

# Method Comparison for the Investigation of Phase Behaviour of Systems CO<sub>2</sub> + Lipid Carrier

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## ABSTRACT

Processing compounds with supercritical fluids requires the knowledge of their behaviour in the presence of the fluid. This work aims the comparison of different methods to study the melting point variation of a lipid carrier in the presence of carbon dioxide by a visual method and by ATR-FTIR *in situ* spectroscopy, and the comparison of the latter with a static analytical method to investigate the solubility of carbon dioxide in the bulk of the same melted matrix. The model lipid studied was glyceryl monostearate (GMS). In the pressure range and temperatures studied, the results obtained were matching for the solubility experiments and followed the same trend for the melting point variation. This last difference was due to the visual aspect of the compound.

## INTRODUCTION

Lipid formulations, composed of either fatty acids, glycerides or waxes, are of particular interest in delivery systems not only for food applications, since they protect the active ingredients and are absorbed in the intestinal track, but in particular for pharmaceutical uses. Due to their physiological composition, they are well tolerated by the human body and this low associated toxicity facilitates regulatory issues.

The feasibility of applying processes with supercritical fluids to these compounds, is strongly dependent on the understanding of the fundamentals, namely on the knowledge of how they behave in the presence of the fluid. This study focuses on the understanding of two phenomena: the melting point variation of the lipid due to the presence of carbon dioxide and the amount of carbon dioxide incorporated in this melted matrix. Among the several methods and techniques available to study high-pressure phase equilibria [1], a comparison between more “traditional methods” (visual and sampling) and FTIR spectroscopy is presented. *In situ* spectroscopy provides an insight into molecular level interactions between compounds or modifications, and with supercritical fluids it has been used to monitor reactions [2] and to study phase modifications mainly in polymers [3-5].

GMS was used as model compound since it generally recognized as safe (GRAS), and has a broad range of applications from stabilizer to release agent in industries like food and personal care, as well as general industrial applications such as adhesives and textiles [6].

## MATERIALS AND METHODS

### Materials

Glyceryl monostearate (Lumulse GMS-K) was used as a gift from Lambent Technologies. Carbon dioxide (99.5%, industrial grade) was purchased from Air Liquide. All chemicals were used without further purification.

### Visual method for melting point measurements

The variation of the melting point of GMS in the presence of carbon dioxide was determined visually in an apparatus previously described [7]. It consists of a high-pressure cell with

sapphire windows, with an internal volume of approximately 4 cm<sup>3</sup>, where a capillary tube containing the sample is inserted. The desired amount of carbon dioxide is pumped into the cell and temperature is continuously increased until the last solid particle melts. Measurements were performed up to 25 MPa. Since it is a visual method, the error was determined by random repetition of some points with a maximum deviation of 0,2 K [8].

#### Sampling method for solubility measurements

The solubility of CO<sub>2</sub> in liquid GMS was determined by a static analytical method in an apparatus described elsewhere [7]. Briefly, a high-pressure cell inside a thermostated air bath loaded with GMS and carbon dioxide at the desired conditions of pressure and temperature. The mixture is then magnetically stirred for one hour, followed by 30 minutes of rest for phase separation. After equilibrium is achieved, samples from the liquid phase are withdrawn through a high-pressure valve system, leading the liquid sample to a trap. The amount of carbon dioxide dissolved is calculated from the increase of sub-atmospheric pressure in a calibrated volume, upon sample expansion [8]. Each point is the average of at least 3 samples with a maximum relative deviation of 7%.

#### ATR-FTIR *in situ* spectroscopy for melting point measurements

A heated “Golden Gate” (Specac, Ltd. UK) support was used to hold a diamond crystal, with ZnSe focusing lenses and an angle of incidence of 45°, and the sample. A specially designed sealed high-pressure cell was used to cover the sample. For these experiments, two spectra were collected as reference for each compound: one of the solid sample (A) and another for a melted sample (B) at atmospheric pressure, to choose which bands should be analysed. The ATR-FTIR spectra were collected using a Bruker Equinox 55 FT-IR spectrometer with a mercury-cadmium-telluride (MTC) detector. A solid sample was then placed in the support and added a certain amount of carbon dioxide. At constant pressure, temperature was set to increase gradually in steps of 1 degree collecting a spectrum on each step until there was no difference in the bands compared with the liquid reference (Figure 1). This approach was developed earlier to study CO<sub>2</sub>-induced reduction in melting temperature of poly(ethylene glycol) [3]. Experimental limitations only allowed acquiring data up to 12 MPa.

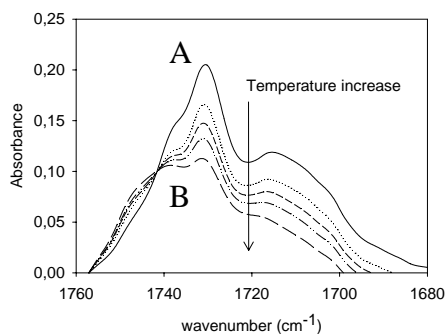


Figure 1: Melting points measurement with ATR – FTIR spectroscopy; (A) – solid reference spectrum of GMS, (B) – liquid reference spectrum of GMS

#### ATR-FTIR *in situ* spectroscopy for solubility measurements

These experiments of solubility were performed at 343 K, in the same equipment previously described, using a method first developed by Flichy *et al.* [9] using attenuated total reflectance (ATR) – FTIR spectroscopy. Briefly, it consists in acquiring a spectrum at different operating conditions and observing the changes in absorbance of the CO<sub>2</sub> band (2333 cm<sup>-1</sup>) (Figure 2), as an indicator for the solubility of carbon dioxide in the matrix. In this case, a melted film of GMS was obtained and slightly pressed to guarantee good contact between the sample and the crystal. At a given temperature, carbon dioxide was added to the cell at defined pressure values, and spectra were collected, two minutes after the pressure was reached. The low volume of the high-pressure cell ensured a fast equilibrium. Quantitative analysis of the solubility of carbon

dioxide was calculated according to Lambert-Beer law. Deviations presented correspond to a variation of the incidence angle of  $\pm 0,5^\circ$ .

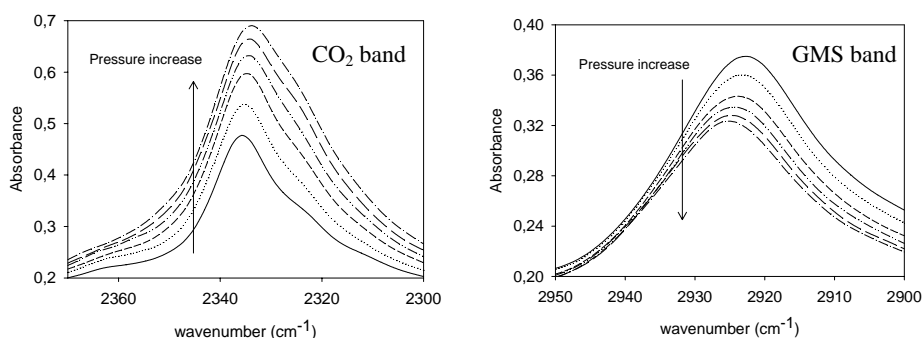


Figure 2: Observations in the solubility measurements using ATR –FTIR spectroscopy – (left) the  $\nu_3$  band of  $\text{CO}_2$ ; (right) GMS band

## RESULTS

### Melting point measurements

In the presence of carbon dioxide, the melting point of GMS has decreased 10K. This behaviour is due to the decrease in density of the bulk compound due to the incorporation of  $\text{CO}_2$ , and is well described in literature [10-12]. The results obtained with ATR-FTIR spectroscopy, presented the same trend but melting temperatures 3 K higher (at 4 and 12 MPa) were achieved. These differences are due to the respective observation levels. In the visual method the melting of the compounds is followed macroscopically and is strongly dependent on the operator. The sample can give the impression of still being solid, when already a viscous liquid or it can look liquid without being completely melted. In the ATR-FTIR spectroscopic method, observations are made microscopically, and once there is a pronounced difference in the IR bands between the solid and liquid references, the melting can be more accurately followed.

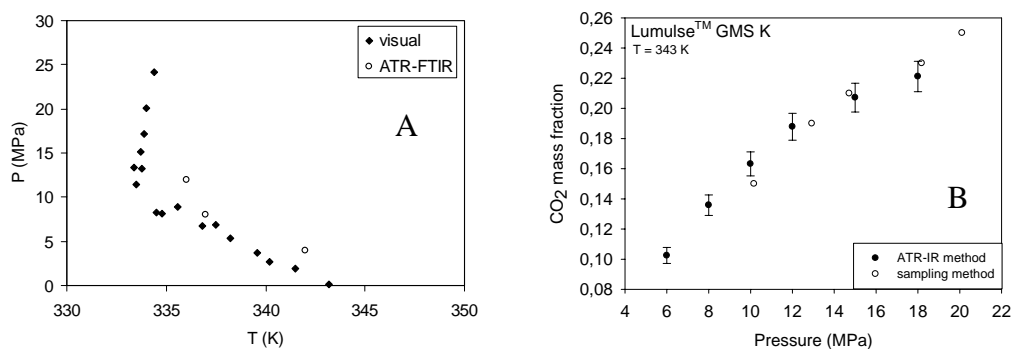


Figure 3: Comparison of the results obtained in both techniques for melting point (A) and solubility of carbon dioxide in melted GMS (B)

### Solubility measurements

The solubility of carbon dioxide in the melted GMS was measured in a pressure range from 6 to 20 MPa, at 343 K to ensure the presence of a total liquid phase. The values obtained range from 0,10 at 6 MPa up to 0,25 at 20 MPa. The  $\text{CO}_2$  composition in this phase increases with pressure, when it is present in larger amounts and higher densities, making it more soluble in the molten GMS. The results obtained are matching for both methods, with the results from ATR-FTIR fitting the experimental error deviations of the values acquired in the sampling method, which presented a maximum relative deviation of 7%.

## CONCLUSION

The methods compared in this study, for the investigation of phase behaviour of systems CO<sub>2</sub> + lipid carrier, gave a 3 K difference in the measurements of the melting point depression and fitted within the experimental error for the composition of the liquid phase, proving the good accuracy of the techniques.

The visual observation of the melting points also proved to be very dependent on the appearance of the compound to be studied. On the other hand, the major challenge encountered in ATR-FTIR spectroscopic measurements was to get a good contact between the sample and the crystal, which sometimes was difficult to achieve during the input of pressure. Nevertheless, this experimental procedure is much faster than the others, requires very small samples and upon further calculations it might be also possible to obtain the volume change of the compound in the presence of carbon dioxide (swelling).

## ACKNOWLEDGMENTS

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