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Soft chemical synthesis, characterization and interaction of ZnO graphene nanocomposite with bovine serum albumin protein

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ABSTRACT

Biofunctionalization of metal oxide nanoparticles (MONs) has enormous impact on biomedical fields. Therefore, the interaction of MONs with a protein must be understood before consideration of the material for a particular biomedical application. This is because the nanoparticles are always enclosed by a protein in biological fluid. In this report, we have studied to understand an interaction between zinc oxide-reduced graphene oxide nanocomposite (ZG, synthesized by soft chemical process) with bovine serum albumin (BSA), a model protein having wide range of physiological functions. Initially, ZnO nanoparticles have been synthesized by polyvinyl alcohol foam based precursor followed by coupling of the nanoparticles with in situ formed reduced graphene oxide (rGO) from as-synthesized graphene oxide in presence of hydrazine. The crystallinity and crystallite size of hexagonal ZnO has been analyzed by X-ray diffraction analysis. In the samples, quasi spherical shaped ZnO/ZnO-rGO (ZG) nanoparticles was observed by field emission scanning and transmission electron microscopes while the FTIR and Raman spectral analyses have been carried out to reveal the existence of chemical interaction/complexation that existed between the oxygen functional groups of rGO and the inorganic moiety (ZnO/Zn^{2+}) present in ZG nanocomposite. With the help of UV–visible and photoluminescence spectral studies, we have established an interaction of BSA protein with ZG nanocomposite. This work can find several applications in biomedical fields such as pharmacology, drug delivery and cell viability.

1. Introduction

Metal oxide nanoparticles (NPs) with improved biocompatibility, capability of biofunctionalization and unique size-selective properties are very much essential for their applications in biological interfaces [1]. In this context, especially, the particle size is important as it influences the particles to penetrate into the surface of biomolecules through an effective interaction [2]. Therefore, the biofunctionalization or surface modification of NPs for obtaining biocompatibility is an utmost requirement for enhancing the biocompatibility of the nanoparticles which could be used in variety of biological studies/applications such as cell viability [3], protein adsorption [4] and antibacterial activity [5] as well as applications in drug delivery [6], bioimaging [7], tissue engineering [8], bioimmobilization [9], biosensor [10] and so on.

Although, the application of nanoparticles in biological interfaces is enormous [3–10] but the main challenge in this aspect is to control the interaction. For safe and effective use of nanomaterial in a particular cell system or biological interface, its interaction study with the cell system is a key issue [11]. It is known that when a nanoparticle enters into a biological interface, a layer of proteins can enclose the surface of the nanoparticle, resulting *protein corona* formation [11] and due to the corona formation, the nanomaterial properties i.e. size, aggregation state etc. can change and the resultant nanoparticle, then acts as biological nanoparticle, rather than pristine nanoparticle [11]. Moreover, the biochemical activity of the protein can vary for the protein corona formation due to some structural change of the protein [12]. It is worthy to note that the consequence of uncontrolled protein-nanoparticle interaction can be a fatal as it directly enters into the blood circulatory system [12]. Therefore, a rigorous study on protein-nanoparticle interaction has enormous importance for understanding the proper function of nanoparticle in biological interface. In this regard, serum albumins are important as these are water soluble and abundant constituents in mammalian plasma cell and have several physiological functions [11]. On the other hand, the ability to a carry drug is one of the major biological issues of albumins [13]. The osmotic pressure and pH of the blood are maintained mainly by the proper functions of albumins [13]. However, the efficacy of an administered drug is strictly depends on the drug-protein interaction. Serum albumins can also help for cellular uptake and internalization of NPs [14]. Thus, any structural change in the protein can directly affect its functions greatly [12]. Hence, it is easy to understand the importance of serum protein-nanoparticle interaction including their behaviour in a particular cellular system.

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Among the serum albumins, bovine serum albumin (BSA) has 76% sequence identity with human serum albumin (HSA) [15]. It is water soluble, highly stable and can easily be isolated in large quantities. BSA is also involved in plenty of physiological functions such as transport and binding of various types of drugs, delivery of fatty acids, etc. [16]. This protein molecule consists of 582 amino acid residues with an average molecular weight of 66,500 in two tryptophan moieties [16]. The structural change for the binding of nanoparticles can be detected by the presence of tryptophan in BSA [2].

Zinc oxide (ZnO) in nano domain is one of such a metal oxide which can be used after adequate biofunctionalization in several biomedical applications including cell viability [17], drug delivery [18], bioimaging [19], biosensor [20], antibacterial activity [21] etc. However, all of these applications mostly depend on ZnO NPs-protein interaction as discussed above. On the other hand, reduced graphene oxide (rGO) has been investigated in biological cell system for numerous purposes such as cell viability [17], protein adsorption [22], drug delivery [23] etc. The reason behind this biocompatibility can reside on the large surface area [17] with unique physiochemical properties [17]. It is expected that if rGO can couple with suitable metal oxide nanoparticles (such as ZnO, Fe_2O_3), then its efficacy can be enhanced. Similar principle can also be adopted for such functional metal oxides [17] for application of the nanocomposite in biomedical applications instead of individual metal oxide or rGO. It is noted that some reports on BSA protein interaction with metal oxides/metal/graphene are available [2,24–25]. In this context, Simonelli and Arancibia [2] had functionalized ZnO nanoparticles with 3-mercaptopropionic acid (3-MPA) for observation of the interaction of BSA with the metal oxide. Also, Karthiga et al. [24] had studied the protein interaction with silver nanorods. It is also noted that Xun et al. investigated the interaction between an organic, varenicline tartrate and the protein [26]. Moreover, the interaction of rGO with the protein [25] that can attribute to the π-π stacking between the aromatic rings of the protein and the graphene sp^2-carbons is also known [25]. Further, a systematic study is greatly needed on the interaction of BSA protein with metal oxide-rGO nanocomposite before considering the nanomaterial for a specific biomedical application. To the best of our knowledge, there is no such report is available on the BSA protein interaction with ZnO-rGO nanocomposite.

In the present work, we report the synthesis of polyvinyl alcohol (PVA) foam based ZnO nanoparticles coupled with rGO (ZG) nanocomposite and the study of interaction between ZG and BSA protein. The BSA has been used as a model protein to study the nanoparticles-protein interaction with the help of UV–visible absorption and photoluminescence spectral analyses.

2. Experimentals

2.1. Synthesis of ZnO (ZO) nanoparticles

ZnO nanoparticles (NPs) were synthesized from polyvinyl alcohol (PVA) based foam precursor. This was the first time report. In this synthesis, 50 ml solution of 0.1 M zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$, E. Merck; purity 98%) was prepared in deionized water. Then, the zinc precursor solution was warmed at 70 °C with continuous stirring for 2 h to obtain a gel-like material. Simultaneously, another aqueous solution of 2 wt% PVA (BDH, average molecular weight ~ 22,000; purity, 98%) was also made in deionized water. In the next step, a fixed amount (7 ml) of PVA solution was added to the precursor gel during vigorous stirring with the help of a magnetic stirrer until a clear liquid is formed. The liquid was again kept for warming at 70 °C with continuous stirring. After nearly 1 h of warming, the liquid was found to form a gel and the gel was noticed to transform into a foam-like material when it was kept at 100 °C in an air oven for 1–2 h duration. The formation of gel-like material would be formed due to the presence of coordinated water molecules in the material. In this respect, we have systematically studied FTIR spectra (Fig. S1, Table S1) to understand the fact. The details are given in the Supplementary materials. To obtain pristine ZnO NPs, the foam was heat-treated at 500 °C in air atmosphere for 60 min to burn away the organics present in the gel. The overall synthesis process of ZnO NPs is illustrated in Scheme 1.

2.2. Synthesis of graphene capped ZnO nanocomposite (ZG)

Modified Hummer's method was adopted to prepare graphene oxide (GO) [27]. In the later, 50 mg of as-prepared GO was initially dispersed in 50 ml of deionized water. Then, the dispersed material was ultrasonically agitated for 2 h to obtain *dispersion A*. The *dispersion B* was prepared by mixing 320 mg of as-synthesized ZnO nanoparticles (ZO) in 100 ml of deionized water through ultrasonication for 2 h duration. Thereafter, *A* and *B* were mixed together and ultrasonicated again for 2 h. After that, the mixed dispersion was kept under continuous stirring for a long time (about 24 h). In the later stage, 500 μl of ammonia solution (28%, v/v) and 100 μl of hydrazine hydrate (H_6N_2O, Merck; purity 99–100%) were added into the mixed dispersion simultaneously and kept it at 90 °C for 1 h under vigorous stirring condition. Finally, the solid particles were separated out by centrifugation and washed the solid material, initially by de-ionized water to remove the water soluble precursors and finally, washed with ethanol for 3 to 4 times. The washed composite material was kept for about 3 h in an air oven at 60 ± 2 °C. The overall synthesis process is described in Scheme 1.

2.3. Characterizations

2.3.1. Materials properties

X-ray diffractometer (Bruker D8 Advance with DAVINCI design X-ray diffraction unit) with nickel filtered CuK_α radiation source ($\lambda = 1.5418$ Å) was used to obtain the X-ray diffraction (XRD) patterns of the samples (2θ range, 5°–80°). Transmission electron/high resolution transmission electron microscopy (TEM/HRTEM) along with TEM-EDS measurements were performed by FEI company make (Tecnai G2 30.S-Twin, Netherlands) instrument at an accelerating voltage of 300 kV. In this measurement, carbon coated 300 mesh Cu grids were used for sample placement. The samples were dispersed in methanol by ultrasonication and the dispersed samples were placed onto separate Cu-grids very carefully. A field emission scanning electron microscope study (FESEM and FESEM-EDS, ZEISS, SUPRA™ 35VP) was performed to analyze the morphology and microstructure of the samples. On the other hand, a Netzsch STA 409 C/CD thermoanalyzer was used for thermogravimetric analysis (TGA) of a representative nanocomposite (ZG) using Al_2O_3 as a reference material maintaining the heating rate of 10 K/min in air atmosphere. For the TG run, a maximum temperature of 800 °C was chosen. Fourier Transform Infrared (FTIR) spectral study was carried out by Thermo Electron Corporation, USA make FTIR spectrometer (Nicolet 5700). For each FTIR experiment, the number of scans was fixed at 100 (wavenumber resolution, 4 cm^{-1}). Moreover, Raman spectra were recorded using micro-Raman (Renishaw inVia Raman microscope) to reveal the presence of rGO as well as its structural change in the nanocomposite. An argon ion laser with 514 nm incident wavelength was used as the excitation source.

2.3.2. UV–visible (UV–vis) and photoluminescence (PL) spectral studies

To understand the interaction that happened between the BSA protein with ZG nanocomposite, the UV–vis spectral study was performed in a systematic way. In this measurement, 100 ml 10^{-6} M bovine serum albumin (BSA) protein stock aqueous solution was prepared using deionized water and kept the solution in stirring condition for an overnight. Afterwards, three different amount such as 1 mg, 2 mg and 5 mg ZG nanocomposite was added with 10 ml of 10^{-6} M BSA protein solution separately and the solutions were termed as *A*, *B* and *C*, respectively. In addition, without BSA, three different solutions (10 ml each) with 1 mg, 2 mg and 5 mg ZG sample in deionized water were also prepared and these were designated as *A1*, *B1* and *C1*, respectively.