

Study of electrochemical interaction between bovine serum albumin and Ti-O based nanotubes

Abstract

Hydrogen Titanate Nanotubes (HTO) were synthesized by hydrothermal process using 10M NaOH and TiO₂ anatase powder. The material was characterized by powder X-ray diffraction (XRD) and infra-red spectroscopy (IR). Structural and morphological details were obtained scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Aqueous solution of bovine serum albumin (BSA) was prepared with deionized water having an arbitrary concentration of the order 10⁻³M. The interaction of (BSA) protein with suspended aqueous solution of titanate nanotubes was investigated using cyclic voltametry. $K_{T(II)}/K_{T(IV)}$ value was calculated to be 1.00.

Keywords: nanotubes, hydrothermal process, bovine serum, albumin, titanate, electrochemical, hydrogen, voltamtrics, enzymes, nanotechnology

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Abbreviations: HTO, hydrogen titanate nanotubes; XRD, X-ray diffraction; IR, infra-red spectroscopy; SEM, scanning electron microscopy; TEM, transmission electron microscopy; BSA, bovine serum albumin

Introduction

Application of nanotechnology in biological field has shown some great perspective in future. This is simply because nano-biology has high potential for catering societal needs compared as to other branches of nano science and technology including healthcare, environmental and industrial applications. The future directions of nanobiology will be seen in the areas of proteomics, medical sciences, immunology, targeted drug delivery and microbiology. All these are the emerging branches of ultra-modern science and can provide better methodologies for medical diagnosis, therapy and biochemical analysis.¹ Therefore accurate and sensitive detection of water-soluble various biomolecules including DNA, proteins and peptides is a highly sought after scientific goal with implications in biology. In this connection the study of interaction between proteins and nanomaterials bears some great importance. The present work deals with the electrochemical interaction between bovine serum albumin and hydrogen titanate nanotubes. Among the one-dimensional oxidic nanomaterials reported Ti-O based nanostructures represent a progressive area of materials chemistry for their wide applications as catalyst supports, semiconductor photocatalysts and sensors in chemical and biochemical fields, DNA sequencing, clinical diagnostics²⁻⁴ etc.

BSA is the most plentiful protein in blood plasma. It is a large globular protein containing 582 amino-acid residues with a molecular weight of 69000 Dalton and two tryptophan moieties at positions 134 and 212 as well as tyrosine and phenylalanine.⁵ Each protein molecule can carry seven fatty acid molecules. They bind in deep crevices in the protein, burying their carbon-rich tails safely away from the surrounding water. Serum albumin also binds to many other water-insoluble molecules. The strong affinity of BSA to the water molecules has made the protein an automatic choice for the interaction studies in the present work. Moreover BSA is used as protein model because of its stability, its lack of effect in many biochemical reactions, and its low cost. BSA has numerous biochemical applications

including enzyme-Linked immunosorbent assay immunoblots, and immunohistochemistry.⁶ It is also used as a nutrient in cell and microbial culture. BSA is used to stabilize some enzymes during digestion of DNA and to prevent adhesion of the enzyme to reaction tubes. This protein does not affect other enzymes that do not need it for stabilization. The binding properties of BSA with various drugs have been fully investigated by many researchers.⁷⁻¹¹

In the present investigation the BSA-HTO interaction was monitored by cyclic voltametric method. The application of electrochemical methods to the study of such interaction between different biomolecules and coordination complexes of metal ions, chelates or metal nanostructures provides a useful complement to the previously used methods of investigation, such as UV-visible spectroscopy.^{12,13} This is because small molecules which are not amenable to such methods, either because of weak absorption bands or because of overlap of electronic transitions with those of the biomolecules, can potentially be studied via voltammetric techniques. Multiple oxidation states of the same species as well as mixtures of several interacting species can be observed simultaneously.

Materials and method

Material

BSA and TiO₂ (AR grade) was purchased from Merck (Mumbai, India). The powder X-ray diffraction (XRD) data were recorded from a PANalytical X'pert Pro diffractometer with Cu K α radiation. The morphology of the nanostructured samples was studied with use of a Jeol JSM-840 scanning electron microscope (SEM) operating at 120keV. TEM studies of nanotubes were carried out with a FEI Tecnai TEM equipped with a LaB₆ filament and operated at 200kV. TEM samples were prepared by placing a drop of the ultrasonically dispersed powder (in ethanol) on a carbon-coated copper grid and drying in air. The reagents for the synthesis of the nanomaterial were obtained from commercial sources and used without further purification.

Hydrothermal synthesis of HTO

Synthesis of HTO was performed hydrothermally following the earlier reports^{14,15} 1.0g of anatase TiO₂ powder was dissolved in 10M

NaOH solution and the resulting suspension was then transferred in an autoclavable reaction chamber. The reaction chamber was autoclaved in an oven at a temperature of 130°C for 22h. The product was filtered after cooling down to room temperature and thoroughly washed with 1M HCl and water to remove any trace of NaCl produced during washing. Subsequently the material was dried at ambient temperature.

Result and discussion

Characterization of HTO by IR spectroscopy and powder XRD

The FTIR spectrum of the pure $H_2Ti_3O_7$ nanotubes (Figure 1) shows a strong and broad O-H stretching mode at 3410cm^{-1} . The IR modes in the $450\text{-}1000\text{cm}^{-1}$ range can be attributed to Ti-O-Ti bending vibrations, while the line at 920cm^{-1} is attributed to a four-fold coordinated Ti-O stretching vibration involving non-bridging oxygen atoms coordinated to H^+ . X-ray diffraction pattern of $H_2Ti_3O_7$ is shown in Figure 2. This diffraction pattern mostly matches with the monoclinic crystal structure of $H_2Ti_3O_7$ (space group $C2/m(12)$) (JCPDS Card no. 41-192).

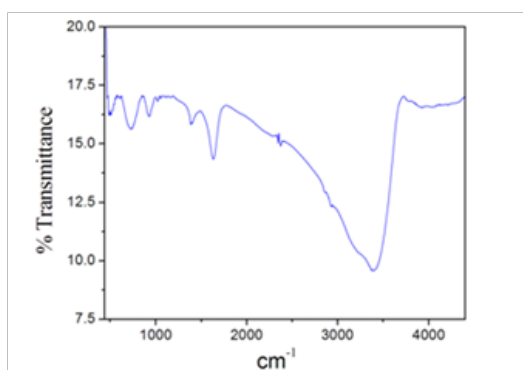


Figure 1 IR spectra of HTO.

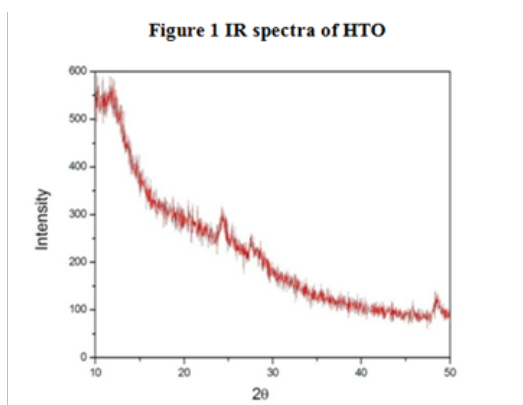


Figure 2 Powder XRD spectra of HTO.

Structural and morphological studies of HTO

Figure 3 shows the SEM images of the nanostructured samples hydrothermally synthesized from anatase powder. The scanning electron micrograph shows the formation of one dimensional (1-D) nanostructures. Further TEM study (Figure 4) clearly shows that all the 1-D units are actually hollow, multiwalled nanotubes and the information is in agreement with the earlier report.¹⁵ The inner diameter of the nanotubes is around 5 nm and the outer diameter is 10 nm.

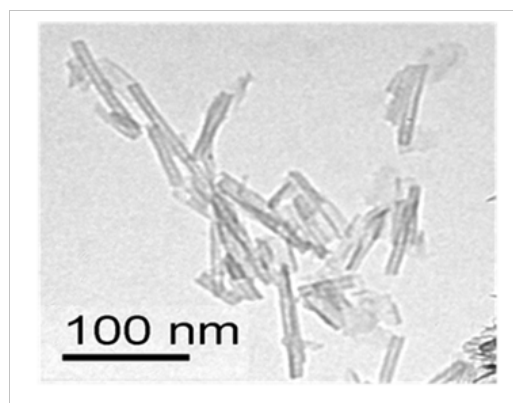


Figure 3 TEM image of HTO.

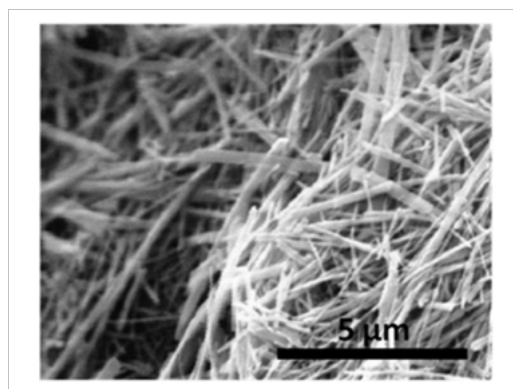


Figure 4 SEM image of HTO.

Study of electrochemical interaction

The study of interaction between the HTO and BSA using cyclic voltammetric technique provides a useful complement to the previously used spectral studies.¹⁶ The cyclic voltammograms of the nano material (Figure 5) in the absence (blue line) and presence (red line) of BSA showed significant shifts in the anodic and cathodic peak potentials followed by decrease in both peak currents, which indicates the interaction existing between the nano material and BSA. The shift in the value of the formal potential (ΔE^0) can be used to estimate the ratio of equilibrium binding constants $K_{Ti(II)}/K_{Ti(IV)}$ according to the model of interaction described by Bard and Carter.¹⁷ From this model one can obtain that:

$$\Delta E^0 = E_b^0 - E_f^0 = 59.15 \log (K_{Ti(II)}/K_{Ti(IV)})$$

Where E_b^0 , E_f^0 are the formal potentials of the bound and free complex forms, respectively, and $K_{Ti(II)}$ and $K_{Ti(IV)}$ are the corresponding binding constants for the binding of reduction and oxidation species to BSA, respectively. Ti(IV) form in HTO interacts with BSA. The $K_{Ti(II)}/K_{Ti(IV)}$ value was calculated to be 1.00. The present study suggested that the Ti(IV) may interact with BSA in the mode of groove binding which is in consistent with the above spectral results. Additionally, upon the addition of BSA, the voltammetric currents of the Ti(IV) decreased. This decrease can be explained in terms of the slow diffusion of the Ti(IV) bound to the large BSA molecule. It was noted from the voltammograms collected at the different scan rates in the range from 0.1 to 1V/s in the presence of BSA, that the peak currents were also directly proportional to the square root of the scan rates, indicating that the electrochemical process of the Ti(IV) bound to BSA is also diffusion controlled.

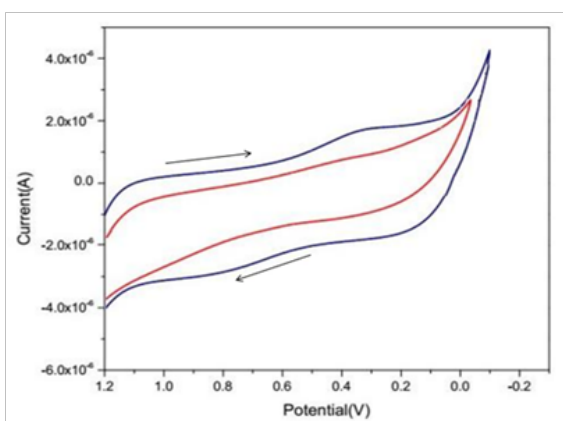


Figure 5 Cyclic voltammograms for the interaction of HTO-BSA.

Conclusion

The present experiment of electrochemical interaction between HTO and BSA using cyclic voltammetry established that the interaction is quite strong which is also revealed by the limiting ratio as mentioned above. This observation can also be considered with importance to investigate such interaction for future application in bio-electrochemistry. At the same time it provides some necessary information for the designing and application of new drugs, ultrasensitive detection of proteins, clinical diagnostics etc.

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Conflict of interest

The author declares no conflict of interest.

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