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Synthesis and characterization of magnetite nanoparticles having different cover layer and investigation of cover layer effect on the adsorption of lysozyme and bovine serum albumin

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ABSTRACT

In this study, differently coated superparamagnetic Fe_3O_4 (magnetite) nanoparticles were synthesized, characterized and used for lysozyme (Ly) and bovine serum albumin (BSA) adsorption. SiO_2, carbon nanotubes (CNTs) and graphene were used for covering the readily synthesized magnetite nanoparticles to elucidate the effect of cover layer on the protein adsorption kinetics and capacities of nanostructure. XRD, FTIR, AFM, SEM, VSM and fluorescence measurements were used for the characterization of the samples and investigating the adsorption kinetics of Ly and BSA by these nanoparticles. The average particle size of the Fe_3O_4 nanoparticles are approximately found as 10 nm and VSM measurement shows that the Fe_3O_4 particles have superparamagnetic behavior with no hysteresis and remnant. The adsorption kinetic of proteins on nanosized material is followed via fluorescence method. All the nanostructures with different cover layers obey pseudo first order kinetics and SiO_2 coated nanoparticles show the fastest kinetics and capabilities for Ly and BSA adsorption.

1. Introduction

Small sized particles, having sizes in the range from 1 to 100 nm are generally known as nanoparticles (NPs). Based on the number of elements involved, nanoparticles may be mono, binary and ternary components system [1–4]. These tiny particles possess numerous catalytic, optic, sensing and electronic characteristics making them superior over the bulk materials. Nanoparticles (natural & synthetic) have widespread applications in many areas of science such as physics, chemistry, biology, medical, environmental and engineering science [5–7]. Nanoparticles possess significant adsorption capabilities due to their high surface area to volume ratio. This property makes them efficient carrier of other molecules such as chemical compounds, drugs, probes and proteins attached to the surface, covalently or through adsorption. Hence, the physicochemical properties of nanoparticles, such as charge and hydrophobicity, can be altered by attaching specific chemical compounds, peptides or proteins to the its surface [8–13].

Among different types of nanoparticles, magnetic nanoparticles (MNPs) were extensively used by many researchers in past few decades because of their exceptional physiochemical, magnetic, and optical properties. These unique properties have enabled MNPs to find potential applications in many research fields including biomedicine, magnetic resonance imaging, catalysis, information technology, telecommunication, and environmental remediation [14–18].

Superparamagnetic iron oxide nanoparticles with an average diameter size of about 10 nm, have proven to be the excellent candidates for biomedical applications among different magnetic materials, because of its good biocompatibility, less cytotoxicity, and stability in physiological environments [19–23]. Biomedically, these nanostructures are utilized in magnetic separation for protein purification, [24] separation of certain cell types, [25] photodynamic therapy (PDT), [26] and as contrast agents in MRI [27]. Magnetic separation is a new developing methodology that is frequently applied in the bio-separation field. In this method, the magnetic nanoparticles are utilized to bind the desired molecules by means of a ligand to form a complex which can be easily separated from the bulk solution with the help of an external magnetic field. The practical applications of these nanostructures include enzyme immobilization [28,29], nucleic acid detachment [30,31], and targeted drug delivery [32]. Magnetic separation is superior to conventional separation due to its speed, accuracy, and simplicity.

Since, magnetic Fe_3O_4 nanoparticles have hydrophobic surfaces with a large surface area to volume ratio, which leads to the agglomeration of the particles and formation of large clusters, resulting in a

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larger particle sizes [33,34]. In the meantime, application of the magnetic particles is restricted by two limiting factors like nonspecific adsorption and slow mass transfer kinetics [35]. However, these limitations could be overcome by coating or modifying the surface of magnetic nanoparticles which makes them colloidally stable, biocompatible and specific in bio recognition [36].

Mesoporous silica with its large surface area and porous nature, narrow pore size distribution, controlled shape, elevated thermal and hydrothermal stabilities has been widely studied due to their extensive potential applications [37–40]. Silica nanospheres are the perfect hosts for proteins and peptides due to its high adsorption capacity, high dispersibility in aqueous media and good compatibility with the neighboring atmosphere [41–44]. These mesoporous silica nanoparticles with small particle sizes are useful for several applications from the view of adsorption equilibrium and kinetics. Nevertheless, the separation of these small nanoparticles from the solution is very difficult and time-consuming. Magnetic nanoparticles, therefore have the advantage as they can be easily separated from an aqueous medium with the help of an external magnetic field. Hence, combining porous materials with magnetic nanoparticles will be an active medium for the separation, thus magnetic separation has attracted widespread attention in recent years [45].

Ferrite nanoparticles have been focused in literature for the adsorption or immobilization studies of lysozyme [46–51]. Additionally, several previous reports are available about the BSA adsorption property which suggest an active solution for the separation of protein with the nanoparticles [52–55].

Bovine serum albumin (BSA) is a globular blood plasma protein which is largely applied to stabilize the enzymes. It is also utilized to evaluate the structures of other proteins, stops the adhesion characters of enzymes and acts as a biosensor [56–58]. Recently, considerable attention has been given to immobilize proteins on the surface of nanomaterials. Nanoparticles have the unique characteristics of retaining the bioactivity of proteins even after adsorption. Adalgisa Tavolaro et al. [59] reported the immobilization of BSA on zeolite inorganic supports. Similarly, Hee Moon et al. [60] recently investigated BSA adsorption on monodispersed hollow silica nanoparticles.

Besides the silica layer, many other covering layers have been used for magnetic nanoparticles in the literature. Carbon nanotubes (CNTs) and graphene are also good candidates for magnetite covering layer because of their unique and novel properties [61–63].

In this study, we describe the synthesis and characterization of superparamagnetic SiO_2@Fe_3O_4, CNTs@Fe_3O_4, and graphene@Fe_3O_4 nanoparticles. After synthesis, we investigated the effect of these different covering layers of magnetite NPs on the adsorption kinetics of lysozyme (Ly) and bovine serum albumin (BSA) using fluorescence technique. This comparative study will help researchers in biomedical sciences as these proteins bonded nanostructures could be applied for the recognition of other important proteins in in-vitro and in-vivo study. Besides, the low less toxicity, strong biocompatibility and high stability in physiological conditions makes these nanostructures, the competent candidate for biomedical applications.

2. Experimental part

2.1. Materials

All the chemicals were of analytical grade and used without further purification by employing pure deionized water as the introductory medium. The lysozyme (Ly), bovine serum albumin (BSA), ammonium hydroxide (NH_4OH), and the precursor salts including Ferrous chloride tetrahydrate and ferric chloride hexahydrate were purchased from Sigma-Aldrich.

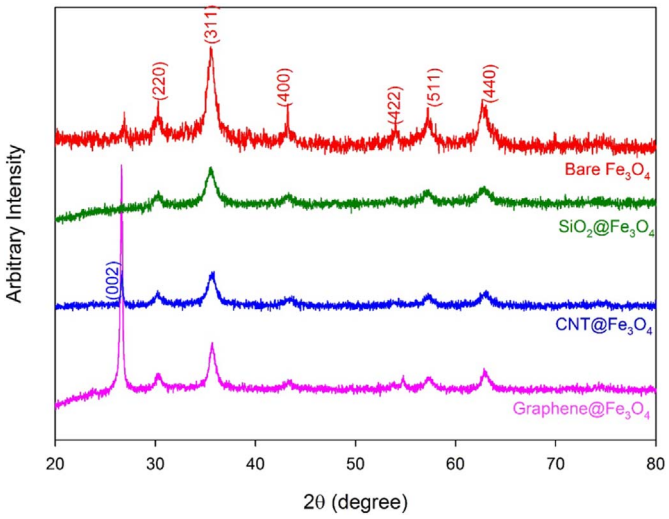

Fig. 1. XRD pattern of bare Fe_3O_4, SiO_2@Fe_3O_4, CNT@Fe_3O_4 and graphene@Fe_3O_4 nanostructures.

2.2. Instrumentation

X-ray diffractometer (XRD) (MMA model X-ray diffractometer GBC Scientific Equipment, Australia), Secondary Electron Microscope (SEM, FEI Nova NanoSEM 450), Vibrating Sample Magnetometer (VSM), Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR, Spectrum One FT-IR, Perkin Elmer), Atomic Force Microscopy (AFM, SPM-9500 J3, Shimadzu), Varian Cary Eclipse Fluorescence Spectrophotometer with a temperature controller and quartz vessel were used during the measurements.

2.3. Synthesis of SiO_2-coated Fe_3O_4 (SiO_2@Fe_3O_4) nanoparticles

Fe_3O_4 nanoparticles were synthesized using the precursor salts $FeCl_2{\cdot}4H_2O$ and $FeCl_3{\cdot}6H_2O$ at 1:2 molar ratio. First, iron (II) chloride tetrahydrate and iron (III) chloride hexahydrate solutions were prepared by weighing exactly 1.075 g (0.054 M) and 2.92 g (0.108 M) respectively in 100 mL pure water. After complete dissolution of salts, 15 mL of 30% NH_3 solution was added to the conical flask containing the mixture. The color of the solution immediately changed from orange to black after the addition of ammonia solution. The mixture was vigorously stirred on mechanical shaker at room temperature for 60 min. The black magnetite precipitates were separated from the solution with the help of an external magnet and washed twice with 0.02 M NaCl solution to remove the unreacted chloride ions. Finally, Fe_3O_4 sample was washed with several times with deionized water and vacuum dried at room temperature.

Fe_3O_4 nanostructures were stabilized by making silica shell around the Fe_3O_4 nanoparticles. Stober procedure was followed for the preparation magnetite silica core-shell nanostructures (SiO_2@Fe_3O_4). For this, 20 mL of Fe_3O_4 nanoparticles aqueous suspension and 100 mL of ethanol were added to 250 mL flask and then the mixture is ultrasonically dispersed for 10 min in an ultrasonic bath. Afterward, 8 mL of tetraethoxysilane (TEOS) in 30 mL ethanol was added to the flask followed by the addition of 10 mL of 30% NH_4OH solution. The reaction was continued at room temperature for 90 min under vigorous mechanical shaking on a mechanic shaker. The reaction was stopped after 90 min and the product was washed 4 times with ethanol and 3 times with pure water and separated from the solution with the help of a permanent magnet and dried at room temperature under vacuum.

2.4. Synthesis of CNT-coated Fe_3O_4 (CNT@Fe_3O_4) nanoparticles

In situ composites of carbon nanotubes (CNTs) with Fe_3O_4 NPs were