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Biocompatibility and antioxidant activity of polypyrrole nanotubes

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ABSTRACT

In the present study antioxidant activity of polypyrrole nanotubes of varying diameter has been investigated. The haemolysis prevention efficiency of the polypyrrole nanotube having highest antioxidant activity has also been studied. Polypyrrole nanotubes have been prepared by chemical polymerization method using $FeCl_3$ as oxidant with methyl orange (MO) and Cetyl trimethylammonium bromide (CTAB). Structural properties of polypyrrole nanotubes with varying CTAB concentrations have been studied by X-ray diffraction pattern and high resolution transmission electron microscopy (HRTEM). The HRTEM micrographs of polypyrrole nanotubes show significant decrease in diameter upon increase in CTAB concentration. Micro-Raman spectroscopy has been performed to understand the polymer conformation. We demonstrate the antioxidant activity of polypyrrole nanotubes by DPPH free radical assay. The biocompatibility of the polypyrrole nanotubes have been investigated via haemolysis assay.

1. Introduction

The past decade has witnessed rapid growth in research on conducting polymer nanostructures motivated by their exceptional electrochemical and optical properties which arise due to their highly π-conjugated polymeric chains [1]. Owing to their good thermal stability and facile synthesis the conducting polymers have been receiving significant attention [2]. The important properties of the conducting polymers stimulated research for exploring these polymers for use in optical and electronic nanodevices and as interesting material for biosensing applications [3]. Considerable efforts have been made to explore the possibility of improved properties by fabricating different nanostructures of conducting polymers. Among the conducting polymers, polypyrrole is by far the most extensively studied in recent years. The intense focus on polypyrrole is attributed to its excellent redox activity, good thermal stability, biocompatibility and low toxicity [4]. Polypyrrole has emerged as a promising material for several applications including batteries, supercapacitors, sensors, light emitting diodes, conductive fabrics and newly in biomedical field as antioxidant or antimicrobial agent [5,6].

Reactive oxygen and nitrogen species are produced in the cell of human body as a consequence of certain undesirable stimuli. This leads to the imbalance between the oxidative and the antioxidant system resulting in tissue damage. This is known as oxidative stress [7]. Oxidative stress has harmful effects on the aging process and is responsible for several diseases like cancer, cardiovascular disease, neurodegenerative disorder, diabetes, etc. Reactive oxygen species, such as superoxide anion ($^{\bullet}O_2^-$), hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) originating from normal metabolic processes are responsible for the peroxidation of membrane lipids which lead to the accumulation of lipid peroxides [8]. Likewise free radicals also cause food degradation. Lipid oxidation by free radicals during storage or food processing is one of the key causes of deterioration of foods [9]. Antioxidants are added to food containing unsaturated fat to increase its shelf life. Polypyrrole can be switched on in different oxidation states, which motivates the use of polypyrrole as antioxidant material. It is understood that electron rich polypyrrole can donate electron to the free radical and is thus capable of inhibiting oxidative degradation. The pronounced antioxidant activity can be achieved by nanostructuring of materials with higher surface to volume ratio with consequent larger active sites for the free radicals.

Several new approaches have been considered in the field of free radicals/antioxidant for the improvement in food storage as well as human health. Many novel approaches are made to enhance the antioxidant activity of conducting polymers and significant findings have come to light in recent few years [10]. Gizdavic-Nikolaidis et al. [11] reported microwave assisted synthesis of copolymers of aniline and 2-aminobenzoic acid or 2-aminosulfonic acid for antioxidant applications. The effects of thermal treatment on antioxidant activity of polyaniline have been investigated by Ashveen V. and his group [12]. The radical scavenging activity of polyaniline decreases slowly upto 200 °C,

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beyond which a rapid fall is observed. This rapid decrease in the antioxidant property of polyaniline is attributed to the oxidation of polymer during heating run. Chu et al. [13] investigated the $ABTS^{\bullet+}$ scavenging activity of polypyrrole, polyaniline and poly(3,4-ethylenedioxythiophene) powders. They have reported that conducting polyaniline showed best antioxidant activity followed by polypyrrole and poly(3,4-ethylenedioxythiophene). The greater radical scavenging activity of polyaniline and polypyrrole than that of poly(3,4-ethylenedioxythiophene) is attributed to the presence of N-H group in their structure [13].

In the present study the effect of diameter on the antioxidant activity of polypyrrole nanotubes has been investigated. Polypyrrole nanotubes of varying diameters have been synthesized by the reactive self degrade methyl orange–ferric chloride (MO-$FeCl_3$) template method in the presence of cationic surfactant CTAB. The antioxidant activity of polypyrrole nanotubes of different diameter have been investigated by DPPH free radical method. Haemolysis assay is employed to assess and compare the human red blood cell compatibility with the polypyrrole nanotubes that exhibit best antioxidant activity.

2. Experimental

2.1. Synthesis of polypyrrole nanotubes

Polypyrrole nanotubes have been synthesized by the reactive self degrade MO-$FeCl_3$ template method discussed elsewhere [14]. MO-$FeCl_3$ complex acts as template in the formation of polypyrrole nanotubes which degrades automatically during polymerization. Polymerization of pyrrole takes place over the template due to the presence of $FeCl_3$ (oxidant). CTAB controls the diameter of nanotubes by partially solubilising the template [15]. Polymerization was carried out for 24 h at room temperature. The resultant product was washed with ethanol several times to remove residual reagents. Finally the product was vacuum dried at room temperature for 48 hrs.

2.2. Apparatus

The structural morphology of polypyrrole nanotubes was visualized by using a HRTEM model JEOL JEM 2100 at an accelerating voltage of 200 kV. For this purpose a drop of prepared sample was placed on a copper grid following solvent evaporation in ambient air at room temperature. X-ray diffractograms were recorded using Rigaku miniflex X-ray diffractometer with Cu Kα radiation (λ = 1.5406 Å). The scan rate, accelerating voltage and current were kept at 5°/min, 30 kV and 15 mA, respectively during the experiment. Micro- Raman spectra were recorded in the range 600–1800 cm^{-1} using Renishaw in-via spectrometer (Rensihaw, Wotton-under-Edge, UK) at a resolution of 0.3 cm^{-1}. Ar^{+} ion laser of 514.5 nm wavelength was used as an excitation source. The UV measurements for both antioxidant and biocompatibility study were carried out using Thermo Scientific spectrometer model UV-10.

2.3. Measurement of antioxidant activity

Antioxidant activity was measured by using the DPPH• free radical method of Serpent et al. [16]. In a typical procedure, an amount of 0.2–0.6 mg of polypyrrole nanotubes were transferred to a test tube and the reaction was started by adding 3 ml of 100 μM DPPH• solution in methanol. The reaction mixture was vortexed for 45 s and stored in dark for the next 20 min and scanning was performed. All measurements for constant amount of 0.4 mg of polypyrrole samples were performed exactly 40 min after the mixing. The time dependent tests were carried out by employing 0.4 mg of polypyrrole sample to the DPPH solution and the absorbance was recorded within the time limit of t = 0–180 min. The scavenging efficiency of DPPH free radicals was calculated as % of free radical scavenging by the following relation:

$$\text{DPPH}^{\bullet}\text{scavenging}(\%) = \left[1 - \frac{A_S}{A_B}\right] \times 100 \quad (1)$$

where, A_S is the absorbance of DPPH with sample and A_B is the absorbance of DPPH without sample.

2.4. Haemolysis assay

The degradation of red blood cell (RBC) membrane against polypyrrole nanotubes was investigated with the help of haemolysis assay using the method by Zhu et al. [17]. Blood was collected into heparinized tube containing 4% Sodium citrate and then centrifuged at 3000 rpm at 4 °C for 20 min. Then erythrocytes were washed twice with a large volume of phosphate saline buffer (PBS, pH 7.4). 5% packed erythrocytes were gently resuspended with PBS. Different concentrations (1.25 mg/ml, 2.5 mg/ml, 5 mg/ml, and 10 mg/ml) of the nanotubes with 2 mM CTAB, dissolved in PBS followed by sonication. Triton X-100 was used as the positive control capable of damaging the red blood cells causing haemolysis and PBS is used as negative control. 100 μl of the dissolved sample was mixed with 1900 μl of haematocrit in different microfuge tubes and incubated at 37 °C for 1 h. RBC cells were subsequently placed in an ice bath for 60 s followed by centrifuging at 3000 rpm for 5 min at 4 °C. Supernatants were used for determining the free haemoglobin concentration as a measure of haemolysis by taking absorbance at 540 nm [18]. The % haemolysis was calculated as follows:

$$\text{Haemolysis percentage} = \frac{A_S - A_N}{A_P - A_N} \times 100 \quad (2)$$

where, A_S, A_P and A_N are the absorbance of the sample, positive control and negative control, respectively.

3. Results and discussion

3.1. Morphological studies

The HRTEM images depicting the tubular morphology of polypyrrole are illustrated in Fig. 1. The average diameter of the nanotubes without CTAB has been measured around 140 nm. It is apparent from the figure that average diameter of the nanotubes decreases continuously with increase in CTAB concentration and is found to be 120 nm and 90 nm with 1 mM and 2 mM CTAB concentration, respectively. The MO-$FeCl_3$ template that forms due to the suppression of the electrostatic repulsive force between negatively charged MO aggregates in solution directs the growth of polypyrrole nanotubes and degrades automatically due to the reduction of oxidising cations. CTAB, a cationic surfactant, adsorbs on the surface of MO-$FeCl_3$ template due to its amphiphilic character with hydrophilic group pointing towards the aqueous solution. The hydrophobic groups of CTAB point towards the MO-$FeCl_3$ template partially solubilising it resulting in decrease in the diameter of the template, which in turn reduces the diameter and surface area of the nanotubes [15]. The diameter distribution of the polypyrrole nanotubes with different CTAB concentration has been depicted using histograms and a Gaussian distribution with standard deviation of the diameters shown in Fig. 2.

3.2. XRD analysis

The XRD pattern of polypyrrole nanotubes (Fig. 3) shows a broad hump that ranges from $2\theta = 20°–30°$ in addition of two sharp peaks