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Radiosynthesis of Gold/Albumin Core/shell Nanoparticles for Biomedical Applications

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ABSTRACT

Gold/albumin *core/shell* nanoparticles (Au/AlbNPs) was prepared by a novel aggregation/crosslinking technique and characterized by several spectroscopic and microscopy methods. Albumin, in presence of gold nanoparticles (AuNPs), is aggregated by the addition of ethanol and further stabilized by radiation-induced crosslinking using a ^{60}Co source. Nanoconstructs are characterized to determine size, morphology and optical characteristics. The Au/AlbNPs were prepared in different ethanol and albumins concentrations. Results showed that it is possible to obtain Au/AlbNPs using ethanol 30 %v/v, albumin in different concentrations and an irradiation dose of 10 kGy. Au/AlbNP plasmon peak shifted to 530 nm, keeping the typical plasmon peak shape. The size of Au/AlbNPs is approximately double respect to the naked AuNPs and they show core/*shell* type morphology. The main amide peaks of albumin in FTIR spectrum can be found in the spectrum of nanoconstructs.

INTRODUCTION

Breast cancer is the tumour with the highest incidence and mortality of women in the world; it is for this reason that many investigations are aimed to therapeutic drug design strategies for diagnosis and treatment. Tremendous advances have been made in the treatment, prevention and early detection of these malignancies; however none of the current therapies are specifically able to cover all the variants of this disease that differ in its histopathology characteristics and genomic and genetic variations [1]. In addition, many of the available drugs are not able to reach the site of metastases [2]. For this reason there is a new approach in the development of therapeutic strategies which allow high degree of specificity and spatial extent of the tumours even after metastasis spread. This approach is addressed by nanotechnology [2].

The use of nanotechnology in medicine, also called nanomedicine, is based on the generation of nanostructures, especially in round shape particles or nanoparticles (NPs). Many characteristics of these nanomedicines will depend on the size and surface properties of the NPs.

NPs size of currently used in anti-cancer therapy varies between 10-100 nm. An advantage of the use of NPs in such therapies is that the tumor vasculature has higher permeation for

macromolecules, in addition to the poorly functionality of lymphatic system in the surround media. NPs accumulate in tumors leading to phenomenon known as '*Effect of enhanced permeability and retention*' also called EPR [3,4].

There are different types of NPs for therapeutic applications according to their chemical composition. The first NPs reported were synthetized by conjugation of synthetic polymers with oncologic drugs, followed by liposomes and Albumin NP (AlbNP) were developed later [5]. In order to enhance the NP entrance into the intracellular milieu, compounds such as PEG were included. There are also NPs developed based on inorganic components such as gold, iron oxide and other metals [6]. Current NP therapeutic strategies are based on multifunctional properties, focused on combining both therapeutic and imaging agents within the same particle. For example, gold NPs (AuNPs) have two major advantages in this context; they are not able to undergo oxidation and can, very efficiently, transform electromagnetic energy (visible/NIR) into thermal energy. Furthermore, as a very stable and human body is capable of tolerating an amount of grams of this material without side-effects [7]. More recently, the possibility of using the isotope 198-Au as raw material to AuNP synthesis, can generate a nanomaterial with radioactive properties, which can emits beta and gamma radiation to the milieu, performing a theranostic tool (therapy and diagnosis properties) [8-10].

Our laboratory has been recently report a novel preparation of protein NPs using globular proteins, more specifically albumin (Alb). Previous results from our research group demonstrated Alb aggregation can be stabilized by intermolecular covalent crosslinking generated by hydrogen abstraction of free radicals generated by ionizing radiation [11]. Tailoring size can be done by changing the reaction medium with a yield higher than 90 %. However, and perhaps the most important property is that AlbNPs maintain the three dimensional structure of constitute proteins according to spectroscopic studies. Therefore, ionizing radiation is able to generate nanostructures in a simple and straightforward manner [12].

The aim of this work is described to novel NP synthesis which involves the preparation of a *core/shell* Au/AlbNPs. Thus, the novel NPs should have advantages of both materials, such as biocompatibility, drug transport and simple detection.

EXPERIMENTAL DETAILS

Gold (III) auric chloride hydrate ($HAuCl_4$. x H_2O) and bovine serum albumin, Fraction V (BSA) was obtained from Sigma Aldrich. Human serum albumin (HSA) was kindly donated by Laboratorio de Hemoterapia from Universidad Nacional de Córdoba. All other reagents were of analytical grade and used as received.

Synthesis of Au/AlbNPs was carried out in several steps. In the first steps, AuNPs are prepared according a standard recipe. Briefly, AuNPs were prepared from a chloroauric solution (1 mM) using as reducing agent sodium citrate, according to the method of Frens [13]. In the second steps, AuNPs were dispersed in different concentrations of albumins (BSA and HSA) from 0, 5, 10, 20 and 30 mg/mL protein. Then, different amounts of ethanol were added drop-wise onto the protein solution, keeping the temperature at 0 ºC under constant stirring. The water/ethanol suspensions containing Alb and AuNPs were irradiated with gamma-rays (10 kGy) of ^{60}Co source (PISI CNEA-Ezeiza) at a dose rate 1 kGy/h and keeping sample temperature in the range of 5-10 ºC during the irradiation.

Particle size was determined for two techniques; dynamic light scattering (DLS) and transmission electronic microscopy (TEM). For DLS, the samples were measured at 25 °C using

a 90Plus/Bi-MAS particle size analyzer, with a light source of 632.8 nm and a 10-mW laser. TEM pictures were captured in Centro de Microscopias Avanzadas (CMA) –FCEyN – University of Buenos Aires. All measurements were carried out on days 1 and 30 after sample preparation.

UV-visible and FTIR spectroscopies were performed to study the NPs. FTIR spectroscopy was run using a multireflexion ATR module. The spectra were recorded with 64 scans and a 1 cm^{-1} resolution. Data was analyzed by IR Solution Software. UV-vis spectroscopy was recorded with Shimadzu UV160U spectrophotometer.

RESULTS and DISCUSSION

The functionalization of the AuNP surfaces gives them solubility, stability and interaction with cells and / or other biomolecules. In addition to improve their physicochemical properties, the surface modification with proteins increases the biocompatibility compared to naked AuNPs. However, most of the proteins partially loss their native conformation when interact with the highly structured AuNP surface [14]. Therefore, the protein recognition sites available in their surface are partially loss by this interaction.

In order to improve the surface of nanostructured materials based on AuNPs, in this work it is proposed the preparation of AuNPs covered with a multilayer shell of albumin. The nanoconstruct is further stabilized by applying ionizing radiation, as is depicted in the Figure 1.

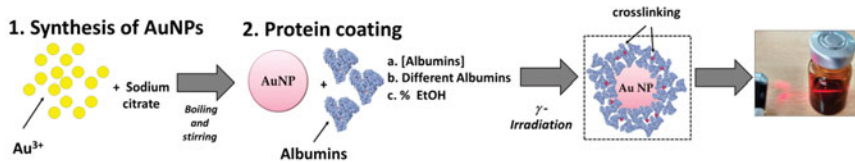

Figure 1. Scheme of the proposed NP preparation to yield a core/shell Au/Alb-NPs

In Figure 2a is shown a typical visible spectrum corresponding to the plasmon absorption band of AuNPs in aqueous solution. In Figure 2b is plotted the DLS histogram corresponding to the same sample. Also a TEM image (Figure 2c) was performed to validate the NP structure formation. The AuNPs size was 33 nm with a plasmon peak at 520 nm.

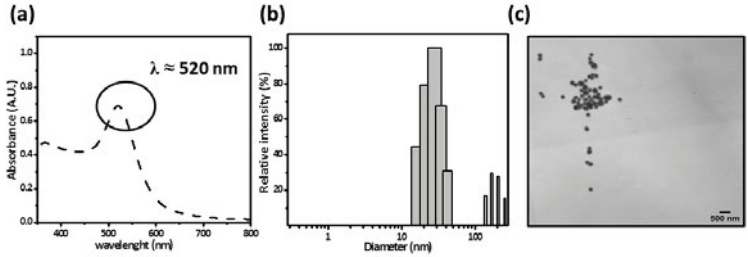

Figure 2. (a) Visible spectrum; (b) histogram of DLS and (c) TEM image of AuNPs

In order to analyse different parameters of core/shell synthesis, visible spectra were performed of ethanol suspensions of Alb and AuNPs after irradiation in a ^{60}Co source. In Figure 3 are shown spectra corresponding to suspension prepared with different ethanol proportions. Spectrum corresponding to 30 %v/v ethanol showed a small shift of the plasmon signal to higher wavelengths than naked (citrate capped) AuNPs. The plasmon peak of Au/Alb NPs has a maximum at 530 nm, approximately. This behaviour is assigned to small changes in the NP size by the adsorption of albumins onto their surfaces. At 40 %v/v ethanol suspension shows also a plasmon peak but with some distortions. By the addition of 50 %v/v of ethanol to the Au/Alb NPs suspension, the characteristic shape is loose. According to these results, only the suspension prepared in 30 %v/v ethanol yield well dispersed NPs. It has also been demonstrated a minimum of 30 %v/v ethanol is required to reach Alb aggregations to reach protein-NPs by the same experimental technique [12].

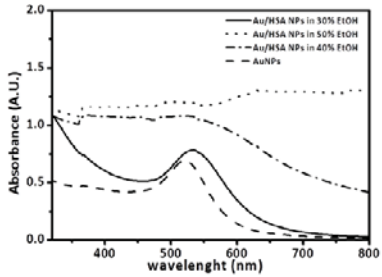

Figure 3. Visible spectra of irradiated Alb/AuNP suspension prepared with different ethanol concentrations. HSA: human serum albumin (Irradiation dose: 10 kGy)

In the next assays was studied the protein concentrations in the NP preparation and the influence of albumins from different sources. Considering the amount of protein added to the AuNP suspension, in Figure 4a are shown the spectra corresponding to irradiated samples prepared using an amount of AuNPs and increasing concentrations of Alb. No peak shifts were found in the range 5 to 30 mg/mL protein concentrations, commonly used in these preparations. Therefore, no change in the Au/proteinNP interaction is expected. However, a reduction in the plasmon intensity was recorded for higher protein concentrations. This behaviour has been also reported for other molecules [15,16]. In Figure 3b are showed the plasmon peaks corresponding to samples prepared with human, bovine and a mixture (1:1 w/w) of both albumins, respectively. Plasmon peaks did not show differences between them.

An Alb and AuNPs suspension prepared with Alb and ethanol (30 %v/v) according to the previous protocol and they were analysed by TEM microscopy and DLS. One sample was irradiated to induce the protein crosslinking. In order to visualize the proteins in TEM pictures, negative staining of uranyl acetate was performed. In Figure 5a is shown a TEM image with many black dots (AuNPs) with a halo around corresponding to proteins; confirming the core/shell type morphology. The average of NPs size determined from the picture analysis was 52 nm for these nanostructures.

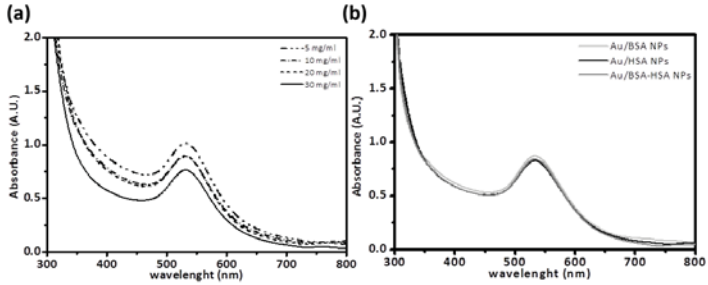

Figure 4. Visible spectra of Au/AlbNPs suspension prepared by irradiation (a) at different initial protein concentrations and (b) different albumin types (Ethanol: 30 %v/v; Irradiation dose: 10 kGy)

The sample analysis by DLS is shown in Figure 5b. The mayor particle distribution peak is centred at 60 nm of hydrodynamic diameter. The NP sizes determined by both techniques can be considered equal, considering in one case the sample is dry and in the other in solution. These results confirmed the presence of an albumin shell which increases the overall size of the NPs. Our approach demonstrated that it is possible to prepare Au/Alb core/shell NPs without the presence of chemical crosslinking agents.

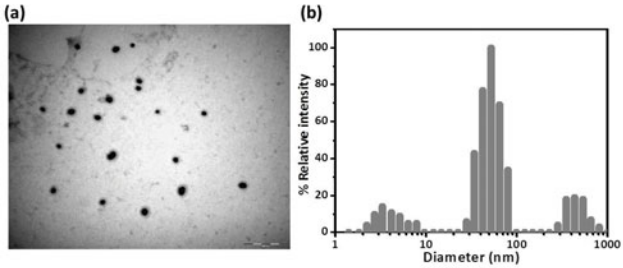

Figure 5. (a) TEM image and (b) histogram of DLS Au/Albumin NPs suspension (Ethanol: 30 %v/v; Irradiation dose: 10 kGy)

In order to analyse the protein shell of Au/AlbNPs, FT-IR spectroscopy were performed. It has been established that this technique is sensitive to detect protein conformational changes. In Figure 6 are showed BSA, AuNPs and Au/AlbNPs spectra. The spectrum of BSA has the characteristic peaks corresponding to amide I and II regions (1651-1604 cm^{-1} and 1539 cm^{-1}). Au/AlbNPs spectrum has the same amide peaks; which are not present in the AuNPs spectrum.

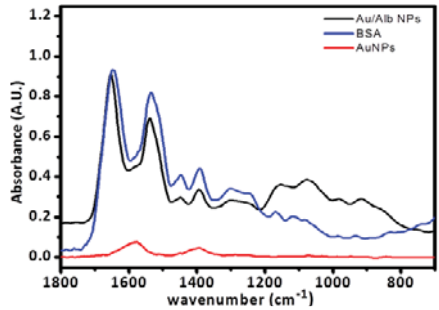

Figure 6. ATR- FTIR spectra of Au/AlbNPs (prepared with 30 %v/v ethanol, 10 kGy doses), AuNPs and BSA

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