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# Adsorption and interactions of the bovine serum albumin-double walled carbon nanotube system

Turkan Kopac [a,*], Kadriye Bozgeyik [a], Emmanuel Flahaut [b]

[a] Department of Chemistry, Bülent Ecevit University, 67100 Zonguldak, Turkey
[b] CIRIMAT, Université de Toulouse, CNRS, INPT, UPS, UMR CNRS-UPS-INP N°5085, Université Toulouse 3 Paul Sabatier, Bât. CIRIMAT, 118, route de Narbonne, 31062 Toulouse cedex 9, France

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ABSTRACT

Adsorption and interactions of Bovine Serum Albumin (BSA) with Double Walled Carbon Nanotubes (DWNT) prepared by catalytic chemical vapor deposition (CCVD) synthesis were studied. Adsorption kinetics and equilibrium were investigated by means of in situ UV-spectroscopy. The extent of adsorption at different temperatures was determined at the end of a 420-min adsorption period. The adsorption equilibrium experiments were performed using various amounts of nanotubes at pH 4 and 40 °C, and the adsorption parameters were evaluated comparing the experimental data with models such as the Freundlich and Langmuir isotherms. The maximum protein adsorption capacity ($Q_0$) of DWNT was determined as 1221 $mg \cdot g^{-1}$. The effect of temperature on the adsorption rate experiments was investigated for constant amount of adsorbent at pH 4. Adsorption kinetics followed the pseudo-first-order rate. Zeta potential measurements were performed with respect to solution pH for understanding the protein-surface interactions. The interactions between positively charged BSA molecules with negatively charged DWNT at pH 4 were found to be electrostatic attractions. Thermodynamic parameters, $\Delta H^0$ and $\Delta S^0$ were found as 9.40 $kJ \cdot mol^{-1}$ and 321.5 $J \cdot mol^{-1} K^{-1}$, respectively. $\Delta H^0$ value indicated that BSA adsorption on DWNT was a physisorption process.



## 1. Introduction

Adsorption of proteins on solid surfaces is important in natural sciences, medicine and industry, and has an important role in numerous applications such as protein chips, biosensors, food industry and medical implants [1,2]. In many applications related with biomedicine, as the interactions between proteins, implants and cells might have undesirable effects, inert surfaces should be used. For this reason, such kind of surfaces have been synthesized with various forms of surface modifications to inhibit the adsorption on the surfaces [3]. Also, there are applications where the surface adsorption and interactions are desired, in that case surface modifications are made in order to enhance the adsorption. In terms of potential toxicity and biodistribution in the body, it is well known that the corona of adsorbed proteins plays a determining role in the fate of nanoparticles [4], and particles in general. Following the discovery of carbon nanotubes (CNT) and because of their many potential uses including different biomedical applications, the interaction of biomolecules with CNT has been investigated in terms of biocompatibility, usefulness as platforms to support growth of nerve cells [5] or bioelectronic sensors [6]. CNTs having unique electronic, mechanical and structural properties are also exploited in the field of materials science [7]. CNTs are in the form of tubular hexagonal carbon structures which are capped by half fullerene molecules. The main types of CNTs are single-walled (SWNT), double-walled (DWNT) and multi-walled (MWNT) carbon nanotubes [8]. DWNT are at the interface between SWNT and MWNT with >2 walls, they combine the morphology and flexibility of the former with the physico-chemical resistance of the latter and can thus be considered as a good mode of "carbon nanotubes" in general. The dimensions of their structures range in between 0.4–2 nm in diameter for SWNT and 2–100 nm for MWNT. Their length typically ranges from 1 to 50 μm. DWNTs have diameters typically ranging in between 1 and 3 nm and lengths from several to tens of micrometers [9]. DWNTs have a strong tendency to agglomerate and are practically not soluble in solvents because of their strong hydrophobicity and van der Waals interactions within bundles. However, for potential applications in the fields of biology and biomedicine, solubility in aqueous solution is important [10]. Chen et al. [11] noted that protein adsorption on SWNT field effect transistors led to appreciable changes in the electrical conductance of the devices that could be utilized for label-free detection of biomolecules. It was also reported that electronic effects occurring at the nanotube-metal interfaces due to protein adsorption constituted a more significant contribution to the electronic biosensing signal than adsorption solely along the lengths of CNTs. Salvador-Morales et al. [12] investigated the protein binding to purified CNTs in human plasma and serum. They reported that when MWNTs were exposed to the

* Corresponding author.
*E-mail address:* turkan.kopac@beun.edu.tr (T. Kopac).

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human plasma and serum, only a few kinds of proteins were able to bind MWNTs. Roman et al. [13] investigated the amino acids adsorption on graphite sheets and CNTs through density functional theory. They reported weak binding of the biomolecules on both CNTs and graphite sheets, but generally favorable adsorption pathways were observed. Zwitterion adsorption through the charged carboxylate and amine groups were bound stronger to the CNT surface as compared to the non-ionic counterparts, phenylalanine, histidine, and cysteine side chain groups. Binding strengths on graphite did not show similar trends for amino acid interactions with the increase in CNT diameter. In order for CNTs to be used as biosensors or drug delivery carriers, information regarding whether protein or enzyme biomolecules could bind with CNT is rather important.

Adsorption of BSA to various surfaces such as gold nanoparticles, gold electrodes, polypyrrole-based adsorbents, neutral and charged hydrophilic and hydrophobic surfaces, silica, poly(hydroxyethylmethacrylate)-Reactive Green 19 cryogel disks, mica, polymeric nanoparticles, chromium(III) oxide suspension, zirconia nanoparticles were investigated in some of the reported literature studies [14–23]. In recent years, there are only a few studies related with the investigation of the adsorption and the attractions between the proteins and carbon based nanomaterials [5,21–29].

In our previous works, the kinetics and equilibrium of BSA adsorption onto several metal oxides ($TiO_2$, $Al_2O_3$, $ZnO_2$) and attraction interactions of surface-protein by zeta potential measurements were investigated [30–32]. The adsorption of BSA on MWNTs and SWNTs were also carried out and compared with the results of metal oxide adsorption studies [33,34]. The optimum conditions for protein adsorption on metal oxides and CNTs were found as pH 4 and 40 °C. In the present study, we focused on the equilibrium and kinetics of adsorption behavior of BSA on clean DWNTs prepared by a catalytic chemical vapor deposition (CCVD) synthesis that involved the use of a MgO-based catalyst [9]. The equilibrium and kinetics of BSA on DWNTs were examined at optimum solution conditions (pH: 4, T: 40 °C) determined in the previous studies [30–34]. The electrokinetic properties of protein adsorption were also examined to determine the interactions between BSA with DWNTs. The protein capacity of DWNTs, at same experimental conditions, was compared with protein capacities of metal oxides and other carbon nanotubes.

## 2. Experimental

DWNTs used in this work were prepared by CCVD synthesis using a MgO-based catalyst containing Co and Mo as the active elements. The details of the synthesis and sample processing were described earlier [9]. Clean CNTs, obtained in gram-scale amounts, contained ca. 80% DWNTs, the rest of the CNTs corresponding to ca. 5% of SWNT and 15% of triple-walled CNTs [9]. Pore characterization and specific surface area of DWNT sample were done using a Quantachrome Automated Gas Sorption Analyzer. Brunauer-Emmett-Teller (BET), Barrett-Joyner-Halenda (BJH) and non-local density functional theory (NLDFT) techniques were used for the surface area, pore size and pore volume analysis of the sample. TGA, operated in air atmosphere at 1°/min, was performed using a SETARAM TAG24 thermobalance. The morphology was observed by transmission electron microscopy (JOEL 2100F) with a maximum acceleration of 100 kV. Raman spectroscopy was performed using a Jobin Yvon LABRAM spectrometer (green laser at 532 nm) with a maximum power of 2 mW.

The effects of working parameters such as adsorbent dosage (0.2–1.0 $g \cdot L^{-1}$) and temperature (25, 30, 37, 40 °C) on BSA adsorption were studied in a batch adsorber for a specific period of contact time. Merck grade BSA (12,657, fraction V) was used as received. The BSA solutions were prepared in 0.15 M NaCl solution at 0.60 $g \cdot L^{-1}$. Adjustment of solution pH was done using a $NaH_2PO_4 \cdot 2H_2O/H_3PO_4$ buffer solution (pH 2.8). For equilibrium and kinetic studies, 100 mL of protein solution at constant initial concentration and at constant pH 4 was taken in a 250 mL flask and agitated in a shaking water bath for 420 min contact time at 100 rpm speed. Equilibrium experiments were conducted with different concentrations of DWNT as adsorbent (0.2–1.0 $g \cdot L^{-1}$) at constant temperature (40 °C). Kinetic experiments were conducted at 25, 30, 37, 40 °C with a constant dose of adsorbent (0.60 $g \cdot L^{-1}$). At various time intervals, the solution sample was filtered and then analyzed using the method of Lowry [35] by a Shimadzu 1700-E spectrometer at a wavelength of 720 nm. The BSA concentration of the adsorbent phase ($q_e$, $mg \cdot g^{-1}$) was determined using,

$$q_e = \frac{(C_o - C)V}{w} \tag{1}$$

where $C_o$ is the initial value of BSA concentrations ($mg \cdot L^{-1}$), $C$ is the BSA concentration ($mg \cdot L^{-1}$) at any time, $V$ denotes the volume (L) and $w$ is the amount of DWNT (g). Some of the experiments were repeated in order to check the reproducibility of the measurements, and the relative errors obtained for the selected runs were found not to be higher than 3%.

Zeta potentials for DWNT were measured at a concentration of 0.02 wt% in 0.6 $mg \cdot mL^{-1}$ BSA solution using a ZetaPlus zeta potential analyzer (Brookhaven Instruments Corporation) by electrophoresis light scattering method. All BSA solutions were prepared using 0.15 $mol \cdot L^{-1}$ NaCl solution in order to keep the ionic strength constant, and the adjustment of the pH of the suspension was done using $NH_4Cl/NH_3$ and $NaH_2PO_4 \cdot 2H_2O/H_3PO_4$ buffers, having pH 10 and 2.8, respectively. The electrophoretic mobility ($\mu_e$) is related to the zeta potential ($\zeta$) as shown by the following Hückel equation:

$$\mu_e = \frac{2e\zeta}{3\eta} \tag{2}$$

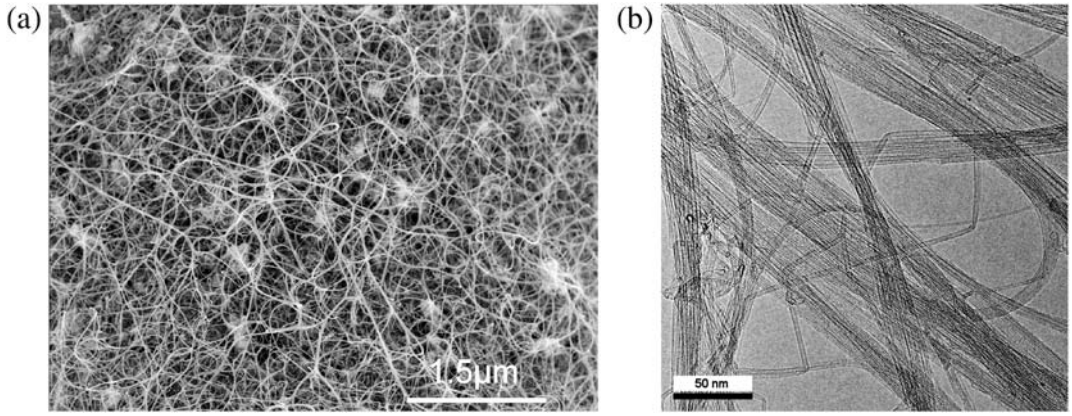


**Fig. 1.** Representative (a). SEM image (b). TEM image of DWNTs.