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Research paper

Preparation of Bovine Serum Albumin (BSA) nanoparticles by desolvation using a membrane contactor: A new tool for large scale production

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ABSTRACT

Albumin nanoparticles are attractive drug delivery systems as they can be prepared under soft conditions and incorporate several kinds of molecules. The aim of this study was to upscale the desolvation process for preparing Bovine Serum Albumin (BSA) nanoparticles using a membrane contactor. At a first step, the BSA nanoparticles were prepared at small scale using a syringe pump. BSA nanoparticles of 139 nm in size, with a polydispersity index of 0.046, were obtained at the optimal conditions: pH 8.2, 100 mg mL^{-1} BSA albumin solution (2 mL), and 1 mL min^{-1} flow rate of ethanol addition (8 mL). The upscaling with a membrane contactor was achieved by permeating ethanol through the pores of a Shirasu Porous Glass (SPG Technology Co., Japan) membrane and circulating the aqueous phase tangentially to the membrane surface. By increasing the pressure of the ethanol from 1 to 2.7 bars, a progressive decrease in nanoparticle size was obtained with a high nanoparticles yield (around 94–96%). In addition, the flow rate of the circulating phase did not affect the BSA nanoparticle characteristics. At the optimal conditions (pH 8.2, 100 mg mL^{-1} BSA albumin solution, pressure of ethanol 2.7 bars, flow rate of the circulating phase 30.7 mL s^{-1}), the BSA nanoparticles showed similar characteristics to those obtained with the syringe pump. Large batches of BSA nanoparticles were prepared up to 10 g BSA. The BSA nanoparticles were stable at least during 2 months at 4 °C, and their characteristics were reproducible. It was then concluded that the membrane contactor technique could be a suitable method for the preparation of albumin nanoparticles at large scale with properties similar to that obtained at small scale.

1. Introduction

Among the available nanosized drug carrier systems, nanoparticles prepared with proteins (e.g., albumin, gelatin) are biocompatible, biodegradable, nonantigenic, and relatively easy to prepare. Further, protein nanoparticles can bind to a large number of drugs in a relatively nonspecific manner. Because of their surface charge, drugs can physically adsorb onto the protein surface or can covalently bind to the matrix [3,4]. Bovine Serum Albumin (BSA) and Human Serum Albumin (HSA) have been widely used over the past 30 years to prepare micro and nanoparticles. More than 100 different active or diagnostic molecules have been incorporated into albumin particles to be administered by different routes including intravenous, intramuscular, nasal, and ophthalmic routes [3,5]. In particular, albumin nanoparticles are used in cancer therapy for the delivery of several anticancer drugs (5-fluorouracil, paclitaxel, doxorubicin) which have a binding affinity to albumin [3,6]. Albumin can penetrate cancerous tissues by two means, active or passive transport [5,7]. The first HSA-based nanoparticle formulation, ABI 007 (Abraxane®), was approved by the FDA in 2005. The incorporation of paclitaxel in albumin nanoparticles was shown to improve drug solubility, with a variety of advantages conferred to the standard paclitaxel therapy [8,9].

Emulsification and desolvation (or coacervation) are the two main methods described for the preparation of albumin particles. The first technique involves emulsification of albumin solution in an oil phase and solidification by heat denaturation or chemical cross-linking using formaldehyde or glutaraldehyde [10]. One limit of the emulsion method is the need of using organic solvents, for the removal of both the oily residues and the surfactants required for emulsion stabilization. Another disadvantage of this method is that it is difficult to control the albumin particle size and to obtain size below 500 nm.

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Desolvation (or coacervation) of albumin with organic solvents followed by chemical cross-linking is a commonly used method for the preparation of albumin nanoparticles. The coacervating agent changes progressively the tertiary structure of the protein to give an increasingly hydrophobic material, which tends to form submicronic aggregates of desolvated albumin (coacervates) [3]. These unstable coacervates have to be hardened by cross-linking with glutaraldehyde where the amino moieties in lysine residues and arginine moieties in guanidino side chains of albumin are solidified by a condensation reaction with the aldehyde-group of glutaraldehyde [4]. The preparation of albumin nanoparticles by desolvation involves a three-step procedure: (1) an unstable dispersed system (coacervates) is obtained by addition of a desolvating agent of albumin (usually ethanol or acetone) to an aqueous solution of the protein; (2) the coacervates are hardened by chemical cross-linking; (3) the resulting nanoparticles are purified and eventually lyophilized or spray-dried. The technique allows a control of the particle size and polydispersity index by varying conditions such as the pH value, the ionic composition, and the concentration of the albumin solution.

Albumin nanoparticles preparation by desolvation has been investigated by several authors. Langer et al. [11,12] systematically studied the desolvation process of HSA from an aqueous solution by addition of ethanol. The preparation conditions were optimized to obtain nanoparticles with a controllable particle size (between 100 and 400 nm) and a narrow size distribution. Using a modified desolvation method, Jun et al. [13] prepared BSA nanoparticles and calcium (Ca)-loaded BSA nanoparticles at the targeted sizes of 100, 400, and 800 nm. The targeted size was obtained by changing pH, protein content, and salt content, which provided different electrostatic and hydrophobic interactions during desolvation of the BSA molecules. The upscaling of the ethanolic desolvation process has been investigated by Wacker et al. [14] for up to 2 g of albumin. Two paddle stirring systems were developed in order to ensure a homogeneous distribution of HSA molecules during the desolvation process. A constant solvent flow was achieved by utilizing a HPLC pump for the addition of ethanol to the protein solution.

One of the major limitations in the manufacture of nanoparticles remains the transfer of scale for the production of large scale batches. The membrane contactor is a relatively simple technique which can be used to produce large volumes of colloidal dispersions. It has been shown previously to be suitable for the preparation of polymeric nanoparticles [15], solid lipid nanoparticles [16], liposomes [17], and nanoemulsions [18]. For the preparation of BSA nanoparticles using the membrane contactor, the ethanol is pushed through the membrane pores allowing the formation of small jets and the BSA aqueous solution flows tangentially to the membrane surface. BSA coacervates are formed when the two phases mixed at the pore outlets. The BSA nanoparticles are obtained by solidification of the BSA coacervates by chemical cross-linkage using a glutaraldehyde solution.

The objective of the present study is the optimization of the membrane contactor technique for the preparation of BSA nanoparticles at large scale. BSA nanoparticles were characterized for their mean size, polydispersity index (PDI), zeta potential, and BSA nanoparticle yield. At a first step, the BSA nanoparticle preparation was investigated at small scale using a syringe pump for the injection of ethanol in the BSA aqueous phase. At large scale, the effect of two process parameters on BSA nanoparticle characteristics has been investigated: the velocity of the circulating phase and the pressure of ethanol. The effect of increasing the amount of BSA nanoparticle prepared was also investigated. Finally, BSA nanoparticles were characterized for their stability, reproducibly and were observed by transmission electronic microscopy (TEM).

2. Materials and methods

2.1. Chemical reagents

BSA was obtained from MP Biomedicals (Illkirch, France). A 8% glutaraldehyde solution was used as the cross-linking agent. It was obtained by dilution of a 25% solution Sigma–Aldrich (France).

The Bicinchoninic Acid Protein Assay Kit (BCA) was provided by Sigma–Aldrich (France). Ethanol 99% analytic grade and sodium hydroxide were obtained from Sigma–Aldrich (France) and ammonium molybdate from Merck (France). All reagents were of analytical grade and used as received. Ultra-pure water was obtained from a Synergy unit system (Millipore, France).

2.2. Materials

2.2.1. Syringe pump

The syringe infusion pump 22 was purchased from Harvard Apparatus (Holliston, Massachusetts, United States).

2.2.2. Membrane contactor

The experimental setup is shown schematically in Fig. 1. It includes a pump (Quatro Flow Fluid Systems, Germany), a Shirasu Porous Glass (SPG) cross-flow-filtration module (SPG Technology Co., Ltd., Japan) with a pressure gauge at the entrance of the module, a valve, a pressure vessel equipped with a pressure gauge (Millipore, France). The BSA solution is stirred continuously under magnetic stirring (RCT basic, IKA, France).

The SPG membrane (SPG Technology Co., Ltd., Japan) is hydrophilic with a mean pore size of 0.2 μm. With an inside diameter of 1×10^{-2} m, a thickness of 1×10^{-3} m, and a length of 0.125 m, the SPG membrane has a surface area of 3.9×10 m^2.

2.2.3. Purification and analysis

Centrifugation of the BSA nanoparticle suspensions was carried out with an Ultracentrifuge Optima™ (Beckman Coulter, France). The measurement of average size, PDI, and zeta potential was realized using a ZetaSizer Nano ZS (model ZEN 3600, Malvern

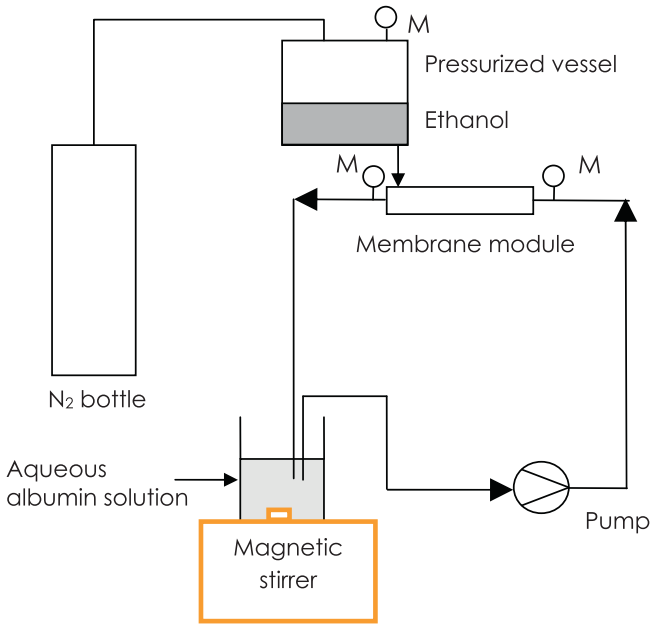

Fig. 1. Schematic representation of the membrane contactor for preparation of large volumes of BSA nanoparticles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)