

## A new concept to measure the mass transfer in SAS technique

R. Adami<sup>1</sup>, S. Dowy<sup>2</sup>, A. Bräuer<sup>3</sup>, E. Reverchon<sup>4</sup>, A. Lejpertz<sup>2</sup>

<sup>1</sup>Università di Salerno, SAOT Fisciano (SA), <sup>2</sup>Lehrstuhl für Technische Thermodynamik (LTT) and SAOT Erlangen, <sup>3</sup>Graduate School Advanced Optical Technologies(SAOT) and LTT Erlangen, <sup>4</sup>Università di Salerno Fisciano (SA)

### Abstract

In this work, a new concept of measuring mass transfer mechanisms related to supercritical antisolvent (SAS) precipitation is presented. It is based on the Förster resonant energy transfer mechanism (FRET) describing energy transfer between two chromophores. It permits the determination of the approach between two molecules within several nanometers (typically less than 10 nm), a distance sufficiently close for molecular interactions to occur. The high sensitivity for intermolecular distances qualifies FRET mechanism to indicate volume expansion and/or evaporation mechanisms. The FRET mechanism can be visualized carrying out fluorescence measurements. After calibration measurements in a cuvette, insitu measurements in a pressurized chamber have been performed. The results obtained are very promising for the study of the mass transfer at the very beginning of the precipitation mechanism, at the exit of the injector.

### Introduction

Supercritical Fluids (SCF) based micronization techniques are promising for the production of particles with controlled size and distribution. The most studied SCF micronization technique is the supercritical antisolvent (SAS) technology, in which SC-CO<sub>2</sub> has the same role than the antisolvent in the classical liquid antisolvent crystallization. The solute is first dissolved within an organic solvent, that is soluble in CO<sub>2</sub> at certain conditions, and then dispersed in the SC-CO<sub>2</sub>. In batch operation (GAS: Gas AntiSolvent) the precipitation vessel is loaded with a given quantity of the liquid solution and, then, the supercritical antisolvent is added until the final pressure is obtained. In the semi-continuous operation (SAS), the liquid solution and the supercritical anti-solvent are continuously delivered to the precipitation vessel in co-current or counter-current mode. Several authors tried to study the process mechanism (Werling 1999, Werling 2000, Elvassore 2001, Lengesfeld 2000, Rantakyla 2002, Reverchon 2010). The process is complex and involves thermodynamics, mass-transfer, jet hydrodynamics and nucleation kinetics.

In this study, mixing conditions below the mixture critical point of the ternary mixture of solute, solvent and antisolvent are relevant, for which a miscibility gap persists in the vapor liquid equilibrium at certain solvent-antisolvent ratios. As reported in the literature (Adami 2007, Reverchon 2008) above the mixture critical point no surface tension between solvent and antisolvent can be observed, whereas, below the mixture critical point the liquid solvent maintains a non-zero surface tension. Consequently, if the solution is injected into the SC antisolvent, droplets are formed after jet break up (Dowy 2009).

Förster Resonance Energy Transfer (FRET) is a mechanism describing energy transfer between two chromophores (Förster 1948). It permits the determination of the approach between two molecules within a distance of typically less than 10 nm, a distance sufficiently close for molecular interactions to occur (Kaminski 2009).

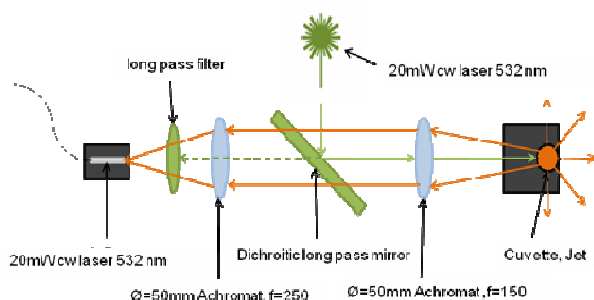
The mechanism of fluorescence resonance energy transfer involves a donor fluorophore in an excited electronic state, which may transfer its excess energy to a nearby acceptor chromophore in a non-radiative fashion through long-range dipole-dipole interactions. In principle, the fluorescence emission spectrum of the donor molecule should overlap the absorption spectrum of the acceptor molecule and both should be within a minimal spatial radius. FRET efficiency is highly sensitive towards the distance between the donor and the acceptor molecules

(Förster 1948) and can be read from the donor acceptor fluorescence emission spectra.

### Materials and methods

Rhodamine B (RhB) and Rhodamine 700 (Rh700), purchased from Radiant Dyes (Germany), were chosen as donor/acceptor (d/a) couple mainly because the RhB emission spectrum and the Rh700 absorption spectrum show a wide overlap, which proves their suitability as a FRET d/a-couple.

In figure 1 there is the schematic representation of the optical setup used for the calibration of the dyes and for the analysis of the volume expansion and spray detection at high pressure. During a FRET measurement, a continuous wave laser beam at 532 nm is focused into the target. The resulting d/a-fluorescence emission is collected in backscattering direction, separated from elastic light scattering interferences and focused onto a glass fiber, which is connected to a spectrometer. The d/a-fluorescence signal is acquired as a d/a-fluorescence emission spectrum.



**figure 1.** Schematic representation of the optical setup for FRET analysis

A high pressure apparatus with an optical accessible high pressure vessel has been used to perform the volume expansion measurements of the liquid EtOH in a cuvette due to the entrainment of CO<sub>2</sub>. The experiments have been performed in batch, filling the windowed vessel with CO<sub>2</sub> at the desired pressure and waiting for the stabilization of temperature and pressure. The pressure has been increased from 0.1 MPa to 8.0 MPa and decreased back to 0.1 MPa and at each pressure FRET measurements have been performed.

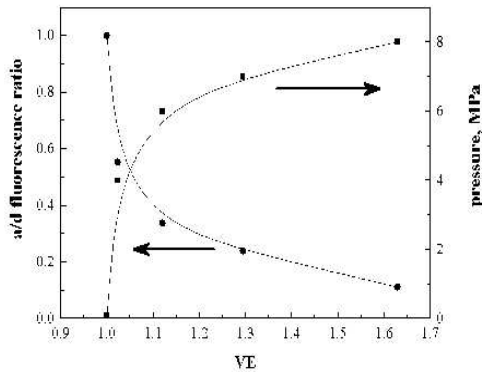
The same equipment has been used for the measurements of the spray at the exit of an injector of 100  $\mu\text{m}$  diameter. The high pressure vessel has been filled with CO<sub>2</sub> at the temperature of 317 K and a pressure of 6.0 MPa. The measurement has been performed in batch. The solution with Rh700 and RhB has been pumped at 2 ml/min and the d/a-fluorescence emission spectra were recorded 3 mm downstream of the injection nozzle exit before the break-up of the jet disperses the liquid phase into droplets.

### Results and discussion

Before starting the volume expansion experiments, solubility of the pure Rhodamine compounds in CO<sub>2</sub> has been qualitatively studied: 10 mg of powder has been put in a cuvette in the vessel, then CO<sub>2</sub> has been pumped in the vessel up to 20 MPa. None of the compounds dissolved in CO<sub>2</sub> and after depressurization, the powder showed no modifications in aspect, colour and consistency. Consequently, it is expected that all RhB and Rh700 molecules remain inside the liquid phase during volume expansion and do not travel across the phase boundary into the SC-CO<sub>2</sub>.

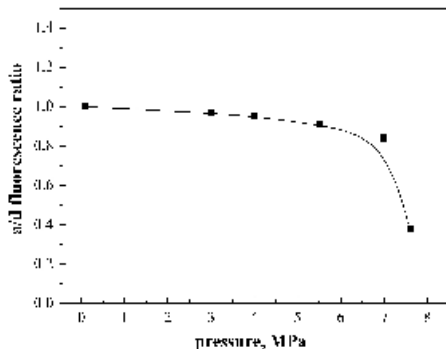
In the first set of experiments a cuvette was filled with 600  $\mu\text{l}$  of a solution acceptor/donor mole ratio 5.33 and put inside the optically accessible high pressure vessel. During pressurization with CO<sub>2</sub> the volume expansion of the solution was measured by photographing the level of the solution meniscus inside the cuvette. Figure 2 shows how the donor fluorescence relative to the acceptor fluorescence, normalized to the original d/a-solution, increases with volume expansion, expressed as the volume of the expanded solution over the volume of

the original solution (VE).  $VE = 1$  represents the normalized d/a-fluorescence emission spectra of the original d/a-solution and  $VE = 1.7$  at 8.0 MPa the pressurized d/a-solution. As expected, the increase of pressure increases the volume (and the height of the liquid in the cuvette), at the same time the efficiency of FRET decreases, indicating that the distance between donor and acceptor molecules increases. Figure 2 shows how the ratio between acceptor and donor (a/d)-fluorescence shifts as a function of volume expansion and pressurization.



**figure 2.** a/d-fluorescence ratio of a d/a-solution in a cuvette as a function of volume expansion (VE) by pressurization with  $CO_2$ .

In a second set of experiments, the original d/a-solution was continuously injected into the optical accessible high pressure vessel, pressurized with  $CO_2$ . Figure 3 shows that the normalized a/d-fluorescence ratio at this measurement position significantly decreases with increasing pressure, especially for the pressures 7.0 and 7.6 MPa. This decrease of the a/d-fluorescence ratio must be assigned to the volume expansion of the liquid phase which results from the absorption of  $CO_2$ . The jet behavior changes from Rayleigh disintegration at 0.1 MPa to atomization close to 8.0 MPa.



**figure 3.** a/d-fluorescence ratio in a continuous jet of d/a-solution as a function of pressurization with  $CO_2$ .

This result is very important: it is possible to evaluate the expansion of a droplet and to describe its behaviour from a spray at high pressure in  $CO_2$  environment with the same rules of volume expansion.

### Conclusions and perspectives

The results on FRET technique applied to EtOH volume expansion detection in a cuvette are very promising for quantitative measurements of SC- $CO_2$  dissolved in a liquid solvent.

The results obtained on the measurements of droplet expansion at the exit of the nozzle, for the effect of SC- $CO_2$  dissolution in the liquid in the pressurized vessel, suggest that the FRET technique can be successfully used to study the mass transfer between solvent and supercritical antisolvent (SC- $CO_2$ ) during SAS.

This preliminary study suggests future developments and applications of FRET technique for the understanding of the complex thermodynamic and fluid dynamic phenomena characteristic of SAS precipitation, such as droplet expansion detection and measurement and nucleation phenomena at the exit of the injector.

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