# PRODUCTION OF ALBUMIN MICROSPHERES FOR DRUG RELEASE BY SUPERCRITICAL ASSISTED ATOMIZATION

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Microparticulate carriers are widely employed as drug controlled release system. In this work, the Supercritical Assisted Atomization (SAA) technique was used to produce microspheres of Bovine Serum Albumin (BSA), selected as carrier and gentamicin sulphate (GS) as a model drug. The processability of BSA and GS by SAA was studied using water as solvent and microspheres were successfully produced. A modification of the protein secondary structure of BSA, that induced a partial hydrophobic behaviour in water, was observed. This fact is not a disadvantage, since it can prolong the stability of BSA microspheres. Composite microparticles of BSA and GS were then produced. Microspheres with a spherical shape and with a mean particle size of 1 μm were obtained. The controlled release properties of the BSA-loaded microspheres took advantage from the protein structural changes since a longer GS release was observed.

## INTRODUCTION

Several biodegradable polymers have so far been used for drug delivery systems; among them very promising are the natural polymers such as: polypeptides and proteins, e.g. albumin[1,2], collagen, gelatin[3]; polysaccharides (e.g. hyaluronic acid, starch and chitosan). Albumin is the most abundant plasma protein (about 55%). It is a water soluble, single stranded protein of about 580 amino acids and has a molecular weight of 66500. The use of albumin as a polymer matrix for the encapsulation of various drug types e.g. anticancer drugs[4,5] and steroids[6] has been studied extensively. The hydrophilic nature of albumin makes it more efficient in the transport of hydrophilic drugs and it has to be stabilized to be used for the controlled release.

Heat treatment on bovine serum albumin (BSA) induces the formation of cross-links, increasing the hydrolytic resistance of the protein. The effect is more evident as the heating time is prolonged and the temperature elevated[7]. Thermal denaturation may affect the secondary structure of this protein, in particular the α-helix and β-sheet structure content of the BSA. The reversible unfolding of the α-helices starts already at 50°C and, increasing the temperature above 65°C[8,9], the unfolding gives easy access to the formation of disulphide bridges. Since disulphide bridges are covalent bonds, this stage is irreversible, the solubility in water decreases and the tendency to form a gel structure increases.

Gentamicin sulphate (GS) is an aminoglycoside and is used in the cure of bacterial infections, e.g. sepsis and septic shock. It is usually administrated in solution with the disadvantage of the systemic toxicity due to high dose administration and of the frequent administrations needed. A biodegradable carrier system can obviate the need to administer high doses and offers the benefit of less frequent administrations.

There are many methods of microsphere preparation, including emulsion oil-in-water (o/w), water-in-oil (w/o)-solvent evaporation, nebulization, coacervation and spray-drying. BSA/GS microspheres have been obtained using spray-drying technique. Particles with a mean size in the range 5-7 μm with a good encapsulation efficiency have been produced[10]. Supercritical fluid technology has been proposed as an alternative to conventional techniques for the production of micro and nano-sized particles. The supercritical assisted atomization (SAA) is an efficient process based on the solubilization of controlled quantities of SC-$CO_2$ in the liquid solutions (formed by organic solvents or water and a solid solute). To date, this process has successfully been tested on some pharmaceutical

compounds, catalyst and superconductor precursors, salts and polymers obtaining micrometric and submicrometric particles of controlled size and distribution[11-15]. Recently, SAA technique has been successfully applied to the production of drug-polymer microparticles for the controlled drug release using a natural polymer such as chitosan [16].
In this work SAA technique is used to produce BSA microspheres loaded with GS, to obtain the controlled release of the drug.

## MATERIALS AND METHODS

Bovine Serum Albumin (BSA) and Gentamicin sulphate (GS) have been supplied by Sigma-Aldrich (Milan, Italy). Water (HPLC grade) with a purity of 99.5% has been supplied by Sigma-Aldrich (Milan, Italy). Carbon dioxide ($CO_2$; purity 99.9%) and Nitrogen ($N_2$; purity 99.9%) have been purchased from SON (Naples, Italy).
SAA laboratory apparatus consists of two high-pressure pumps (mod. 305, Gilson) delivering the liquid solution and liquid $CO_2$ to a heated bath (Forlab mod. TR12, Carlo Erba) and, then, to the saturator. The saturator is a high pressure vessel (I.V. 25 $cm^3$) loaded with stainless steel perforated saddles which assure a large contact surface between liquid solution and $CO_2$. The solution obtained in the saturator is sprayed through a thin wall 80 μm diameter injection nozzle into the precipitator (I.V. 3 $dm^3$) operating at atmospheric pressure. A controlled flow of $N_2$ is taken from a cylinder, heated in an electric heat exchanger (mod. CBEN 24G6, Watlow) and sent to the precipitator to facilitate liquid droplets evaporation. The saturator and the precipitator are electrically heated using thin band heaters. A stainless steel filter located at the bottom of the precipitator allows the powder collection and the gaseous stream flow out. SAA apparatus layout and further details on the experimental procedures were published elsewhere[17,18].
Morphological characteristics of BSA and GS-BSA loaded particles have been analysed by a Scanning Electron Microscope (SEM, mod. 420, LEO). Particle size (PS) and the particle size distribution (PSD) have been evaluated from SEM images using the Sigma Scan Pro Software (rel. 5.0, Jandel Scientific). Histograms representing the PSDs have been best fitted using Microcal Origin Software (rel. 7.0, Microcal Software Inc.).
Drug release profiles over the time were obtained using a physiological saline solution (pH 7.2) as the dissolution medium. 100 mg of microspheres were pre-suspended in 2 mL of physiological saline solution and charged into dialysis sack (cut off 9-10 Kdaltons). Then the GS release profiles were determined in 200 mL of physiological saline solution continuously inverted at 20 rpm in a 37°C incubator to maintain adequate sink conditions. GS released was determined by measuring the absorbance at 257 nm (UVvis mod. Cary 50, Varian, California, USA).

## RESULTS

GS and BSA have been separately processed by SAA using water as the liquid solvent to verify the feasibility of the micronization of the two compounds. BSA micronization has been performed using a mass flow ratio between $CO_2$ and water R=1.8, temperature in the saturator: 85°C, pressures between 105 and 110 bar and temperature in the precipitator of 100°C. The effect of the concentration of BSA in water has been studied in the range 5-40 mg/mL. Spherical well separated particles have been obtained in all the experiments. At low BSA concentrations it could be noted that several particles are collapsed, as shown in **Figure 1**. These results suggest that at low BSA concentrations some of the particles are formed by an empty shell of solute.

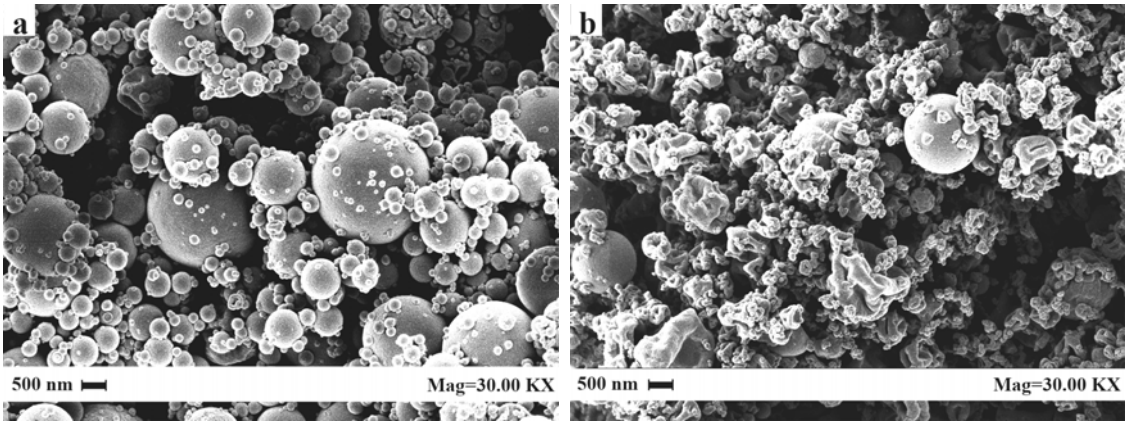


**Figure 1.** SEM photomicrographs of BSA precipitated from water at 100°C, 20 mg/mL (a), 5 mg/mL (b).

The variation of concentration did not have a relevant effect on the mean diameter of the particles, as can be observed in the particle size distributions presented in terms of number of particles in **Figure 2**. Though, increasing the concentration, the number of particles with a larger diameter increases. This is more evident in the calculations in terms of volume percentages: $D_{90}$, that is the maximum diameter corresponding to 90% of particles volume, moved from 1.09 μm to 1.95 μm increasing the concentration form 5 to 40 mg/mL of BSA in water.

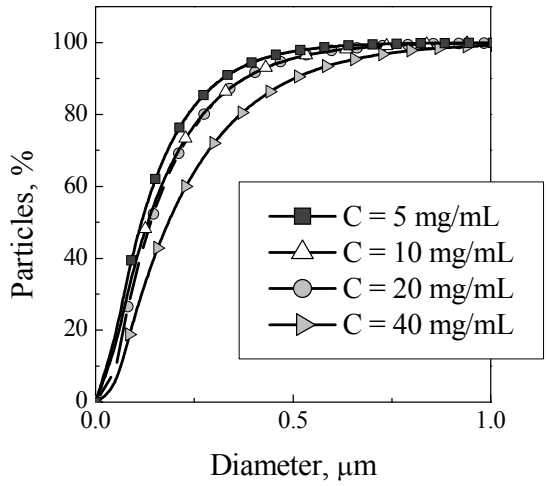


**Figure 2.** PSDs of BSA powders precipitated by SAA at different concentrations of BSA in water. Calculations in terms of cumulative particle number percentages.

SAA process also influences the structure of BSA. Untreated BSA solubility in water was measured at room temperature and was about 50 mg/mL; after SAA micronization water-solubility of the BSA was significantly decreased, and the microspheres had a tendency to produce hydrophobic aggregates. This fact suggests that because of the process temperature, the irreversible unfolding of the protein was obtained. This modification can be an advantage for the application as controlled release device: the hydrolytic resistance and the cross-linked structure lead to a different mass transfer mechanism for the drug dissolution in the microspheres: water slowly penetrates the structure solubilising the loaded compound.

GS was micronized using a concentration of 10 mg/mL of drug in water, a mass flow ratio between $CO_2$ and water R=1.8, temperature in the saturator: 90°C, pressure: 105 bar and temperature in the precipitator of 130°C. The precipitated powder had a yellow colour compared to the untreated GS that is a white powder, suggesting that the drug was sensitive to heat. SEM photomicrographs of the product showed (**Figure 3**) that the particles obtained were spherical, with a size of about 2-5 μm. The

coprecipitation of GS with the BSA, could stabilize it, acting as a protection against the heating of the molecule and protecting it from possible thermal degradation.

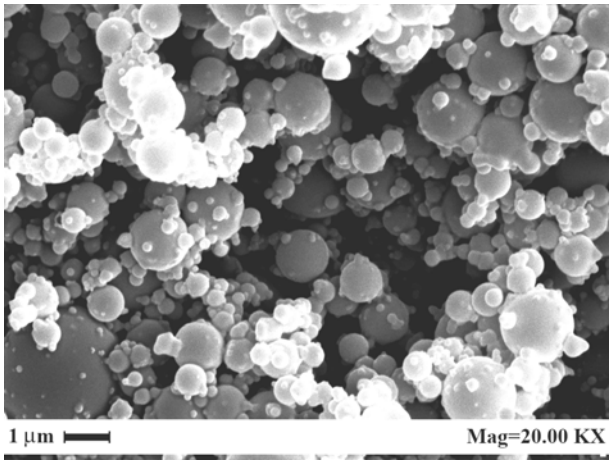


**Figure 3.** SEM photomicrograph of GS precipitated from water at 130°C, 10 mg/mL.

Therefore, GS loaded microspheres were prepared from a solution of 20 mg/mL of solute in water, with a carrier/drug weight ratio (r=BSA/GS wt/wt) of 1, 4 and 8. The process conditions were selected based on the micronization tests of BSA. In all the experiments a white powder was obtained as a product, suggesting that, as expected, the BSA acts as a protecting agent for the GS. SEM photomicrographs (**Figure 4**) show that spherical particles were obtained in all the cases.

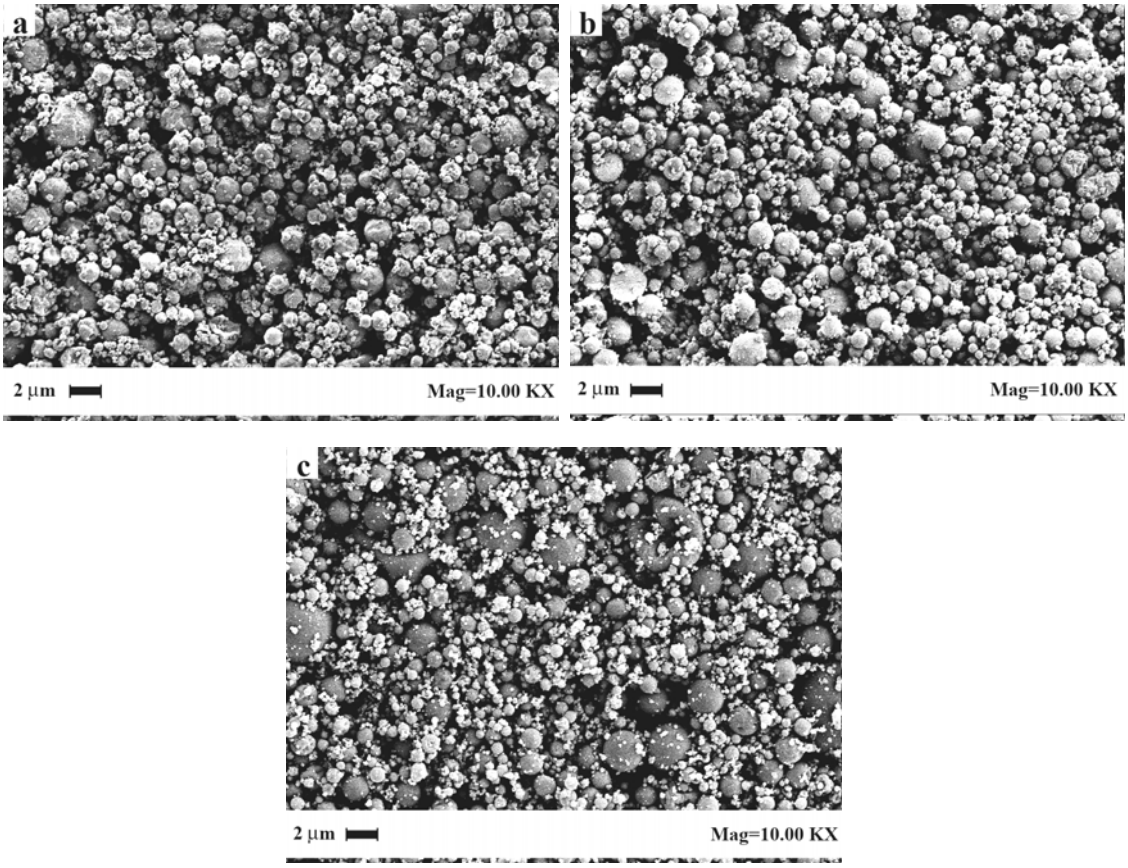


**Figure 4.** SEM photomicrographs of BSA/GS precipitated from water at 100°C, 20 mg/mL r=1 (a), r=4 (b), r=8 (c).

The PSDs reported in **Figure 5** show that the mean size of the microspheres was less than 3 μm. $D_{90}$, was in all the experiments in the range 3.5-4 μm.

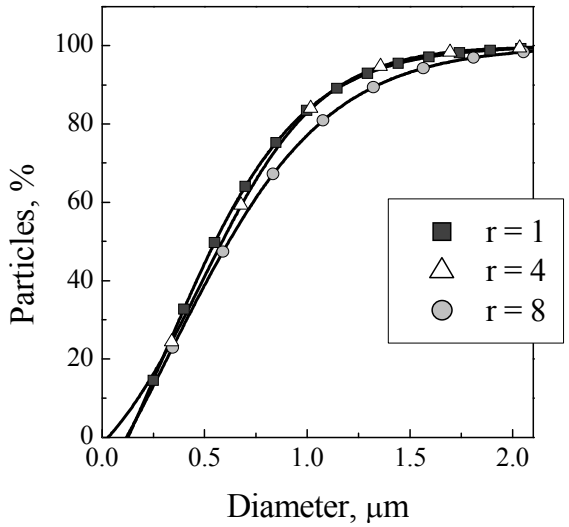


**Figure 5.** PSDs of BSA/GS coprecipitated by SAA at different BSA/GS weight ratio (r). Calculations in terms of cumulative particle number percentages.

To verify the loading of GS in BSA microspheres, drug release experiments were performed using dialysis bags for the r=8. The efficiency of the coprecipitate as controlled release device was verified comparing the in vitro release profiles of the GS loaded BSA microparticles and the pure drug. **Figure 6** shows that the SAA coprecipitate exhibits a slow drug release rate: pure GS diffused through the dialysis bag after 15.5 h, whereas during the same time, SAA coprecipitates released about 60% of the drug. GS was totally released from the BSA after 116.6 h.

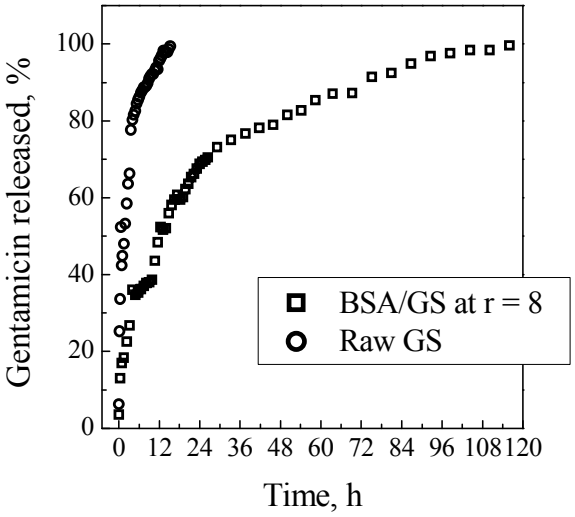


**Figure 6.** In vitro GS release profiles from: raw GS and BSA/gents SAA coprecipitates at the ratio r=8. Drug release rates are reported up to 120 h.

## CONCLUSION

BSA microparticles obtained using SAA show a modification of the protein secondary structure, that increases the hydrolytic resistance of the polymer. The protein structural changes give to the microspheres properties of a carrier for drug release because allows the water to slowly penetrate the structure solubilising a loaded compound. Coprecipitation of BSA, as carrier, and GS, as model drug,

was successfully obtained and BSA showed to be a stabilizing agent for GS, protecting it from thermal effects. Well defined spherical microspheres, with a uniform morphology and a narrow distribution were obtained. The coprecipitates exhibit a slow drug release rate.
Further analyses on the coprecipitates BSA/GS, are necessary to confirm that the drug is efficiently entrapped into the polymeric matrix. Moreover, experiments at different polymer/drug ratios and varying the operating parameters have to be performed to identify the controlling parameter for drug release. At last, the anti-microbial activity of GS loaded BSA microspheres has to be verified to attest efficiency of the product for pharmaceutical applications.

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