NANOPARTICLES OF ZnO - CHANGE OF ZETA POTENTIAL AND ADSORPTION OF BSA

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Abstract

This study was focused on interaction ZnO nanoparticles with protein. Bovine Serum Albumin (BSA) was chosen as a model protein to show how successful is the adsorption of protein on our prepared nanoparticles. Particles of ZnO were prepared by direct precipitation method from $Zn(NO_3)_2$ by addition of urea and further treatment. These particles were dispersed in water and characterized by distribution of size and compared with commercial ZnO. Average size of dispersed prepared particles was around 75 nm. Afterwards the zeta potential of particles was measured in different conditions (change of pH, concentration, ionic strength) and the isoelectric point was determined. These results were used for optimization the appropriate conditions for adsorption of BSA on ZnO nanoparticles. The changes of zeta potential were measured during adsorption of BSA on ZnO nanoparticles. These results can help to understand the interaction of ZnO nanoparticles with protein which is important to know for use their antibacterial effect in application in medicine.

Keywords: Zinc oxide, bovine serum albumin, zeta potential, adsorption

1. INTRODUCTION

Nanoparticles are still very interesting materials in terms of their several functional properties which can be used in different applications. ZnO is one of the well-known materials. It is a II-IV semiconductor with 3.37eV band gap and large excitation binding energy (60 meV) compared to other widely used semiconductors at room temperature and it has several useful properties such as luminescence, piezoelectricity, high chemical stability, high electrochemical coupling coefficient, broad range of radiation adsorption, high photostability [1,2] etc. These properties depend on physical and chemical properties especially on size, shape and crystal structure [3]. There are various methods of preparation, such as physical and chemical vapor deposition [4, 5], hydrothermal growth [6, 7], sol gel [8], mechanochemical methods [9], pulsed-laser deposition [10], microwave synthesis [11] and each of them can produce nanoparticles of ZnO with different morphology. ZnO nanoparticles are widely used in several areas such as UV light-emitting devices, gas sensors, photocatalysis, biosensors, pharmaceutical and cosmetic industries [1, 2, 12, 13]. They are also non-toxic, photochemically stable, show high biocompatibility and selective cytotoxicity [1, 9, 12]. These properties make them interesting for use in medicine.

There are several possible methods of preparation. Some methods are not cost effective, include very high temperatures or low pressures, some of them include precursors which are unstable and can cause environmental hazards. In this work, we try to use simple and effective method for produce suitable ZnO nanoparticles for our measurements and experiments. We decided to use a simple precipitation method [14] based on reaction of zinc nitrate and urea. The adsorption of proteins at solid-liquid interfaces is an important process for biomedical technologies and in our work we focus on possible functional properties for medical applications.

For our experiments we used the Bovine Serum Albumin (BSA). This protein is often used as a model protein for studying the interactions. It is one of the most abundant proteins in plasma and also it is a multifunctional protein with extraordinary ligand binding capacity. This protein is similar to human serum albumin (HSA). BSA (molecular weight 66 463 Da) consists of three intrinsic fluorophores: thyptophan (2 residues), tyrosine (20 residues) and phenylalanine (30 residues) [2]. In solution, BSA can form different conformations, which are

modified by changes of pH or ionic strength [15, 12]. Nanoparticles interact with protein in solution and around nanoparticles there is forming a layer of proteins which is usually called as “protein corona” [12].

2. MATERIALS AND METHODS

2.1. Materials

We used Zinc nitrate hexahydrate (Sigma Aldrich), urea (Lachema), Bovine Serum Albumin (Sigma Aldrich), Zinc oxide nanoparticles (Sigma Aldrich), Hydrochloric acid (Sigma Aldrich) and Sodium hydroxide (Sigma Aldrich).

2.1.1. Preparation of Zinc oxide nanoparticles

ZnO nanoparticles were synthesized by simple direct precipitation method. 50 ml of 0.5M $Zn(NO_3)_2$ solution was heated at 70 °C and dissolved in a distilled water under constant stirring. In the same way was prepared 50 ml of 1M urea solution. After 30 minutes of stirring and heating was the solution of urea (precipitation agent) added dropwise into solution of $Zn(NO_3)_2$ under continual stirring at 70 °C. After 2 hours of growth nanoparticles the solution turned to whitish cloudy. The solution was cooled down, centrifuged at 8000 rpm for 10 min and washed several times with distilled water to remove all impurities. The product was calcinated at 500 °C for 3 hours in muffle furnace.

2.1.2. Preparation of solutions for measurement

ZnO was prepared for measurement as a dispersion in distilled water in several concentrations and all samples were sonicated (30 minutes) for achievement homogenous solutions. BSA was dissolved in distilled water in required concentrations. There were Hydrochloric acid and Sodium hydroxide used for adjusting the pH.

2.2. Methods

2.2.1. Dynamic light scattering

The size distribution of used particles was determined by the dynamic light scattering (DLS) technique. The measurements were accomplished using a Zetasizer Nano ZS (Malvern Instruments Ltd., GB). The 12 mm cell (DTS 0012) was used. The suitable parameters were chosen for each sample of ZnO (Refractive index: 2.003; Absorption: 0.1) or BSA (Refractive index: 1.450; Absorption: 0.001) and dispersant (water; Refractive index: 1.333; Viscosity: 1.0031 cP, Dielectric constant: 80.4). All measurements were performed several times and finally was taken an average value. All measurements were taken at 20 °C.

2.2.2. Zeta potential measurement

The zeta potential was obtained by measurement of electrophoretic mobility using a Zetasizer Nano ZS (Malvern Instruments Ltd., GB) in folded capillary cells (DTS1070). The electrophoretic mobility was converted to the zeta potential using a Smoluchowski equation. Firstly, the dependence of zeta potential on pH was measured for ZnO and BSA in range of pH 3 - 13. The automatic Autotitrator (Malvern MPT-2) was used for this measurement to adjust pH by using Hydrochloric Acid (0.25 and 0.025 mol/L) and Sodium Hydroxide (0.25 mol/L). Then the change of zeta potential was measured with the other parameters (temperature and ionic strength) and it was found that the change of zeta potential with the change of temperature and ionic strength is negligible in comparison to change with the pH. For other measurement was considered only influence of pH on zeta potential of particles.

The samples with the most suitable zeta potential was chosen for adsorption. To determine protein adsorption, 5 ml of 400 ppm ZnO and 5 ml of BSA in six different concentrations (10, 20, 30, 50, 75 and 100 ppm) was added and mixed in pH 7. The zeta potential of each sample was measured after 5 minutes.

3. RESULTS AND DISCUSSION

3.1. Particle size distribution

Figure 1 shows the size of used samples. The average size for BSA is 4.8 nm, for ZnO(com) 85 nm and for our prepared ZnO 78 nm. There can be seen that prepared ZnO was comparable with the commercial sample. The size of particles is almost the same and particles show good size distribution in the sample after ultrasound treatment. We measured also a size of particles after adsorption and the size of ZnO(com) with 25 ppm BSA is 105nm. It confirms that the adsorption was performed.

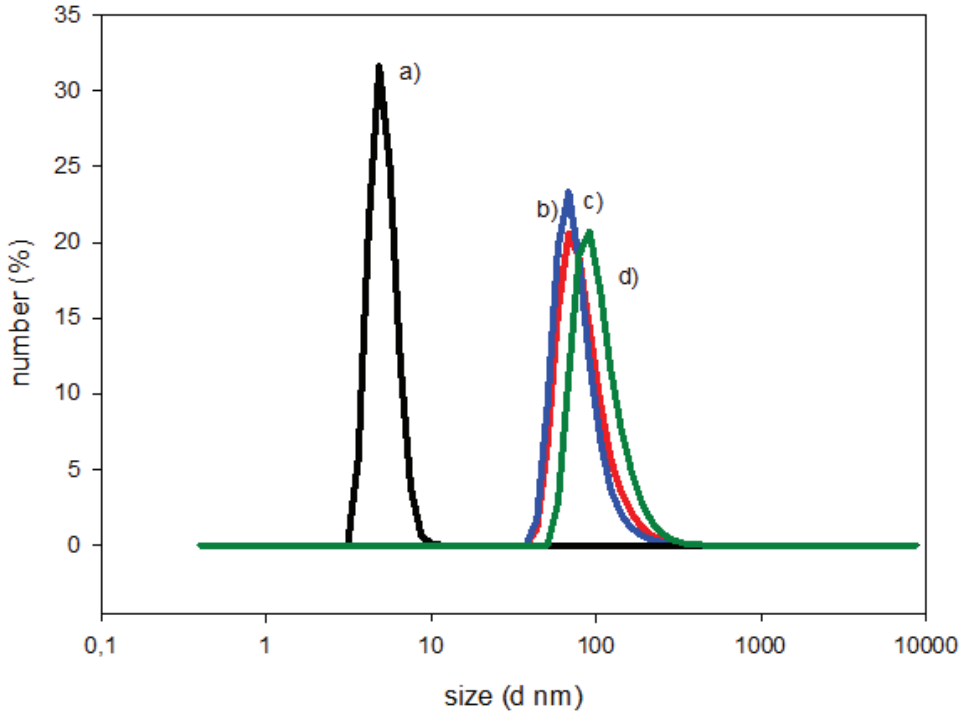

Figure 1 a) distribution of BSA size (black curve); b) distribution of prepared ZnO size (blue curve); c) distribution of commercial ZnO size (red curve) d) distribution of commercial ZnO + BSA (green curve)

3.2. Zeta potential

Figure 2 shows the change of zeta potential during change of pH. Measured points were fit with four parameter sigmoid curve ($f= y_0+a/(1+\exp(-(x-x_0)/b))$). These curves have a typical shape and show that in this range zeta potential of measured particles goes through an isoelectric point (IEP). The isoelectric point is the value of pH when the zeta potential is zero. For the suspension, it means that this system is unstable and it is prone to agglomerate or flocculate. For pH from 3 to 12 the zeta potential of BSA changes from +28 to -33 mV. For prepared ZnO from 20 mV to -60 mV and for commercial ZnO from 36 to -33 mV. The shift of prepared ZnO zeta potential values and commercial ZnO(com) is due to presence of impurities in prepared ZnO and because of the process of preparation. IEPs for each sample show **Table 1**.

Table 1 IEPs for ZnO, ZnO (com) and BSA

	IEPs [pH]
ZnO	7.4
ZnO (com)	8.4
BSA	4.8

The zeta potential which had been measured was used for decision which pH is the most suitable for adsorption of BSA in ZnO. For adsorption is the best to take particles with opposite zeta potential. So from the measured samples was chosen the sample of ZnO (com) with IEP in pH 8.4. In pH 7 the BSA has zeta potential -29 mV and ZnO (com) +22 mV. For adsorption of our prepared ZnO we should choose different pH, for example pH 6. It will be the subject of further studies.

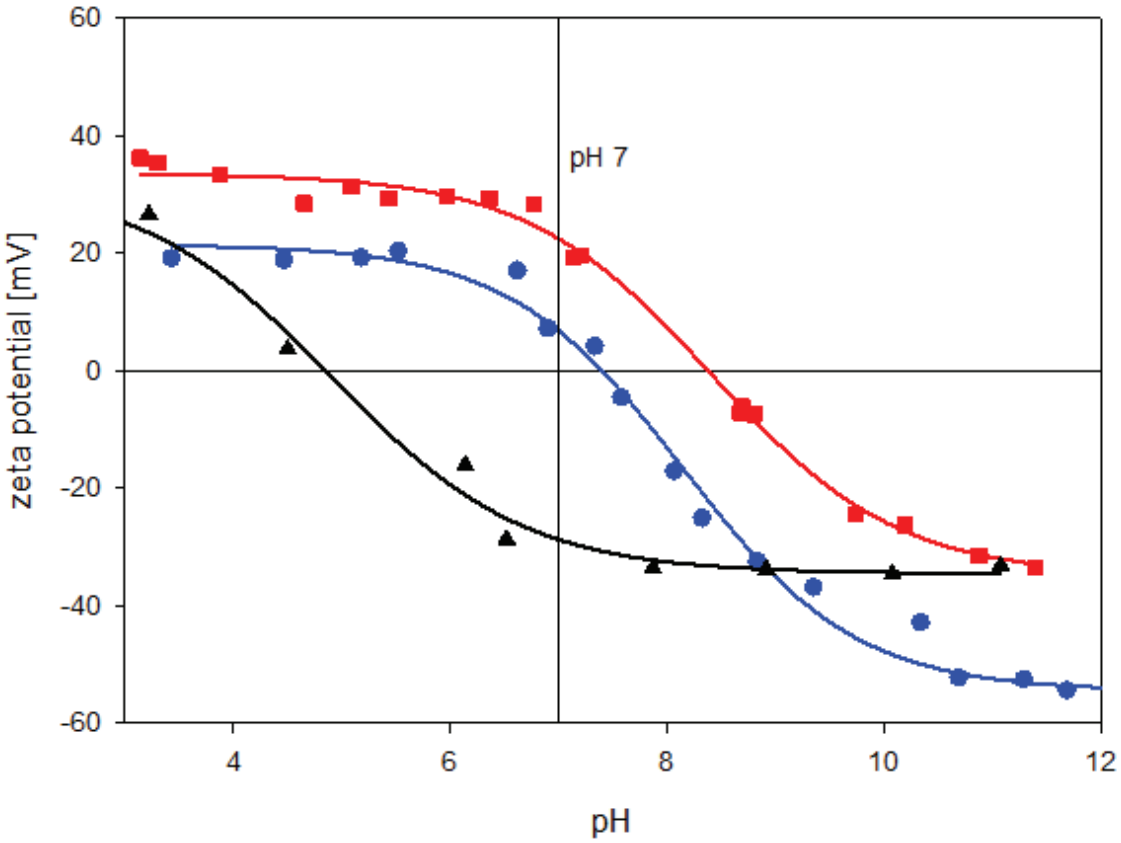

Figure 2 The dependence of zeta potential on pH of prepared ZnO (●), commercial ZnO(com) (■) and BSA (▲)

3.2.1. Zeta potential after adsorption

The zeta potential was measured again after adsorption of BSA on ZnO in several concentrations. The results are shown at **Figure 3**. When is the curve fit on our measured data, it can be seen that the required concentration BSA for full neutralization of the charge on 200 ppm ZnO(com) is 16 ppm. When the concentration of BSA is lower, we can see a positive resultant zeta potential. That is because of surplus of ZnO with positive zeta potential. When the concentration of BSA is higher, the resultant zeta potential is negative, because of surplus of BSA with negative zeta potential. Our curve of dependence zeta potential on amount of BSA is very similar to Langmuir adsorption isotherm and will be used for further study of adsorption parameters.

The same experiments were done with prepared ZnO but the results were unsatisfying. This sample has different surface properties and in this pH is not suitable for adsorption. We need to do several other experiments and determine the value of pH with larger difference of zeta potential ZnO and BSA suitable for adsorption. On this we will focus in our future work.

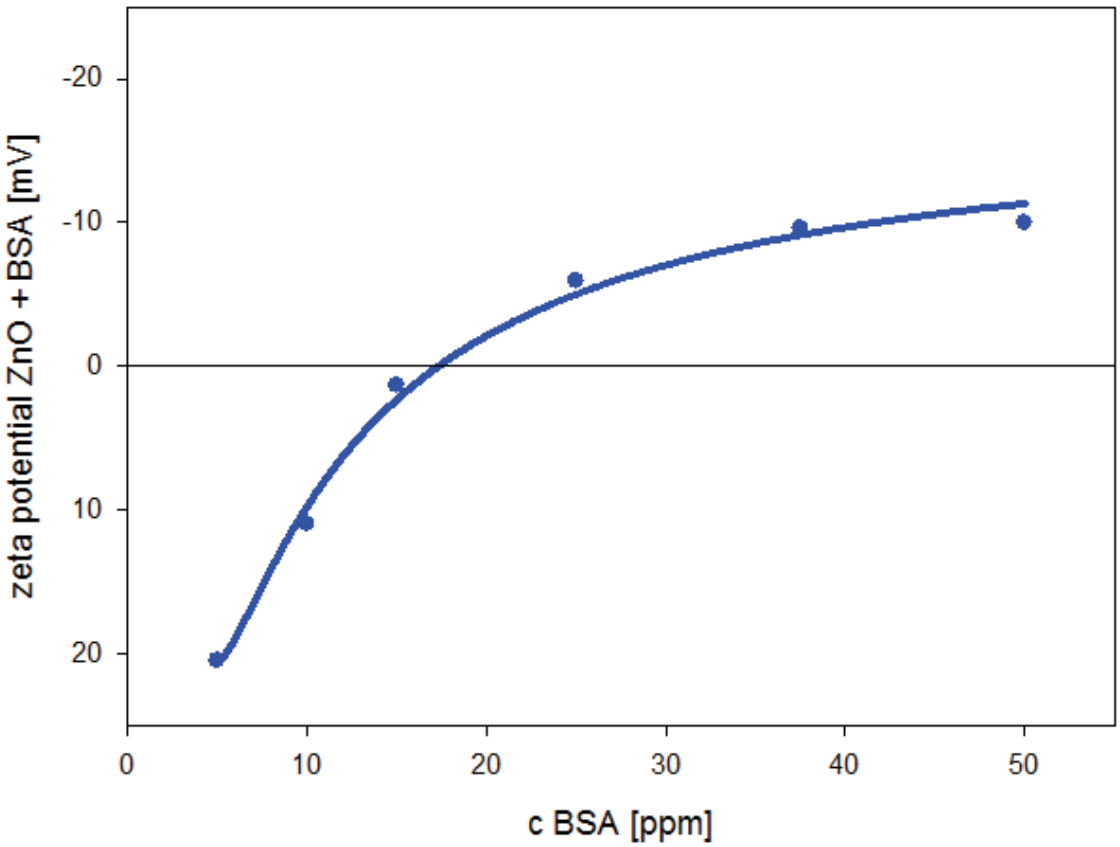

Figure 3 Zeta potential of 200 ppm ZnO(com) + 5, 10, 15, 25, 37.5 and 50 ppm BSA

4. CONCLUSION

The adsorption of BSA on ZnO was performed. Firstly, the zeta potential of ZnO, ZnO(com) and BSA was measured. Secondly, ZnO(com) was chosen for adsorption because of suitable values of zeta potential. Then the adsorption was performed and the change of zeta potential was measured. It can be seen from results, that adsorption was successful and it was determined the amount of BSA on ZnO(com) for fully covered surface of ZnO. This results will be used in further experiments especially for optimization of adsorption of our prepared ZnO samples.

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