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Albumin-based nanoparticle trehalose lyophilisation stress-down to preserve structure/function and enhanced binding

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ABSTRACT

The aim of this study was to preserve albumin nanoparticle structure/function during the lyophilisation process. Bovine serum albumin nanoparticles were obtained by γ-irradiation. Nanoparticles were lyophilised in buffer, miliQ water or in trehalose/miliQ solution. The size and charge of the nanoparticles were tested after lyophilisation by light scattering and Z potential. The most relevant results in size of BSA nanoparticle were those lyophilised in PBS between 20 and 350 nm, assembled in different aggregates, and negative Z potential obtained was 37 ± 8 mV in all, and those nanoparticles lyophilised with trehalose had a size range of 70 ± 2 nm and a negative Z potential of 20 ± 5 mV.

Structure determination of surface aminoacids SH groups in the BSA NP lyophilised in PBS showed an increase in the free SH groups. Different aggregates had different amount of SH groups exposure from 55 to 938 (from smaller to bigger aggregates), whereas BSA NP lyophilised with trehalose showed no significant difference if compared with BSA NP.

The binding properties of the BSA nanoparticle with a theragnostic probe (merocyanine 540) were studied after lyophilisation. Results showed more affinity between the BSA NP lyophilised with trehalose than that observed with non lyophilised BSA NP.

As a result, the lyophilisation condition in trehalose 100 μM solution is the best one to preserve the BSA NP structure/function and the one with the enhance binding affinity of the BSA NP.

1. Introduction

Bovine serum albumin (BSA) is widely used as drug delivery system because of its abundance, ease of purification, low cost, and ligand-binding properties [1]. Because of their physicochemical and biological properties, BSA nanoparticles (BSA NP) are considered a potential drug delivery system. BSA NPs obtained by Soto Espinoza et al. [2] by γ irradiation are 17–20 nm size. To date no lyophilisation process has been tested on the irradiated BSA NP. Other authors have reported lyophilisation of glutaraldehyde BSA NPs in presence of sugars like galactose and mannitol [3]. Others had obtained BSA or HSA nanoparticles lyophilised, but these were no clean nanoparticles [4]. Nevertheless, these authors use lyophilisation process as a mean of disposing off the solvent present, not as a way of long-term storage [5].

Lyophilisation, the process by which a frozen liquid is removed by sublimation, is used as a method to stabilise materials such as protein drugs and biotechnology products also used in long-term storage [6,7]. Proteins may suffer degradation or structure alteration during the process. To prevent this stabilisers are used, mainly, sugars [7,8]. These molecules are used to decrease stress during the lyophilisation process severe conditions [8]. Our purpose is to achieve a way of long-term storage, but imposing a condition that would maintain the functionality of the nanoparticle.

In this work, lyophilisation is considered successful when the NP preserved its structure/function after the process.

Different lyophilisation conditions are described to stress down the process conditions which is the objective of this study; (i) BSA NP in buffer solution without the use of any cryoprotector and (ii) BSA NP in miliQ water and (iii) BSA NP with increasing trehalose concentration solution. If condition (i) is an efficient way to lyophilise the BSA NP preserving its structure, it will be the best option to be considered being the simplest of them all.

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Kumar et al. [8] proposed the use of trehalose in order to preserve the structure/function of albumin by lyophilisation. Considering that our structure is a complex one and considering the exposure of certain groups, in this study we propose the use of trehalose in different concentrations based on the relation of the interaction of trehalose OH group and NH surface groups in the NP that do not produce BSA NP aggregation or water crystals formation giving rise to a hygroscopic sample.

Trehalose is a disaccharide formed by an α,α-1,1-glucoside bond between two α-glucose units. Not only does this sugar have the advantage of protecting cells from disruption, but can also act as an antioxidant. It is used as a cryoprotector for nanoparticles, because of its ability to preserve their original size and structure after freezing [6].

The cryoprotection property is given by its several polymorphisms in both solid and liquid state. Trehalose has two polymorphisms: an amorphous and a dehydrate trehalose state. The latter can be dehydrated into two different anhydrous molecules: α o β. Each of the non-water states are capable of hydration going back to the dehydrated trehalose, characteristic of a sugar useful as a cryoprotector. Moreover, trehalose can rearrange the water molecules and decrease the freezing-temperature point, allowing more stability to the molecule in question. Mechanisms are Vitric, Exclusion and Water Replacement Theories;

Vitrification Theory: trehalose has a transition temperature to a more vitric phase where no crystal is formed during the process. It is here, inside the lattice formed, that the molecule is trapped gaining stability.

Preferential Exclusion Theory: there is no direct interaction between the NP and sugar. Instead, the trehalose molecules entrap bulks of water in order to stabilise the molecule.

Water Replacement Theory: the interaction is given between the molecule and trehalose. Here the latter is able to replace the water molecules forming hydrogen bonds with the stabilized molecule.

The next step after confirming the NP structure preservation will be to study its functionality. A theragnostic probe like Merocyanine 540 (MC540) will be useful to answer questions on functionality [8]. This experiment also aims to study the binding properties of the NPs through their interaction with MC540.

MC540 is an anionic cyanine dye which absorbs light in visible region (main peak in 532 nm in water solutions). Moreover, it has a dual role of photosensibiliser as a bactericidal agent and an immune reaction regulator [8]. Also, owing to its structure and function, MC540 is used as an optical and fluorescent probe in biomembranes and proteins. The interaction between protein and probe is mainly given by its fluorescence intensity. In our study, BSA NPs were freeze dried in the presence of phosphate buffer or miliQ water (with and without trehalose), and after reconstitution were evaluated regarding their physicochemical characteristics. The working hypothesis is that BSA NP lyophilised in buffer PBS (pH 7.0; 30 mM) and/or BSA NP lyophilised with trehalose has well preserved structure/function.

2. Materials and methods

2.1. Materials

The BSA 98% (agarose gel electrophoresis) powder, lyophilised ~7.1% in 0.15 M NaCl was acquired from Sigma-Aldrich, Co., St. Louis, USA. The Merocyanine 540 was from Molecular Probes, Inc., Eugene, Oregon, USA. The buffer used was phosphate buffer saline (PBS). Ethanol HPLC grade was acquired from Sintorgan®, SA, Buenos Aires, Argentina.

The lyophilisation of the BSA nanoparticle was performed in a Labconco Freeze Dry System/Freezone 4, 5 (La Química Quirúrgica, Buenos Aires, Argentina). The light scattering and Z potential measurements were obtained in a Zetasizer Nano ZS Malvern Instruments Ltd., Worcs, Malvern, UK. The UV–vis measurements were obtained in a UV–160 A UV–vis Recording Spectrometer, Shimadzu Corporation, Nakagyo-ku, Japan. Graphic analyses were carried out with GraphPad Prism v.5.0.

2.2. Methods

2.2.1. Nanoparticle preparation

BSA NPs were prepared according to Soto Espinoza et al. [2]. Briefly, BSA was dissolved in PBS (pH 7.0; 30 mM). Ethanol aliquots were added to the protein solution. The solution was then irradiated by γ-irradiation in a ^{60}Co source (PISICNEA-Ezeiza, Arg.) 1 kGy/h. The irradiation process lasted 10 h at an irradiation rate of 1 kGy/h, with a final dose of 10 kGy.

2.2.2. Lyophilisation procedure

The samples were lyophilised in two different ways:

a. A millilitre of BSA NP lyophilised in buffer PBS (pH 7.0; 30 mM) solution 450 μM in buffer 35% ethanol solution was eluted with a size exclusion column (Sephadex 50 PD-10) in order to exchange the solvent to a 100% PBS (pH 7.0; 30 mM) solution. After that, the BSA NPsolution was left in freezer at −80 °C for 24 h. Then, they were lyophilised for another 24 h. The powder obtained was resuspended in buffer PBS (pH 7.0; 30 mM).
b. The BSA NP obtained from γ-irradiation, was then passed through a size exclusion column (GE- Healthcare PD-10) in order to exchange the solvent from PBS/EtOH 35% v/v, to miliQ water.

Samples were prepared according to the molar ratio relationship R = [Trehalose]/[BSA NP], keeping the BSA NP concentration at 0.30 μM. Trehalose concentration varied from 0.0 to 400.0 μM (R = from 0.0 to 1000). After that, the BSANP solution was left in freezer at −80 °C for 24 h. Then, they were lyophilised for another 24 h. The powder obtained was resuspended in buffer PBS (pH 7.0; 30 mM).

The lyophilisation process for both protocols lasted for 24 h, reaching a vacuum under 133 μbar.

2.2.3. Characterization of nanoparticles

To characterise the structure/function of NPs lyophilisation process, before and after, DLS, Z Potential and MC540 affinity experiments were carried out. The lyophilised nanomaterial was rehydrated in buffer PBS (pH 7.0; 30 mM) up to initial volume to maintain its concentration.

2.2.3.1. Particle size and Z potential. Size distribution and Z potential of NPs were measured by a commercial zeta-potential and particle size analyser size measurement, 90Plus/Bi-MAS particle size analyzer, 1 ml of the NPs in PBS was prepared. Subsequently 100 μl volume from the original concentration was taken up to 1 ml final volume with PBS. The particle size was measured at 25 °C with a scatter angle of 90°. For the Z potential measurement, the NPs were dispersed in 1 ml of PBS, and measured at 25 °C.

2.2.3.2. Microscopy.

2.2.3.2.1. Scanning electron microscopy (S.E.M). The lyophilised powder was analysed under a SEM Carl Zeiss NTS SUPRA 40 electronic microscope (Centro de Microscopía Avanzada, Pabellón I, Facultad de Ciencias Naturales y Exactas, Ciudad Universitaria, UBA, Buenos Aires, Argentina). Microscopies of randomly selected field of the sample were recorded in order to have a view of what was