Solid-Liquid Equilibrium of Binary and Ternary Mixtures of Lipids Under Higher Pressure of CO$_2$ for PGSS Process Development

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Solid-liquid equilibria of solid mixtures in compressed CO$_2$ are very important in assessing the feasibility of gas-assisted micronization processes such as PGSS (Particles from Gas-Saturated Solution). The determination of solid-liquid transition temperatures, the eutectic temperature and the associated composition of the melt can be problematic and time-consuming. Such data are required in the construction of solid-liquid phase diagrams. This information is useful for the selection of appropriate operating conditions in the PGSS process.
The use of high pressure differential scanning calorimetry (DSC) for the determination of solid-liquid transition temperatures of solid mixtures in high pressure CO$_2$ provides a rapid and indirect method for the determination of the liquid-phase composition. A simple thermodynamic model was developed to predict the phase behaviour.

INTRODUCTION

Lipid nanoparticles, or solid lipid nanoparticles (LNP), are colloidal dispersions which are useful in pharmaceutical and cosmetic formulations [1, 2], such as stabilization and controlled/prolonged release of bioactive compounds. The conventional techniques for producing SLN include high pressure homogenization, high shear homogenization and ultrasound [3]. More recently, the gas-assisted micronisation process known as Particles from Gas-Saturated Solutions (PGSS) [4] has been considered for the preparation of SLN [5, 6].
The PGSS technique [7] is solvent-free: the lipid mixture is melted and then saturated with CO$_2$ under suitable pressure. Then, this gas-saturated solution is atomized by a special nozzle in a semi-continuous apparatus and particles of sub-micronic size can be obtained [6].
The CO$_2$ dissolution into materials leads to a reduction of the viscosity, melting and solidification temperature of the mixture. The ability of the high pressure CO$_2$ to reduce the melting point permits to operate at lower temperature with respect to classical methods [3, 5].
The dissolution of CO$_2$ in the lipidic mixture could shift the eutectic composition and so change the characteristics of the final product. For this reason, the knowledge of solid-liquid equilibrium is important: the operative pressure and temperature of the micronization process determine the final composition of solid nanoparticles produced.
In this work, phase equilibrium data have been measured by Differential Scanning Calorimetry (DSC) technique, and a simple thermodynamic model has been developed to correlate and predict the phase equilibrium behaviour. According to our approach, the subcooled liquid is considered as the reference state for the solid-phase fugacity, so only the heat of fusion and the melting temperature of the solid are required; both of these properties can be determined directly from DSC measures. The vapour phase is considered pure because the solubility of heavy components in compressed CO$_2$ rarely exceeds 1 mol% [8], and it is of

minor importance in the PGSS. The regular solution theory is applied for the calculation of activity coefficients to quantify the non-ideality in the liquid phase.

Ceramides, fatty acids and cholesterol are examples of epidermal lipids that find application in the preparation of LNP. In the present study, we examine the solid-liquid phase behaviour of mixtures of ceramide 3A, cholesterol and fatty acids in CO_2 in the range of pressure from 0.1 to 6.0 MPa. For thermodynamic modelling purposes, solid-liquid equilibrium data are reported for the pure lipids and their mixtures in CO_2.

MATERIALS AND METHODS

The lipids: Ceramide, Cholesterol and Radiacid® (a commercial mixture of fatty acids, mainly composed of stearic and palmitic acid) are kindly provided by UNI.FAR.CO Belluno (Italy). Carbon dioxide is supplied by Sapio Srl (Monza, Italy).

Differential scanning calorimetry (DSC) is performed using a Q10P/PDSC (TA Instruments) equipped with a pressure cell system that allows operating at high temperature. About 10 mg of materials is analyzed up to 160°C at a rate 1 °C/min and under different pressure values (from 1 up to 60 bar).

Binary Systems of two lipids
The equilibrium of the two lipids is modelled as the solubility of a solid solute (2) in a liquid solvent (1) by the following equation [9]:

$$\ln\left(\gamma_{21}x_2\right) = -\frac{\Delta H_m}{RT_t}\left(\frac{T_t}{T}-1\right) + \frac{\Delta c_p}{R}\left(\frac{T_t}{T}-1\right) - \frac{\Delta c_p}{R}\ln\frac{T_t}{T} \qquad (1)$$

where all the parameters are referred to the solid component: γ_{21} is the activity coefficient of the solute in the liquid, x_2 is its molar concentration, ΔH_m is the melting enthalpy at the triple point (T_t), which can approximate with the melting enthalpy at melting temperature (T_m), while $\Delta c_p = c_{p(liquid)} - c_{p(solid)}$ is the difference in specific heat capacity (c_p) of the substance between the liquid and solid states. As a first approximation, it is possible to neglect the terms containing Δc_p and to substitute T_t with T_m, so equation (1) becomes:

$$\ln\left(\gamma_{21}x_2\right) = -\frac{\Delta H_m}{RT_m}\left(\frac{T_m}{T}-1\right) \qquad (2).$$

The activity coefficient of the solute in the solvent was estimated by the Scatchard-Hildebrandt relation [13]:

$$\ln\gamma_2 = \frac{v_2^L\left[\left(\delta_1-\delta_2\right)^2 + 2l_{12}\delta_1\delta_2\right]\Phi_1^2}{RT} \text{ , with } \Phi_1 = \frac{x_1 v_1^L}{x_1 v_1^L + x_2 v_2^L} \qquad (3)$$

where v_2^L is the molar volume of subcooled liquid, δ_1 and δ_2 are the solubility parameters of the solvent and the subcooled liquid respectively, l_{12} is the interaction parameters and Φ_1 is the volume fraction of the solvent. The values of the solubility parameter for the lipid is calculated as proposed by van Krevelen [10].

Binary Systems: lipid with CO_2
The solubility of carbon dioxide in a melt lipid at the pressure P (bar) was modelled as a gas solubility in liquid considering the regular-solution to determine the activity coefficient [9]:

$$\frac{1}{x_{CO_2}} = \frac{f_{CO_2}^L}{f_{CO_2}^G}\exp\left[\frac{v_{CO_2}^L\left(\delta_1-\delta_{CO_2}\right)^2\Phi_1^2}{RT}\right] \qquad (4)$$

where x_{CO2} is the molar concentration of CO_2 in the mixture, v_{CO2}^L is the molar volume in liquid state [9] (55 cm^3/mol at 25°C), δ is the solubility parameter, $f_{CO2}{}^G$ and $f_{CO2}{}^L$ are the fugacity of CO_2 in the gas and in a hypothetic liquid status, respectively. The last fugacity value can be calculated by:

$$f_{CO_2,P}^L = f_{CO_2,1bar}^L \exp\left[\frac{v_{CO_2}^L (P-1.013)}{RT}\right] \tag{5}$$

with $f_{CO2,1bar}{}^L$ determined by the diagram reported in Prausnitz *et al.* [13].

Ternary SLV equilibria: 2 lipids + CO$_2$

The melting behaviour of two lipids in the presence of high pressure CO_2 is modelled using equation (2) to represent the dissolution of each solid in the liquid phase. This approach again assumes that each solid phase remains pure. Equation (4) was used to calculate the solubility of CO_2 in the molten lipids. The use of regular solution theory to describe the liquid-phase activity coefficient can be generalized for mixtures containing more than two components as follows:

$$RT\ln\gamma_k = \nu_k \sum_{i=1}^{N}\sum_{j=1}^{N}\Phi_i\Phi_j\left[D_{ik} - \frac{1}{2}D_{ij}\right] \tag{6}$$

$$D_{ij} = (\delta_i - \delta_j)^2 + 2l_{ij}\delta_i\delta_j \tag{7}$$

$$\Phi_j = \frac{x_j v_j}{\sum_{i=1}^{N} x_i \nu_i} \tag{8}$$

where N is the number of components and for every component i, $l_{ii} = D_{ii} = 0$.

RESULTS

In the PGSS process, the materials are melted at suitable temperature and pressure of CO_2 before to be atomized by a nozzle. The solubilization of CO_2 in the substance can reduce the melting point up to 40°C depending on its chemical structure [11]. The materials employed in this study have different melting points of the pristine materials: 55°C for Radiacid®, 145°C for Cholesterol and 124°C for Ceramide.
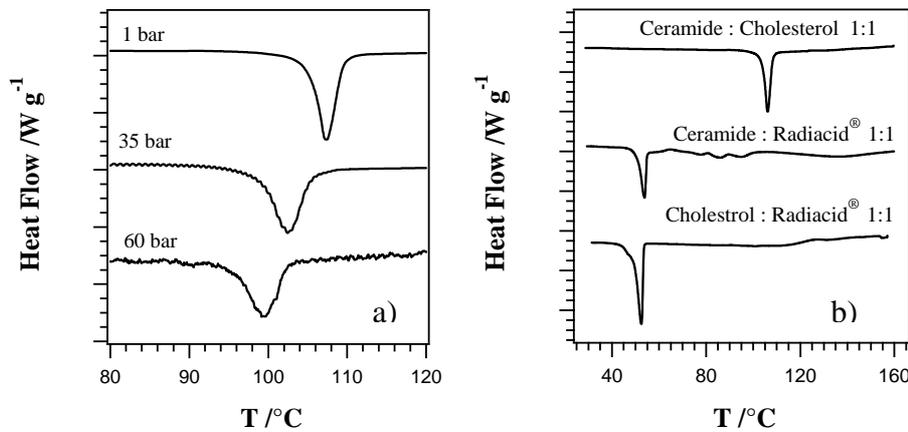

Figure 1. DSC measurement carried out on a) Ceramide as function of CO$_2$ pressure and on b) binary mixtures at ambient pressure.

The DSC experiments carried out under CO_2 pressure showed the reduction of the melting temperature and heat of melting (Figure 1a).

In Figure 1b, the results of the DSC measurements carried out on the binary mixtures at ambient pressure during the heating step are reported. It is possible to observe that the melting point of binary systems is lower than those of the pure substance.
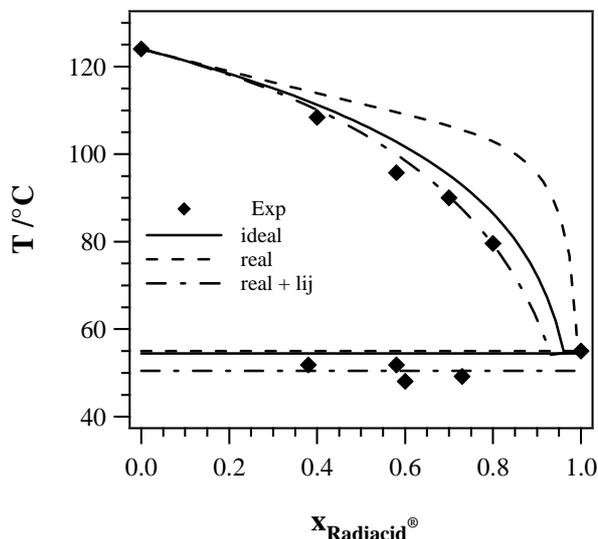

Figure 2. *T-x diagram of Ceramide-Radiacid* $^{®}$ *at ambient pressure obtained by equation(2).*

All the three binary lipid mixtures presented an eutectic. Note that in the mixtures made with Radiacid$^{®}$, the melting temperature is near to that of Radiacid$^{®}$. In Figure 2, the behaviour of Ceramide-Radiacid$^{®}$ was modelled by equation (2) considering the system as ideal, real and with binary interaction parameter. This parameter is determined by fitting the experimental data with equations (2) and (3). Figure 2 shows the good correlation obtained.
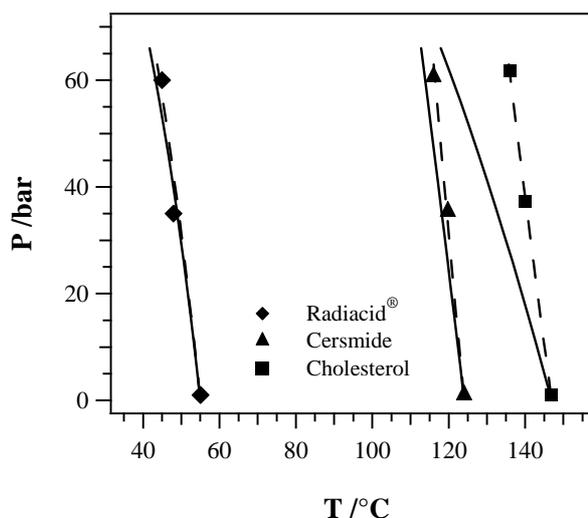

Figure 3. *P-T diagram of lipid-CO_2 modelled by equation (2) and (4): the continuous lines are obtained with $l_{ij} = 0$, while the broken ones with $l_{ij} < 0$.*

The effect of CO_2 on the melting temperature of lipid is reported in Figure 3. The system was modelled by equation (2) for lipid and (4) for the CO_2. A good correlation with the experimental data was obtained considering interaction parameters (l_{ij}) equal to -0.05 for Radiacid®-CO_2, -0.095 for Ceramide-CO_2 and -0.0025 for Cholesterol-CO_2.
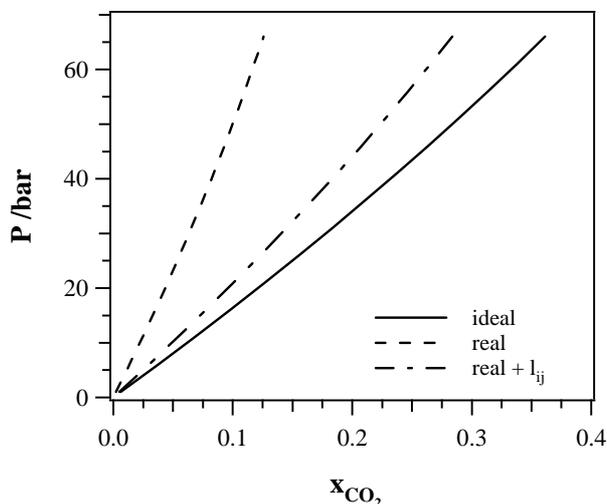

Figure 4. *P-x diagram of Ceramide-CO_2 modelled by equation (2) and (4).*

In Figure 4, the P-x diagram is reported for Ceramide-CO_2 system. It is possible to observe the large difference in the CO_2 concentration in the liquid phase, depending on the activity coefficient considered. For this system, the ideal model predicts the higher amount of CO_2 concentration, unlike the lower is obtained with the use of solubility parameters. The use of l_{ij} shifts the equilibrium in a sensitive way. In conclusion, the determination of CO_2 concentration inside the lipidic phase is very sensitive on the interaction parameter.
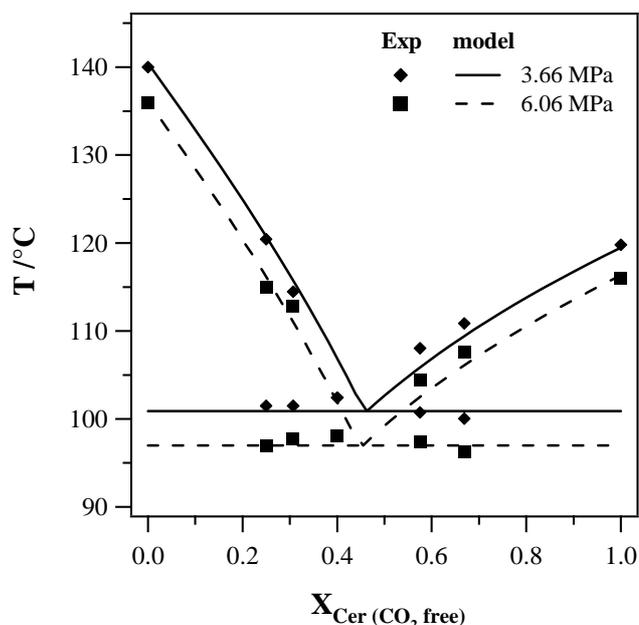

Figure 5. *T-x behaviour of (Ceramide-Cholesterol)-CO_2 behaviour modelled with equation (2) and (4) considering lij ≠0.*

The ternary system made of Radiacid®-Cholesterol-CO_2, at the pressure of 3.66 MPa and 6 MPa, is modelled considering only these three binary interaction parameters (l_{ij}). The results are reported in Figure 5, where it is possible to observe the good prediction of the experimental data obtained by DSC measurements.

CONCLUSION

The solid-liquid equilibrium of epidermal lipids in the presence of dense CO_2 was analyzed by DSC measurements and modelled on the base of the pure materials properties. The model developed predicted the presence of eutectics in all binary lipid mixtures. Ternary and quaternary systems were modelled using only pure material data and binary interaction parameters.

The DSC measurement carried out on the lipid under CO_2 pressure showed a reduction on melting temperature, which were correlated by a simple model of gas-liquid mixtures with good results. This model allowed to calculate the interaction parameters of lipid with CO_2. The determination of CO_2 concentration inside the lipidic phase is very sensitive on this interaction parameter.

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