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Poly (m-chloroaniline) and poly (1-naphthylamine) based conjugated polymer for enhanced fluorescence imaging in diverse cell types

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ABSTRACT

Fluorescent probes, based on doped polyaniline, poly(m-chloroaniline) and poly(1-naphthylamine) have been tailored for in vitro cell imaging application. Its structure was characterized by FTIR, UV–visible, fluorescence, atomic force microscopy and BET surface area analysis. The intensity of fluorescence in poly(m-chloroaniline) and poly(1-naphthylamine) was much stronger compared to polyaniline alone. The optimal emitting properties of poly(m-chloroaniline), poly(1-naphthylamine) and polyaniline were related to both structure and shape of the particle and behave according to concentration dependent manner. The poly(m-chloroaniline) and polyaniline emits greenish blue color while poly(1-naphthylamine) exibits intense red and greenish blue color emission respectively. Poly(m-chloroaniline) and poly(1-naphthylamine) demonstrate highly enhanced signals for fluorescence based cell imaging in K-562, U2-OS and MCF-7 cells. Above compounds are cytocompatible, and with no significant loss of viability in K-562, U2-OS and MCF-7 cells following treatment. Higher concentration of the material however, causes moderate loss in viability of the cell besides inducing cytotoxicity. Luminescent micro particles of doped poly(m-cholroaniline) and poly(1-naphthylamine) showed significant potential for biological and diagnostic application.

1. Introduction

Now a day's fluorescent materials are performing impressive role in biological diagnosis and treatment. Fluorescent materials upon photo excitation re-emit light of less energy at higher wavelength. Several aromatic groups with π bonds show fluorescent behavior and have myriad of potential applications such as its usefulness as marker for biological reagents (peptides, antibiotics, nucleic acids etc.) and staining tissues and cells [1–5]. Polymeric materials have strongly attracted to material and biological scientists because of its better performance than organic dyes with respect to photo, thermal and environmental stability [6–9]. Polyaniline act as fluorescent material with low manufacturing cost, biocompatible, easy to synthesize, non-toxic and having good environmental as well as thermal stability [10–13]. Polyanilines are electroactive [14,15] polymers with rich electrical property, optical property and sensing behavior. Polyaniline is also used in multiple other applications like antioxidant, [16] biosensor, [17] and detecting nucleic acids [18–20]. Sun et al. demonstrated that polyaniline nano fibers could serve as a novel fluorescent sensing platform in detecting the nucleic acid with high selectivity [21]. Yu et al. showed that emeraldine base of polyaniline (PANI), could be reduced by biological antioxidants [22].

Polyaniline shows photo-luminescence in their reduced state which underwent quenching following oxidation of polymer (emeraldine state) and subsequent improvement in conductivity from insulator to semiconductor, when electron is delocalized. Fernando V. Molina has studied the fluorescence in polyaniline film and observed that the film is composed of domains of crystalline structure with intervening amorphous regions, besides having efficient quenching of emission surrounding those ordered domains [23]. In spite of its application as fluorescent probe in reduced state, polyaniline suffers from poor environmental stability and become insoluble and nonfusible either in water or in organic solvents. For enhancement of the processsability of polyaniline as a fluorescent probe in biomedical field, several halo, benzo and alkoxy derivatives of aniline were made which are free from such problems. Copolymers, composites and nitrogen substituted polymers were prepared to address the processability problem [23–27]. Among the various modifications, chloro and benzo derivatives of

Abbreviations: PANI, polyaniline; 3ClP, poly (m-chloroaniline); NPNA, poly (1-napthylamine)

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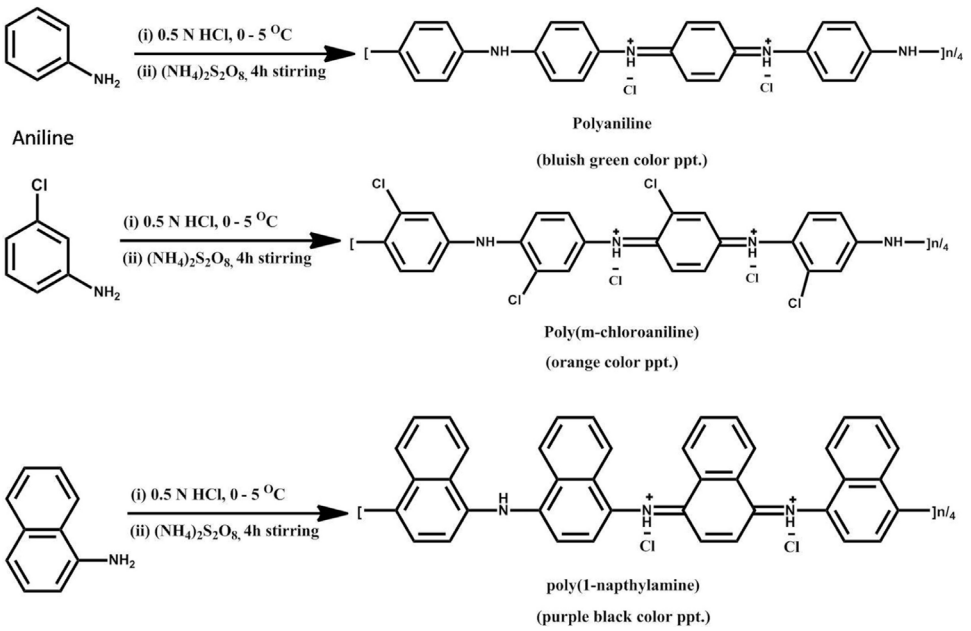

Scheme 1. Synthesis of polyaniline, poly(m-chloroaniline) and poly(1-naphthylamine).

aniline have received great attraction due their enhanced processabilty with improved optical property.

Herein, we have fabricated effective strategies for better luminescent features of polyaniline by preparing poly(m-chloroaniline) (3CIP) and poly(1-naphthylamine) (NPNA) as depicted in Scheme of synthesis (Scheme 1). In our knowledge, the role of poly(m-chloroaniline) or poly (1-naphthylamine) in cell imaging was not investigated. The poly(m-chloroaniline), poly(1-naphthylamine) and polyaniline were analyzed by FT-IR, UV–vis, Fluorescent spectra and atomic force microscopy (AFM). The material's surface area was analyzed by nitrogen adsorption using the Brunauer-Emmett-Teller (BET) method. In the present report, we have demonstrated the application of conjugated doped poly(m-chloroaniline), poly(1-naphthylamine) and polyaniline for cellular uptake studies. Fluorescent spectra of poly(m-chloroaniline), poly(1-naphthylamine) and polyaniline were determined and interpreted in DMSO solution at various concentration using fluorescent spectrophotometer. Significant uptake of 3CIP and poly(1-naphthylamine) was observed by various cell lines (K-562, U2-OS and MCF-7) of diverse origin, demonstrating the possible application of these compounds for cell imaging analysis. We also observed that poly(m-chloroaniline) and poly(1-naphthylamine) were tolerated by the above cell types although higher concentration of the material causes moderately affects viability of cells and induces cytotoxicity. There was a significant uptake of the compounds by the cells and appear to label the cell's nucleus, suggesting its incorporation to DNA.

2. Experimental section

2.1. Materials

Aniline, m-chloroaniline, 1-napthylamine, ammonium persulphate were procured from SD fine-chemical. Purification of Aniline and m-chloroaniline was performed by double distillations. Other reagents used were procured as analytical grade.

2.2. Sample characterizations

IR analysis for the samples was performed in the range 400–4000 cm^{-1}. The IR analysis was performed using Perkin Elmer Spectrum and the FTIR Spectrometer as KBr pellets. Atomic force microscopy was employed for determining the size of particle and morphology of composites. AFM images of different samples were recorded by NT-MDT atomic force microscope (model Solver NEXT) by preparing thin film on cover slip. Surface roughness was assessed by NOVA Px 3.1.0 rev 3880 software. Polymers in DMSO were used for recording of UV-Visble absorption spectra (Shimadzu UV-1700). Fluorescence spectra was taken in a JY Horiba fluorescence spectrophotometer. The fluorescence spectra of polyaniline, poly(m-chloroaniline) and poly(1-naphthylamine) were taken at slit width 5, 3 and 4, respectively. Surface area of poly(m-chloroaniline), poly(1-naphthylamine) was measured by nitrogen adsorption and desorption at 77.3 K using NOVA Quanta Chrome 347.

2.3. Synthesis of polyaniline

2.33 g aniline (0.025 mol) in 100 mL 0.5 N HCl was dissolved and placed on ice bath. Pre-cooled ammonium persulphate solution (7.0 g) in 50 mL 0.05 N HCl was added gently by a burette to aniline solution. Polymer with bluish green precipitation was formed. The reaction was continued up to four hours, and kept in normal temperature for 24 h. Following polymerization, a blue green precipitation was formed. Following filtration and washing with HCl (0.05 N), distilled water and methanol. The precipitate become free from the oligomers and unreacted reagents. The blue green precipitate was dried for twenty four hours in an oven at 60 °C to make it dry. Structure of polyaniline emeraldine salt is exhibited in scheme of synthesis.

2.4. Poly(m-chloroaniline) synthesis

3.19 g m-chloroaniline (0.025 mol) was mixed in 100 mL HCl (0.5 N) and kept on ice bath. Pre-cooled ammonium persulphate solution (7.0 g) was mixed in 50 mL 0.05 N HCl and was gently added by burette in m-chloroaniline solution. An orange precipitation of polymer was obtained. After four hours, the mixture was left in normal temperature for twenty four hours. After polymerization, an orange precipitate was observed. The polymer then filtered, dried and become brown in color. Formation of poly(m-chloroaniline) emeraldine salt is depicted in scheme of synthesis.

2.5. Synthesis of poly(1-naphthylamine)

1-napthylamine (0.025 mol) was mixed with100 mL methanol and kept on ice bath. Ammonium persulphate solution (pre-cooled) of (7.0 g) was mixed to 50 mL 0.05 N HCl and was gently added by a