

Study of the nanostructure and formation mechanism of composite microparticles produced by SAA

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ABSTRACT

Objects of nanometric dimension have peculiar physic-chemical properties, different than traditional materials and many industrial applications take the advantage of the characteristics of nanomaterials. For example in biomedical field nanoparticles are used for the administration of active principles and nanomaterials have often a composite structures, in which nanometrics objects are dispersed on a support that allows the driving of the drug and the controlled release.

SAA (Supercritical Assisted Atomization) technique is a well-established process for the production of nanoparticles. The knowledge of the SAA process and, in general of spray drying technique, has a significant gap: in literature there are no data related to the structures in which the compounds are organized in the composite particles. The coprecipitated compounds are usually an active principle and a polymer that has the function to protect and control the release of the other principle in the particle.

This work proposes the study of the nanostructure inside the composite microparticles produced by SAA technique and a classification of the different possible structures of the composite microparticles.

Accurate analyses using Scanning Electron microscope (SEM) with secondary and back scattering electron technique have evidenced the influence of polymer-solute solubility properties on the particle nanostructures. Considering polymer A and compound B, two possible structures have been found: nanometric clusters of B dispersed in a matrix A; structure core-shell of B covered by A.

INTRODUCTION

Composite microparticles can be classified depending on the morphology and the composition of the structure formed by the loaded compound B and the polymer A. If B is encapsulated in the core of the microparticle surrounded by a shell of B, a microcapsule is obtained; if B is dispersed in the matrix A, then a microspheres is formed [1, 2]. These two different kind of structure are extremely interesting, since the characteristics of the microparticles are strongly related to the distribution of the loaded compound in the matrix. They find the main application in pharmaceutical field as controlled delivery systems, in which microparticles of polymers loaded with an active principles are used to control the dissolution rate of the active principle or to improve its bioavailability [3, 4]. The modification of particle surface is important for the proper introduction of the active principle in the blood stream, in order to make the active principle invisible to the body's natural defense system. For example nanoparticles of active principles can be covered by a protective hydrophilic layer of polymer [4].

Supercritical Assisted Atomization (SAA) is very effective for micronization of pure compounds [5-7] and for the production of composite microparticles formed by a polymeric carrier loaded with a drug homogeneously dispersed in the matrix [8-15]. In some cases the target was to obtain formulations for the controlled release of drug, in other cases to improve the bioavailability using a water soluble polymer to increase the active principle dissolution rate.

The controlled release studies showed that the dissolution behavior of a drug is not easily correlated to the polymer used as a carrier or to the active principle loaded. Indeed, using the same carrier the dissolution rate in the same environment increases or decreases depending on the system studied [14, 15]. The dissolution rate seems to depend from the chemical characteristics of the compound and the polymer forming the composites and this might be attributed to the structure of the microparticles. If the microparticles are in form of capsules, the release of the active principle is controlled by the swelling or erosion of the polymer shell, if they are in form of spheres, the active principle dissolves together with the polymeric matrix, with a rate related to the efficiency of dispersion in the polymer. The analysis of the internal structure of the particles can help to understand the shape of the dissolution curves in the controlled release and the mechanism of the release of the active principle. The aim of this work is to study of the nanostructure inside the composite microparticles produced by SAA technique, to classify the different possible structures of SAA composite microparticles by SEM analysis and to measure the size of the particles and of the nanostructures forming the particles.

APPARATUS, MATERIALS AND METHODS

Carbon dioxide (CO₂; purity 99.9%) was purchased from Morland Group and Nitrogen (N₂; purity 99.9%) from SON. The materials used in this work are: polyvinylpyrrolidone (PVP, Mw: 10,000) as carrier for microcapsules and microspheres, silver acetate (AgAc) and Sodium Molybdate (Na₂MoO₄) as active principle have been bought from Sigma Aldrich.

SAA laboratory apparatus consisted of two high-pressure pumps delivering the liquid solution and liquid CO₂ to a saturator. The saturator is a high pressure vessel (25 cm³) loaded with stainless steel perforated saddles thus assuring the formation of an expanded liquid. The solution obtained is sprayed through a thin wall (80 μm ID) injection nozzle into the precipitator (3 dm³) operating at atmospheric pressure. A controlled flow of N₂ is taken from a cylinder, heated and sent to the precipitator to assist liquid droplet evaporation. A stainless steel filter located at the bottom of the precipitator allows powder collection and the gaseous stream can flow out. SAA apparatus layout and further details on the experimental procedures were published elsewhere [16].

The morphology of SAA powders was observed by a scanning electron microscope (SEM, mod. EVO-HD, Carl Zeiss). Back scattered and secondary electron detection was used to analyse the particle structure. Particle size distribution (PSD) of the particles were measured from SEM photomicrographs using the Sigma Scan Pro Software (release 5.0). Histograms representing the PSD were fitted using Microcal Origin Software (release 8.0). Particle size (PS) and particle size distribution (PSD) were measured by dynamic laser scattering (DLS) using a Nanosizer (NanoZS Malvern Instrument, UK) equipped with a He-Ne laser operating at 5.0 mW and 633 nm, that measures the hydrodynamic diameter of the particles. Microparticles were dispersed in acetone to measure the external PS and 1-butanol to measure the internal PS. DLS analysis was performed setting temperature at 25°C. 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 quantity of salts encapsulated in the polymeric matrix was determined by thermogravimetric analysis (TGA) (SDT Q600, TGA/DSC, TA Instruments), as the solid residue remaining after the combustion of the polymeric component of the particles. The sample was placed in alumina crucibles and heated up to 650°C at a rate of 10°C/min, under a constant air flow of 100 Ncm³/min).

RESULTS AND DISCUSSION

Composite microparticles of Na₂MoO₄-PVP and AgAc-PVP were produced operating at 105 bar and 90°C in the saturator, mass flow ratio between CO₂ and liquid solution GLR=1 and a precipitation temperature of 110°C. For the both systems, spherical and well separated particles were obtained. This can be noted in the SEM photomicrographs reported in figure 1 and figure 3, that show the

morphology of the particles after the sample has been covered by gold. In order to study the nanostructure and the way in which the compound loaded is distributed in the polymeric matrix, EDX analysis and SEM with secondary and back scattering electrons. The presence of the compounds in the polymer composite particles was verified using EDX technique, but it was not possible to attest the distribution of the molecules in the matrix, because of the resolution limits of the technique. Using SEM technique with the back scattering detector, without covering the powders with conductive gold, it was possible to recognize the compounds loaded from the polymer. The elements with high atomic number gives higher intensity of backscattered electron with a consequent brighter image compared to elements with low atomic number. The salts used in this work as loading agents have been chosen with a higher atomic number compared to the polymer, therefore in the samples they can be detected with a great contrast compared to the matrix.

In case of Na_2MoO_4 -PVP, from the photomicrographs reported in figure 2, it is possible to note that the salt is present as a spherical core in the particles, surrounded by a shell of polymer. The nanostructure is typical of microcapsules.

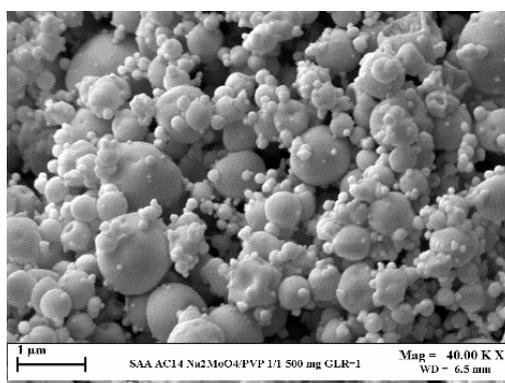


Figure 1. SEM photomicrograph of SAA particles of PVP- Na_2MoO_4

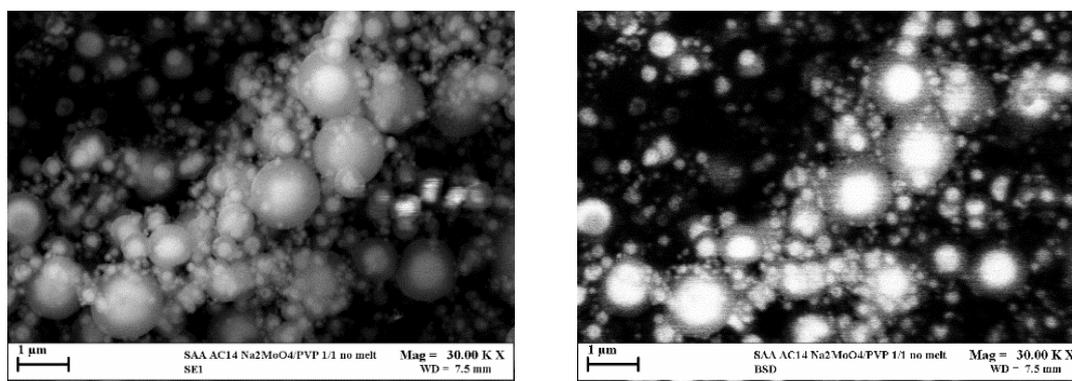


Figure 2. SEM photomicrographs of SAA particles of Na_2MoO_4 -PVP detected with secondary electrons (left) and back scattering electrons (right).

The PSD are reported in table 1. The elaboration of the SEM images showed a PSD with a mean diameter of the microcapsules of about 320 nm and a core mean diameter of about 220 nm. For the DLS analysis, the particles have been first suspended in acetone, in which the PVP is not soluble and the particle diameter measured was of about 520 nm; then the particles have been dissolved in 1-butanol in which PVP is soluble, and Na_2MoO_4 is not, with the consequent suspension of the salt. The diameter measure for the core is of about 350 nm. The results of the two measurement technique show a difference of about 100 nm, with larger particle size for the DLS technique.

Technique	Diameters	D ₁₀	D ₅₀	D ₉₀	SD
		<i>nm</i>	<i>nm</i>	<i>nm</i>	<i>nm</i>
SEM	External	142.5	318.8	710.4	259.3
	Internal	104.4	220.7	464.3	179.0
DLS	External	407.5	520.3	635.6	220.4
	Internal	202.5	350.7	550.6	170.4

Table 1. PSDs data of SAA microcapsules PVP-Na₂MoO₄ in terms of particle number. Comparison of SEM and DLS technique. SD=standard deviation

In case of AgAc-PVP, the photomicrographs reported in figure 4, show that the salt is present as small clusters distributed on the surface and we suppose also inside the spherical polymeric particles. Very bright points can be noted in the dark structure of the particles, showing that the nanostructure is the one of microspheres.

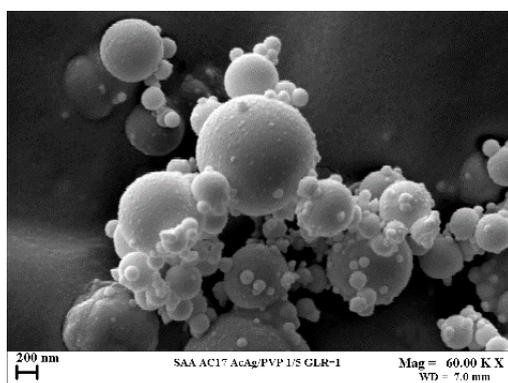


Figure 3. SEM photomicrograph of SAA particles of AgAc-PVP

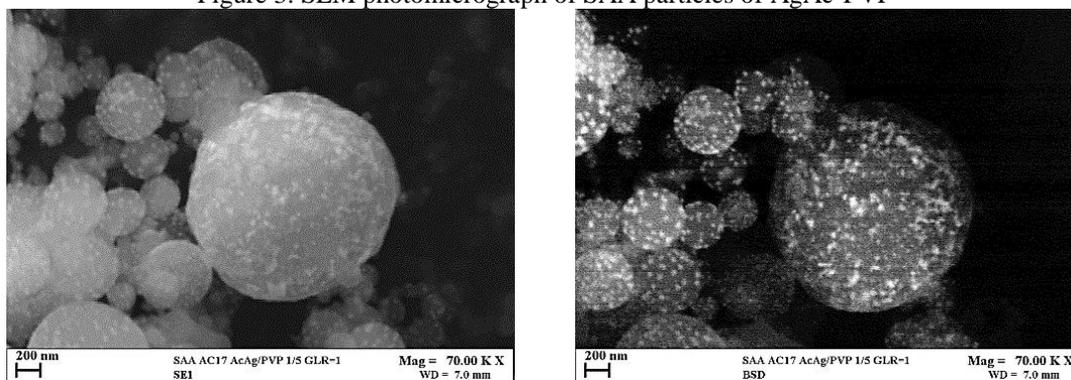


Figure 4. SEM photomicrographs of SAA particles of PVP- AgAc detected with secondary electrons (left) and back scattering electrons (right).

The PSD are reported in table 2. From the elaboration of SEM photomicrographs, the mean particle diameter measured was of about 375 nm and the diameter of the AgAc loaded was of about 40 nm. For DLS analysis, acetone has been used to suspend the whole particles and 1-butanol to solubilize the PVP and suspend the salt loaded. In this case, the DLS technique gave comparable results of diameter size, maybe because of a better interaction of the compounds with the used solvent.

Technique	Diameters	D ₁₀	D ₅₀	D ₉₀	SD
		<i>nm</i>	<i>nm</i>	<i>nm</i>	<i>nm</i>
SEM	External	196.4	375.9	800.7	180.1
	Internal	27.7	39.3	52.6	15.3
DLS	External	230.5	400.6	580.1	180.1
	Internal	25	39	53	15.3

Table 2. PSDs data of SAA microspheres AgAc-PVP in terms of particle number. Comparison of SEM and DLS technique. SD=standard deviation

TGA analysis showed that the loading efficiency of the salt in PVP is different in case of microcapsules and microspheres: in the microcapsules, the encapsulation of Na₂MoO₄ in PVP was of about 100%, in the microspheres, AgAc loading in PVP was of about 40%. This difference can be related to the interaction between the salt and the polymer: if the interactions of the loaded compound B and the polymer A have higher energetic stability compared to the interactions A-B, a microsphere structure is obtained, with B dispersed in A; on the other hand, if the interactions A-A and B-B have higher energetic stability, the dispersion of B in A is difficult and B tends to form a nucleus that A covers as a shell forming a microcapsule. In case of microcapsules, the entrapping of the loaded compound is more effective than in the case of microspheres, in which it is possible that part of the material is not entrapped in the polymeric matrix.

CONCLUSION

Using SAA technique it is possible to obtain two different kind of composite particles: microcapsules and microspheres. The two typologies are related to the kind of salt used as principle loaded in the particles: the system Na₂MoO₄-PVP produces microcapsules, the system AgAc-PVP produces microspheres. The nanostructure is strongly dependent from the chemical species in the system, that is related to the compatibility of the principle with the polymeric carrier and probably to the crystallization rate of the principle. The evaporation rate of the solvent during the atomization in the precipitator can affect the nanostructure and it can be modified by the precipitation temperature and the gas to liquid ratio between SC-CO₂ and liquid solvent.

The DLS is an effective analytical method to measure both the size of the particles and the size of the principles loaded in the particles, when the right solvent is used to suspend different compounds.

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