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Impact of lysozyme on stability mechanism of nanozirconia aqueous suspension

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ABSTRACT

The effect of lysozyme (LSZ) presence on the zirconium(IV) oxide (ZrO_2) aqueous suspension stability was examined. The applied zirconia contains mesopores (with a diameter about 30 nm) and its mean particle size is about 100 nm. To determine the stability mechanism of ZrO_2 suspension in the biopolymer presence, the adsorption and electrokinetic (surface charge density and zeta potential) measurements were performed in the pH range 3–10. The lysozyme adsorption on the nanozirconia surface proceeds mainly through electrostatic forces. Under solid-polymer repulsion conditions, there is no adsorption of lysozyme (pH < 6, C_{NaCl} 0.01 mol/dm^3). The increase of solution ionic strength to 0.2 mol/dm^3 causes screening of unfavourable forces and biopolymer adsorption becomes possible. The LSZ addition to the ZrO_2 suspension influences its stability. At pH 3, 4.6 and 7.6, slight improvement of the system stability was obtained. In turn, at pH 9 considerable destabilization of nanozirconia particles covered by polymeric layers occurs.

1. Introduction

Protein adsorption on the metal oxide surface plays an essential role as for many practical applications of such systems. In recent years great scientific interest has focused on the possibility of mineral oxides usage in drug-delivery systems [1] in pharmacy, in dentistry, orthopedics and cardiology [2–4], as well as in implantation process in medicine [5]. The protein adsorption occurs immediately after the implantation and it enables the cell interaction with the implant surface (implant acceptance or rejection). The excellent biocompatibility with the organism, corrosion resistance, high mechanical strength and low chemical activity of oxide materials [6] contribute to their wide application in those areas of human activity.

Metal oxides are also used in food processing as adsorbents of undesirable proteins in the clarification of beer and wine. Their application effectively improves the quality of product leading to significant reduction of its turbidity [7].

Additionally, the adsorption of proteins is an important process in biogeochemical cycles. The main source of proteins in soil is degradation of organic residues by microorganisms [8].

Classification of proteins taking into account their functions in the organism, includes the following types of these biomolecules: enzymatic (e.g. lysozyme), structural (e.g. keratin), transport (e.g. hemoglobin), motor (e.g. actin), storage (e.g. ovalbumin), signal (e.g. hormones), regulatory (e.g. troponin) and receptors (e.g. thyroid hormone receptors) [9]. Almost all proteins include twenty different amino acid types connected by peptide bonds. Their sequence in the linear polypeptide chains is different for various proteins. These biomolecules form specific three-dimensional structures which are responsible for essential biological functions in organism [10].

Lysozyme (LSZ), used in the present study as an adsorbate, is the enzymatic protein of cationic character. It hydrolyzes the β-1,4-glycosidic bonds between *N*-acetylmuramic acid and *N*-glucosamine (bacterial cell wall degradation). Lysozyme occurs mainly in polynuclear granulocytes, monocytes and macrophages (in most tissue fluids such as tears, saliva) [11]. Because of antibactericidal properties, lysozyme is used as a food preservative [12] and as an ingredient of medicines [13,14].

The aim of the present paper is determination of stability mechanism of mesoporous zirconia nanoparticles dispersed in aqueous solution after the lysozyme addition. For this purpose, the biopolymer adsorption as well as the parameters characterizing the electrical double layer formed on the ZrO_2 nanoparticles in the LSZ presence were specified. The influence of pH and ionic

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strength of the solution was examined. In the literature there are many reports describing lysozyme adsorption on the solid surfaces [15–20]. Nevertheless, the application of turbidimetry method for suspension stability determination and explanation of stability mechanism of nanozirconia suspension containing lysozyme is novel. This method enables suspension stability estimation on the basis of Turbiscan Stability Index TSI, which is calculated from light backscattering data. On the other hand, application of zirconia characterized by nanoscale size of pores and solid grain (about 30 and 100 nm, respectively) is important for the practical use of such systems. Such size of pores enables protein molecules penetration into their interior leading to LZS adsorption increase in relation to nonporous and microporous materials [21]. The obtained results may be helpful in the development of modern implant coatings (as scaffold for tissue regeneration) [22,23]. Moreover, they may be essential in environmental engineering for development of new procedures of undesirable macromolecules removal from wastewaters. Other mesoporous materials play a significant role in ecology as effective adsorbents of poisonous gases and substances [24–27].

2. Methods

Zirconium(IV) oxide (ZrO_2, zirconia), produced by Sigma-Aldrich, was used as an adsorbent. It is a mesoporous material with the average pore diameter 31 nm, specific surface area – 21.7 m^2/g and the particle average size about 100 nm (nanooxide). The adsorbent pore diameter and surface area were determined by the BET method using the ASAP 2405 analyzer (Micrometritics) whereas the particle size was established using a mastersizer 2000 (Malvern Instruments).

Lysozyme (LSZ) from chicken egg white (Sigma-Aldrich) was used as an adsorbate. This is an enzymatic protein with the molecular weight 14.3 kDa and the isoeleectric point (pI) about 11 [28,29]. It means that almost in the whole range of examined pH (3–10), the lysozyme macromolecules are positively charged. LSZ is classified as a protein of high internal stability (so called "hard" protein) [29].

All measurements were performed at 25 °C using sodium chloride as a supporting electrolyte. The adsorption and stability measurements were made as a function of solution pH value (3; 4.6; 6 and 9) and at the NaCl concentration 0.01 mol/dm^3. The adsorption experiments were also performed with higher ionic strength of solution (i.e. 0.1 and 0.2 mol/dm^3).

The adsorbed amount of biopolymer on the zirconia surface was determined based on the protein concentration difference in the solution before and after the adsorption process. At the beginning, the samples containing 0.045 g of ZrO_2 and LSZ (50, 100, 150, 200, 300, 400 and 500 ppm) were prepared. Then the appropriate suspension pH was adjusted using a pH-meter (Beckman Instruments). The adsorption process was conducted for eight hours (until the equilibrium was reached) under the conditions of continuous shaking (shaker Unimax 1010, Heidolph). Kinetic measurements of lysozyme adsorption on the zirconium(IV) oxide surface showed that at neutral pH (about 5) the examined system reaches equilibrium after 8 h. After that the samples were centrifuged twice using a microcentrifuge (MPW Med. Instruments) and the supernatants were collected for quantification. The LSZ concentration was determined spectrophotometrically (spectrophotometer UV–vis Cary 100, Agilent Technology) at the wavelength 280 nm. A single result was the average of three repetitions. The measurement error did not exceed 5%.

The zirconia surface charge density (σ_0) without and with lysozyme was calculated using the computer program "titr_v3" developed by W. Janusz from potentiometric titration data. The measuring set consists of: teflon thermostated vessel, water thermostat RE 204 (*Lauda*), glass and calomel electrodes (*Beckman Instruments*), pH-meter PHM 240 (*Radiometer*), automatic microburette Dosimat 765 (*Metrohm*), PC and printer. The surface charge density was determined from the difference in the base volume added to a suspension with polymer and that containing supporting electrolyte, to achieve a given pH value [30].

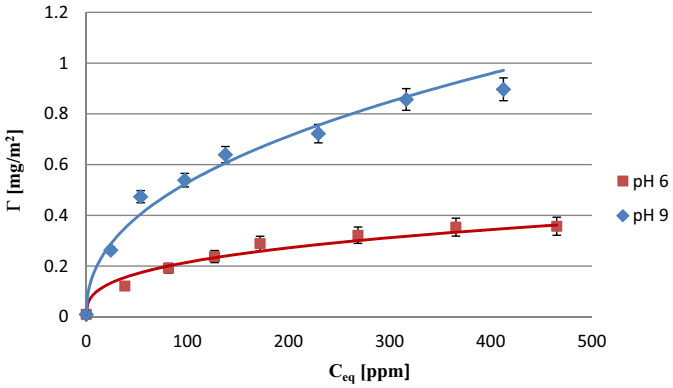

Fig. 1. Adsorption isotherms of LSZ on the ZrO_2 surface at various pH values (C_{NaCl} 0.01 mol/dm^3).

Initially the potentiometric titration of the supporting electrolyte was performed (the reference curve was obtained). Then the zirconia suspensions in the absence and presence of LSZ (with the concentrations 50 and 100 ppm) were titrated. The thermostated vessel was filled with 50 cm^3 of the appropriate solution and then 0.8 g of the solid was added. The systems were titrated using NaOH with the concentration 0.1 mol/dm^3. These experiments were performed in the pH range 3–11.

The electrokinetic (zeta) potential measurements were performed using a zetameter Nano ZS (Malvern Instruments) with the automatic titrator MPT-2.

First, zeta potential of the zirconia particles in supporting electrolyte was measured. Next the suspensions containing the protein (with the concentration 100 or 500 ppm) were examined. All analyzed systems were prepared by adding 0.0075 g of the adsorbent to the appropriate solution. Each sample was sonicated for 3 min applying an ultrasonicator XL 2020 (Misonix) and then the solution pH was adjusted. The examined pH range was 3–11.

The stability measurements were performed using a turbidimeter Turbiscan Lab^{Expert} with a cooling module TLab Cooling. The obtained results are presented as curves of transmission and backscattering of light beam (850 nm) passing through the sample during the measurement. Based on these data the stability coefficient – Turbiscan Stability Index (TSI) was calculated numerically from the following equation:

$$TSI = \sqrt{\frac{\sum_{i=1}^{n}(x_i - x_{BS})^2}{n-1}} \quad (1)$$

where: x_i – the average backscattering for each minute of measurement, x_{BS} – the average x_i value, n – the scans number.

The single stability measurement lasted 3 h during which the relevant data (so called scans) was recorded every 5 min. First 0.015 g of ZrO_2 was added to the supporting electrolyte solution. In the next step, the system was sonicated for 3 min. LSZ was added to the suspension and the solution pH value was adjusted just before the measurement. The final LSZ concentration was 100 ppm.

3. Results and discussion

Fig. 1 presents the adsorption isotherms of LSZ on the nanozirconia surface for the supporting electrolyte concentration equal to 0.01 mol/dm^3. As can be seen biopolymer adsorption reaches the level about 0.35 mg/m^2 at pH 6, whereas at pH 9 the adsorbed