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# Functional short-chain zwitterion coated silica nanoparticles with antifouling property in protein solutions

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ABSTRACT

A new functional nanoparticle, consisting of a silica core onto which short-chain zwitterions are chemically connected, was successfully prepared and showed excellent antifouling performance to protein solutions. These nanoparticles (NPs) own excellent stability even in 1 M NaCl solutions for at least 48 h. The interaction between these "zwitterated" NPs and proteins were investigated by dynamic light scattering (DLS), turbidimetric titration, and isothermal titration calorimetry (ITC). The results demonstrated that the zwitterated NPs had antifouling property both in single protein solutions and serum (fetal bovine serum, FBS). The zwitterated NPs also own abundant functional groups which could conjugate with biomolecules for future applications in therapeutic and diagnostic field.



## 1. Introduction

Functional nanoparticles (NPs) have many potential applications in biomedical fields such as drug delivery, imaging, bioanalysis and so on [1–6]. Surface modification is generally required for all these applications since it essentially affects the bio-distribution, toxicity, and clearance of NPs [7,8]. When NPs are exposed to complex media like serum and plasma, protein coronas [9–11] caused by protein adsorption to NPs are always formed and mask the functional surface of NPs, leading to adverse effect like NPs aggregation and deactivation of active sites. Protein corona attributes mostly to nonspecific adsorption and should be minimized. Thus, NPs with antifouling property, which means low adsorption to untargeted proteins and other biomolecules, attract great interest of researchers [12–17].

Up to now, grafting antifouling polymers to the surface of NPs by physical adsorption or chemical conjugation is the most commonly used approach to reach antifouling NPs. Polyethylene glycol (PEG) [18] and zwitterionic polymers, such as poly(carboxybetaine methacrylates) (PCBMA), poly(sulfobetaine methacrylates) (PSBMA), and poly (carboxybetaine acrylamide) (PCBAA) [19,20] are two kinds of most widely used antifouling polymers [21]. In previous studies [13,22–24], NPs coated with antifouling polymers were confirmed having excellent antifouling property and possessed long-term stability in both negative and positive protein solutions. However, polymer grafting remarkably increased the hydrodynamic size of NPs and would affect its use in size sensitive applications such as drug delivery [8,25].

Oligomeric ethylene glycol (OEG) self-assembled monolayers (SAMs) have been demonstrated owning a rather strong interaction with water around OEG, yielding large repulsive forces to repel proteins [26,27]. OEG SAMs on nanoparticles increased the hydrophilicity, colloid stability, and decreased the non-specific protein absorption [18,28]. However, short OEG can stabilize only small nanoparticles (<10 nm) due to the van der Waals interaction increase with the size and the OEG SAM on the colloid surface can be destroyed by long-term aging or dialysis [29,30]. Therefore, short-chain zwitterion coating approach was developed to overcome above shortcoming. Schlenoff et al. reported a novel approach to modify silica nanoparticles (SiNPs) with a small sulfobetaine siloxane. The modified NPs were proved to have long-term stability under challenging conditions, such as 3 M NaCl and 50% fetal bovine serum (FBS) [31]. It was a very simple and effective strategy which did not significantly change the NPs size, but sulfonic acid group is difficult for further functionalization in real applications. Subsequently, Frank et al. achieved a low-fouling zwitterionic surface by using a small amino acid cysteine, and the modified SiNPs possessed excess amine and carboxyl functional groups to conjugate biomolecules for nanomedicine applications [32].

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Nevertheless, this two-step process has to control the reaction conditions carefully to achieve selective attack by the sulfhydryl group on the epoxide ring. Thus, current approaches are either hard for further functionalization or with complicated preparation process.

In this work, a novel functional zwitterion organosiloxane (ZWS) owning carboxyl group was synthesized by ring-opening addition reaction, and then ZWS functionalized silica nanoparticles ($SiO_2$-ZWS) were synthesized via a simple silanization process. The novel zwitterion structure with carboxyl end group endows antifouling property to SiNPs and also could be helpful for further functionalization like bio-probe conjugation. The properties of NPs in salt and protein solutions were investigated by DLS, turbidimetric titration, and ITC analysis.

## 2. Experimental

### 2.1. Materials

(N,N-dimethyl-3-aminopropyl)trimethoxysilane was purchased from Gelest Inc., β-propiolactone were obtained from J&K Chemical. Albumin bovine V from bovine serum (BSA, MW = 66.2 kDa, p*I* = 4.6) was from Roche. Lysozyme (LYZ, MW = 14.0 kDa, p*I* = 11.2) was from Amresco. Fetal bovine serum was from Gibco and stored at −20 °C. Both were used as received. The ingredients of phosphate buffer saline (PBS) were 137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, and 2 mM $KH_2PO_4$ with pH 7.40. Acetone was dried with anhydrous $CaSO_4$ and distilling before use. Other reagents were the products of China National Medicines Group, Shanghai Chemical Reagents Company and used without further purification.

### 2.2. Synthesis of zwitterion organosiloxane 3-(dimethyl(3-(trimethoxysilyl)propyl)-ammonio)-propanoate (ZWS)

The synthesis of zwitterion organosiloxane, ZWS, was as follows. A dose of 1.87 g (N,N-dimethyl-3-aminopropyl)trimethoxysilane in 10 mL of anhydrous acetone under nitrogen atmosphere was added to 1.12 g of β-propiolactone dropwise. The mixture was then stirred at ambient temperature in nitrogen atmosphere for 12 h. The mixture was filtered by vacuum filtration and washed twice with anhydrous acetone to yield a white solid compound (90%) and stored under $N_2$.

$^1$H NMR (400 MHz, $D_2O$): $\delta$ 3.65–3.35 (m, 2H), 3.27 (s, 9H), 2.96 (s, 6H), 2.83 (s, 2H), 2.69–2.41 (m, 2H), 1.81 (s, 2H), 0.67 (s, 2H). *m*/*z* (ESI) 265.1, 237.9.

IR ($cm^{-1}$): 1729, 1591, 1485, 1385, 1036, 930.

### 2.3. Synthesis of zwitterion organosiloxane functionalized $SiO_2$ nanoparticles ($SiO_2$-ZWS)

$SiO_2$ nanoparticles (SiNPs) with a diameter of 130 nm (measured by dynamic light scattering at 633 nm) were prepared by the Stöber procedure [33]. ZWS (200 mg) was added into 20 mL suspension of SiNPs in water (containing 1 g of SiNPs). After stirring for ten minutes, ammonia (0.1 mL) was added to keep the pH about 9.0. The mixture was heated to around 80 °C under nitrogen and stirred for another 24 h. The product was washed with water for four times and obtained as white particles suspended in 20 mL water.

### 2.4. Nanoparticle characterization

$^1$H NMR (400 MHz) spectra were recorded on a Bruker 400 UltraShield spectrometer at 25 °C using $D_2O$ as a solvent. Mass spectrometry (MS) was performed on a Pegasus 4D GCxGC-TOFMS mass spectrometer. Thermogravimetric analysis (TGA) was performed on a Netzsch (TG 209 F1 Iris) thermogravimetric analyzer in air at a heating rate of 10 °C/min. Zeta potential measurements were performed on a Malvern Zetasizer Nano ZS instrument.

### 2.5. Turbidimetric titration

Turbidimetry was used to study the protein adsorption on NPs [10]. Transmittance (*T*%) was monitored with a Brinkmann PC 950 colorimeter (420 nm filter), connected to a 2 cm path length optical probe. The turbidity was reported as 100 − *T*%, and the fluctuations (±0.1%) of transmittance were treated by consistently selecting the average transmittance. The single protein or serum solutions were dispersed in PBS with pH 7.40. The dependence of solution turbidity on time was obtained by observing the change of turbidity upon addition of 2.0 mg/mL protein solutions at once into a $SiO_2$-ZWS/PBS mixture. The $SiO_2$-ZWS concentration was 0.5 mg/mL (equal to concentration of $1.04 \times 10^{-7}$ M) and the protein concentration was 1.0 mg/mL ($1.52 \times 10^{-5}$ M for BSA and $7.2 \times 10^{-5}$ M for LYZ). Nanoparticle-free blanks and protein-free blanks were subtracted to eliminate the effect of free protein and NPs scattering. A Mettler Toledo Seven Easy S20 pH meter with a combination electrode was used to monitor the solution pH.

### 2.6. Dynamic light scattering (DLS)

The effect of adding salt or protein on the hydrodynamic diameter of the NPs was assessed using dynamic light scattering (DLS). The experiments were carried out at 25 °C with a Malvern Zetasizer Nano ZS instrument, with measurement duration of 600 s. The measurement angle was 173°. For data processing, the analysis model was "general purpose (normal resolution)" based on CONTIN. Intensity particle size distribution (PSD) was chosen for plotting in order to provide the original particle information of the mixture. Assay solutions (1.0 mL) were taken from turbidimetric titration samples. The mean value of *Z*-ave was used as hydrodynamic diameter ($D_h$).

### 2.7. Isothermal titration calorimetry (ITC)

The thermodynamic quantities of protein binding onto colloidal particles were determined by ITC (MicroCal iTC200, GE Healthcare). Proteins and $SiO_2$-ZWS solutions were mixed in PBS with the concentration 10 mg/mL (0.15 mM for BSA, 0.70 mM for lysozyme) and 1 mg/mL ($2.1 \times 10^{-4}$ mM), respectively. After instrument stabilization at 25 °C, 200 μL $SiO_2$-ZWS solution was titrated by 18 successive injections of protein solutions (each 2 μL, overall 40 μL). The interval between injections was 180 s. The solution was stirred at 1000 rpm in the sample cell during the experiments. Prior to data analysis, heats of dilutions were corrected by subtracting values for nanoparticle-free and protein-free blank solutions assays.

## 3. Results and discussion

### 3.1. Surface functionalization of silica nanoparticles

The synthetic route toward $SiO_2$-ZWS is illustrated in Scheme 1. The novel functional zwitterion organosiloxane ZWS was synthesized via attacking the carbonyl group of β-propiolactone, **2**, by the nitrogen atom of (N,N-dimethyl-3-aminopropyl)-trimethoxysilane, **1**, to obtain carboxyl end group. The ZWS is of good solubility in water, and it will become transparent after about 5 min exposure to the air.

$SiO_2$-ZWS were synthesized via a simple silanization process at 80 °C. Ammonia was used as the catalyst which is benefit for a higher surface coverage [34,35]. Besides, excess ZWS was added to the SiNPs aqueous solution to ensure an adequate reaction.