INTERACTION BETWEEN BOVINE SERUM ALBUMIN AND CeO_2

MARŠÁLEK Roman, BUKÁČKOVÁ Marta

Faculty of Science, University of Ostrava, Ostrava, Czech Republic, EU
roman.marsalek@osu.cz

Abstract

Cerium oxide nanoparticles were prepared by the hydrothermal method. Simultaneous measurements of the bovine serum albumin adsorption and zeta potential determination of the (adsorption) suspensions were carried out. The samples of nanoceria with positive zeta potential adsorbed more bovine serum albumin while on the other hand, the samples with negative zeta potential exhibited little or no protein adsorption. Particle size distribution and the influence of pH and temperature on the zeta potential of the prepared CeO_2 and BSA were determined. The adsorption isotherms of BSA on CeO_2 were found to be of the typical Langmuir type; values of the bovine serum albumin adsorption capacities were calculated. Increasing pH led to a decrease in zeta potential and decrease in the adsorption capacity of cerium oxide nanoparticles. It was proved that the adsorption capacity of BSA on nanoceria particles increased with a rise in temperature. The maximum adsorption capacity was found for an acid (pH 4) suspension and temperature of 34 °C (a_m = 124 $mg.g^{-1}$). Calculated thermodynamic parameters indicate that the adsorption of BSA on the ceria is an endothermic ($\Delta H_{ads} > 0$) and spontaneous ($\Delta G_{ads} < 0$) process. The kinetics of adsorption best fit the pseudo-second-order.

Keywords: Nanoceria, albumin, adsorption, zeta potential, kinetics, thermodynamics

1. INTRODUCTION

Nanoparticles exhibit exceptional physicochemical properties such as large surface area, surface reactivity, charge, shape, etc. These interesting functions and properties of nanoparticles have led to their expansion into many fields of human activity. With their extensive applications, serious concerns have been raised over their health risks once they are released into the environment. Nanoparticles (NPs) can be transported into blood or across cell membranes into cells, and may interact with proteins in blood or cytoplasmic proteins. Protein adsorption on a solid surface may induce changes in their structures and functions, even the entire protein molecules. Hence, protein adsorption could result in adhesion, proliferation, and differentiation of cells, as well as affect foreign body response and inflammatory processes. Adsorption of protein molecules is generally governed by Columbic forces, van der Waals forces, hydrophobic interactions, and the sorbate conformational stability. The surface area provides possible sorption spaces for protein adsorbed on particle surfaces [1]. Cerium oxide nanoparticles are very useful in biomedical applications. Cerium oxide nanoparticles (nanoceria) are a unique nanomaterial because they exhibit anti-inflammatory properties. Cerium oxide nanoparticles can act as direct antioxidants to limit the amount of reactive oxygen species required to kill hippocampal nerve cells [2]. The following research found that cerium oxide nanoparticles exhibit catalase mimetic activity and can act as a catalyst that mimics superoxide dismutase and catalase [3]. 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 subject to further review. Both of these interactions were going on, and inter alia were monitored with a focus on the changing zeta potential [4]. It is believed that the zeta potential is one of the decisive factors in the adsorption of proteins on the surface of nanoparticles. Our goals in this study were to synthetize cerium oxide nanoparticles and determine how the surface charge modification (measured by the zeta potential) of cerium oxide nanoparticles (nanoceria) affects the bovine serum albumin adsorption.

2. MATERIALS AND METHODS

2.1. Materials and Methods

2.1.1. Preparation of Cerium Oxide Nanoparticles

Cerium nitrate (Sigma Aldrich) was dissolved in distilled water to obtain a 0.1 M solution. An 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 to a Teflon-lined stainless-steel autoclave containing 2.0 N sodium hydroxide solution (Sigma 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 [4].

2.1.2. Zeta potential

The zeta potential was measured by analysing 0.1 g of CeO_2 in 10 mL of distilled water using the Zetasizer Nano ZS (Malvern Instruments Ltd., GB). Before the zeta potential measurements, all samples were sonicated for 5 minutes. The influence of pH in the range 2-11 was determined. The dynamic method consisted of using the ZS Malvern Zetasizer device coupled with an automatic titrator (Malvern MPT-2). The pH of the suspensions was automatically adjusted by this automatic titrator using hydrochloric acid (0.25 and 0.025 $mol.L^{-1}$) and sodium hydroxide (0.25 $mol.L^{-1}$). To determine changes on the CeO_2 surface caused due to adsorption of BSA, the zeta potential of the CeO_2-BSA system was carried out.

2.1.3. Protein Adsorption

To determine protein adsorption, 10 mg of nanoceria was weighed in a flask and 10 mL of a BSA (Sigma Aldrich) solution of a known concentration (3, 6, 12, 25, 50, 100, 200 $mg.L^{-1}$) was added. The solutions were stirred vigorously with a magnetic stirrer for 8 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 wavelength 280 nm. The pH value of each suspension was adjusted by adding either NaOH or HCl when the influence of pH on the adsorption capacity was followed.

2.1.4. Thermodynamics and kinetics

The amount of nanoceria particles and concentration of BSA were the same as mentioned above, which means 10 mg of CeO_2 in 10 mL of BSA solutions, concentrations varied from 3 to 200 $mg.L^{-1}$. In the next step we adjusted pH, the previous results indicate that a pH of 4 is most suitable for BSA adsorption. The suspension was put into a thermostatic bath at four different temperatures (22 °C, 28 °C, 34 °C and 40 °C) and the flasks were constantly shaken. The experiments proved that an 8 hour period is needed to reach equilibrium.

The fact that the adsorption of BSA on nanoceria was carried out at four different temperatures allows us to calculate the thermodynamic parameters. From the dependence of the equilibrium constant (K_c^0) on temperature, the change in the adsorption enthalpy (ΔH_{ads}) and adsorption entropy (ΔS_{ads}) can be determined.

$$\ln K_c^0 = -\frac{\Delta H_{ads}}{R}.\frac{1}{T} + \frac{\Delta S_{ads}}{R} \quad (1)$$

The change in Gibbs free energy (ΔGads) can be calculated from the following well-known equation:

$$\Delta G_{ads} = \Delta H_{ads} - T.\Delta S_{ads} \quad (2)$$

For the kinetics study, the experiments were carried out at a temperature of 22 °C with a constant shaking speed and a medium pH of 4.0. 10 mL of the BSA solution with an initial concentration of 200 mg.L^{-1} and 10 mg CeO_2 was agitated until the adsorption equilibrium was reached. Samples were pipetted out at different time intervals for BSA assay. After that, different kinetic equations were used for modelling the kinetics of BSA adsorption. Two kinetic models were used to explain the mechanism of the adsorption processes. The pseudo-first-order kinetic model is given by the Lagergren equation [5, 6]:

$$log(a_e - a_t) = -\frac{k_1}{2{,}303}t + log a_e \quad (3)$$

where a_e and a_t are the amounts of BSA adsorbed (mg.g^{-1}) at equilibrium time and any time t (hour), respectively, and k_1 is the rate constant of adsorption (min^{-1}). The plot of log (a_e − a_t) versus t gives a straight line for the first-order adsorption kinetic which allows computation of the rate constant k_1.

The pseudo-second-order model based on equilibrium adsorption is expressed as [5, 6]:

$$\frac{t}{a_t} = \frac{1}{k_2 a_e^2} + \frac{1}{a_e} \quad (4)$$

where k_2 is the pseudo-second-order rate constant (g.mg^{-1}.min^{-1}). The equilibrium adsorption capacity (a_e) and the model constants (k_2) can be determined experimentally from the slope and intercept of plot t/at versus t.

3. RESULTS AND DISCUSSION

3.1. Adsorption of BSA onto nanoceria and zeta potential

Table 1 summarizes the results of the adsorption experiments and zeta potential measurments. These measurements were carried out at a temperature of 295.15 K and at different pH values. The adsorption isotherms proved to be consistent with the Langmuir model as deduced from the calculated r-square value, which is closer to 1 in comparison with the Freundlich results. As mentioned, parameter *b* (binding constant or adsorption equilibrium constant) is sometimes used as a reaction equilibrium constant and thermodynamic parameters are calculated. For example, Gibbs free energy using the following equation:

$$\Delta G_{ads} = -RT.lnK_c^0 \quad (5)$$

The binding constant values of our measured and calculated isotherms are lower than 1 (**Table 1**), it means that logarithm will be negative. Such values of binding constants will be resulted to positive value of Gibbs free energy. Experience and many experiments indicate that adsorption of BSA is mostly a spontaneous process, the determined value of G_{ads} is negative [5-9]. With the fact that the equilibrium constant should be dimensionless, we used a different way in which to determine the thermodynamics parameters.

Table 1 Langmuir and Freundlich data obtained from the adsorption measurements and values of the zeta potential

	Langmuir				Freundlich			Zeta potential		
pH	a_m (mg.g^{-1})	b (L.mg^{-1})	Theoretical calculate isotherm	R^2	R^2	k (mg.g^{-1}) /(mg.L^{-1})^n	n	CeO_2 (mV)	BSA (mV)	CeO_2+ BSA (mV)
4	107	0.14	a=14.58 x c_e/(1+0.14 x c_e)	0.99	0.92	10.93	0.59	16	10	13
6	93	0.06	a=5.35 x c_e/(1+0.0.06 x c_e)	0.99	0.94	5.29	0.67	5	-16	-15
8	60	0.09	a=4.10 x c_e/(1+0.0.09 x c_e)	0.98	0.89	4.62	0.58	-14	-34	-24

Adsorption capacity and zeta potential values are strongly dependent on the pH of the suspension. The data in **Table 1** show that a pH value increase leads to a reduction in the zeta potential, and at the same time to a reduction in the adsorption capacity of cerium oxide nanoparticles. BSA is preferentially adsorbed onto the positively charged surface of the cerium oxide nanoparticles. Adjusting pH is one way to modify the surface of nanoparticles and thus affect their adsorption capacity for proteins. Albumin adsorption on the surface of nanoparticles is affected by two main factors. The first is the size and shape of the protein at a certain pH. The second important factor is the surface charge of nanoparticles and proteins. The highest amount of BSA macromolecules gets adsorbed at pH 4, such pH of the solution not being far off the BSA isoelectric point (4.7). Under these conditions, the net charge of the macromolecules is slightly above zero-the number of positive groups is higher probably due to the adsorption of hydrogen ions from hydrochloric acid. The zeta potential of cerium oxide nanoparticles is also positive (see **Table 1**). The reciprocal electrostatic repulsions between a solid surface and BSA macromolecules are not so strong and albumin is adsorbed on the nanoparticles' surface via the hydrophobic parts of the molecule. At isoelectric point, the BSA shows a highly compressed structure and the size (hydrodynamic radius) of the macromolecule is smaller than at pH six or eight. Charges (CeO_2 and BSA) and conformation of BSA allow the adsorption of its maximum amount and the formation of a consistent film. The higher adsorption level at pH 6 compared to pH 8 is again a result of different BSA conformation and surface charges. Increase in pH causes growing BSA hydrodynamic radius, and secondly, due to the adsorption of hydroxide ions the values of the zeta potential are negative. The absorption on the nanoceria surface changes its zeta potential over the whole pH range. When the adsorption process is completed the adsorbate masks the adsorbent charge and the cerium oxide surface exhibits properties typical of BSA. A similar behaviour was also observed during interaction between BSA and silicon dioxide [10] or between BSA and chromium (III) oxide [11].

3.2. Thermodynamics and kinetics

Table 2 Pseudo-first-order and pseudo-second-order kinetic parameters for adsorption of BSA by cerium oxide nanoparticles.

	Pseudo-first-order constant			Pseudo-second-order constant		
conditions	k_1 (10^{-3} min^{-1})	a_e ($mg.g^{-1}$)	R^2	R^2	k_2 (10^{-3} g mg^{-1} min^{-1})	a_e ($mg.g^{-1}$)
pH 4, 295 K, 200 $mg.L^{-1}$ BSA, 10 ml, 10 mg CeO_2	5.26	28.79	0.90	0.99	9.88	109.15

Previous studies [5, 6, 12, 13] show that adsorption of BSA onto various adsorbents is mostly described as a process of the pseudo-second order. In our study we compared only two popular kinetic models, pseudo-first-order and pseudo-second-order kinetic models. From **Table 2** we can see that the calculated linear regression correlation coefficient (R^2) is closer to one in the case of pseudo-second-order model. The calculated values of the adsorption amount also agree better with experimental data (**Table 1**). These results indicate that the adsorption of BSA on cerium oxide followed pseudo-second-order kinetics.

Finally, the thermodynamics parameters enthalpy, entropy and free Gibbs energy were calculated. Firstly, we used the following relation to determine the equilibrium constant (distribution coefficient):

$$K_c = \frac{a_e}{c_e} \quad (6)$$

K_c values at different temperatures were determined from the graphs plotted $\ln(a_e / c_e)$ versus a_e and extrapolation of a_e to zero [14-16]. As constant K_c^0 was used and from the slope and intercept of the Van´t Hoff equation changes of enthalpy and entropy were calculated. The change in Gibbs free energy was determined for all temperatures.

Table 3 Langmuir data obtained from the adsorption measurements and values of thermodynamics parameters

	Langmuir constants			Thermodynamics		
T (K)	a_m (mg.g^{-1})	B (L.mg^{-1})		ΔG_{ads} (kJ.mol^{-1})	ΔH_{ads} (kJ.mol^{-1})	ΔS_{ads} (J.mol^{-1}.K^{-1})
295.15	107	0.14		-2.60	5.31	26.80
301.15	121	0.14		-2.76		
307.15	124	0.17		-2.92		
313.15	114	0.19		-3.08		

Meanwhile, an increase in temperature up to 307.15 K deals with an increase in the immobilization capacity of BSA adsorption at equilibrium, suggesting that the system followed an endothermic process. This idea is also supported by the positive value of adsorption enthalpy (**Table 3**). A slightly different behaviour was seen in the case at a temperature of 313.15 K. At this temperature, adsorption capacity decreased. We assume that there are changes in the structure of the BSA molecules. Higher temperature causes the denaturation of proteins and affects its conformation. Thermal denaturation and structural changes in BSA have already been published. The helicity of the protein sharply decreased with a rise in temperature beyond 30 °C [17]. It is obvious that the adsorption of BSA on cerium oxide is an endothermic (positive values of ΔH_{ads}) and spontaneous (negative values of ΔG_{ads}) process. The positive value of change in entropy then corresponds with the increasing randomness at the solid - liquid interface during the adsorption.

CONCLUSIONS

Linear regression confirmed that the adsorption of BSA on cerium oxide nanoparticles can be described by the Langmuir model while the kinetics can be expressed by the pseudo-second order. The corresponding thermodynamic data were calculated to give the Gibbs free energy, enthalpy and entropy of the adsorption. The overall adsorption process was endothermic and spontaneous in nature. The results show that the effects of pH and temperature are very important. pH strongly influences the zeta potential of BSA and the zeta potential of cerium oxide nanoparticles, respectively. Temperature affects the conformation of BSA, and the kinetic and thermodynamic equilibrium of the adsorption process. Maximum adsorption capacity was found near the isoelectric point of BSA at a temperature of 34 °C.

ACKNOWLEDGEMENTS

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 Environmental Protection against Negative Impacts", and project SGS06/PrF2016-2017 "Zeta potential determination of Bovine Serum Albumin and study of its (BSA) interaction with nanoparticles".

REFERENCES

[1] SONG, L., YANG, K., JIANG, W., DU, P., XING, B. Adsorption of bovine serum albumin on nano and bulk oxide particles in deionized water, *Colloid and Surfaces B: Biointerfaces,* 2012, vol. 94, pp. 343-346.

[2] SCHUBERT, D., DARGUSCH, R., RAITANO, J., CHAN, S.W. Cerium and yttrium oxide nanoparticles are neuroprotective, *Biochemical and Biophysical Researches Communications,* 2006, vol. 342, no. 1, pp. 86-91.

[3] PIRMOHAMED, T., DOWDING, J. M., SINGH, S., WASSERMAN, B., HECKERT, E., KARAKOTI A.S., KING, J. E., SEAL, S., SELF, W. T. Nanoceria exhibit redox state-dependent catalase mimetic activity, *Chemical Communication,* 2010, vol. 46, no. 16, pp. 2736-2738.

[4] PATIL, S., SANDBERGB, A., HECKERTC, E., SELÍC, W., SEAL, S. Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential, *Biomaterials,* 2007, vol. 28, no. 31, pp. 4600-4607.

[5] WANG, Z., YUE, T., YUAN, Y., CAI, R., NIU, C., GUO, C. Kinetics of adsorption of bovine serum albumin on magnetic carboxymethyl chitosan nanoparticles, *International Journal of Biological Macromolecules*, 2013, vol. 58, pp. 57- 65.

[6] RATHINAM, L. ZOUB, Biosorption of bovine serum albumin by Ulva lactuca biomass from industrial wastewater: Equilibrium, kinetic and thermodynamic study, *Journal of Hazardous Materials*, 2010, vol. 184, no. 1-3, pp. 597-602.

[7] VALERO VIDAL, C., OLMO JUAN, A., IGUAL MUNOZ, A. Adsorption of bovine serum albumin on CoCrMo surface: Effect of temperature and protein concentration, *Colloids and Surfaces B: Biointerfaces*. 2010 vol. 80, no. 1, pp.1-11.

[8] SHI, N., GAO, B. J., YANG, Q. Adsorption Characteristics of Bovine Serum Albumin on Cationic Grafted Particles QPDMAEMA/SiO2 with Brush Structure, *Acta Physico -Chimica Sinica,* 2014, vol. 30, no. 11, pp. 2168-2176.

[9] LIU, X., DENG, H., WANG, Y., LU, Z., ZENG, X., WANG, X., ZIU, P., RAO, H. Thermodynamics Studies on the BSA Adsorption onto Zinc Oxide Surfaces with Different Morphologies, *Chemical journal of chinese universities*. 2014, vol. 35, no. 10, pp. 2156 -2163.

[10] WISNIEWSKA, M., SZEWCZUK-KARPISZ, K., STERNIK, D. Adsorption and thermal properties of the bovine serum albumin-silicon dioxide system, *Journal of Thermal Analysis and Calorimetry,* 2015, vol. 120, no. 2, pp. 1355 -1364.

[11] SZEWCZUK-KARPISZ, K., WISNIEWSKA, M. Adsorption properties of the albumin-chromium (III) oxide system-effect of solution pH, and ionic strength, *Soft Materials,* 2014, vol. 12, no. 3, pp. 268-276.

[12] SASIDHARANA, P., CHANDRANB, P., KHANA, S. S. Interaction of colloidal zinc oxide nanoparticles with bovine serum albumin and its adsorption isotherms and kinetics, *Colloids and Surfaces B: Biointerfaces*. 2013, vol. 102, pp. 195- 201.

[13] HWANG, M. J., KIM, O. H., SHIM, W. G., MOON, H. Adsorption of BSA on monodispersed hollow silica nanospheres, *Microporous and Mesoporous Materials*. 2013, vol. 182, pp. 81-86.

[14] LEMRASKI, E. G., SHARAFINIA, S. Kinetics, equilibrium and thermodynamics studies of Pb2+ adsorption, *Journal of Molecular Liquids*. 2016, vol. 219, pp. 482-492.

[15] KOPAC, T., BOZGEYIK, K. EQUILIBRIUM, Kinetics, and Thermodynamics of Bovine Serum Albumin Adsorption on Single-Walled Carbon Nanotubes, *Chemical Engineering Communications*. 2016, vol. 203, no. 9, pp. 1198-1206.

[16] TARABA, B. Immobilisation of lead(II) ions from aqueous solutions on natural coal. Comparison of calculated and measured adsorption enthalpies, *Journal of Thermal Analysis and Calorimetry,* 2013, vol. 112, no. 3, pp. 1559-1563.

[17] MORIYAMA, Y., KAWASAKA, Y., TAKEDA, K. Protective effect of small amounts of sodium dodecyl sulfate on the helical structure of bovine serum albumin in thermal denaturation, *Journal of Colloid and Interface Science*. 2003, vol. 257, no. 1, pp. 41-46.