PRODUCTION OF SOLID-LIPID-MICROPARTICLES BY HIGH-PRESSURE GAS ASSISTED TECHNIQUE

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A new technique that uses high-pressure gasses for melting and atomising lipids is applied to produce solid lipid micro- and nano-particles. A hydrodynamic analysis was used to correlate the process variables with the dimension of the final particles. An equation for two phase jet disgregation was used, all the parameters were estimated and drops diameter obtained was related with particles one.

Micro-particles of lipids and lipid mixtures were successfully produced. The effects of temperature and pressure on the particles dimension and their stability in water where investigated. In addition, the experiments were carried out at temperatures as low as 315 K, so that it would be possible to process biological active substances without losing their biological activity. This technique is particularly promising as it avoids almost completely the use of organic solvent and permits to easily scale up the method to the industrial production level.

INTRODUCTION

Micro- and nano-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. Micro and nano solid-lipid particles [1] offer important advantages: enhancement of stability and relative high loadings of either lipophilic or hydrophilic bio-active substances. Classical processes for producing solid-lipidic-nanoparticles are: high shear homogenisation, ultrasound, high-pressure homogenisation, micro-emulsion and solvent emulsification/evaporation [2]. However, these methods suffer of a number of disadvantages related to high process temperature necessary for melting the lipids, high shear and pressure stresses, the use surfactant and/or organic solvents (solvent emulsification/evaporation) [2] and the lyophilization procedure need to obtain dry powder from a dilute aqueous solution.

To avoid these problems a high-pressure technique, named particle from gas saturated solution (PGSS), was used. This method provides a dry product and avoids completely the use of organic solvents. In particular, it shows great advantages when applied to lipid systems, because lipids can easily melt at mild temperature and pressure conditions with low risks of drug degradation.

In our 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. Leading to the formation of solid particles or liquid droplets. CO_2 is generally used as gas.

Because the drug release depend also on particles diameter, a hydrodynamic analysis was carried out to study the influence of process variables on product dimension.

Another important property of a pharmaceutical formulation is stability; for this reason the zeta potential of products obtained in our laboratory was measured using a dynamic light scattering.

I - MATERIALS AND METHODS

99.95% CO_2 was purchased from Air Liquide (Padova, Italy). Tristearin (molecular weight of 891.51 Da), phospatidylcholine (phospatidylcholine) and (sodium salt, 99%) were purchased from Fulka Chemie AG (Switzerland). Both pure tristearin or mixture of tristearin/phospatidylcholine/dioctyl sulfosuccinate were used.

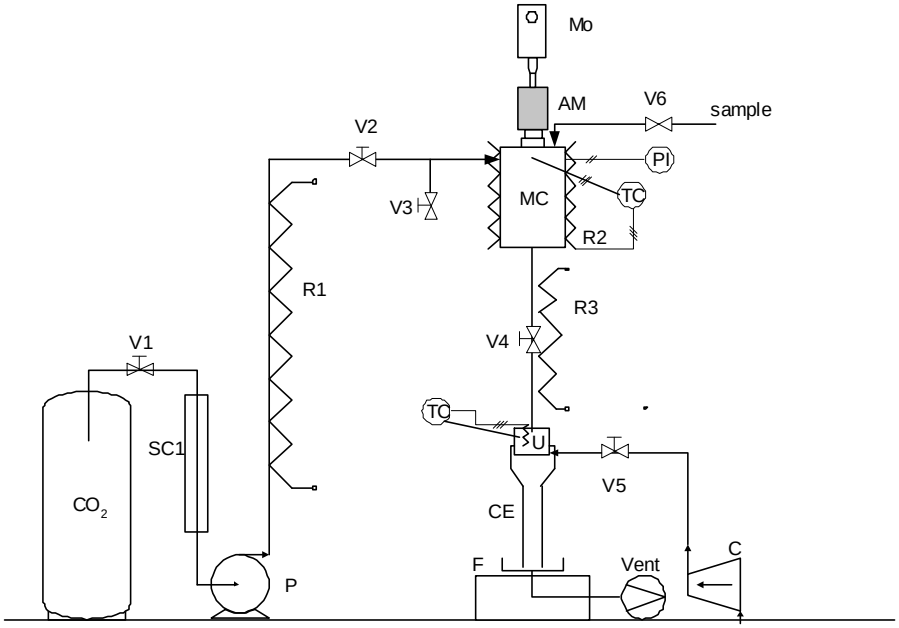

Figure 1: *Schematic of our experimental set-up: MC: mixing chamber; U: nozzle; CE: expansion chamber; F: filter; Mo: engine; AM: magnetic mixer; R1, R2, R3 e R4 resistances; SC: heater exchanger; P: CO_2 high pressure pump; V1, V2, V3, V4 and V5: on-off valves; PI: pressure gauge; TC: temperature control.*

The schematic plant of our experimental set up is reported in Figure 1. The solid lipids or lipid mixture was initially charged into the mixing chamber heated at 313-333K depending on the formulation used. The gas was filled until the desired pressure was reached (10-20 MPa). CO_2 caused a melting temperature depression so in the chamber we obtain a CO_2 saturated liquid mixture. The CO_2-lipid system was mixed at fixed rate (150-200 rpm) for about 30 minutes in order to reach the equilibrium condition. At this point valves V5 and V4 were opened and the mixture was atomised through a micrometric nozzle (180 μm). The solid particles were collected on a 50 μm stainless steel filter. The auxiliary air flow (about 4.3 Nm^3/h, at standard condition of 0.1MPa and 298K) supplied by compressor has the function of delivering the entire product on the filter device.
Products diameters were measured using an optical microscope (LABOR LUX S LEITZ, LAIKA) and a dynamic light scattering (NICOMP 380). *z* potential was measured by using the zeta potential analyser (NICOMP 380).

HYDRODYNAMIC MODELING

Supposing that dimension of final solid products is the same of the liquid drops obtained after the jet disgregation, the diameter of the particle can be estimated using Jasuja equation [3]. This empirical model that was successfully used to predict particle diameter in other high-pressure process [4], follows:

$$d_{32} = 0.17\left(s / r_G\right)^{0.45}\left(1/U_{GL}\right)^{0.9}\left(1+L/G\right)^{0.5} d_{nozzle}^{0.55} + 0.015\left[m_L^2/\left(s r_L\right)\right]^{0.5} d_{nozzle}^{0.55}\left(1+L/G\right) \quad (1)$$

where d_{32} is the main drops diameter, s is liquid surface tension, r_G and r_L (kg/m^3) are gas and liquid density, U_{GL} is the difference between gas and liquid velocity, L/G is liquid and gas mass flow rate fraction, d_{nozzle} is nozzle diameter and m_L is liquid viscosity.

Parameter estimation

The composition of saturated CO_2-lipid mixture was calculated as reported by Elvassore [5]. The surface tension it was measured for different mixture of tristearin and phospatidylcholine with D 2578-84 A.S.T.M. method at temperature of 313K ad pressure of 0.1 MPa. This information was necessary to predict the surface tension for CO_2-lipid system by the equation proposed by Dittmar [6]:

$$s_m = 1.759 \cdot 10^{-4} r_{CO_2}^2 - 0.1227 r_{CO_2} + s_{lipid} \quad (2)$$

where s_m is saturated mixture surface tension, s_{lipid} is lipid surface tension as measured experimentally and r_{CO2} is gas-phase density.

For the viscosity we used the empirical correlation of Lewis-Squires [7] that give temperature dependence of viscosity:

$$m_L^{-0.2661} = m_K^{-0.2661} + \frac{T - T_K}{233} \quad (3)$$

where m_L is the unknown viscosity at temperature T and m_K is the experimental value measured at temperature T_K.

Tristearin viscosity was measured using by reometer at T=58°C and has a value of 0.0306 P. CO_2 viscosity is estimated using a value of pure CO_2 at the operative temperature and at molar volume equals to partial molar volume of CO_2 in the saturated liquid mixture at operative T and P.

The viscosity for the CO_2-lipid system was obtained using the mixing rule proposed by Grunberg and Nissan equation [7].

Flow rate and velocity: we have considered G as the solubilised CO_2 flow rate and L as the liquid lipid mixture flow rate. Fixing a gas saturated liquid flow rate equal to 1 cm^3/s we have calculated both volumetric and mass flow rate for CO_2 and lipids. At this point is also possible to evaluate U_{GL}, in our case we have chosen a nozzle diameter of 180μm corresponding to nozzle used in our experiments.

RESULTS

In Figure 2 are reported results of our hydrodynamic analysis for pure tristearin. The dot-dash curve indicates drops diameter when operative T and P correspond to solid-liquid-fluid equilibrium condition as indicated in the work of Elvassore [5]. This curveshows the lower limit of particle diameter that can be obtained by PGSS process.

As it is evident in Figure 2, diameter decreases with an increase of operative pressure, this behaviour is due to two different causes: first of all at higher pressure CO_2 concentration in liquid rises up and the viscosity fall down, that brings to an easier jet disgregation; second a variation in pressure influences much more gas density than liquid one so that ratio L/G decreases. Both these effects became less significant at high pressure.

Temperature instead has the opposite effect on drops diameter: an increase in operative T brings to bigger particles. Although viscosity of single component decreases with T, variation

of CO_2 concentration causes a decrement of mixture viscosity. Moreover, the reduced quantity of CO_2 solubilizzed in the liquid phase brings a decrease in *G* flow rate and so the ratio *L/G* increases. This result agrees with the study realised by Knez and Novak [8].

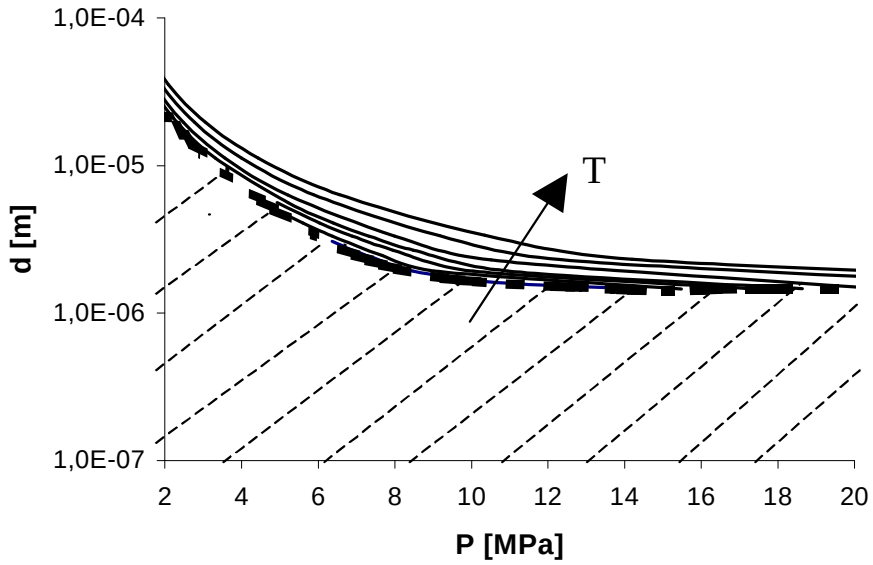

Figure 2: *Results of hydrodynamic model: Tristearin particles diameter as function of operative pressure for temperature between 315K and 355K*

To validate the model some experimental runs with apparatus in Figure 1 were carried out using pure tristearin. Optical microscope gives a mean dimension of these products between 2 and 3 μm. Figure 3 shows as, with a quite simple model, it is possible to obtain a good approximation of particles diameter.

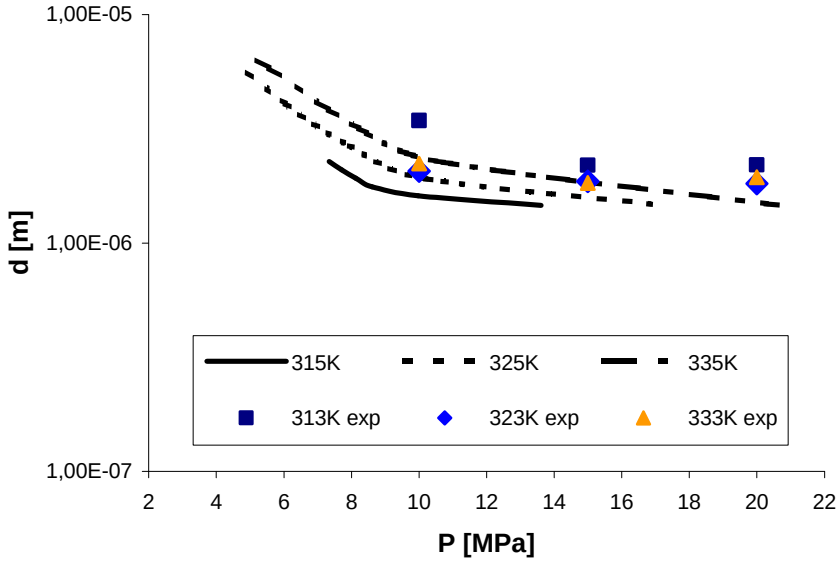

Figure 3: *Comparison between experimental data and model simulation*

To obtain a products that gives stable suspension in water we have added surfactants to tristearin: phospatidylcholine and dioctyl sulfosuccinate.
Mixture of different compositions were micronised in order to optimise the product in term of particle dimension and stability. Experiments at different *T* and *P* were carried out. In each case particles obtained were analysed in dimension and *z* potential.

The addition of phospatidylcholine and dioctyl sulfosuccinate in the formulation results in a decrement of products diameter up to 0.7μm. For the 40:40:20 w/w tristearin/phospatidylcholine /dioctyl sulfosuccinate mixture experimental runs at 15 MPa and at different temperature were carried out. Some results are presented in Table 1

Table 1: *Dimension of particles of 40/40/20 mixture at 15MPa.*

Temperature [K]	Dimension [m] light scattering	Dimension [m] optical microscope
313	0.73	1.34
323	0.99	1.32
333	0.78	1.18

By combining both microscope and light scattering we can provide analysis on a wide dimension range. For example, the use of microscope allows to observe presence of some particles aggregates in the range of 5-10 μm.
Typically, the average diameter is 0.8 μm with standard deviation values between 0.20 and 0.30μm and an example of particle size distribution is showed in Figure 4.

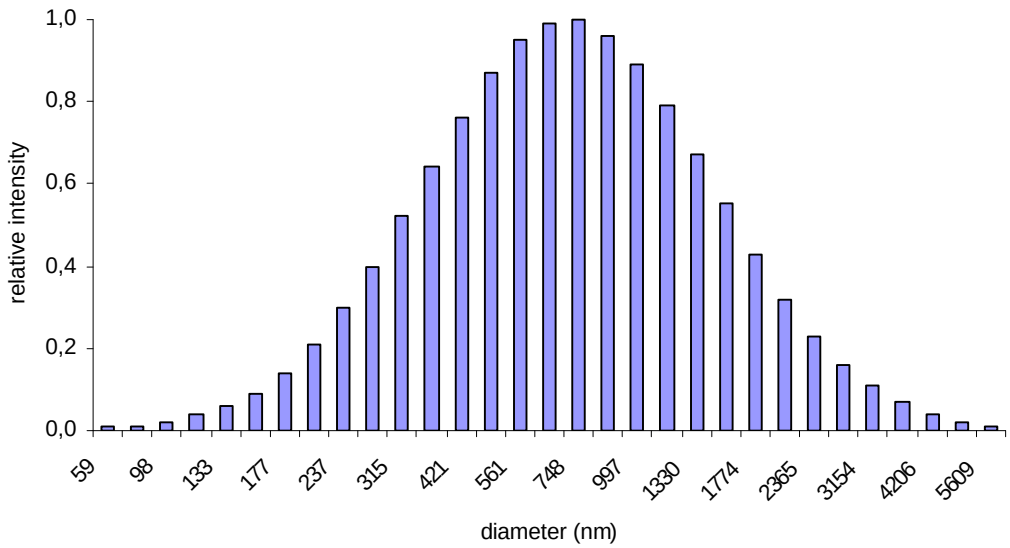

Figure 4: *Particle size distribution for a mixture 40/40/20 w/w processed at 323K and 15MPa*

Values of *z* potential were between –13.24 mV and –41.09 mV depending on composition: an addition on the percentage of phospatidylcholine or dioctil leads to decrease the values of the *z* potential. The products containing mixture of lipids have shown an almost constant value of *z* potential that ranges between –28.91mV and –37.63mV depending on operative *T*.
As reported by Heurtault [9] *z* potential indicates the stability of a suspension. When *z* potential is lower than –30mV, suspension can be considered completely stable, whereas, suspension that can create some small aggregates has a value of –15 / -30mV. Our lipids micro- and nano-particles produced in this work can be successfully dispersed in distilled water and create a stable suspension.

CONCLUSION

A hydrodynamic analysis of the gas-assisted process (PGSS) for production of solid-lipid micropartilces was carried out considering two phases jet in the nozzle. All the parameters

necessary for this study were measured when possible, or estimated with empirical equations. Results of this model were validated by measurement of diameter of particles produced with the apparatus here presented.
Products obtained using different lipid mixtures and at different experimental conditions were analysed in dimension and stability in water. The mean dimension was about 1μm and *z* potential was around –30mV. Both these results made our products suitable for pharmaceutical application. Moreover microparticles can be obtained at moderate condition of temperature and pressure, without use organic solvent. For this reason our high-pressure process can be applied also at mixture containing drugs without losing their biological activity.

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