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Original Research Article

Rapid capture and exemplary detection of clinical pathogen using surface modified fluorescent silica coated iron oxide nanoparticles

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ABSTRACT

Rapid, sensitive and selective detection of pathogenic bacteria is very important for treatment of diseases like foodborne illness, sepsis and bioterrorism. The silica coated iron oxide nanoparticles (SIO) were synthesized using simple, cost-effective method and used for the rapid capture and detection of clinical pathogen. The surface modification of nanoparticles was carried out using 3-aminopropyltriethoxy silane. The scanning electron microscopy image results showed the slightly agglomerated spherical shaped nanoparticles. Transmission electron microscope result showed the polydispersed particles in the size ranges from 5 to 12 nm. The EDAX results confirmed the coating of silica with iron oxide particles. The SAED pattern confirmed the crystalline nature of iron oxide nanoparticles and also indicated the presence of silica. The FTIR spectrum of the nanoparticles confirmed the functional groups of the iron oxide and surface modified fluorescent silica coated iron oxide nanoparticles (SFSIO). This work provides a very effective method for controlling the growth, capture and detection of pathogenic bacteria.

1. Introduction

Nanotechnology is a formation of functional materials, devices and systems through manipulation of materials in the nanometer scale and development of novel phenomena and properties which arise because of the nanometer scale. Hence, it is possible to create materials of required optical, magnetic, and chemical properties by controlling the reaction conditions [1]. The fundamental building blocks of nanotechnology are nanoparticles and due to the special properties nanoparticles have attracted considerable interest in the scientific field [2,3].

Nanostructured materials habitually have unique electrical, chemical, structural and magnetic properties allowing for use in a various novel applications such as information storage, biosensing applications and biomedical applications. The magnetic properties of nanoparticles provide advantages for the selective attachment to functional molecules and

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bestow the magnetic properties to the target furthermore permit manipulation and transportation to craving site via the control of magnetic field formed by electromagnet or a permanent magnet [4]. Nanoparticles are having a large surface area and highly active surface sites thus it has a variety of applications such as shape selective catalysis, chromatographic separations, sorption of metal ions, enzyme encapsulation, DNA transfection and drug delivery [5].

Iron oxide nanoparticles (IO NPs) are being either paramagnetic or superparamagnetic particles, mostly superparamagnetic particles (Fe_2O_3 and Fe_3O_4) are of interest for in vivo applications [6]. The magnetic nanoparticles are presently being extensively studied through the rapid growth of nanotechnology. Due to the diverse properties (small size, superparamagnetism and low toxicity) of superparamagnetic iron oxide (Fe_3O_4) nanoparticles have trapped researchers in many fields including physics, medicine, biology and materials science [7]. IO NPs are the primary choice in biological and biomedical applications, because of its biocompatibility and chemical stability. Various methods have been used for the preparation of iron oxide nanoparticles; the coprecipitation is the most effective method for preparing aqueous dispersions of iron oxide nanoparticles [8].

Currently, fluorescent silica coated magnetic nanoparticles (FSIO) gained more and more attention. Magnetic nanoparticles were extensively examined for in vivo and in vitro biomedical applications including magnetic resonance imaging, target drug delivery and so on. On the other hand dye doped silica nanoparticles were used for bio-labeling and bioimaging applications because they have many advantages such as photostable, sensitive, water soluble and easy surface modification, thus these bi-functional nanoparticles have fluorescent and magnetic properties concurrently which make them useful in biomedical applications [9,10]. There is a great need for an effective technique for microbial decontamination and rapid detection without time consuming cell culturing method. Magnetic field measurements are an alternative technique for detection of pathogen to optical read-out [11,12].

The present work investigates the synthesis of FSIO and used for the capture and detection of bacterial pathogen. The FSIO were synthesized and surface modified with 3-(aminopropyltriethoxysilane) and characterized using X-ray diffractometer (XRD), scanning electron microscope (SEM) and energy dispersive X-ray spectra (EDAX), transmission electron microscope (TEM), Fourier infra-red spectrophotometer (FT-IR) and fluorescence microscopy. The SFSIO were used for the detection of pathogens using fluorescence microscope and the capture efficiency of the nanoparticles was examined using UV-spectrophotometer.

2. Experimental

2.1. Chemicals and reagents

Ferric chloride ($FeCl_3{\cdot}6H_2O$), ferrous chloride ($FeCl_2{\cdot}4H_2O$), ammonia solution, tetraethoxyorthosilane (TEOS), fluorescein isothiocyanate, dimethyl formamide, 3-aminopropyltriethoxy silane (APTES), isopropyl alcohol, nutrient agar, nutrient broth, phosphate buffer (PBS) were purchased from Himedia Pvt. Ltd., Mumbai, India. The model bacterial pathogen *Escherichia coli* was collected from Microlabs, Institute of Research and Technology, Arcot.

2.2. Preparation of IO nanoparticles

Magnetic nanoparticles were prepared using the coprecipitation method. Ferric chloride (0.074 g) and ferrous chloride (0.190 g) were dissolved in 20 ml deionized water, which was then stirred and heated to 80 °C. Subsequently 5 ml of 2.5 M NaOH solution was added at 80 °C and the reaction continued at 80 °C for 20 min followed by the temperature was cooled to 0 °C. After 1 h of stirring, the iron oxide nanoparticles were isolated by centrifugation and used for the preparation of surface modified fluorescent silica coated iron oxide nanoparticles (FSIO).

2.3. Preparation of SFSIO

A mixture of the above iron oxide dispersion (0.75 ml) was mixed with 1.5 ml of distilled water, 0.6 ml of ammonia, and 10 ml of isopropyl alcohol with magnetic stirring and 10 μl of TEOS were added together. Then it was stirred for 3 h. Subsequently, 20 μl of APTES, 5 ml of FITC (0.1 M) solutions in isopropyl alcohol and 20 μl of TEOS were added into the mixture, after 0.5 h, 5 ml of isopropyl alcohol, 2.5 ml of H_2O and 0.25 ml of ammonia was added dropwise into the reaction mixture simultaneously. After 24 h of reaction the mixture was centrifuged, and the nanoparticles were collected, then the nanoparticles were dried in hot air oven and used for the characterization and detection of pathogens.

2.4. Characterization of synthesized nanoparticles

The morphology of the SFSIO was observed using scanning electron microscopy (Hitachi, Model: S-3400N), and the size of the nanoparticles was detected using transmission electron microscopy (Philips CM200). The functional groups of the nanoparticles were identified using FT IR (Thermo Nicolet Model: 6700). The UV–vis spectrophotometer (Perkin) was used to examine the capture efficiency of the prepared nanoparticles. The UV spectrofluoremeter is used to examine the fluorescence excitation and emission of nanoparticles. The fluorescence microscopy (Nikon) was used to check the fluorescence of nanoparticles and also to identify the microorganisms.

2.5. Control the growth of pathogen

The antibacterial activity of the SIO was examined against *E. coli* in Luria Bertani broth (LB). The 24 h old bacterial cultures were inoculated into the broth with various concentrations (20 μl, 50 μl, and 100 μl) of SIO NPs. The nanoparticles free LB broth was used as a control, then the flasks were incubated at room temperature for 24 h and the absorption was taken at 600 nm for various time intervals.

2.6. Bacterial capture and detection efficiency of SFSIO

E. coli culture was grown at 37 °C on an orbital shaker at 150 rpm for 16 h, and then the culture was centrifuged at