World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:8, No:12, 2014

Adsorption of Bovine Serum Albumin on CeO_2

Roman Marsalek

***Abstract*—**Preparation of nanoparticles of cerium oxide and adsorption of bovine serum albumin on them were studied. Particle size distribution and influence of pH on zeta potential of prepared CeO_2 were determined. Average size of prepared cerium oxide nanoparticles was 9 nm. The simultaneous measurements of the bovine serum albumin adsorption and zeta potential determination of the (adsorption) suspensions were carried out. The adsorption isotherms were found to be of typical Langmuir type; values of the bovine serum albumin adsorption capacities were calculated. Increasing of pH led to decrease of zeta potential and decrease of adsorption capacity of cerium oxide nanoparticles. The maximum adsorption capacity was found for strongly acid suspension (am = 118 mg/g). The samples of nanoceria with positive zeta potential adsorbed more bovine serum albumin on the other hand, the samples with negative zeta potential showed little or no protein adsorption. Surface charge or better say zeta potential of CeO_2 nanoparticles plays the key role in adsorption of proteins on such type of materials.

***Keywords*—**Adsorption, BSA, cerium oxide nanoparticles, zeta potential.

I. Introduction

INTERACTION between nanoparticles and animal cells takes place on the imaginary axis: nanoparticle - protein - cell. This is a very difficult and complex problem, which is still not clearly understood. Firstly, it is necessary (using all available techniques) as accurately describe the physical and chemical properties of nanoparticles. Secondly, it is the adsorption of proteins on the surface of the nanoparticles that follows.

The authors of one of reviewed articles described the adsorption of proteins on the surface of nanoparticles, creating the so-called corona. This complex is then captured on the cell surface, it can also penetrate inside and in case that the release of the metal ion leads to the oxidative stress it may inherently result in damage to the cell [1].

The adsorption of bovine serum albumin to the nanoparticles of cerium oxide, and also studies of the adsorption of these nanoparticles on the surface of lung cancer cells A 549 were the subjects of further review. Both of these interactions were going on, and inter alia were monitored with a focus on the changing zeta potential [2].

The zeta potential is a parameter which is considered by many authors as very important for the characterization of nanoparticles, but not only that. It is believed that zeta potential is one of the decisive factors in the adsorption of proteins on the surface of nanoparticles. It may also play a role in the interactions between the nanoparticles and the cell.

Importance of charge, or better said the zeta potential for adsorption of nanoparticles of cerium oxide on different types of cells were discussed in an article of which the authors came to a conclusion that the particles with a positive or neutral charge are preferentially adsorbed on the surface of the "normal" cells, whereas particles with a negative charge on the surface of cancer-causing cells. The work also includes the possibility of modification of the surface of nanoparticles and thus their charge [3].

Current knowledge about the possible use of zeta potential for "axis": nanoparticles - a chemical substance (e.g. drug) - cells are summarized in next reviewed articles. The authors consider the particle size and zeta potential to be the main factors influencing behaviour in the given axis [4], [5].

It seems that even relatively small differences (40 and 60nm) in the nanoparticle size (specifically, the cerium oxide) can lead to relatively large differences in the effect on the cellular structure [6].

Zeta potential as a tool to monitor the interactions between nanoparticles FeOx and normal breast epithelial cells (MCF10) and malignant breast epithelial cells (MCF -7) were used in another work. Furthermore in terms of comparison of theoretical models and experimental results of the measurement of zeta potential, authors also point out the potential use of zeta potential in diagnosis and therapy [7]. Measurement of zeta potential of human cells (leukocytes, erythrocytes, line MCF -7) was the subject of a further study. The authors observed changes in the values of the zeta potential of these cells after exposure to elevated temperature, and after interaction with various polymers [8].

Our goals in this study were to synthetize cerium oxide nanoparticles and determine how the surface charge modification (measured by zeta potential) of cerium oxide nanoparticles (nanoceria) affects the bovine serum albumin adsorption.

II. Experimental

A. Preparation of Cerium Oxide Nanoparticles and Their Characterization

Cerium nitrate (Sigma Aldrich) was dissolved in distilled water to obtain 0.1M solution. Equal volume of 0.5 N ammonium hydroxide solution (Sigma Aldrich) was then added with stirring at about 300 rpm. The solution was then heated in an oven at 110°C to evaporate all the water in the solution and the cerium oxide powder obtained was then heated at 300°C for an hour and then furnace cooled. The powder was then transferred into a Teflon-lined stainless-steel autoclave containing 2.0 N sodium hydroxide solution (Sigma

R. Marsalek is with the Faculty of Science, University of Ostrava, 30. Dubna 22, Ostrava, 701 03, Czech Republic (phone: 402-737-443663; e-mail: roman.marsalek@ osu.cz).

This work was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic in the "National Feasibility Program I", project LO1208 "Theoretical Aspects of Energetic Treatment of Waste and Environment Protection against Negative Impacts".

Aldrich) up to 80% of its total volume and heated at 120°C for 24 h under autogenous pressure. The system was then allowed to cool down to room temperature. Finally, the resulting solution was titrated with HCl (Sigma Aldrich) to bring the pH of the solution to 7.0. Then the excess liquid was evaporated off resulting in hydrothermal ceria nanoparticles [1].

1) Particle Size Distribution

For dynamic light scattering measurements 12 mm cell (DTS 0012) was used. One millilitre of distilled water was added to the cell and then 50 µl from stock dispersions (1% wt.) were added. Samples were sonicated for 5 minutes. Size distributions of the nanoparticles were determined with a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., GB) by the DLS technique. The suitable parameters (viscosity, absorption and refractive index) were chosen for each CeO_2 and dispersant. For CeO_2 sample: absorption 0.1, refractive index 2.0, for water: viscosity 1.0031cP, refractive index 1.33. The pH value of each suspension was adjusted by adding either NaOH (Sigma Aldrich) or HCl (Sigma Aldrich). Histograms with the distributions of sizes were recorded.

2) Zeta Potential Measurements

The zeta potential was measured by analyzing 0.1 g of CeO_2 in 10 ml of water using the Zetasizer Nano ZS (Malvern Instruments Ltd., GB). Before zeta potential measurements all samples were sonicated for 5 minutes. Zetasizer Nano ZS uses Laser Doppler Velocimetry to determine electrophoretic mobility. The zeta potential was obtained from the electrophoretic mobility by the Smoluchowski equation.

The dynamic method consisted of using ZS Malvern Zetasizer device coupled with an automatic titrator (Malvern MPT-2). pH of the suspensions was automatically adjusted by this automatic titrator using hydrochlorid acid (0.25 and 0.025 mol/l) and sodium hydroxide (0.25 mol/l).

3) Microscopy

The obtained nanoceria particles were characterized using scanning transmission electron microscopy (SEM). The SEM images were obtained with JEOL (JSM-6610LV) scannig electron microscope operated at 30 kV.

B. Protein Adsorption

To determine protein adsorption, 10 mg of nanoceria was weighed in the flask and 10 ml of the BSA (Sigma Aldrich) solution of a known concentration was added. Solutions were stirred vigorously with a magnetic stirrer for 2 h. The nanoceria particles were centrifuged and the concentration of BSA was determined in the supernatant using UV–visible spectroscopy (Cary 1E UV-Visible Spectrophotometer, Varian Analytical Instruments) by determining the absorbance maximum at 280nm wavelength.

The amount of BSA adsorbed (a) was determined from the change in the solution concentration before and after equilibrium, according to:

$$a = \frac{(c_0 - c_e)V}{m} \quad (1)$$

where c_0 is the initial concentration of BSA solution, c_e the concentration of BSA solution at the adsorption equilibrium, V the volume of BSA solution and m the mass of the nanoceria.

III. Results and Discussion

A. Particles Size Distribution and SEM Analysis

The following pictures show the particle size distribution of CeO_2 in water (Fig. 1) and photo from SEM analysis (Fig. 2).

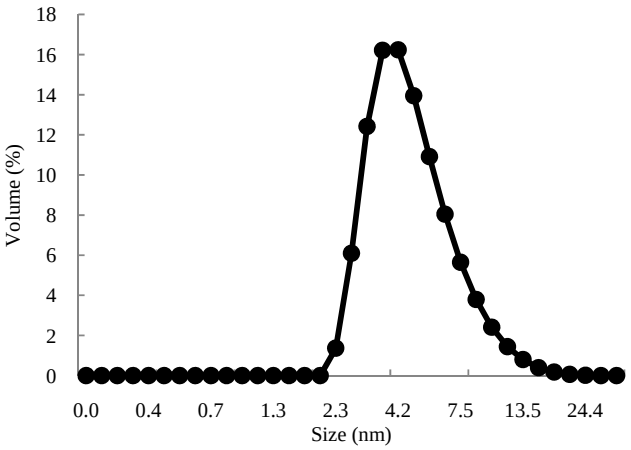

Fig. 1 Particle size distribution of CeO_2 by Volume

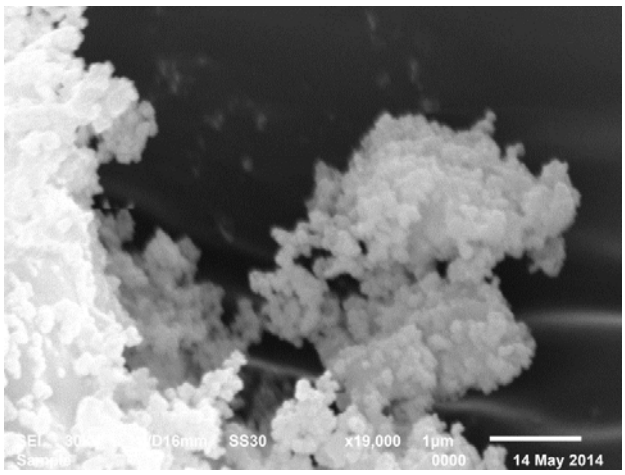

Fig. 2 SEM image of CeO_2 nanoparticles

The value of Z-average was 9 nm parameter Number mean was 3.7nm. Such values of average size of our nanoparticles well correspond with data obtained from SEM and also with value in articles [1].

B. Influence of pH on Zeta Potential

Fig. 3 shows values of zeta potential as a function of pH. The aim was detected IEPs of used nanoparticles.

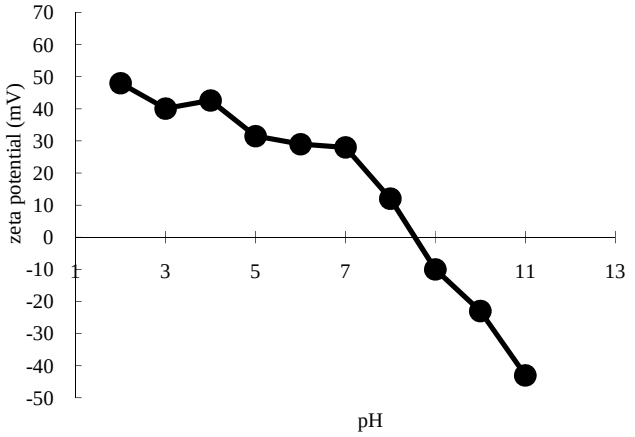

Fig. 3 The influence of pH on zeta potential of CeO2. The value of IEPs 8.8

The shape of curve is typical and the mail role plays the adsorption of hydrogen ions in acid range of pH and the adsorption hydroxide ions in the basic range of pH, respectively. In many applications the so-called isoelectric point plays an important role. It is such pH when the zeta potential equals zero. Such system (suspension) is very unstable and particles often agglomerated. Generally, high value of zeta potential (positive or negative) prefers adsorption of hydrophilic compounds with opposite charge.

The correlation of zeta potential to pH is important to know so that one can predict how the varying pH inside the human body will affect the surface charge of the nanoceria particles. Because of the positive zeta potential, the particles can be expected to enhance the removal of negatively charged substances from the liquid solution when the pH will lay between pH 2 and 8.

C. Adsorption of BSA onto Nanoceria

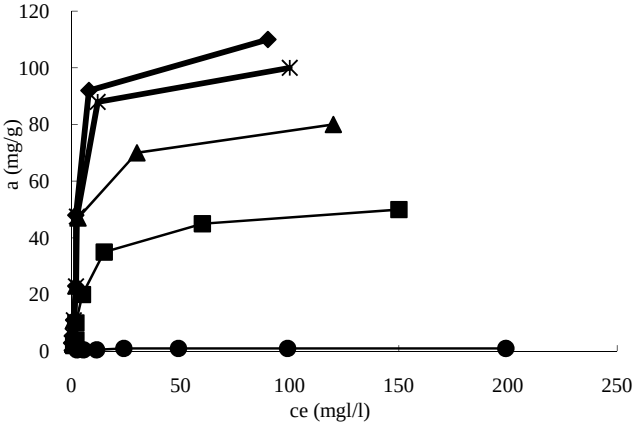

Fig. 4 BSA adsorption isotherms on nanoceria (-◆- pH=2, -✳- pH=4, -▲- pH=6, -■- pH=8, -●- pH=10)

Fig. 4 depicts the typical adsorption isotherms obtained from the experimental data. The shape of isotherms indicates that the adsorption data could be well fitted by the Langmuir adsorption model of monolayer coverage. In a linear form, the Langmuir equation is given by

$$\frac{c_e}{a} = \frac{c_e}{a_m} + \frac{1}{a_m b} \quad (2)$$

where a is the amount of BSA adsorbed, c_e is an equilibrium concentration of BSA in solution, b represents a monolayer binding constant and a_m is the monolayer adsorption capacity.

The adsorption isotherms proved to be consistent with the Langmuir model as deduced from the calculated r-square values close to 1. The most informative parameter in the Langmuir equation is a_m, providing information on an adsorbed amount at the monolayer surface coverage. The values obtained for nanoparticle sorbent is compiled in Table I.

TABLE I
LANGMUIR DATA OBTAINED FROM THE ADSORPTION MEASUREMENTS

pH	Zeta potential (mV)	a_m (mg/g)	b
2	48	118	58
4	42	107	54
6	29	87	39
8	12	56	22
10	-23	1	79

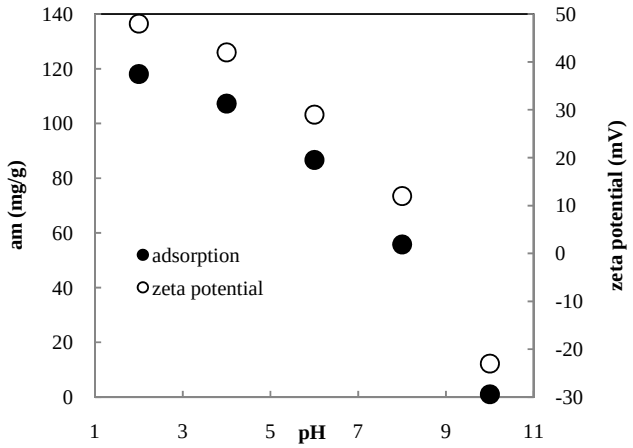

Fig. 5 Dependence of zeta potential (blank symbols) and adsorption of BSA (full symbols) on cerium oxide nanoparticles on pH

Fig. 5 shows the noteworthy similarity between the adsorption capacities (a_m) and zeta potential of the adsorption systems.

Both values are strongly dependent on the pH of the suspension. The data in Table I and Fig. 5 show that pH value increase leads to a reduction of the zeta potential, and at the same time to reduction of the adsorption capacity of cerium oxide nanoparticles. BSA is preferentially adsorbed onto the positively charged surface of the cerium oxide nanoparticles. Adjustment of pH is one of the ways how to modify the surface of nanoparticles and thus affect their adsorption capacity for proteins.

IV. Conclusions

Cerium oxide nanoparticles have been prepared by hydrothermal method. Size of the prepared nanoparticles was 9nm, which was confirmed by DLS measurements and scanning electron microscopy. In thus prepared nanoparticles a zeta potential depending on the pH value was measured, isoelectric point was 8.8. Then the adsorption of bovine serum albumin on these particles at different pH was carried out. Adsorption can be described by Langmuir adsorption theory, using linear regression were calculated maximum adsorption capacities for each pH. Results showed that there was a direct correlation between pH, zeta potential and adsorption capacity. BSA binds preferentially to cerium oxide nanoparticles which have a positive charge. The interaction between the nanoparticles of cerium oxide and BSA performed at acidic pH, which is essential for the application of these materials in living systems.

References

[1] M. Horie, H. Kato, H. Iwahashi, „Cellular effects of manufactured nanoparticles: effect of adsorption ability of nanoparticles" in *Arch Toxicol* vol. 87, 2013, pp. 771–781.

[2] S. Patila, A. Sandbergb, E. Heckertc, W. Selíc, S. Seal, "Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential" in *Biomaterials* vol. 28, 2007, pp. 4600–4607.

[3] A. Asati, S. Sandra, Ch. Kaittanis, J.M., Perez, "Surface-Charge-Dependent Cell Localization and Cytotoxicity of Cerium Oxide Nanoparticles" in *ACS Nano* vol. 4, 2010, pp. 5321–5331.

[4] S. Honary, F. Zahir, "Effect of Zeta Potential on the Properties of Nano-Drug Delivery Systems - A Review (Part 1)" in *Tropical Journal of Pharmaceutical Research*, vol. 12, 2013, pp. 255-264.

[5] S. Honary, F. Zahir. "Effect of Zeta Potential on the Properties of Nano-Drug Delivery Systems - A Review (Part 2)" in *Tropical Journal of Pharmaceutical Research* 12, , vol. 12, 2013, pp. 265-273.

[6] G. Zhou, G. Gu, Y. Li, Q. Zhang, W. Wang, S. Wang, J. Zhang, "Effects of Cerium Oxide Nanoparticles on the Proliferation, Differentiation, and Mineralization Function of Primary Osteoblasts In Vitro" in *Trace Elem Res*, vol. 153, 2013, pp. 411–418.

[7] Y. Zhang, M. Yang, N.G. Portney, D. Cui, G. Budak, E. Ozvat, M. Otkán, C.S. Otkan, "Zeta potential: a surface electrical characteristic to probe the interaction of nanoparticles with normal and cancor human breast epithelial cells" in *Biomed Microdevices*, vol. 10, 2008, pp. 321–328.

[8] O.V. Bonsae, D.V.Saifullina, I.I. Shakkmaeva, I.I. Mavlyutova, T.I. Andulin, "Monitoring of the Zeta Potential of Human Cells upon Reduction in Thein Viability and Interaction with Polymers" in *Acta naturae*, vol. 4, 2012 pp. 78-81.