

SAA produced nanostructured polymer microparticles loaded with magnetic nanoparticles

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Supercritical Assisted Atomization (SAA) is one of the most efficient supercritical fluid (SCF) based micronization techniques proposed in the literature. The SAA working principle suggests the possibility of producing composite microparticles, since multi-component solutions can be processed.

Therefore, in this work, SAA has been applied to the formation of polymer microparticles loaded with magnetic nanoparticles starting from suspensions of nanoparticles in polymer-solvent solutions. Magnetic nanoparticles possess the ability to work at the cellular and molecular level for biological interactions; the encapsulation in a polymeric coating makes the particles biocompatible and provides a steric barrier to prevent nanoparticle agglomeration and avoid opsonization.

Dextran and chitosan have been used as dispersing matrices for magnetic nanoparticles. Nanostructured microspheres ranging between 0.2 and 1.5 μm with nanoparticle loading up to 20% have been obtained. They have been characterized by SEM, EDX, TGA, obtaining information on the morphology, particle size distribution, nanostructure and loading of nanoparticles in the polymeric matrices.

INTRODUCTION

Using traditional micronization processes, it is very difficult to obtain the required particle size distribution (PSD). Therefore, micronization techniques based on SCFs have been proposed that are promising for the production of nano and micro particles with controlled size and distribution [1]. Supercritical Assisted Atomization (SAA) is one of the most efficient Supercritical fluid (SCF) micronization techniques proposed in the literature [2]. The process is based on the solubilization of controlled quantities of SC-CO₂ in liquid solutions containing a solid solute and on the subsequent atomization of the ternary solution through a nozzle. In this case, SC-CO₂ plays both as co-solute being miscible with the solution to be treated, as well as atomization agent. A two steps atomization is obtained: the primary droplets produced at the outlet of the injector (pneumatic atomization) are further divided in secondary droplets by CO₂ expansion from the inside of the primary ones (decompressive atomization). SAA process has been applied to the micronization of different kinds of compounds, among them pharmaceutical compounds [3-6], polymers and biopolymers [7-9]. The SAA working principle suggests the possibility of producing composite microparticles, since multi-components solutions can be processed; indeed, SAA has been applied to the production of drug-polymer microparticles for the controlled release of active principles from nanostructured polymer microparticles [10-11].

Magnetic nanoparticles (MNPs) possess the ability to work at the cellular and molecular level for biological interactions; the encapsulation in a polymeric coating makes the particles biocompatible, stable, and may serve as a support for biomolecules. Furthermore, polymeric coatings provide a steric barrier to prevent nanoparticle agglomeration and avoid opsonization. The magnetic properties of nanostructured polymer-iron oxide microparticles enable their use in numerous applications: as magnetic contrast agents in magnetic resonance imaging (MRI), as hyperthermia agents, where the magnetic particles are heated selectively by the application of an high frequency magnetic field and as magnetic vectors that can be directed by a magnetic field gradient towards a given location in the body, for targeted drug delivery [12-14].

In this work SAA has been applied to the formation of polymer microparticles loaded with nanometric iron oxide starting from suspensions of iron oxide nanoparticles in polymer-solvent solutions. Chitosan and dextran have been used as polymer considering their biocompatibility for pharmaceutical formulations or local targeted drug delivery [10].

1 - MATERIALS AND METHODS

Low molecular weight chitosan, dextran (mw 43000), magnetite (Fe_3O_4) nanoparticles, water, acetic acid were used for the experiments.

SAA laboratory apparatus consists of two high-pressure pumps delivering the liquid solution and liquid CO_2 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 $80 \mu\text{m}$ diameter injection nozzle into the precipitator (I.V. 3 dm^3) operating at atmospheric pressure. A controlled heated flow of N_2 is sent to the precipitator to assist liquid droplets evaporation. 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 [2]. The compounds to micronize were dissolved in 1% v/v acetic acid aqueous solution and, then, delivered to the SAA apparatus.

Nanostructured microparticles were observed by a Scanning Electron Microscope (SEM) coupled with a field emission source (FE-SEM). Particle size (PS) and particle size distribution (PSD) were measured from SEM photomicrographs using the Sigma Scan Pro Software. Fourier transform infrared (FT-IR) spectra were obtained via M2000 FTIR, at a resolution of 0.5 cm^{-1} . Microanalysis was used to investigate the particle chemical structure. Elemental analysis and element mapping were conducted with the field emission-scanning electron microscope (FESEM) equipped with an energy dispersive X-ray spectroscopy (EDX). The loading of MNPs in the polymeric matrix was determined by thermogravimetric analysis (TGA). Solid state analysis of the samples (XRPD=X-ray powder diffraction) was performed using an X-ray powder diffractometer (Bruker) with a Cu sealed tube source. The measuring conditions were: Ni-filtered $\text{CuK}\alpha$ radiation, $\lambda=1.54 \text{ \AA}$, 2θ angle ranging from 5 to 70° with a scan rate of 3 s/step and a step size of 0.02° .

2 – RESULTS AND DISCUSSION

Microparticles were produced operating at 100 bar and 85°C in the saturator, mass flow ratio between CO_2 and liquid solution $R=1.8$ and a precipitation temperature of 110°C . The particles were spherical and amorphous at all the percentages of Fe_3O_4 loaded in the carrier. This can be observed, for example, in the SEM photomicrographs in figure 1, that reports the particles with a starting loading of MNPs of 10 and 30% (MNPs wt/polymer wt).

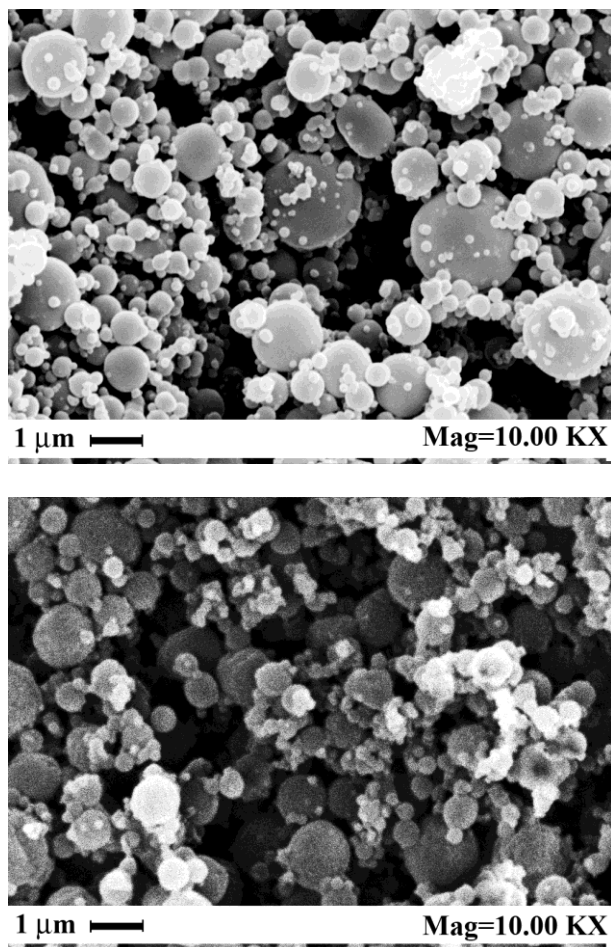


figure 1. SEM photomicrographs of chitosan microparticles loaded with 10% (top) and 30% (down) of Fe_3O_4 MNPs

Uniformly nanostructured microparticles ranging between 0.2 and 1.5 μm with Fe_3O_4 loadings have been obtained. Several concentrations of MNPs in the polymer in the starting solution have been processed, to understand the relation between the loading in the starting solution and the loading in the obtained particles (table 1).

Fe_3O_4 /polymer, wt%	Fe_3O_4 theoretic, wt%	Fe_3O_4 effective, wt%	encapsulation, %
5	4.76	3.50	73.50
10	9.09	6.20	68.20
15	13.04	7.60	58.27
20	16.67	11.17	67.02
30	23.08	17.80	77.13
40	28.57	19.20	67.20

table 1. loading of Fe_3O_4 in chitosan microparticles produced by SAA

TGA analysis showed that the encapsulation efficiency of Fe_3O_4 in chitosan varies from 58 to 77%. It is possible to note that there is not a trend in the encapsulation efficiency depending from the Fe_3O_4 loaded as a suspension in the starting polymer solution, therefore, the efficiency might be strictly related to the preparation of the suspension. The MNPs loaded in the polymeric microparticles also affect the PSD: increasing the Fe_3O_4 percentage, the size of the particles decreases (figure 2). In particular, in the case of a loading of 30%,

corresponding to an effective loading of MNPs of 17.8% wt/particle wt, the particles are smaller than 1 μm .

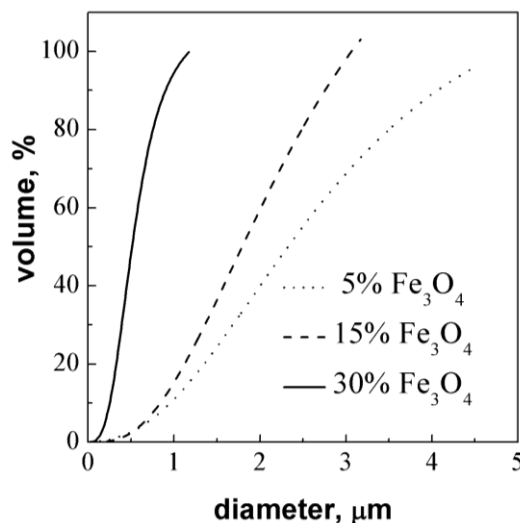


figure 2. PSDs in terms of volumetric cumulative percentage of chitosan microparticles loaded with Fe_3O_4 MNPs

This result can be explained with a decrease of viscosity of the polymeric solution with the increase of MNPs loading: the increase of nanoparticles percentage suspended and uniformly distributed reduces the viscosity of the liquid solution, resulting in the formation of smaller primary droplets, that also influence the dimensions of the secondary droplets, improving the atomization of the solution [8-9].

XRPD analyses confirmed the presence of the nanoparticles in the polymeric matrix and FTIR showed no bounds between the polymer and the Fe_3O_4 . The uniform dispersion of MNPs in the polymer microspheres was verified using EDX technique.

Very interesting results have been obtained also using dextran as polymer. Loadings of Fe_3O_4 MNPs of 23.47% wt/particle wt have been obtained, with a very high encapsulation efficiency. In figure 3 a SEM photomicrograph of dextran particles produced operating at 84 bar and 85°C in the saturator, mass flow ratio between CO_2 and liquid solution $R=1.8$ and a precipitation temperature of 110°C is reported.

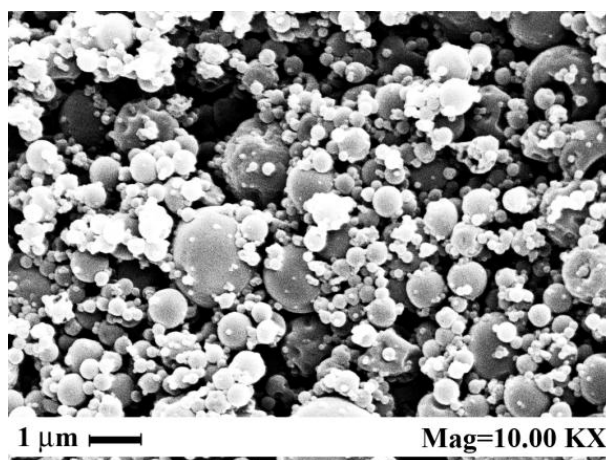


figure 3. SEM photomicrographs of dextran microparticles loaded with 23.47% of Fe_3O_4 MNPs

The particles were spherical and XRPD analyses showed that they are amorphous and the Fe_3O_4 peaks confirmed the presence of the nanoparticles that did not change their structure. Also in the case of dextran loaded microparticles, increasing the effective loading of Fe_3O_4 , the PSD strongly decreases (figure 3). This confirms that the MNPs act as viscosity reducer, helping the rupture of the jet at the exit of the nozzle.

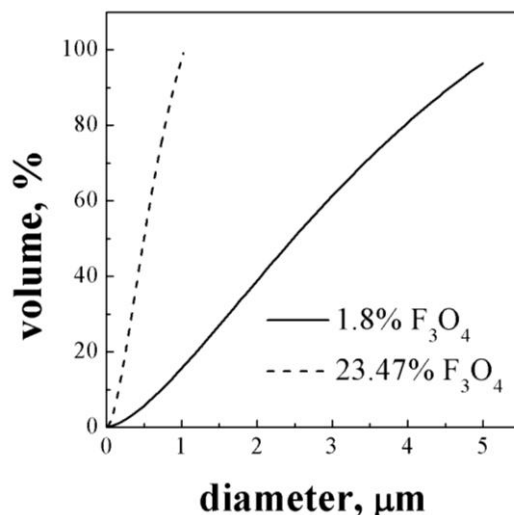


figure 4. PSDs in terms of volumetric cumulative percentage of dextran microparticles loaded with Fe_3O_4 MNPs

Also in the case of dextran-MNP microparticles, EDX confirmed that the Fe_3O_4 nanoparticles were uniformly distributed in the polymeric matrix.

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