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Protocols

Halloysite nanotubes with immobilized silver nanoparticles for anti-bacterial application

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ABSTRACT

Halloysite nanotubes (HNTs) with immobilized silver (Ag) nanoparticles (NPs) were prepared by methods of wet chemistry and were characterized by using the transmission electron microscopy, x-ray diffraction, optical spectroscopy and experiments with *E. coli* bacteria *in-vitro*. It was found that Ag NPs with almost perfect crystalline structure and sizes from ~9 nm were mainly attached over the external surface of HNTs. The optical absorption measurement revealed a broad plasmonic resonance in the region of 400–600 nm for HNTs with Ag NPs. The later samples exhibit bactericidal effect, which is more pronounced under illumination. A role of the plasmonic excitation of Ag NPs for their bioactive properties is discussed. The obtained results show that Ag NPs–decorated HNTs are promising agents for the antibacterial treatment.

1. Introduction

An ability to integrate nanotechnology with biotechnology ensures the greatest impact in biology and biomedicine, since the convergence of these two fields gives rise to a combinatorial field of nanobiotechnology [1]. As the noble metal nanoparticles demonstrate unique optical, electronic, photonic, and catalytic properties, thus integration of these nanoparticles (NPs) with biocompatible clays produces novel hybrid nanocomposites to be explored in different fields. In noble metal nanoparticles, the fascinating optical properties originates from resonant oscillation of their free electrons in the presence of light, also known as localized surface plasmon resonance, which can be visualized from their bright intense color [1,2]. In the recent years, anti-bacterial materials have widely been used in our day-today life as they effectively protect our health. A wide variety of anti-bacterial materials have been reported to prevent attachment and proliferation of microbes [3–6]. However, their usage sometime is limited owing to the concerns of antibiotic resistance, environmental pollution, relatively complex processing, and high cost [7,8]. Therefore, it is an urgent need to develop an anti-bacterial material which will be devoid of these concerns and at the same time has low toxicity, high thermal stability and low volatility.

Halloysite nanotubes (HNTs) are a kind of kaolinite clay with a hollow tubular structure produced by the surface weathering of aluminosilicate minerals, having chemical structure of $Al_2Si_2O_5(OH)_4{\cdot}nH_2O$. The adjacent alumina and silica layers with their water of hydration, give rise to a packing disorder and help the nanotubes to curve and roll up to form multilayers [9,10]. As the internal and external surfaces of HNTs consist of gibbsite-like array of Al-OH groups and Si-O-Si groups respectively, they possess positive and negative charge at the inner and outer surfaces which result in different inner/outer surface chemistry as well as help to manipulate their chemico-physical properties through the control of the chemistry of the constituent elements [11,12]. According to the state of hydration, HNTs are of two types: hydrated halloysite–10 °A with one layer of water molecules between the multi-layer and dehydrated halloysite–7 °A achieved by an irreversible phase transition with loss of adsorbed water [10,13,14].

Modification of HNTs by different metal NPs is a promising approach to form new nanocomposites with interesting optical, sensor and biochemical functionalities [15,16]. For example, HNTs

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with deposited gold (Au) NPs can exhibit surface plasmon resonance (SPR) [17,18], which is widely used in surface-enhanced Raman scattering (SERS) for detection of small amount of molecules [19]. We have also shown that silver (Ag) NPs deposited on the surface of HNTs could result in SPR and SERS [20]. In the present paper, we report the synthesis of a nanocomposite, consisting of Ag NPs immobilized on HNTs and study the antibacterial application of the prepared nanocomposites for *E. coli* bacteria. We have also demonstrated the role of the plasmonic excitation of Ag NPs for their bioactive properties.

2. Experimental

2.1. Materials

All chemicals were used as received. HNTs, silver nitrate ($AgNO_3$, 99.9999%) and sodium borohydride ($NaBH_4$) were purchased from Sigma-Aldrich. (3-aminopropyl) triethoxysilane (97%) was received from Alfa Aesar. Toluene was obtained from Merck.

2.2. Sample preparation

Prior immobilization of Ag nanoparticles (NPs) over the surface of halloysite nanotubes (HNTs), the outer surface of HNTs was modified with an aminosilane, (3-aminopropyl) triethoxysilane through grafting reaction [21]. The reaction was performed under nitrogen atmosphere using standard air-free technique. A three-necked round bottom flask containing 3.0 g of HNTs and 20.0 mL of toluene was fitted with a condenser, rubber septum, thermocouple adaptor, and a quartz sheath through which a thermocouple was inserted. The reaction mixture was then heated with a heating mantle; once the reaction temperature reached to 60 °C, 3.0 mL of (3-aminopropyl) triethoxysilane was injected to the reaction flask and the temperature was increased to 120 °C, followed by refluxed the reaction solution for 12 h. After the completion of the reaction, the product was washed several times with toluene and ethanol respectively to remove excess aminosilane and then dried at 100 °C under vacuum. The aminosilane modified HNTs were referred to as HNTs-NH_2.

HNTs loaded with Ag NPs have been prepared based on the immobilization of silver precursors over the surface of HNTs-NH_2 followed by the reduction with ice cold aqueous solution of sodium borohydride. First, 2.0 g HNTs-NH_2 were taken in beaker containing 25 mL 10^{-2} M $AgNO_3$ solution. The reaction mixture was now stirred on a magnetic stirrer for 10 h to complete immobilization of Ag ions onto the surface of HNTs-NH_2. Once the HNTs-NH_2 gets saturated with $AgNO_3$, the product was washed several times with Mili-Q water to remove the unadsorbed $AgNO_3$ if any. After complete loading of Ag ions onto HNTs, dilute HCl solution was added to the supernatant solution, which produces a white precipitation of silver chloride, demonstrating the presence of excess Ag ions in the supernatant solution even after complete immobilization. Finally, HNTs-NH_2 loaded with Ag ions was reduced with ice cold aqueous solution of $NaBH_4$ to produce Ag loaded HNTs, which in turn changes the colourless halloysite into yellow, owing to the immobilization of Ag NPs over the surface of HNTs-NH_2 (see Scheme 1). At the end of the reduction reaction, nanotubes decorated with Ag NPs were washed several times with de-ionized water to remove the excess borohydride and finally dried in air to study their optical properties and bioactivity.

2.3. Sample characterization

The morphology of HNTs before and after immobilization of Ag NPs was investigated using a transmission electron microscope (TEM: FEI TECNAI G2 F20-ST) operating at 200 kV, after drop casting a drop of solution of the sample onto a carbon coated copper grid. High resolution transmission electron microscopy (HR-TEM) and Energy dispersive X-ray spectroscopy (EDX) have been performed in the above mentioned TEM operating at 200 kV. Field emission scanning electron microscopy has also been performed for HNTs and HNTs-NH_2 (FESEM: FEI QUANTA FEG 250) by drop casting a drop of sample solution on silicon wafer. CHN analysis was done by using a PerkinElmer 2400 Series II CHNS Elemental Analyzer.

Fourier transform infrared (FTIR) spectra were recorded in the range of 500–4000 cm^{-1} by using a JASCO FT/IR 6300 apparatus. Powder X-ray diffraction (XRD) analysis was performed by a RIGAKU MiniFlex II powder diffractometer using Cu Kα radiation with 35 kV beam voltage and 15 mA beam current. Measurements of the total (specular together with diffusive) reflectance/transmission spectra in the region from 200 to 1500 nm were carried out with a Perkin Elmer Lambda 950 spectrometer equipped with an integrating sphere. Samples for the optical measurement were prepared in the form of 100 μm-thick layers deposited on optically polished quartz substrates by using spin coating from aqueous suspensions. The suspensions were formed by mixing HNT powder in de-ionized water followed with sonification in an ultrasonic bath for 30 min the initial concentration of HNTs in suspension was 10 g/L. After the spin coating the layers were dried in air for1 h.

2.4. *Experiments* in-vitro

Bacteria *Escherichia coli* (*E. coli*) were grown by the standard method in agar medium at 37 °C. For the experiment with HNTs loaded with Ag NPs, the bacterial cell suspension was used at concentration of 10^8 per 1 mL. The experiment was carried out in several stages. At the first stage, 1 mL of the bacteria suspension was mixed with 1 mL of the aqueous suspension of HNTs-Ag with concentration of 0.4–2 g/L and the mixture was put in optically transparent plastic cuvette. At the second stage, a part of the obtained mixtures were homogeneously illuminated by white light at room temperature. The light of a tungsten lamp was passed through the filter to get the spectral band from 400 to 800 nm. The light intensity and illumination time were 100 mW/cm^2 and 10 min, respectively. Additionally, the experiments were carried out with laser irradiation at 488 nm with intensity 10 mW/cm^2 for 15 min. A special attention was paid to keep the constant temperature of the bacteria cell suspension during the illumination with an accuracy of 1–2 °C. Another part of the samples was kept in darkness for the same time. At the third stage, all the samples were transferred in Petri dishes and incubated at 37 °C for 12 h. Finally, the result of bacterial growth was evaluated by comparing the number of colony formed in the experimental (with HNTs-Ag) and control dishes. The results were averaged for 3 series of the samples.

3. Results and discussion

The grafting of aminosilane, (3-aminopropyl) triethoxysilane over the outer surface of HNTs was confirmed by Fourier transform infrared (FTIR) spectroscopy. FTIR spectra of HNTs and HNTs-NH_2 are shown in Fig. 1A. The observed three new peaks at 1563, 2933 and 3452 cm^{-1} in case of HNTs—NH_2 are attributed to the N—H deformation, stretching vibration of C—H, and the stretching vibration of N—H respectively, demonstrating the grafting of the aminosilane onto the surface of HNTs [22,23]. Additionally, presence of two well-defined bands at 3621 and 3697 cm^{-1} in both HNTs and HNTs-NH_2 owing to the stretching vibrations of inner hydroxyl group and inner surface hydroxyl group respectively,