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# Surface functionalization of zirconium dioxide nano-adsorbents with 3-aminopropyl triethoxysilane and promoted adsorption activity for bovine serum albumin

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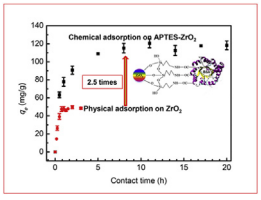
## HIGHLIGHTS

- APTES chemically immobilized on $ZrO_2$ nanoparticles via Zr—O—Si bond.
- Enhanced adsorption capacity of BSA was observed on APTES-$ZrO_2$.
- Chemical adsorption character of BSA on APTES-$ZrO_2$.
- Adsorption/release of BSA on APTES-$ZrO_2$ accomplished by adjusting pH value.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Surface functionalization of zirconium dioxide ($ZrO_2$) nano-adsorbents was carried out by using 3-aminopropyl triethoxysilane (APTES) as the modifier. The addition amount of APTES was varied to determine the optimum modification extent, and the bulk $ZrO_2$ microparticles were also modified by APTES for comparison. Some means, such as TEM, XRD, FT-IR, XPS and TG-DSC were used to character these $ZrO_2$ particles. The results showed that the APTES molecules were chemically immobilized on the surface of $ZrO_2$ nanoparticles via Zr—O—Si bonds, and the nano-$ZrO_2$ samples showed larger special surface area. In the adsorption of bovine serum albumin (BSA), nano-$ZrO_2$ samples exhibited enhanced adsorption activity, and APTES modified nano-$ZrO_2$ with proper APTES content presented the best adsorption property. Under the same adsorption conditions, the equilibrium adsorption capacity of BSA on APTES-$ZrO_2$-2 was almost 2.3 times as that on pristine nano-$ZrO_2$ and 3.0 times as on bulk $ZrO_2$ microparticles. The increased adsorption capacity of APTES-$ZrO_2$ nano-adsorbents can be attributed to the chemical interaction between amino and carboxyl groups at APTES-$ZrO_2$/BSA interface. The pH-dependent experiments showed that the optimum pH value for the adsorption and desorption was 5.0 and 9.0, respectively, which suggested that the adsorption and release of BSA could be controlled simply by adjusting the solution pH condition.



## 1. Introduction

Protein adsorption at solid/liquid interfaces is a widespread phenomenon in both natural and man-made systems, which is becoming the subject of intense studies due to its important role

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and applications in the design of biocompatible material [1–3], biosensors [4] and drug delivery systems [5]. The driven forces in protein adsorption may arise from electrostatic, hydrophobic or chemical forces among the side chains of proteins and the reactive groups at the solid/liquid interfaces. Because of the complexity of protein adsorption, in-depth understanding of the protein adsorption mechanism is important to well control the interaction between proteins and adsorbents and obtain the best adsorption performances [6–8].

Serum proteins have important physiological functions, which can maintain the osmotic pressure and the pH value of blood, and transport a wide variety of endogenous and exogenous compounds [9]. Bovine serum albumin (BSA) has been widely used as a model protein in various experiments for its structural homology with human serum albumin, easy preparation, high purity and good dissolubility in water [9–11]. BSA is a globular protein with dimensions of 4 nm × 4 nm × 14 nm, and its isoelectric point ranges of pH 4.5–5.0, which means that BSA molecules have the ability to be bound on the charged substances reversibly by changing the pH value [2]. There are extensive studies focused on the serum protein adsorptions, and it was reported that many factors, including electrostatic interaction, surface hydrophilicity or hydrophobicity may affect the adsorption performance [12]. However, some opposite trends were reported. For example, some studies showed that albumin was adsorbed much more on the hydrophobic surfaces, such as polystyrene and polyurethane, than on hydrophilic surfaces; while others reported that hydrophobic surfaces, such as polyethylene oxide or polyethylene glycol presented an opposite adsorption activity [8]. These results indicated that the different adsorption mechanism may play a role at the different solid/aqueous interfaces. Therefore, the fundamental studies about protein adsorption process are still necessary and important in protein engineering.

Nano-adsorbents show excellent surface adsorption activity because of its large specific surface area and high interfacial activity. Zirconium dioxide ($ZrO_2$) is a significant material due to its good chemical and thermal stability, high mechanical strength and less toxicity. Moreover, $ZrO_2$ is a biological inert material, which shows good cytocompatible and hemocompatible [13] properties and has been used as implant [14] and dentistry materials [15]. Furthermore, $ZrO_2$ nanoparticles have also been applied in the immobilization of enzyme and proteins [16–18].

The surface modification is important for nano-adsorbents not only in controlling interfacial properties, such as wetting, adhesion and friction, but also in providing active surfaces to attach designed molecules and improve its adsorption performances [19]. Anchoring organic small molecules, such as amino, thiol, carboxyl, phosphate or cyanide groups on the surface of adsorbents is proven to be a powerful way to control protein-material interaction [20,21]. Among various surface modification methods, surface silylation is undoubtedly a most widely used technique to introduce surface functional groups on the surface of some inorganic solids [21]. For example, silanes were introduced into zirconia materials to improve its chemical interaction with dental substrates [22], and silane-modified $ZrO_2$ can readily adsorb cytochrome C [23]. 3-aminopropyl triethoxysilane (APTES) has also been used in various interfacial process for its functional $-NH_2$ group [20,24]. Based on above literature works, it is prospective that APTES-modified $ZrO_2$ nanoparticles may have good interfacial adsorption activity toward some proteins. However, related research has not been reported to the best of our knowledge.

In this paper, functionalized $ZrO_2$ nano-adsorbents were fabricated by the surface modification with APTES. BSA was chosen as the target molecule to explore the interaction between protein and modified surface. The adsorption and desorption behaviors of BSA on the functionalized $ZrO_2$ were explored, and the effects of different conditions, such as solution pH value, initial concentration of BSA and contact time were examined to optimize the adsorption conditions. Furthermore, the adsorption mechanism was also discussed.

## 2. Materials and methods

### 2.1. Materials

Bulk $ZrO_2$ microparticles, $ZrOCl_2 \cdot 8H_2O$ and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Sinopharm Chemical Reagent Co., Ltd. BSA (fraction V) and the protein marker was obtained from Aladdin Corporation. 3-aminopropyl triethoxysilane (APTES, 99%) was purchased from Shanghai Yuanye Biotechnology Corporation. The other chemicals and organic solvents were all of analytic grade and used as received. Distilled water was used throughout the experiments.

### 2.2. Synthesis and characterization of $ZrO_2$ samples

$ZrO_2$ nanoparticles were synthesized through a hydrothermal process [25]. Typically, 1.5 M $NH_3 \cdot H_2O$ was added into 50 mL of 0.2 M $ZrOCl_2$ aqueous solution till the pH value reached 8.0, then the solution was transferred into a Teflon reactor sealed in an autoclave and maintained at 180 °C for 2 h. The solid was centrifuged and washed with deionized water to remove residual $Cl^-$.

APTES functionalized $ZrO_2$ nanoparticles were prepared by redispersing the $ZrO_2$ nanoparticles (2 g) in 100 mL of ethanol containing certain volume of APTES. The solution pH was adjusted to 5.0 using acetic acid. A sonication treatment was followed at 40 °C for 2 h. After that, the solid was centrifuged, washed with ethanol and dried under vacuum at 60 °C for 24 h. The obtained samples were labeled as APTES-$ZrO_2$-*x*, and the *x* value indicated the addition volume (mL) of APTES.

For comparison, the bulk $ZrO_2$ was also functionalized by APTES using above method, and the addition volume of APTES was 2 mL. The sample was signed as APTES-(B)$ZrO_2$.

X-ray diffraction pattern was taken with a Rigaku XRD D/max-2500PC instrument (CuKα, tube voltage of 50 KV and tube current of 100 mA). The morphology investigation was performed by using a Transmission electron microscopy (TEM, FEI TECNAI20); suspension of samples was casted on a copper grid with a carbon support membrane. The specific areas of $ZrO_2$ particles were determined by nitrogen adsorption-desorption isotherm measurement using a Micromimeritics ASAP 2020M system. FT-IR analyses were performed on a Perkin Elmer FT-IR (Spectrum One) spectrometer, and the samples were dispersed in anhydrous KBr. Thermo gravimetric curves were taken on a TA Instrument (TGA/DSC/1600LF, Mettler Toledo, Switzerland), the sample was heated to above 800 °C under nitrogen with heating rates of 10℃/min. Zeta potential measurement upon different pH values was conducted on a Nano-ZS laser particle size analyzer (Malvern, U.K.) using 2.5 mL aqueous suspension of $ZrO_2$ samples with 0.15 M NaCl, while solution pH was adjusted by 0.1 M HCl or 0.1 M NaOH respectively. X-ray photoelectron spectra (XPS) was conducted on an ESCALAB 250Xi (Thermo Fisher Co., USA) using an Al-Kα monochromatic x-ray (1486.6 eV) as source.

### 2.3. Adsorption and desorption experiments of BSA

Batch experiments were carried out by mixing a certain amount of $ZrO_2$ samples, 8 mL BSA aqueous solution in desired initial concentrations, and right amount of EDC (mass radio of EDC:BSA was 1.0). The soution pH value was adjusted by 0.1 M HCl and 0.1 M