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Antibacterial and hemolysis activity of polypyrrole nanotubes decorated with silver nanoparticles by an in-situ reduction process

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ABSTRACT

Polypyrrole nanotube–silver nanoparticle nanocomposites (PPy-NTs:Ag-NPs) have been synthesized by in-situ reduction of silver nitrate ($AgNO_3$) to suppress the agglomeration of Ag-NPs. The morphology and chemical structure of the nanocomposites have been studied by HRTEM, SEM, XRD, FTIR and UV–vis spectroscopy. The average diameter of the polypyrrole nanotubes (PPy-NTs) is measured to be 130.59 ± 5.5 nm with their length in the micrometer range, while the silver nanoparticles (Ag-NPs) exhibit spherical shape with an average diameter of 23.12 ± 3.23 nm. In-vitro blood compatibility of the nanocomposites has been carried out via hemolysis assay. Antimicrobial activity of the nanocomposites has been investigated with *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) bacteria. The results depict that the hemolysis and antimicrobial activities of the nanocomposites increase with increasing Ag-NP concentration that can be controlled by the $AgNO_3$ precursor concentration in the in-situ process.

1. Introduction

Combinations of electrical, optical, and biological properties of multifunctional nanomaterials have shown immense potential for biosensing, optoelectronics, energy conversion and storage, and biocidal applications [1–3]. Successful application of nano-materials into functional nanodevices requires controlled design at nano level. The characterization of these nanostructures is also a vital issue in fundamental research as well as for technological applications. Conducting polymers are emerging as a promising material for their synthesis as nanostructured form as they exhibit electrical, magnetic, electronic, and optical properties analogous to that of metals or semiconductors while retaining polymer like properties such as their flexibility, ease of processing, low toxicity and modifiable electrical conductivity [4]. The nanostructures of electrically conducting polymer can be synthesized by chemical and electrochemical techniques. Fine metal nano particles are of particular interest because of their extraordinary size dependent optical, electrical, catalytic and biological properties. The current progress in research on metal nanoparticles has revived the use of silver nanoparticles for antibacterial application. The use of excellent antibacterial activity of nano-silver is reasonably new in the field of biotechnology [5]. Silver-based antibacterial materials captured much attention because of their being a long lasting biocide with high temperature stability and low volatility [6]. The main problem associated with nano-sized silver particles is that they form clusters and agglomerate due to high surface energy which reduces the possibility of commercialization of Ag-NPs in terms of reusability [7]. The antibacterial activity of Ag-NPs is size dependent and once they aggregate, their activity decreases noticeably. To overcome this difficulty several techniques such as dispersion of the nanoparticles in different matrices or stabilizing metal nanoparticles by ligands have been considered [8]. The nanostructures of conducting polymers such as polyaniline (PAni) or polypyrrole (PPy) have proven to be good matrices for loading metal nanoparticles to form conducting polymer–metal nanocomposites. The entrapment of metal nanoparticles within the conducting polymer matrix is an alluring aspect as it increases the processability of the nanoparticles due to high thermal stability of conducting polymers. It has been reported that conducting polymers such as PAni and PPy also possess antibacterial activity [9,10]. Therefore, the synergy between 1D conducting polymers and metal nano-particles provides resultant nanocomposites with additional functionalities over individual materials with the possibility of designing device functionality. Several new approaches have been considered in synthesis, characterization and applications of nanocomposites with diverse combinations of properties of conducting polymers (PAni, PPy) and metallic nanoparticles. S. Fujii et al. have developed polypyrrole-coated silver nanocomposites with core shell morphology by aqueous chemical oxidative dispersion polymerization of PPy with silver nitrate as oxidant [11]. The synthesis of polypyrrole silver nanocomposites has been reported by P. Dallas et al. by interfacial polymerization method at water chloroform interface using sodium dodecyl sulfate (SDS) and dodecyl trimethyl ammonium

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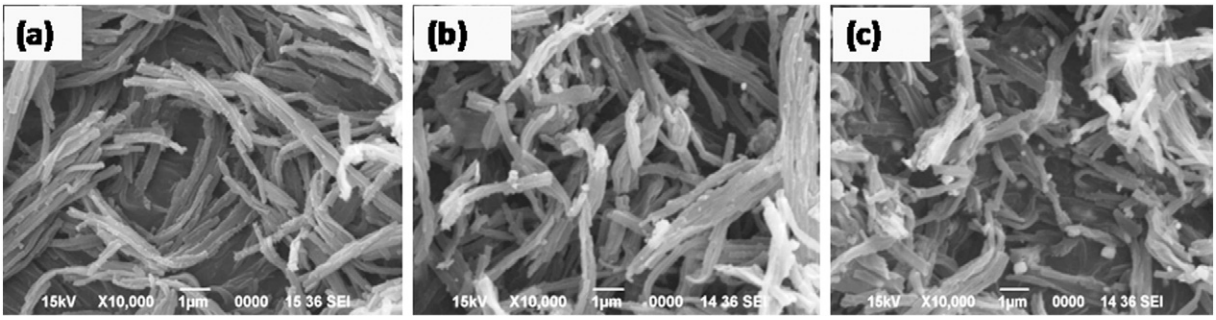

Fig. 1. SEM images of (a) PPy-NTs and PPy-NT:Ag-NP nanocomposites with (b) 6 wt.% and (c) 15 wt.% of Ag-NPs.

bromide (DTAB) as surfactants [12]. J. Zhang et al. [13] reported the synthesis of PPy-NTs and metal nanoparticle nanocomposites via MnO_2 nanowires as a reactive template. MnO_2 nanowires induce the 1D polymerization of pyrrole and the simultaneous dissolution of the templates gives the hollow tube-like structure.

In spite of the antibacterial activity of Ag-NPs, safety of their use needs to be carefully investigated since the expanding applications of nano-silver may also increase the risk of overexposure to human cells [14]. Quantification of hemolysis activity of a material is considered as one of the fundamental tests in determining the safety of a blood contacting biomaterial. If hemolysis occurs to a significant extent it might lead to dangerous pathological conditions. The side effects of Ag-NPs or silver based nanocomposites on mammalian cells have been reported by various groups [15]. J. Choi et al. compared the hemolysis activity of silver micro and nano particles and reported that silver nanoparticles are more hemolytic as compared to micron-size particles due to their high activity owing to higher surface to volume ratio. According to their report the nano particles exceed the limit of 5% hemolysis at a concentration of 70 μg/ml [16]. In other report, Ag-NPs of average diameter of 30 nm at a concentration of 200 μg/ml cause 14.2% hemolysis [17].

In the present study, PPy-NT:Ag-NP nanocomposites have been synthesized by in-situ reduction of $AgNO_3$ and their antibacterial and hemolysis activities have been investigated as a function of varying silver nanoparticle concentrations. Antibacterial activity of the nanocomposites has been investigated as a function of silver content by the diffusion plate method. In-vitro hemolysis activity measurements of the nanocomposites have been carried out against mammalian red blood cells (RBC).

2. Experimental

2.1. Materials and methodology

Pyrrole (Sigma Aldrich) was vacuum dried prior to use. Ferric chloride ($FeCl_3$), $AgNO_3$, methyl orange (MO) and $NaBH_4$ from Sisco Research Laboratory were used as received.

2.2. Preparation of polypyrrole nanotubes

PPy-NTs were synthesized using the method by T. Dai et al. [18]. 0.243 g of $FeCl_3$ was added to 30 ml of 5 mM MO solution. After the formation of flocculent precipitation 105 μl of pyrrole were added to the mixture and stirred for 24 h. The black precipitate formed was thoroughly rinsed three times with DD water and ethanol, and separated by centrifugation. Subsequently, the PPy-NTs were dried in a desiccator overnight.

2.3. Preparation of polypyrrole nanotube–silver nanoparticle nanocomposites

70 mg of PPy-NTs were dispersed in 25 ml DD water and ultrasonicated for 20 min. A calculated amount of $AgNO_3$ was mixed with PPy-NT solution by stirring up to 2 h. Sodium borohydride solution was prepared separately by dissolving in 75 ml of DD water. The silver nitrate solution was mixed with sodium borohydride solution dropwise and magnetically stirred for 24 h at room temperature. The molar ratio of silver nitrate to sodium borohydride was maintained at 1:2 during the synthesis process. Finally the filtrate was thoroughly washed in ethanol and DD water, and dried. Four different compositions with different Ag-NP concentration were prepared by varying $AgNO_3$ concentration as 6, 9, 12 and 15 wt.% with respect to PPy-NTs.

2.4. Characterization techniques

The morphology of the nanocomposites was visualized using scanning electron microscopy (SEM, Jeol 6390 LV) and high resolution transmission electron microscopy (HRTEM, Jeol JEM 2100, 200 kV). For HRTEM imaging, a small drop of the prepared sample was placed on a copper grid following solvent evaporation in ambient air at room temperature. The crystalline nature of the nanocomposites was investigated using Rigaku miniflex X-ray diffractometer with Cu K_α radiation ($\lambda = 1.5406$ Å). The scan rate, accelerating voltage and current were kept at 5°/min, 30 kV and 15 mA, respectively during the XRD recording process. Optical absorption in the range of 200–800 nm was measured

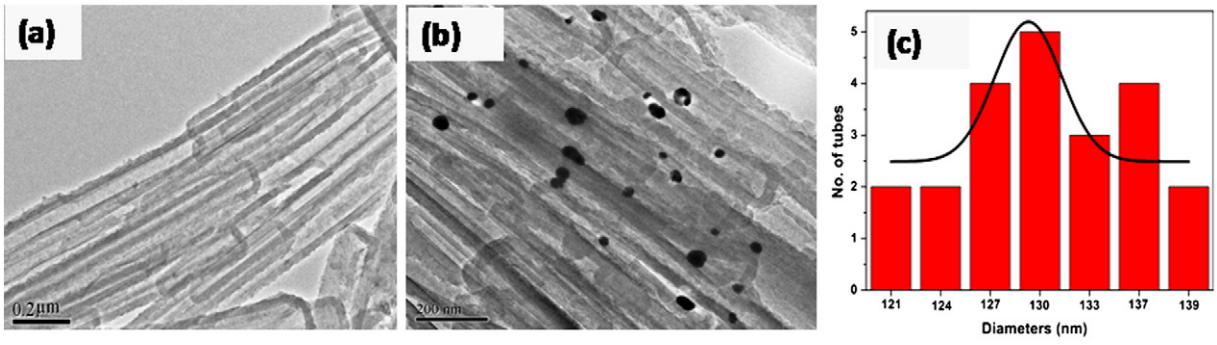

Fig. 2. HRTEM images of (a) PPy-NTs and (b) PPy-NT:Ag-NP nanocomposites with 15 wt.% of Ag-NPs. (c) Gaussian distribution of diameters of PPy-NTs.