complexes were obtained by using electron beam energy of 30 keV.

2.3.3. Energy dispersive X-ray (EDX) spectroscopy

For the EDX spectral analysis LINK Analytical 2000 QX microprobe mounted on a JEOL JSM 5300 scanning electron microscope was employed. The same samples prepared for SEM analyses were used for obtaining EDX spectra.

2.3.4. X-ray diffraction (XRD)

The XRD technique was used for determination and confirmation of the crystal structure of silver nanoparticles. The sample for XRD analysis was prepared by press and pull method in top-loading specimen plate [14]. The diffractogram was recorded in Brag–-Brentano θ: 2θ geometry using a conventional powder diffractmeter, Seifert V-14, employing Cu $K\alpha$ radiation (λ Cu $K\alpha_1 = 1.5406$ Å, Ni filter, generator settings: 30 kV, 30 mA). LaB_6 was used as an external standard for peak position calibration and for determination of instrumental peak broadening. XRD data were collected over the 2θ range of 5–90° with a step size of 0.02° and an exposition time of 2 s per step.

2.3.5. Fourier transform infrared (FTIR) spectroscopy

The samples for FTIR spectroscopy have been prepared by the method of pressing substance with KBr. FTIR spectra of solid samples have been recorded immediately after pastille preparing at room temperature on BOMEM MB-100 (Hartmann & Braun, Canada) spectrometer equipped with a standard DTGS/KBr (deuterated triglycine sulphate/KBr) detector. Spectra were collected in the mid-IR region from 4000 to 400 cm^{-1} with the resolution of 2 cm^{-1}. Reference pastille was prepared from pure KBr (150 mg, Merck). The computer processing of the recorded FTIR spectra has been performed by using Win-Bomem EasyTM 3.01C Level II software application (Copyright© 1991–1994, Galactic Industries Corporation) under Windows XP OS platform.

2.4. Antibacterial and antifungal activity of AgNPs - DS

The antibacterial and antifungal activity of the DS stabilized AgNPs were measured by agar disc diffusion method. Nine bacterial strains and one fungal strain: (Gram-positive) *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Bacillus luteus in haus strain*, *Bacillus subtilis* ATTC 6633, *Listeria monocytogenes* ATCC 15313, (Gram-negative) *Escherichia coli* ATTC 25922, *Pseudomonas aeruginosa* ATTC 27853, *Klebsiella pneumoniae* ATTC 700603, *Proteus vulgaris* ATTC 8427, and fungal strain *Candida albicans* ATTC 2091 were used as indicator strains for this analysis. Suspension preparation was performed by method previously described by Ref. [15]. Bacterial and yeast suspensions were prepared by direct colony method. The colonies were taken directly from the plate and suspended in 5 mL of sterile 0.85% saline. The turbidity of the initial