Investigation of interaction between biogenic selenium nanoparticles and human serum albumin using microwave plasma optical emission spectrometry operating in a single-particle mode

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Abstract Characterization of selenium nanoparticles and selenium nanoparticle–human serum albumin conjugates prepared in nanopowder form, their elemental composition, and particle size distribution were investigated with a microwave plasma optical emission spectrometry operating in a single-particle mode. This new analytical technique was used for the first time to examine the molecular interaction between selenium nanoparticles and human serum albumin regarding potential biomedical applications of selenium nanoparticles. Nanopowder sample was introduced to a helium plasma by pneumatic nebulization based on fluidized bed approach and measured with a time resolution of 20 ms. Both selenium nanoparticles and selenium nanoparticle–human serum albumin conjugates were characterized by observation of synchronous signals from different particle components. Plots of the time correlation between Se and C signals for all particles in selenium nanoparticles and selenium nanoparticle–human serum albumin conjugates samples differed from each other in degree of correlation and synchronicity of recorded signals. The interaction between selenium nanoparticles and human serum albumin was confirmed using Bradford assay. For selenium nanoparticles synthesized using yeast cells, the percentage of bound protein was only of 4%, whereas for selenium nanoparticles synthesized using yeast extract as a stabilizing agent it was 16%.

Graphic abstract

Keywords Nanochemistry · Peptides · Bonding · Green chemistry

Introduction Selenium nanoparticles have attracted significant interest as a potential source of the element for living organisms being an alternative to common forms of selenium. It is noteworthy that the bioavailability reported for both inorganic selenium species and selenium-containing amino acids is definitely lower than that for nanometer-sized elemental selenium. Moreover, selenium nanoparticles exhibit lower toxicity comparing with other common selenium chemical forms [1–3]. Recent studies reported that selenium nanoparticles are more attractive than any other nanoparticles due to their potential application including drug delivery, targeted therapy, and heavy metal detoxification [4–6].

The interaction between these particles and serum proteins leads to the formation of protein corona on selenium
nanoparticle surface. Due to their interaction with proteins, the changes in core and surface chemistry of selenium nanoparticles can be observed. A wide range of applications of selenium nanoparticles in biomedical field calls for both understanding its physicochemical properties in biological fluids and investigating the effect of protein corona formation on particle surface. The nanoparticle–protein conjugates may increase the bioavailability and mobility, and decrease the toxicity of native nanoparticles in biological fluids [7–9].

Human serum albumin is biodegradable, biocompatible, and non-immunogenic protein carrier, which protect drug molecules from degradation, increase drug absorption, and improve cellular uptake and intracellular distribution. To understand the biocompatibility of nanoparticles, potential interactions of human serum albumin with nanoparticles should be investigated [10, 11].

Due to ability of human serum albumin to deliver drugs to their target cells, human serum albumin has been used as a model protein in many different studies. Monteiro-Riviere et al. [12] have demonstrated the human serum albumin corona formation on citrate-stabilized and silica-coated silver nanoparticles of different size, and determined cellular uptake in human epidermal keratinocyte cells. Another study showed that cellular uptake of nanoparticles strongly depends on the presence and the physicochemical properties of a protein adsorption layer covered these nanoparticles. For example, human serum albumin modified by succinic anhydride showed a threefold decreased binding affinity toward dihydrolipoic acid-coated ZnS quantum dots, whereas human serum albumin modified by ethylenediamine increased binding affinity towards the nanoparticles. These physicochemical changes of protein corona affect biological responses [13]. Hosseinifar et al. reported that human serum albumin is an ideal component to fabricate nanocarriers for drug delivery systems. These type of nanoparticles can be accumulated in tumor interstitium due to the enhanced permeability and retention effect [14]. In turn, Chakraborty et al. found that the application potential of selenium nanoparticles depends on their surface functionalization, thus net predominant negative surface potential of human serum albumin promoted their binding to positively charged CTA + group of selenium nanoparticles stabilized by cationic cetyltrimethylammonium bromide. Moreover, they showed that human serum albumin corona enhanced the antioxidant activity of selenium nanoparticles. Additionally, protein corona formation over functionalized selenium nanoparticles causes increasing of their size [15, 16]. On the other hand, Prasanth and Sudarsanakumar elucidated the interaction of L-cysteine-capped selenium nanoparticles and human serum albumin using spectroscopic and thermodynamic analysis. They observed a change in the microenvironment and the conformation of human serum albumin due to the addition of selenium nanoparticles [17].

While the interaction of nanoparticles with human serum albumin has been long reported, little has been published about the characterization of selenium nanoparticle–human serum albumin conjugates. Moreover, the characterization of nanoparticles–protein interaction still requires advanced analytical methods which allow for the determination of both elemental composition and structural information of selenium nanoparticles.

The goal of this study was to investigate the chemical composition and size distribution of selenium nanoparticle–human serum albumin conjugates prepared in nanopowder form and subsequently characterized by single particle microwave plasma optical emission spectrometry [18]. The feasibility of single particle microwave plasma optical emission spectrometry as a promising tool for detection and sizing of selenium nanoparticles and selenium nanoparticle–human serum albumin conjugates, providing information about their elemental composition, size, dispersity, and agglomeration or aggregation is described for the first time.

Results and discussion

Optimization of measurement conditions

The single-particle microwave plasma optical emission spectrometry technique allows complete characterization of selenium nanoparticles and selenium nanoparticle–human serum albumin conjugates after their preparation as nanopowders. Particle identification and determination of its elemental composition for both selenium nanoparticle and conjugate can be achieved under optimized experimental conditions. The nanoparticles transport efficiency and signal integration time are the most important parameters affecting data acquisition. To obtain representative signal for a single selenium nanoparticle, an integration time ranging from 5 to 50 ms should be used and transport efficiency should be lower than 50 particles per second [18]. The carrier gas flow rate directly affects nanoparticles transport efficiency. For helium, which was used as a carrier gas, the effect of gas flow rate on transient signal was examined in the range 70–190 cm³ min⁻¹. The representative, short transient signal as generated by single-particle microwave plasma optical emission spectrometry was obtained at 120 cm³ min⁻¹. The integration time of 20 ms was selected as a compromise to keep satisfactory temporal resolution, avoid split particle events and obtain suitable signal intensity.

For a given element being a nanoparticle component, the signal intensity at the selected wavelength can be used for converting single-particle microwave plasma optical emission spectrometry data into the nanoparticle size. Next, raw data of pulse intensity versus time are converted to determine particle size distribution. A plot of the time correlation
between signals for two different elements in a sample enables determination of elemental composition of each particle passing through the plasma by the examination of synchronicity of signals from selected elements.

Quantification of protein corona formation on functionalized selenium nanoparticles: preliminary study using Bradford assay

In this study, biogenic selenium nanoparticles were incubated with human serum albumin for 3 h, according to the previous report where a progress in corona formation was observed within 4 h, and there was no significant change during further incubation [15]. Protein adsorption in terms of percentage efficiency and unbound protein concentration were determined using Bradford assay. In Table 1, the concentration of bound protein in mg cm\(^{-3}\) represents the protein corona bound to different functionalized selenium nanoparticles.

Human serum albumin binding to biogenic selenium nanoparticles synthesized using yeast extract as a reducing and stabilizing agent (Synthesis 1) was more effective than that to biogenic selenium nanoparticles synthesized using living yeast cells (Synthesis 2), and the percentage efficiency of adsorbed protein on selenium nanoparticles surface was 16% and 4%, respectively. In similar reports, it was suggested that in the case of protein corona formation onto selenium nanoparticles under physiological conditions, the variation in the adsorption of protein over functionalized selenium nanoparticles was due to electrostatic attraction and other adsorption mechanisms, such as Van der Waals interactions, hydrogen bonding, and direct chemical bonding that can influence the protein corona formation [15,19,20].

Yeast extract is a mixture of amino acids, peptides, water soluble vitamins, and carbohydrates, which can play an important role in reducing and stabilizing process of selenium precursor, and subsequently they functionalize the selenium nanoparticles surface [21,22]. For biogenic selenium nanoparticles synthesized using living yeast cells, both proteins present in the cell and other cellular biomolecules such as saccharides can cover selenium nanoparticles surface and determine their physico-chemical properties [21,23–25]. The human serum albumin adsorption on such functionalized selenium nanoparticles is lower comparing with selenium nanoparticles functionalized by yeast extract compounds. This is likely because the selenium nanoparticles functionalized by cell proteins are unable to multiple layering of proteins [15]. Moreover, the proteins covering selenium nanoparticles after biosynthesis can express negative charge which subsequently enhances the electrostatic repulsion with negatively charged human serum albumin protein. On the other hand, higher binding of human serum albumin to selenium nanoparticles synthesized using Synthesis 1 protocol, comparing with Synthesis 2, may be explained due to the presence of different stabilizing agents on selenium nanoparticles surface which can exhibit more stabilizing effect on the protein structure.

To investigate the interaction between selenium nanoparticles synthesized using Saccharomyces cerevisiae var. boulardii yeast cells and human serum albumin, optimization of selenium nanoparticles concentration and their incubation time with human serum albumin are required. Thus, selenium nanoparticles synthesized using yeast extract have been chosen as a model objects for both selenium nanoparticles characterization by single-particle microwave plasma optical emission spectrometry and investigation of their interaction with human serum albumin.

Characterization of functionalized selenium nanoparticles using single-particle microwave plasma optical emission spectrometry

The size, elemental composition, and macroscopic properties of the single nanoparticle including agglomeration were evaluated using single particle microwave plasma optical emission spectrometry. Most of the biomolecules present in the yeast extract, which can functionalize selenium nanoparticles surface, contain carbon in their structure. Thus, the light emission from carbon atoms can be used for identification of functionalizing groups and for determination of elemental composition of functionalized selenium nanoparticles. Each element of the particle can be determined at its characteristic wavelength. The emission signals for Se and C from functionalized selenium nanoparticles have been measured at their specific wavelengths and acquired with a time-resolved manner, where each pulse corresponds to one particle only. Typical time scans for Se and C in selenium nanopowder sample recorded in a single-particle mode and at the integration time of 20 ms are shown in Fig. 1. In both cases, regular pulse distribution was observed.

The nanopowder composition was evaluated by examining the synchronicity of Se and C pulses. By comparing Se/C intensity ratio for all synchronous pulses, it is possible to confirm the reproducibility of elemental composition particle-by-particle for the whole sample. A plot of the time correlation between Se and C signals for all particles

| Selenium nanoparticles | \(C\) (human serum albumin)/mg cm\(^{-3}\) | Initial | Unbound | Protein corona |
|------------------------|------------------------------------------|---------|---------|----------------|
| Synthesis 1            | 5                                       | 4.2     | 0.8 (16%)|                |
| Synthesis 2            | 5                                       | 4.8     | 0.2 (4%) |                |
in selenium nanoparticles sample shows that the signals are synchronous, well correlated and size dependent for all particles (Fig. 2a). Obtained results indicate that each selenium nanoparticle was covered by molecules containing carbon atoms in their structure and that the amount of functionalized groups strongly depends on the selenium nanoparticle size.

The use of scanning electron microscopic images of the nanoparticles enables determination of their mean size, and subsequently the correlation between the particle size and the signal intensity measured by optical emission spectrometry. Then, the particle size can be evaluated by conversion of signals intensities after entering of detection threshold value to differentiate particles from the background using scanning electron microscopic images to verify the mean particles size. The particle size determined as a mean diameter, since all nanoparticles were nearly spherical, was to be 70 nm. The C signal intensity distribution displayed in Fig. 2b implies that the particle size distribution is monodisperse, because C signal intensities, which determine nanoparticle hydrodynamic diameter, were in the narrow range from 950 to 1250 cps (the signal intensity I is a function of particle diameter $d$ as $I \approx d^2$).

**Characterization of selenium nanoparticle–human serum albumin conjugates using single-particle microwave plasma optical emission spectrometry**

To determine elemental composition of selenium nanoparticle–human serum albumin conjugates, the correlation between Se and C as an indicator of both functionalizing groups and human serum albumin on the selenium nanoparticle surface, was examined. Typical time scans for Se and C are shown in Fig. 3. The elemental composition and presence of selenium nanoparticle–human serum albumin conjugates were evaluated by the observation of synchronized pulses distribution. The raw data for selenium nanoparticle–human serum albumin conjugates sample indicate both the presence of synchronous pulses with intensities similar to those recorded for biogenic selenium nanoparticles (1400–3000
counts for Se and up to 1250 for C as shown in Fig. 1) which represent the selenium nanoparticles in the sample, and a number of large pulses with intensities of up to 5000 cps for Se and up to 1550 cps for C, which are related to selenium nanoparticle–human serum albumin conjugates and agglomerates (Fig. 3). These results confirmed corona protein formation on selenium nanoparticles surface. Moreover, several reports suggest that formation of protein corona can increase the size of core nanoparticles depending on both nanoparticles–protein interaction and protein–protein interaction [15,16,26]. The increase of size of core nanoparticles can be confirmed by observation of higher Se-based intensity. On the other hand, Machat et al. observed [27] the signal enhancement for Se in the presence of carbon when determined by ICP-OES caused by efficient energy transfer from C ions to Se atoms. Thus, to identify the presence of selenium nanoparticle–human serum albumin conjugates, the C signal intensity distribution was used.

A plot of the time correlation between Se and C signals for all particles in selenium nanoparticle–human serum albumin conjugates sample shows that the signals are synchronous and size dependent. However, the correlation for selenium nanoparticle–human serum albumin conjugate sample is worse (the correlation factor $R^2$ is 0.6449) than that for biogenic selenium nanoparticles sample (the correlation factor $R^2$ is 0.7391), likely, due to accidental attachment of human serum albumin proteins onto the nanoparticles surface. Moreover, data points from selenium nanoparticles and selenium nanoparticle–human serum albumin conjugates are both displayed in Fig. 4a that also worsens the correlation for synchronous pulses. The slopes of the two correlations graphs (Figs. 2a, 4a) do not differ significantly from each other because carbon present in protein structure is usually more difficult to atomize and excite due to the complex protein structure. On the other hand, the shift factors of presented correlations (829.26 vs. 1007.3) being significantly higher in the presence of selenium nanoparticle–human serum albumin conjugates due to the higher number of C atoms per selenium nanoparticle. The data points observed clearly above trend line displayed in Fig. 4a and occurred

![Fig. 3](image1) Time scans based on Se (left) and C (right) signals for sample containing selenium nanoparticle–human serum albumin conjugates

![Fig. 4](image2) a Correlation between C and Se events for selenium nanoparticle–human serum albumin conjugates nanopowder sample; b carbon signal intensity distribution plot for selenium nanoparticle–human serum albumin conjugates nanopowder sample
in the entire intensity range for Se, come from the selenium nanoparticle–human serum albumin conjugates. In particular, the data points of relatively low Se signal intensity between 2000 and 3500 cps and relatively high C signal intensity exceeding 1200 cps has to be attributed to the conjugates. Interestingly, the formation of conjugates does not depend on selenium nanoparticle size.

It is clear from the C signal intensity distribution displayed in Fig. 4b that human serum albumin molecules interact with selenium nanoparticles and the equivalent carbon particle size of selenium nanoparticle–human serum albumin conjugates is bigger comparing with selenium nanoparticles (C signal intensities are observed in the range between 1100 and 1550 cps). In addition, the signal intensity distribution shows bimodal shape with low peak at about 1400 cps indicating possible agglomeration of particles as a potential side-effect of selenium particle–human serum albumin interaction.

The use of multi-element specific detection by microwave plasma optical emission spectrometry enables determination of elemental composition of complex nanomaterials. Thus, single particle microwave plasma optical emission spectrometry was also applied to investigate the differences in nanoparticles composition after incubation under physiological conditions in the presence of Ringer’s solution. Both, monovalent Na+ and divalent Ca²⁺ cations exhibit ionic interactions with selenium nanoparticle–human serum albumin conjugates and subsequently balance the charge of the particles. Signals correlation between sample components show that both Ca and Na signals are synchronous with Se signal, whereas size-dependent effect was observed for correlation between Ca and Se only (Fig. 5). On the other hand, competition effect between cations can be observed. Similar effect between different cations was observed by Jain et al. who reported that Zn²⁺ ions adsorption on the selenium nanoparticles was largely unaffected by the presence of Na⁺, whereas the presence of Ca²⁺ inhibited adsorption of Zn²⁺ [28].

Conclusion

The characterization of functionalized nanoparticles and examination of interaction between selenium nanoparticle and human serum albumin is feasible, due to capability of single particle microwave plasma optical emission spectrometry for the simultaneous multi-element detection. The plots of the time correlation between Se and C signals for all particles in selenium nanoparticles and selenium nanoparticle–human serum albumin conjugates samples showed the differences in correlation and synchronicity of recorded signals between them. The application potential of selenium nanoparticles strongly depends on their surface functionalization and ability to form protein corona. However, different functionalizing species may compete on the nanoparticle surface and affect protein corona formation considerably. Biogenic selenium nanoparticles produced in microwave-assisted synthesis using yeast extract can form conjugates with human serum albumin four times more effectively than selenium nanoparticles synthesized using living yeast cells. Thus, the former show potential application in many biomedical fields including drug delivery, targeted therapy, and heavy metal detoxification.

![Fig. 5](image_url) Correlation between Se and both Ca (left) and Na (right) events for selenium nanoparticle–human serum albumin conjugates nanopowder sample

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Experimental

Human serum albumin, selenium dioxide, yeast extract, and Bradford reagent were purchased from Sigma-Aldrich. For synthesis of selenium nanoparticles using yeast *Saccharomyces cerevisiae var. boulardii* and yeast extract, Se(IV) stock solution (4 mg cm⁻³) was prepared by dissolving selenium dioxide in water with small amount of NaOH. The solution was slightly alkaline with an approximate pH value in the range 10–12, and the pH was not further adjusted. Ringer’s solution used as a simulated body fluid for incubation of human serum albumin with selenium nanoparticles was obtained by dissolving in water the aliquots of NaCl, KCl, and CaCl₂ (POCh, Poland) to obtain the final concentration of 8.6, 0.3, and 0.33 g dm⁻³, respectively. Pure helium (99.999%) (Multax, Warsaw, Poland) was used as plasma and carrier gas.

Microwave-assisted synthesis of biogenic selenium nanoparticles (Synthesis 1)

Selenium nanoparticles were synthesized in slightly alkaline conditions using microwave-assisted approach based on the reduction of selenite with yeast extract which also acted as stabilizing agent. Specifically, 0.2 g of yeast extract was placed in a 100 cm³ PTFE vessel and 9.6 cm³ of distilled water was poured in. Next, 0.4 cm³ of 4 mg cm⁻³ Se(IV) standard solution was added. A microwave-assisted procedure involving sample heating to approximately 110 °C in one step within 10 min. Under experimental conditions, the initial reaction mixture was lightly yellow due to the yeast extract presence. After reaction, the color turned red, indicating the initial formation of selenium nanoparticles. After microwave heating, the sample suspension was allowed to cool to room temperature and subsequently centrifuged at 4000 rpm for 20 min and the obtained pellet was washed two times with distilled water. One portion of the obtained pellet was subjected to investigate the interaction with human serum albumin. The second portion was lyophilized and subjected to single particle microwave plasma optical emission spectrometry analysis.

Biogenic selenium nanoparticles synthesis using yeast cell *Saccharomyces cerevisiae var. boulardii* (Synthesis 2)

Biogenic selenium nanoparticles were synthesized, extracted, and purified according to approach described previously [21]. Briefly, selenium nanoparticles were synthesized using living yeast cells *Saccharomyces cerevisiae var. boulardii*. The color of the culture changed from colorless to orange-red after addition of selenium solution with a final concentration of selenium equal to 158 mg dm⁻³. Yeast cells were harvested by centrifugation (10000 rpm for 10 min). The obtained pellets were washed three times with water. Next, the yeast cells were disrupted by sonication for 15 min and selenium nanoparticles were extracted and purified using organic-aqueous approach (n-hexane–water). Purified selenium nanoparticles were subjected to investigate the interaction with human serum albumin.

Analysis of protein corona formed on selenium nanoparticles surface

Selenium nanoparticles, protein, and Ringer’s solution were mixed together with final concentration of 5 mg cm⁻³ for human serum albumin and incubated at 37 °C for 3 h. Next, sample was centrifuged at 4000 rpm for 10 min. The obtained pellets were washed two times with distilled water, then lyophilized and subjected to single particle microwave plasma optical emission spectrometry analysis and the supernatant was collected for determination of unbound human serum albumin by Bradford assay.

Determination of unbound proteins using Bradford assay

The amount of unbound protein in the supernatant was quantified after centrifugation and dilution by adding Bradford reagent and reading the absorbance at 595 nm. The functionalyzed selenium nanoparticles solution without any protein addition was similarly treated as the control. Briefly, protein standards of appropriate concentration were prepared by serial dilution of the 2 mg cm⁻³ human serum albumin solution. The Bradford assay was performed directly in a cuvette by adding 1.5 cm³ of Bradford reagent to 50 mm³ of sample solution. Next, the sample was gently mixed and incubated at room temperature for 10 min and the absorbance was measured.

Instrumentation

Absorbance measurements for Bradford assay were done using a V-730 UV–Vis spectrophotometer (JASCO, Germany) with plastic cuvettes of 1 cm optical path. Selenium nanoparticles and selenium nanoparticle–human serum albumin conjugates were characterized using single-particle microwave plasma optical emission spectrometry described in detail in our previous study [18]. The single-particle microwave plasma optical emission spectrometry experimental set-up consisted of six-phase rotating field 2.45 GHz microwave plasma source (Ertec-Poland, Wroclaw, Poland) as an excitation source and miniature optical spectrometer AvaSpec-2048XL with Optical Fiber FC-UV800-2-ME-SR...
(Avantes, Netherland) operating in time resolved analysis (TRA) mode as an OES detection system. The helium microwave plasma source was operated at optimized power of 300 W and gas flows were at the following settings: plasma 700 cm$^3$ min$^{-1}$, carrier 120 cm$^3$ min$^{-1}$. Single-particle data, acquired with the integration time of 20 ms, were processed in Microsoft Excel for the determination of particle size and selection of synchronous signals from different particle components. The selected wavelengths for monitored analytes were 203.98, 247.76, 317.95, and 330.21 nm for Se, C, Ca, and Na, respectively.

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