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Mechanistic insights into the interactions of magnetic nanoparticles with bovine serum albumin in presence of surfactants

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ABSTRACT

This work reports the physicochemical parameters and the nature of association between magnetic nanoparticles and bovine serum albumin (BSA) in presence of cationic and anionic surfactants. Magnetic iron oxide nanoparticles (MNPs) are first synthesized using chemical co-precipitation method and subsequently characterized by FTIR, XRD, DLS, TEM and Zeta potential. The bare nanoparticles are then coated with BSA and their interactions studied using fluorescence spectroscopy, dynamic light scattering and circular dichroism techniques. The spectroscopic investigation sheds light into various aspects of binding and size variation during the molecular association of BSA with the MNPs in absence and presence of cationic and anionic surfactants. Isothermal titration calorimetry was used to probe the thermodynamic parameters of the systems. MNPs-BSA system was found to be more stable in presence of cationic surfactant. This study provides valuable mechanistic insights into the interactions taking place at the interface of the nanoparticles which further helps in designing a stable colloidal MNPs systems.

1. Introduction

Magnetic nanoparticles (MNPs) are the next generation high-performance nanomaterials in the area of biomedical research, having the unique property of being able to be guided by an external magnetic field. MNPs are commonly composed of magnetic elements, such as iron, nickel, cobalt and their oxides. Among them, iron oxide nanoparticles have been extensively used in biomedical applications due to their proven cyto-compatibility. Organic and inorganic polymers are usually used to coat the magnetic core to achieve improved biocompatibility by protecting biological entities from adverse toxic reactions. In this study, iron oxide nanoparticles are referred to as MNPs. By virtue of their ultrafine size, magnetic properties and biocompatibility, they play an important role in biomedical applications such as magnetic resonance imaging (MRI), targeted drug and gene delivery, tissue engineering, cell tracking and bioseparation [1–3]. As a pre-requisite, MNPs, especially for *in vivo* use, should be coated with appropriate materials to ensure colloidal stability and bio-compatibility. Use of coating materials on nanoparticles addresses various issues like aggregation, stability, shielding the nanoparticle's corrosive body from exposing it to plasma fluids and cells, thereby reducing osponization [2,3]. Therefore, need for a biocompatible coating on MNPs is crucial. Various coating materials such as proteins, sugars, polymers [4–6] have been used and their effects on cells and biological environment are studied [7,8]. The studies on surface properties of the coating material help decode the fate of the nanoparticles in human body. Selection of a coating material on the nanoparticles depends on the required end application. However, before choosing a coating material, it is important to understand the mechanism of molecular interactions occurring at the interface of nanoparticles. Surface thermodynamics provides us opportunity to understand the balance of energetic forces driving binding interactions between the nanoparticles and the coating materials. Thorough understanding and optimizing molecular interactions are required for designing a stable colloidal nanoparticle system.

In literature, there is a paucity of information on the binding affinities and other physicochemical parameters of various coating materials with respect to MNPs. Handful of studies are done on zinc, silver and gold nanoparticles. Mehta et al. [9] studied the effect of different types of surfactants head groups on agglomeration behavior of ZnS nanoparticles. On similar lines, interaction of ZnO nanoparticle with plasma enzymes were studied by Chakraborti et al. [10]. Interactions of surface modified iron oxide nanoparticles with proteins have been reported, however,

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there are no reports on the interaction/binding of bare iron oxide nanoparticles with proteins. Zhang et al. [11] studied interaction of oleic acid coating with MNPs and derived their binding energies using thermogravimetric analysis (TGA), however, detailed study of protein binding as core/shell interaction with MNPs is not documented.

The understanding of physico-chemical characteristics of MNPs-protein systems, and retention of the protein structure on binding with nanoparticles is important and needs to be addressed [12]. Hence we investigated the parameters involved in the interactions of bare and surfactant modified MNPs with protein-bovine serum albumin (BSA) where BSA can be viewed as a potential biocompatible coating on MNP. Besides, BSA is a model protein due to its structural homology to human serum albumin [13], and imparts the necessary biocompatibility to MNPs. There are reports of biomimetic studies on interaction of BSA with surface coated MNPs [14–17]. In these studies, BSA was used as a plasma protein to detect the fate of coated MNPs *in vivo*. However, there are no studies on the binding affinity and evaluation of various binding parameters for BSA as coating material with uncoated and surfactant modified MNPs.

In the current study, bare iron oxide nanoparticles (MNPs) were synthesized and characterized using various characterization techniques. Interactions of MNPs with serum protein (BSA) were studied using fluorescence spectroscopy, dynamic light scattering and circular dichroism. The spectroscopic investigation helped in understanding the interactions and stability of the molecular association of BSA with the MNPs in terms of binding and variation in size. The thermodynamic parameters were determined using Isothermal titration calorimetry technique [18]. Further, the above system was studied in presence of a cationic and anionic surfactant. Surfactants are known to stabilize the nanoparticles and stop aggregation. In this study a cationic surfactant, cetyltriammonium bromide (CTAB), which has a positive-polar head group and hydrocarbon-non polar tail group is studied. BSA is known to have a pI of 4.7 [19] making it negatively charged at neutral pH. Hence expecting a fair amount of interactions due to opposite charges, MNPs were modified with CTAB and BSA was incorporated for coating these nanoparticles. The study provided valuable insights into the interactions at the interface of MNPs. Interaction studies were conducted in presence of anionic surfactant, sodium dodecyl sulphate (SDS). An understanding of the dynamics would enable a design of efficient MNPs for applications requiring a biocompatible protein cover.

2. Materials and methods

2.1. Reagents

Ferrous chloride tetrahydrate [$FeCl_2{\cdot}4H_2O$] ≥ 99%, Ferric chloride hexahydrate [$FeCl_3{\cdot}6H_2O$] ≥ 98%, fatty acid free BSA ≥ 98%, CTAB and SDS with ≥99% were procured from Sigma–Aldrich. 25% aqueous ammonia was obtained from S.D. Fine Chemicals. All other chemicals were of analytical grade and used as received. Deionized (DI) water from Elga Labwater was used for all the experiments.

2.2. Synthesis and characterization of bare MNPs

Bare MNPs were synthesized following the conventional co-precipitation method [20,21]. Briefly, 6 g of $FeCl_3{\cdot}6H_2O$ and 2.1 g of $FeCl_2{\cdot}4H_2O$ were dissolved in 100 ml of deionized water in a flask to maintain a Fe^{3+}/Fe^{2+} molar ratio of 2:1. The temperature was gradually increased to 80 °C in nitrogen atmosphere with constant mechanical stirring. After 30 min, pH of the mixture was increased rapidly using 25% aqueous ammonia and further kept for 30 min of digestion with vigorous stirring. Black precipitates formed were rinsed 5 to 6 times with water and decanted using a permanent magnet. Subsequently it was kept for drying in a vacuum oven at 45 °C.

Fourier Transform Infrared Spectroscopy (FTIR) was carried out using Jasco, FTIR 300E spectrometer and the spectra were recorded from 4000 to 400 cm^{-1}. To evaluate the crystal structure, X-ray diffraction (XRD) of the MNPs were performed on XRD—PANalytical X'Pert Pro using CuKα radiation. Size of the MNPs was evaluated by transmission electron microscopy (TEM) using JEOL, Phillips CM 200. The surface charge of the nanoparticles were determined by measuring their ζ-potential values in different pH, using phase analysis light scattering of Nano-ZS 90 instrument from Malvern. The pH of the solution was adjusted using 0.1 M HCl and NaOH.

2.3. Interaction studies

Colloidal stability of the MNPs were carried out in aqueous, CTAB surfactant and buffer (pH 7.4) solution. Aggregation of nanoparticles leads to sedimentation and reduced dispersion, which may in turn affect the actual concentration of the nanoparticle in a solution [22]. Colloidal stability studies are important to identify the optimum concentration of nanoparticles that can remain stable in the solution during the course of experiment. Dispersion of various concentrations of MNPs were studied wherein 30 μg/ml of MNPs showed the aqueous stability of more than 24 h and hence was fixed as the maximum MNPs stock concentration for further studies. Good colloidal stability was observed for MNPs dispersed in post micellar surfactant solutions. The MNPs in CTAB solution (MNPs/CTAB) prepared in post micellar concentration range of 1.5–2.2 mM was stable for more than two months. Experiments in buffer solutions showed poor stability due to particle sedimentation and thus studies in buffer solutions were not carried further. All interaction studies of the nanoparticle solution with BSA protein were performed in two sets—one in aqueous as MNPs-BSA and other in CTAB solution as MNPs/CTAB-BSA. Studies of MNPs/SDS-BSA were also carried out and are listed at the end of the discussion with figures in supporting information.

2.3.1. Fluorescence studies

The fluorescence experiments were carried out on Cary Eclipse spectrofluorimeter with a quartz cell of 1 cm path length. For fluorescence measurements, concentration of the nanoparticle varied from 0 to 25 μg/ml. The BSA concentration in all the fluorescence experiments was kept constant at 1 mg/ml. The CTAB concentrations were also kept constant in MNPs/CTAB-BSA experiments, ranging from 1.5 to 2.2 mM (post micellar concentration). The excitation wavelength was selected at 295 nm to selectively excite the tryptophan residues of BSA molecules and corresponding emission spectra were recorded in the range of 300 nm to 450 nm. The excitation and emission slit widths were fixed at 5 nm. Each set was carried out in triplicate for $n \geq 3$, standard deviations are represented by the error bars in the graphs.

2.3.2. DLS studies

Dynamic light scattering (DLS) measurements were performed on a Nano-ZS 90 from Malvern Instruments at 25 °C. Nanoparticle concentration of 30 μg/ml was monitored in aqueous and CTAB solution [2.2 mM] for a time period of 0 to 24 h with respect to their size. The aqueous MNPs and MNPs/CTAB solutions were monitored after the addition of BSA (1 mg/ml) and the change in size recorded for MNPs-BSA and MNPs/CTAB—BSA systems. The DLS results are expressed by plotting scattering intensity *versus* hydrodynamic size.