LIPID SYSTEM MICRONIZATION FOR PHARMACEUTICAL APPLICATIONS BY PGSS TECHNIQUES

Nicola Elvassore*[1], Marina Flaibani[1], Keti Vezzù[1], Alberto Bertucco[1], Paolo Caliceti[2], Alessandra Semenzato[2], Stefano Salmaso[2]

[1]*Department of Chemical Engineering – University of Padua*
Via Marzolo, 9 - I-35131 Padova PD ITALY
[2]*Department of Pharmaceutical Sciences -- University of Padua*
Via Marzolo, 5 - I-35131 Padova PD ITALY
*e-mail: nicola.elvassore@unipd.it

ABSTRACT

A technique named particle from gas saturated solution (PGSS) is used to produce micro-particles of lipids. A thermodynamic study of gas solubility and melting temperature depression is carried out by a perturbed-hard-sphere-chain equation of state. This equation fairly describes both fluid-liquid and solid-fluid-liquid equilibria and provides the enthalpy of the gas-lipid mixture at high pressure. Using this information, the relation between the initial and final thermodynamic properties of the PGSS process were calculated by solving simultaneously the energy balance and the equation of state. The pressure-temperature charts show three regions above the *P-T* solid-liquid-fluid coexistence curve, from which solid, solid-liquid or liquid products can be obtained.
Micro-particles of lipids and lipid mixtures were successfully produced by PGSS. The effects of temperature and pressure on the particle dimension and their diameter distribution size were investigated. In addition, the PGSS process was carry out at temperatures as low as 310 K, so that it was possible to process biological active substances without losing their biological activity. In particular, the PGSS technique was used to embody biological active molecules within a solid micro-particulate lipid matrix. This technique is particularly promising as it avoids almost completely the use of organic solvent and allows to easily scale up the method to the industrial production level.

INTRODUCTION

Micro-particulate solid lipidic systems represent one of the most recent innovative formulations in pharmaceutical and cosmetic fields. It is well know that the therapeutic performance of a drug is not only related to the drugs itself but it also depends on the type of formulation. A class of drug carriers, Solid Lipid Nanoparticles (SLN) [1] offers important advantages such as: an increase of stability and relative high loadings of either lipophilic or hydrophilic bio-active substances. Classical processes for producing solid-lipidic-nanoparticles are: high shear homogenization, ultrasound, high pressure homogenization, micro-emulsion and solvent emulsification/evaporation [2]. However, these methods suffer a number of disadvantages related to high process temperature necessary for melting the lipids, high shear and pressure stresses, the use of surfactant and/or organic solvents (solvent

emulsification/evaporation) and the lyophilization procedure needed to obtain dry powder from a dilute aqueous solution.
On the other hand, in order to produce micro-particulate lipid products, an alternative technique, called *Particle from Gas Saturated Solution* (PGSS) [3], was recently proposed. This method provides a dry product avoiding completely the use of organic solvents. In particular, PGSS shows great advantages when applied to lipid systems, because lipids can easily melt at mild temperature and pressure conditions.
In the PGSS process, a solid is melted in a high-pressure vessel pressurized by a compressed gas. Under these conditions, the gas dissolution into the liquid phase causes the formation of the so-called gas-saturated solution. This solution is expanded through a nozzle where, due to the Joule-Thompson effect and the gas evaporation, it is cooled down; thus leading to the formation of solid particles or liquid droplets. CO_2 was used as the gas.
The characteristics of the system CO_2-lipids depend on operative conditions (temperature and pressure of mixing chamber) and nature of lipids and, for these reason, a thermodynamic study was carried out by a perturbed-hard-sphere-chain equation of state (PHSC EOS).

MATERIALS AND METHODS

99.95% CO_2 was purchased from Air Liquide (Padova, Italy). The lipids used in this work, triestearin (molecular weight of 891.51 Da) and phospatidylcholine (lecithin), were purchased from Fulka Chemie AG (Switzerland).

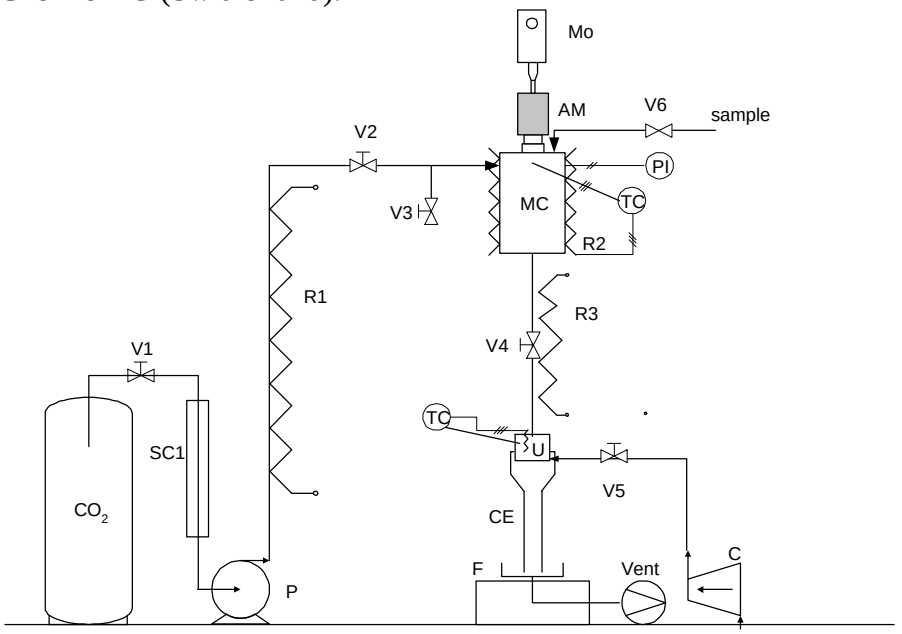

Figure 1: S*chematic of the PGSS set-up: MC: mixing chamber; U: nozzle; CE: expansion chamber; F: filter; Mo: engine; AM: magnetic mixer; R1, R2, R3 e R4 resistances; SC: heater exchanger; P: CO_2 high pressure pump; V1, V2, V3V4 and V5: on-off valves; PI: pressure gauge; TC: temperature control.*

The schematic of the PGSS set-up is reported in Fig. 1. The lipids or lipid mixtures were initially charged into the mixing chamber heated at 331K. The gas was filled until the desired pressure was reached (10-20MPa). At this point the CO_2-lipids system was mixed at fixed rate (150 rpm). The temperature of mixing chamber was then reduced to 313-323K depending on the formulation used. When the system reached the saturation, normally after 30 minutes,

valves V5 and V4 were opened, so the mixture was atomized through a micrometric nozzle (50, 100 e 180 μm).. The micro-particles were collected on a 75 μm stainless steel filter.

THERMODYNAMIC MODELING

The expansion of the lipid-CO_2 saturated solution in the PGSS process leads to final products that can be solid, liquid or solid-liquid with different fractions of solid. The Joule-Thompson effect and the evaporation of CO_2 cause cooling down of the lipid depending of the initial temperature and pressure. The energy balance between the conditions before and after the expansion nozzle allows predicting the properties of the product for given initial conditions. In the case of adiabatic expansion through the nozzle, the energy balance is:

$$E^{f}_{mix} - E^{i}_{mix} = 0 \tag{1}$$

where E^{f}_{mix} and E^{i}_{mix} are the total energy of system at the initial and final conditions (after and before the expansion).

Under the hypothesis of negligible potential and kinetics energy changes, we obtain:

$$\Delta H^{f-i} = \left[x_{CO_2} H^{f}_{CO_2} + \left(1 - x_{CO_2}\right) H^{f}_{lipid}\right] - H^{i}_{mix} = 0 \tag{2}$$

where ΔH^{f-i} is the system enthalpy change, $H^{f}_{CO_2}$ and H^{f}_{lipid} are, respectively, the enthalpy of pure CO_2 and lipid after the expansion, x is component mole fraction in the starting mixture and H^{i}_{mix} is the saturated liquid enthalpy of this mixture before expansion.

To solve the energy balance, expressions for the solubility of CO_2 in lipids and for the residual enthalpy of CO_2-lipid system are required. These values can be calculated by a proper equation of state (EOS); the equation used was the perturbed-hard-sphere-chain equation of state (PHSC EOS) [5,6]. A relation between the initial and the final thermodynamic state of the PGSS process can be obtained by solving the system:

$$\begin{cases} \Delta H^{f-i} = \left(x_{CO_2} H^{f}_{CO_2} + \left(1 - x_{CO_2}\right) H^{f}_{lipid}\right) - H^{i}_{mix}\left(T^{i}, x_{CO_2}\right) = 0 \\ \\ P^{i} = EOS\left(T^{i}, x_{CO_2}\right) \end{cases} \tag{3}$$

where P^{i} and T^{i} are pressure and temperature of the saturated solution before expansion. Solving equations (3) it is possible to trace the *P-T* values that satisfy a given final state condition. For example, the operative conditions leading to saturated solid products is needed, it is necessary to use the proper values of final enthalpy and, for a given initial pressure, P^{i}, to solve the system (3) with respect to the two unknowns T^{i} and x.

Because in the PGSS process it is important to obtain solid material, we have calculated the initial *P-T* conditions that lead to a saturated solid product at the final process condition of 0.1MPa and lipid melting temperature. Similar calculations of initial *P-T* conditions can be repeated for saturated liquid product.

RESULTS

The results of thermodynamic analysis of tristearin-CO_2 system are reported on Figure 2. The initial operative condition of PGSS techniques is that of a saturated (melted) lipid solution: the area above the solid-liquid-fluid coexistence curve (continue line). This area can be divided into three regions according to the characteristic of the final products. In fact, depending on the initial pressure and temperature, the expansion of the system can lead to either solid or liquid or solid-liquid lipid particles. Region I includes the initial operative

conditions from which a complete solid product can be obtained. On the other hand, starting from regions II and III, partially solid or totally liquid products are obtained. The dashed line that separates region I from II and the dot/dashed line dividing region II from III represent the condition at which saturated-solid and saturated-liquid lipid products are obtained, respectively. In region II the thin lines indicate the initial *P-T* conditions from which a product with a given fraction of solid can be obtained. Figure 2 shows also the effect of temperature changes at constant pressure by the "operative line" that represents a link between the initial and final state properties of PGSS process. Starting from region I, an increase of initial temperature leads to higher final temperature of the solid product. On the other hand, when increasing the initial temperature from 327 to 334 K in region II, the fraction of solid phase in the final product decreases from 80% to 50 %.

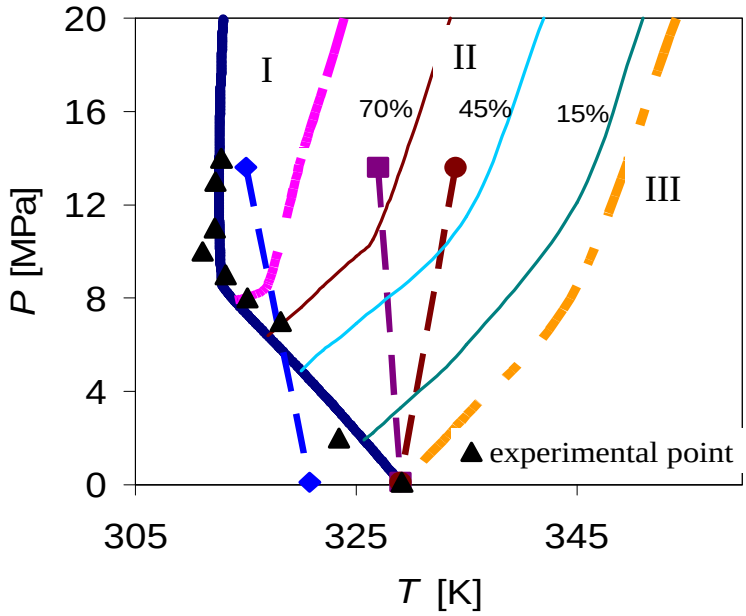

Figure 2: *P-T* chart of tristearin-CO_2 system. The continue line describes the solid-liquid-fluid coexistence curve; the dashed line is the curve of saturated-solid product, the dot-dashed the curve of saturated-liquid product. The thinner lines in region II represent the condition from which a product with given fraction of solid can be obtained. Points (?,¦ ,?) represent the start and end point of operative lines. Triangles (?) are solid-liquid-fluid equilibrium experimental points obtained by visual observation in a high-pressure windowed cell.

On the basis of these results, same experiments were carried out for pressure above 130 bar and for temperatures ranging between 311 and 323 K. It was experimentally observed that increasing the pressures the particle size distribution could be reduced. This effect is probably related to the efficiency of the atomization process that becomes higher when the pressure of the system is increased. Also a decrease of temperature reduces the dimension of product.
The effect the nozzle (silica-capillary tube of 50 and 100 µm diameter and 180 µm sapphire nozzle) was studied. Unfortunately, even if few microns sized particles could be obtained using 50 µm capillary tube nozzle, the use of such small nozzle can not be practically considered because it always leads to occlusion problems.

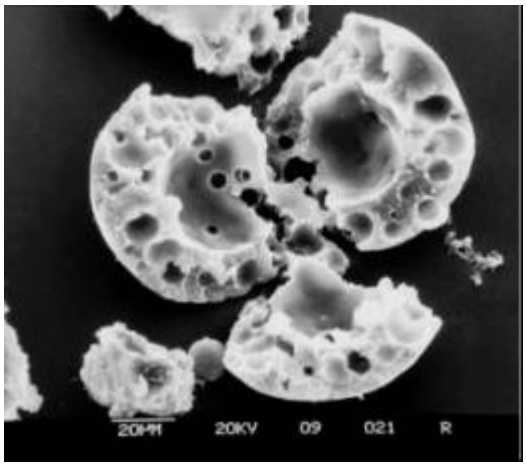

Figure 3: Fractured micro-particles of tristearin obtained by PGSS

For tristearin micro-particles, the best result was obtained using a pressure of 13MPa and 311K in the mixing chamber and with a 100 μm nozzle (Figure 3a). Figure 3b shows an internal view of broken lipid particle obtained at 13MPa and 314K with a 100 μm-nozzle. It is interesting to observe that capsule-like particles can be produced. They present an internal cavity, high porosity on the internal shell and compact and homogeneous external surface.
For the mixture tristearin-lecithin the better operative conditions were 13MPa, 331K and 180 μm-nozzle and the micro-particle dimension was lower than that obtained for tristearin.

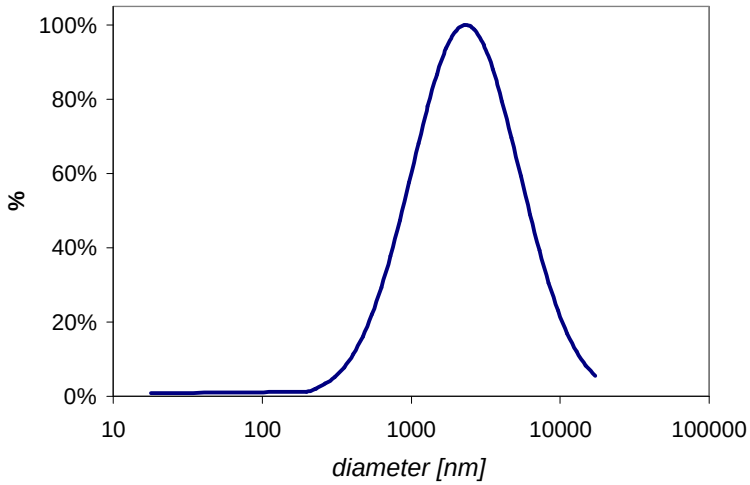

Figure 4: *Particle size distribution of tristearin/phospatidylcholine (50:50 w/w) micro-particles obtained at 20MPa, 333K, 180 mm-nozzle.*

Typical particle size distribution (based on particle volume) produced by PGSS process are reported on Figure 4. The average particle diameter is 2292 nm.

CONCLUSION

A thermodynamic study of solid-liquid-fluid equilibria of lipids-CO_2 system was carried out by a perturbed-hard-sphere-chain equation of state. The operative condition to obtain solid lipid micro- and nano- particles were evaluated.

The effect of operative temperature, pressure and type of nozzle on the resulting product was investigated. The dimension was reduced by increasing pressure and decreasing temperature. The mean dimension of particles was about 2 μm.

Micro-particles of lipids and lipid mixtures were obtained by PGSS process at moderate conditions. It was possible to use PGSS to process biological active substances without losing their biological activity.

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