ELSEVIER

International Journal of Pharmaceutics 243 (2002) 83–91

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Rifampicin microparticles production by supercritical antisolvent precipitation

Ernesto Reverchon *, Iolanda De Marco, Giovanna Della Porta

Dipartimento di Ingegneria Chimica ed Alimentare, Università di Salerno, Via Ponte don Melillo, 84084 Fisciano (SA), Italy

Received 2 May 2002; received in revised form 3 May 2002; accepted 6 May 2002

Abstract

Semi-continuous supercritical antisolvent (SAS) precipitation has been used to produce Rifampicin micro- and nanoparticles with controlled particle size (PS) and particle size distribution (PSD). SAS experiments were performed using different liquid solvents. The best micronization results have been obtained using dimethyl sulfoxide (DMSO); using this solvent and operating at 40 °C, we obtained nanoparticles with mean diameters ranging from 0.4 to 1 μm at a pressure of 120 bar or more, and microparticles with mean diameters ranging from 2.5 to 5 μm at pressures between 90 and 110 bar. The morphology of Rifampicin precipitates was different too. Nanoparticles connected in small aggregates were obtained at pressures higher than 120 bar, whereas, spherical single microparticles were obtained operating at lower pressures. We also investigated the effect of the concentration of Rifampicin in the liquid solution on particles diameter: we observed that, increasing the liquid concentration, the mean PS increased and the PSD enlarged. XRD and HPLC analysis on treated Rifampicin showed that particles are amorphous and no degradation occurred as a consequence of supercritical processing. We attempted an explanation of the different morphologies observed considering the modification of the high pressure vapor–liquid equilibria of the ternary system Rifampicin–DMSO–CO_2 with respect to the behavior of the binary system DMSO–CO_2.

Keywords: Rifampicin; Supercritical fluids; Microparticles; Antisolvent

1. Introduction

The micronization based on the use of supercritical antisolvents (SAS) has been suggested during the last years in alternative to traditional liquid antisolvent processes. Supercritical fluids in this process substitute the liquid antisolvent to induce the precipitation of microparticles with controlled diameter and particle size distribution (PSD). This technique has been applied by various research groups to explosives, catalysts, superconductor precursors (Gallagher et al., 1992; Reverchon et al., 1998, 1999), polymers (Dixon et al., 1993), biopolymers (Yeo et al., 1993; Rever-

* Corresponding author. Tel.: +39-089964116; fax: +39-089964057

E-mail address: ereverchon@unisa.it (E. Reverchon).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00261-2

chon et al., 2000a), and some pharmaceutical compounds (Reverchon and Della Porta, 1999; Shekunov and York, 2000; Reverchon et al., 2000b; Chattopadhyay and Gupta, 2001; Velaga et al., 2002).

Rifampicin is an antibiotic that is mainly used for the treatment of tuberculosis; but, it is also used in the therapy of the meningitis and in the infections of the biliary ways. It acts preventing DNA transcription in the cells, a process in which the genetic information of DNA are transcribed in form of RNA. In particular, it interacts with RNA polymerase holoenzyme, resulting in abortive initiation and extension of transcription (Cole, 1996; Singh et al., 2001). This kind of antibiotics can attack either bacteric cells or guest organism cells. For this reason, they can be toxic for the human organism too. Rifampicin has a reduced toxicity for the human specie since it stops only the bacterial RNA-polymerase, the enzyme that in the bacteria gives rise to DNA transcription. Rifampicin is practically insoluble in water, thus, its dissolution in biological liquids is particularly difficult; therefore, its micronization can have a great impact on the effectiveness and duration of therapy. The production of micro and nanoparticles of controlled size and PSD of this antibiotic can reduce the therapeutic dosage and can avoid the effects correlated to the toxicity of the drug. Aerosolized microparticles could also be used to target the delivery of Rifampicin to alveolar macrophages trying a different approach to tuberculosis therapy. Suitable micronized Rifampicin particles will deposit on the lung periphery, where they can be ingested by alveolar macrophages and dissolution will occur (O'Hara and Hickey, 2000).

Therefore, the aim of this study is to ascertain the feasibility of SAS processing for this antibiotic to prepare micronic and submicronic particles. The powder morphologies, the role of SAS process parameters and the influence of different liquid solvents on the particle size (PS) and PSD are also studied. A windowed precipitation vessel has also been used to analyze the interactions of the high pressure vapor–liquid equilibria (VLE) with the precipitation process.

2. Experimental apparatuses, materials, procedures and methods

2.1. Apparatuses

Our SAS apparatus consists of two High-Performance Liquid Chromatography (HPLC) pumps (Gilson, mod. 305) used to deliver the liquid solution and supercritical CO_2, respectively. A cylindrical vessel of 500 cm^3 I.V. (I.D. = 5 cm) is used as precipitation chamber. The liquid mixture is delivered to the precipitator as a rule through a 60 μm diameter stainless steel nozzle. Supercritical CO_2 is delivered through another inlet port located on the top of the chamber. Before entering the precipitator, CO_2 is heated at the process temperature. The precipitation vessel is electrically heated using thin band heaters (Watlow, mod. STB3J2J1). The pressure in the chamber is measured using a test gauge manometer (Salmoiraghi, mod. SC-3200) and regulated by a micrometering valve (Hoke, mod. 1315G4Y) located at the exit (bottom) of the chamber. This valve is heated by a cable heater (Watlow, mod. 62H24ASX) connected to a controller. A stainless steel frit (pore diameter of 0.1 micron) located at the bottom of the chamber is used to collect the produced powder. A second collection vessel located downstream the micrometering valve is used to recover the liquid solvent. A backpressure valve (Tescom, mod. 26-1723-44) regulates the pressure in this vessel. At the exit of the second vessel a rotameter (Matheson, mod. 605) and a dry test meter are used to measure the CO_2 flow rate and the total quantity of antisolvent delivered, respectively. More information on the SAS apparatus was published elsewhere (Reverchon et al., 1998).

The transparent SAS apparatus differs from the other one only for the precipitator (NWA, Germany) that consists of a stainless steel cylindrical vessel (375 cm^3 I.V.) with two quartz windows put along all the longitudinal section (Fig. 1). Therefore, it is possible to visually follow the macroscopic evolution of the precipitation process from the liquid jet break-up to the deposition of precipitated particles.

2.2. Materials

Rifampicin with a purity of 99.9%, dimethyl sulfoxide (DMSO), *N*-methyl 2-pyrrolidone (NMP), methyl alcohol (MeOH), ethyl acetate (EtAc) and dichloromethane (MeC) with a purity of 99.5% were supplied by Sigma–Aldrich (Italy). CO_2 (purity 99.9%) was purchased from SON (Naples, Italy). The approximate solubilities of Rifampicin in DMSO, NMP, MeOH, EtAc and MeC were measured at room temperature and are 120, 80, 60, 40 and 60 mg/ml, respectively. The untreated material was formed by irregular crystals with a mean PS ranging between 20 and 100 μm (Fig. 2). All materials were used as received.

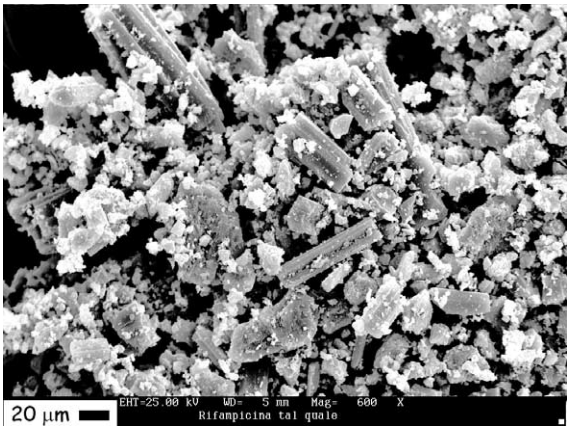

Fig. 2. SEM image of unprocessed Rifampicin.

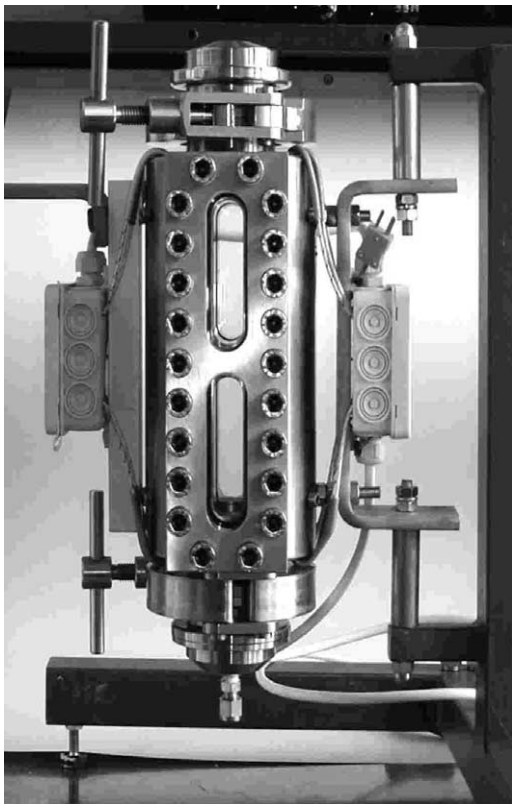

Fig. 1. Transparent SAS precipitator.

2.3. Experimental procedures

A SAS experiment begins by delivering supercritical CO_2 to the precipitation chamber until the desired pressure is reached. The antisolvent steady flow is then established. Then, pure solvent is sent through the nozzle to the chamber with the aim of obtaining steady state composition conditions during the solute precipitation. At this point, the flow of the liquid solvent is stopped and the liquid solution is delivered through the nozzle. The experiment ends when the delivery of the liquid solution to the chamber is interrupted. However, supercritical CO_2 continues to flow to wash the chamber for the residual content of liquid solubilized in the supercritical antisolvent. If the final purge with pure CO_2 is not done, solvent condenses during the depressurization and can solubilize the collected powder. More details were given elsewhere (Reverchon et al., 1998).

2.4. Analytical methods

Samples of the powder precipitated on the metallic frit were observed using a Scanning Electron Microscope (SEM) mod. LEO 420. SEM samples were covered with 250 Å of gold using a sputter coater (Agar mod. 108A). The PS and the PSD were measured using the Sigma Scan Pro software (Jandel Scientific) and about 1000 particles were considered in each calculation of PSD.

X-ray diffraction pattern (XRD) analysis was performed using a Philips PW 1050 XRD apparatus to ascertain if changes occurred in the crystal habit of Rifampicin as a consequence of the SAS process.

HPLC was performed to test if the SAS process modified the antibiotic. The HPLC system (Hewlett Packard series 1100) consisted of a micropump (mod. G1311A) and a UV detector set at 220 nm. The column used was a Zorbax RX C_8, 150×4.6 mm I.D., 5 μm PS. The mobile phase was 0.05 M potassium dihydrogen phosphate–acetonitrile (55:45 v/v) with a flow rate of 1 ml/min at ambient temperature. A standard solution of Rifampicin was prepared by dissolving 2 mg/ml of Rifampicin in MeOH (Lau et al., 1996).

3. Results and discussion

As a first step we tested SAS Rifampicin precipitation process from some solvents: DMSO, NMP, EtAc, MeOH and MeC using the stainless steel precipitator. The best results were obtained using DMSO since a powder formed by small particles precipitated in the collection vessel. In the other cases most of solute was recovered in the liquid collection vessel; i.e. the drug was partly extracted by the solution formed by the liquid solvent and supercritical CO_2 (Reverchon, 1999). In the experiments performed using NMP, MeOH and MeC, Rifampicin was precipitated in form of tightly networked nanoparticles, while using EtAc we mostly obtained some millimeters long needle-like crystals (Fig. 3). Based on these results, we decided to continue to test Rifampicin, using only DMSO as the liquid solvent.

The starting range of operating conditions used in our experiments was selected on the basis of our previous experiences on this process (Reverchon, 1999; Reverchon and Della Porta, 1999; Reverchon et al., 2000b). Thus, we performed a first set of experiments at a pressure of 120 bar. The other operating conditions were temperature 40 °C, 1 ml/min liquid solution flow rate. The ratio between CO_2 and liquid flow rates was set equal to 20 on a volumetric basis at the process

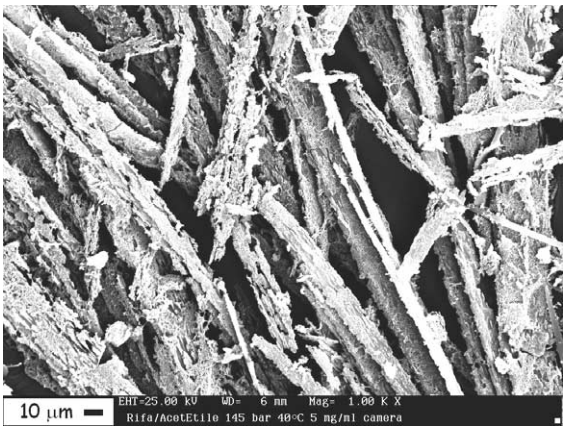

Fig. 3. SEM image of Rifampicin precipitated from EtAc at 145 bar, 40 °C and 5 mg/ml.

operating conditions. At these conditions we varied the concentration of the Rifampicin–DMSO solution from 10 to 70 mg/ml. SEM analysis of the powders precipitated in this set of experiments showed that Rifampicin was precipitated in form of nanoparticles coalescing in small groups. An example of this morphology is reported in Fig. 4.

Since the groups of particles show an irregular geometry, to describe these particles we decided to consider two characteristic dimensions of the aggregates: diameter and length. Then, we calculated the diameter of the spherical particles having

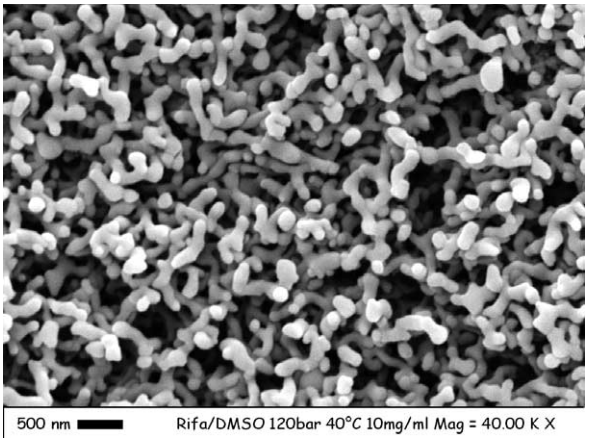

Fig. 4. SEM image of the micronized Rifampicin precipitated from DMSO at 120 bar, 40 °C and 10 mg/ml.

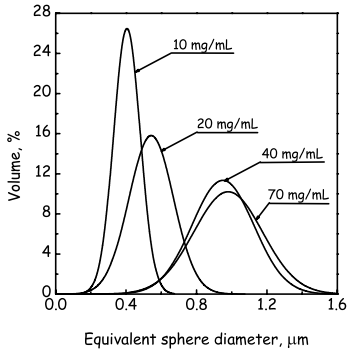

Fig. 5. PSDs of Rifampicin powders precipitated from DMSO. Calculations in terms of particle volume percentages.

the same volume as the correspondent aggregates. This data was used to evaluate the PSD of the powders in terms of volume percentage, the most representative distribution from the pharmaceutical point of view, since it is directly correlated to the drug dosage. PSDs obtained at different concentrations of Rifampicin in DMSO are reported in Fig. 5 and show that by increasing the solute concentration the mean PS increases and the PSD enlarges. The distributions are symmetric (Gaussian) and their mean ranges from 0.4 to 1 μm. Rifampicin particles obtained operating at 10 mg/ml DMSO are of particular interest since the PSD is very sharp and practically all particles fall within the range of injectable suspension drugs.

The second set of experiments was performed fixing the concentration of the liquid solution at 10 mg/ml and varying the precipitation pressure from 90 to 180 bar. For pressures higher than 120 bar, we obtained results similar to the ones obtained at 120 bar; whereas, in the tests performed at 90, 100 and 110 bar, we observed that, surprisingly, a different morphology of Rifampicin particles was produced: neat, spherical, micronic particles were obtained. An example of this morphology obtained operating at 100 bar is reported in Fig. 6. This morphology has been observed in some other cases of SAS precipitation (Reverchon et al., 1998, 2000b); but, this is the first time that it has been observed in connection with the one observed at pressures equal or larger than 120 bar; i.e. this change in morphology with precipitation pressure was not expected. Therefore, we decided to perform a new set of experiments at a pressure of 90 bar, varying the liquid solution concentration from 10 to 70 mg/ml to evaluate again the influence of concentration of Rifampicin on the morphology and PS. The results are exemplified in Fig. 7a and b, that allow a qualitative evaluation of the particle dimensions. The morphology does not change with concentration but, as for the experiments performed at 120 bar, the mean PS increases and the PSD enlarges. The quantitative PS analysis performed on SEM images showed that the mean particle diameter ranges in this case from 2.5 to 5 μm when evaluated on the volume basis. The PSDs are well represented by log-normal curves (Fig. 8) when calculated on the basis of the particle number percentages, and by a GCAS distribution (Fig. 9) when calculated on the basis of particle volume percentages. GCAS is an asymmetric curve commonly used in chromatography. The mode (the most frequent PS) of number of particles based distribution (Fig. 8) is always around 1 μm and varies only slightly with concentration, whereas the span of the distribution increases with this parameter. Volume based distributions (Fig. 9) move towards larger diameters with the increase of Rifampicin concentration in DMSO since the largest particles in the distribution assume a major relevance when computed in terms of volume.

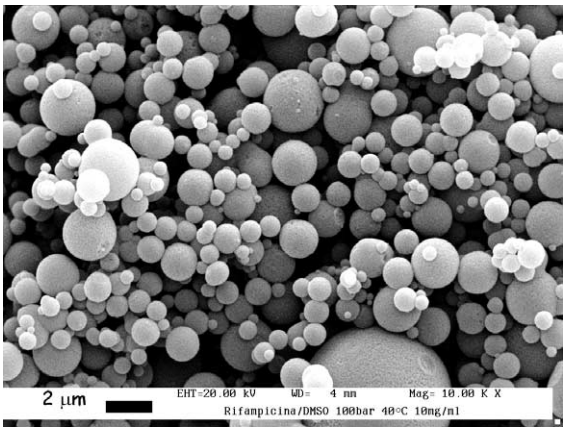

Fig. 6. SEM image of Rifampicin micronized from DMSO at 100 bar, 40 °C and 10 mg/ml.

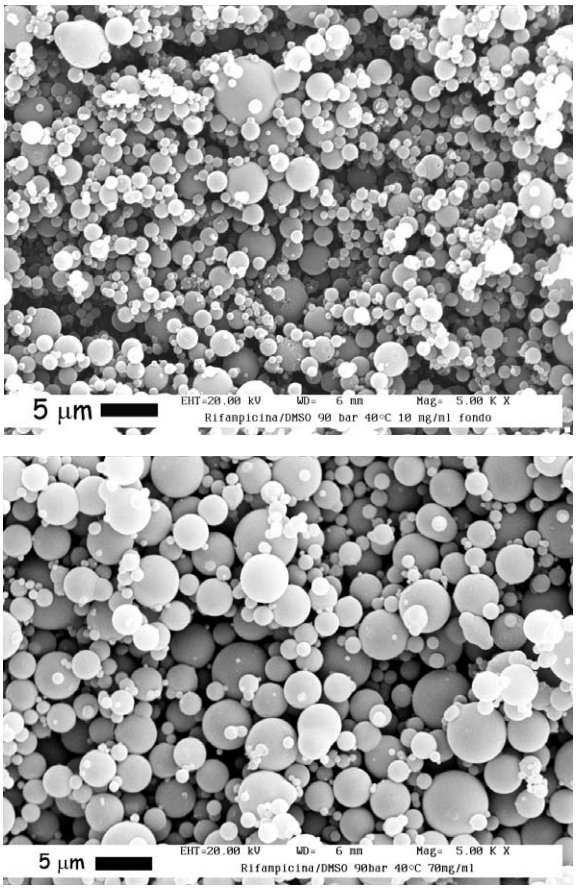

Fig. 7. SEM images taken at the same enlargement of micronized Rifampicin at 90 bar, 40 °C; (a) 10 mg/ml DMSO; (b) 70 mg/ml DMSO.

On the basis of results reported in Fig. 9, Rifampicin particles produced at concentrations of 10 and 20 mg/ml in DMSO could be good candidates for aerosol delivery since the PSD is narrow and under 5 μm in diameter; moreover, particles with diameters lower than 1 μm give a very small contribution to the overall PSD.

Occasionally, during SEM observations, we observed that some spherical particles randomly distributed in the sample were partly destroyed (Fig. 10). In these cases it was possible to observe the inner of these particles. The conclusion is that Rifampicin particles are not continuous, but formed by nanopieces strictly connected all together. This indication could be useful in the

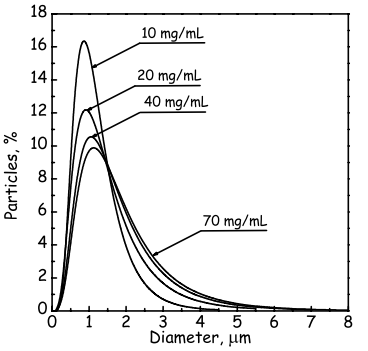

Fig. 8. PSDs of Rifampicin powders precipitated from DMSO at 90 bar, 40 °C; calculations in terms of particle number percentages.

understanding of the mechanism of particles growth during the SAS precipitation: micronic particles precipitated by SAS are tight aggregates of nanoparticles instead of the result of a single growth process.

The comparison of Rifampicin XRD patterns showed that Rifampicin was crystalline before processing and amorphous after SAS (Fig. 11). This difference can be explained by the very fast precipitation that can characterize the SAS that does not allow the organization of the compound in a crystalline form.

We also performed HPLC analysis on untreated and SAS processed Rifampicin: no modification occurred in HPLC retention peaks; therefore, the SAS process has not induced degradation of the drug (Fig. 12).

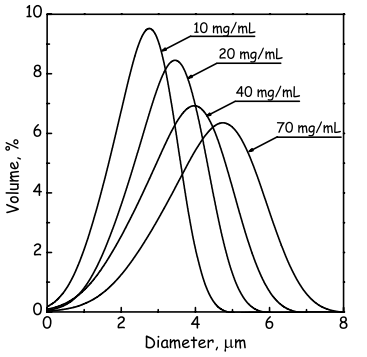

Fig. 9. PSDs of Rifampicin powders precipitated from DMSO at 90 bar, 40 °C; calculations in terms of particle volume percentages.

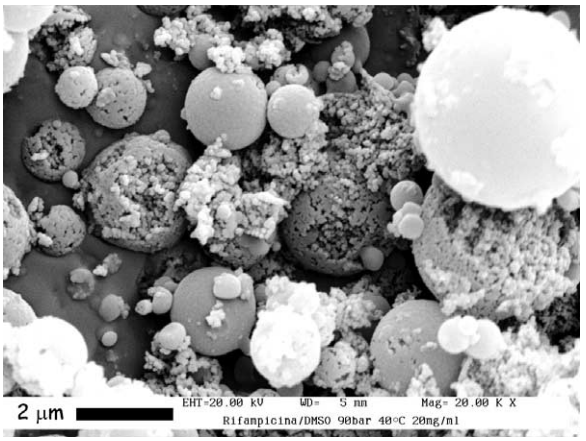

Fig. 10. SEM image of Rifampicin micronized from DMSO at 90 bar, 40 °C, 20 mg/ml.

In the attempt to clarify how different Rifampicin morphologies have been obtained operating SAS below or above 120 bar, we decided to repeat a selected series of experiments on the quartz windowed precipitator.

First, some experiments were performed to confirm information on the binary DMSO–CO_2 system behavior. We used the windowed precipitator and when we operated in the two phase conditions; i.e. at conditions in which the binary system liquid–supercritical CO_2 is not completely miscible, we observed a neat separation between the liquid and supercritical phase represented by a meniscus and the formation of a liquid jet that entered deeply in the fluid phase before its disappearance. When we repeated the same experiment in the single-phase region; i.e. at pressure and

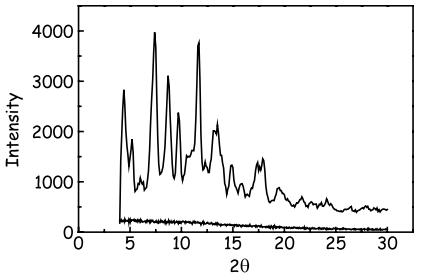

Fig. 11. Comparison of Rifampicin XRD patterns before and after SAS process. Upper trace: unprocessed. Lower trace: SAS processed.

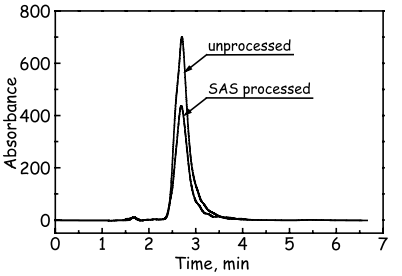

Fig. 12. Comparison of HPLC traces of untreated and SAS precipitated Rifampicin.

temperature conditions for which the binary system exhibits complete miscibility, the liquid jet was visible only in the immediate proximity of the injector. A further increase of pressure produced its almost complete disappearance. These results demonstrate that in the single-phase region the dissolution of the liquid into the supercritical phase is extremely fast and that the higher is the pressure the faster is the kinetics of liquid dissolution in the SCF. Therefore, the limiting step of the precipitation could not be the mass transfer but the thermodynamics; i.e. VLE at high pressure.

At 40 °C single phase behavior of the binary system starts at pressures slightly higher than 90 bar as previously observed on the basis of liquid expansion curves (Reverchon et al., 1998). Whereas, during the tests performed using the ternary system Rifampicin–DMSO–CO_2 and using the windowed vessel, we observed at 90 bar, 40 °C the formation of two phases almost as soon as we started the injection of the liquid solution. Although the injection of the liquid solution was continuous, this situation was stable; i.e. the volume occupied by the two phases was constant. The steadiness of the volumes allows us to suppose that the system was very close to thermodynamic equilibrium. Thus, despite the binary system DMSO–CO_2 is completely miscible at these conditions, the presence of Rifampicin induces the formation of a fluid and a liquid phase. The upper phase was transparent, while the lower one was red (the typical color of Rifampicin). The two phases were separated by a sharp and defined interface, Rifampicin precipitated from the lower phase starting from the separation meniscus.

Thus, two phases are formed, due to the presence of solute that modifies the high pressure VLE moving the mixture critical point (MCP) (i.e. the pressure at which the mixture is supercritical) towards higher pressures. It is possible to visualize this situation using Fig. 13. In this figure we report a semi-quantitative solubility diagram for the system DMSO–CO_2 (represented with a solid line; data adapted from Reverchon et al., 1998) and its hypothesized modification (represented with a dashed line) for the ternary system with the shift of the MCP to higher pressures, as our visual observations suggested when Rifampicin was added. However, Fig. 13 is only a simplification of the real behavior of the ternary system that should correctly be represented on a triangular diagram. Point A in Fig. 13 is located at conditions where we observed the two phases. Indeed, the mixture A splits in two parts in equilibrium, whose composition is given by two points on the boundaries of miscibility curve: a supercritical phase containing part of the liquid solvent and negligible quantities of Rifampicin, and a liquid one, containing part of CO_2 and almost all the solute. When Rifampicin precipitates from this expanded liquid phase, the morphology of spherical non-aggregated particles is produced. Increasing the pressure (departing from 90 bar), we observed that the higher was the pressure, the smaller was the level occupied by the lower phase. When the operating pressure was 120 bar, the lower phase vanished and the upper phase filled the whole volume of the chamber. At these conditions, Rifampicin precipitated from this unique fluid phase. Thus, when we operated at pressures larger than about 120 bar, we worked on point B in Fig. 13; i.e. above the MCP of the ternary system; we were at supercritical conditions with respect to the whole system and nanometric connected particles were produced (Fig. 4). Thus, the parameter controlling the observed change in SAS precipitates morphology is of thermodynamic nature and is connected to the high pressure phase equilibria characteristic of the Rifampicin–CO_2–DMSO system.

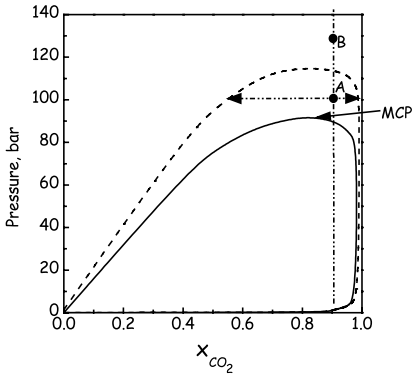

Fig. 13. High pressure solubility diagram at 40 °C for the binary system DMSO–CO_2 (solid line) and its hypothesized modification due to the presence of Rifampicin (dashed line). MCP is the mixture critical point, A is an operating point in the two phases region and B is an operating point into the one phase region.

4. Conclusions

Using SAS we produced micronic and nanometric particles of Rifampicin by varying the pressure of the process. Moreover, we can control the PS and the PSD of the particles by varying the concentration of the liquid solution.

At low pressures and concentrations (for example, at 10 mg/ml) only the 4.5% of the particles on volume basis (see also Fig. 9) has a diameter lower than 1 μm and no particles have a diameter larger than 5 μm; i.e. 95.5% of the volume of micronized particles ranges between 1 and 5 μm, that is the range suitable for aerosol delivery.

It is also possible to produce nanometric Rifampicin particles working at high pressure and low concentration of the liquid solution. For example, at 120 bar and 10 mg/ml (see also Fig. 5), only 12% of the particles has an equivalent diameter larger than 0.5 μm (on the volume basis). Therefore, it could be possible to consider these particles for the production of injectable suspensions.

Acknowledgements

The authors acknowledge the financial support for this research given by MiUR (University and Research Ministry), Italy, PRIN 2000.

References

Chattopadhyay, P., Gupta, R.B., 2001. Production of griseofulvin nanoparticles using supercritical CO_2 antisolvent with enhanced mass transfer. Int. J. Pharm. 228, 19–31.

Cole, S.T., 1996. Rifamycin resistance of mycobacteria. Res. Microbiol. 147, 48–52.

Dixon, D.J., Johnston, K.P., Bodmeier, R.A., 1993. Polymeric materials formed by precipitation with a compressed fluid antisolvent. AIChE J. 39, 127–139.

Gallagher, P.M., Coffey, M.P., Krukonis, V.J., Hillstrom, W.W., 1992. Gas anti-solvent recrystallization of RDX: formation of ultra-fine particles of a difficult-to-comminute explosive. J. Supercrit. Fluids 5, 130–142.

Lau, Y.Y., Hanson, G.D., Carel, B.J., 1996. Determination of rifampin in human plasma by high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. B 676, 147–152.

O'Hara, P., Hickey, A.J., 2000. Respirable PLGA microspheres containing Rifampicin for the treatment of tuberculosis: manufacture and characterization. Pharm. Res. 17, 955–961.

Reverchon, E., 1999. Supercritical antisolvent precipitation of micro- and nano-particles. J. Supercrit. Fluids 15, 1–21.

Reverchon, E., Della Porta, G., 1999. Production of antibiotic micro- and nano-particles by supercritical antisolvent precipitation. Powder Technol. 106, 23–29.

Reverchon, E., Della Porta, G., De Rosa, I., Subra, P., Letourneur, D., 2000a. Supercritical antisolvent precipitation of some biopolymers. J. Supercrit. Fluids 18, 239–245.

Reverchon, E., Della Porta, G., Falivene, M.G., 2000b. Process parameters and morphology in amoxicillin micro and submicro particles generation by supercritical antisolvent precipitation. J. Supercrit. Fluids 17, 239–248.

Reverchon, E., Della Porta, G., Pace, S., Di Trolio, A., 1998. Supercritical antisolvent precipitation of submicronic particles of superconductor precursors. Ind. Eng. Chem. Res. 37, vol. 3, 952–958.

Reverchon, E., Della Porta, G., Sannino, D., Ciambelli, P., 1999. Supercritical antisolvent precipitation of nanoparticles of a zinc oxide precursor. Powder Technol. 102, 127–134.

Shekunov, B., York, P., 2000. Crystallization process in pharmaceutical technology and drug delivery design. J. Cryst. Growth 211, 122–136.

Singh, S., Mariappan, T.T., Sankar, R., Sarda, N., Singh, B., 2001. A critical review of the probable reasons for the poor/variable bioavailability of rifampicin from anti-tubercular fixed-dose combination (FDC) products, and the likely solutions to the problem. Int. J. Pharm. 228, 5–17.

Velaga, S.P., Ghaderi, R., Carlfors, J., 2002. Preparation and characterization of hydrocortisone particles using a supercritical fluids extraction process. Int. J. Pharm. 231, 155–166.

Yeo, S.D., Lim, G.B., Debenedetti, P.G., Bernstein, H., 1993. Formation of microparticulate protein powders using a supercritical fluid antisolvent. Biotechnol. Bioeng. 41, 341–346.