UNDERSTANDING SOLUBILITY OF PEPTIDES IN LOW VISCOSITY MEDIUM USING HIGH-PRESSURE NMR

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The commercial available carboxylic acid end capped fluorinated oil, Fluorolink 7004® (Solvay-Solexis) was tested successfully, for the encapsulation of peptides in water-in-carbon dioxide microemulsions (W/C). A high pressure experimental apparatus containing a stainless-steel cell, equipped with two sapphire windows, was specially developed for the solubility tests and the preparation of the NMR samples. The solubility of a small peptide, Gly-Gly-Try-Arg (Sigma) in $scCO_2$ was studied by visual inspection of the mixtures through the sapphire windows. The surfactant was tested successfully, allowing the encapsulation of the test molecule in water-containing reverse micelles, suspended in the apolar supercritical solvent. NMR measurements confirmed the solubility of the peptide in $scCO_2$/Fluorlink/D_2O. Preliminary NMR studies for the characterization of the solvent/surfactant/peptide interactions were also performed.

INTRODUCTION

Nuclear Magnetic Resonance (NMR) is a powerful tool to determine the structure of proteins and peptides as well as their dynamics in solution. The NMR spectra of biopolymers present several degrees of complexity, because they reflect the molecular complexity of the biopolymers microstructures and are usually limited in their resolution due to the high transverse relaxation rates.
The transverse relaxation is directly proportional to the rotational correlation time and this can be reduced by employing low viscosity solvents or high temperatures [1]. When compared to water, supercritical carbon dioxide ($scCO_2$) is a very low viscosity medium, and therefore it allows the modification of the correlation times of large proteins. Unfortunately, CO_2 is non-polar and ionic substrates are generally very insoluble in this media. One approach to overcome this limitation is to employ $scCO_2$ soluble surfactants that will induce the formation of micelles with high-density aqueous cores. Particular success has been obtained using fluorinated surfactants to generate microemulsions of water-in-CO_2 that were used for the solvation of proteins and ionic compounds [2,3]. It is then possible to suspend peptides in the aqueous core without irreversibly denaturing the peptide [4].

MATERIALS AND METHODS

Materials

Commercial carboxylic acid end capped fluorinated oil (Fluorolink 7004®, avg. MW600, Solvay-Solexis), commercial peptide Gly-Gly-Try-Arg ($C_{19}H_{29}N_7O_6$, MW451.26, Sigma), ammonium hydroxide aqueous solution (Sigma) and deuterated water (D_2O, Aldrich) were used without further purification. Carbon dioxide was supplied by Air Liquid, with purity better than 99.998%.

Formation of water-in-scCO_2 (W/C) microemulsions

Carboxylic acid end capped fluorinated oil was converted to its corresponding ammonium salt according to the already described procedure [5].
Encapsulation of peptides in water-containing reverse micelles (W/C) was carried out in a 10 mL stainless steel high pressure cell equipped with two sapphire windows for visual inspection. The visual cell was loaded with modified Fluorolink 7004®, peptide and deuterium water and was immersed in a thermostatised water bath. The temperature was measured by a thermocouple, inside the bath, connected to a temperature controller. The cell was internally stirred with a magnetic Teflon bar induced by a stir plate. A digital transducer measured the pressure. CO_2 was added at the required pressure by means of high pressure compressor (New Ways of Analytics) and dried with molecular sieves before the introduction into the stainless steel cell. A schematic diagram of the experimental apparatus is shown in Figure 1.

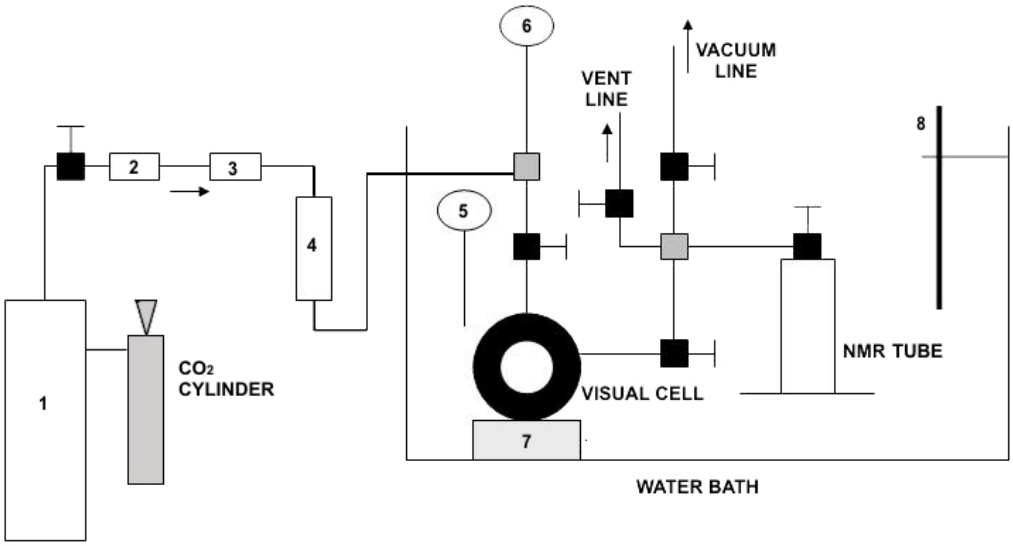

Figure 1: Schematic diagram of the experimental apparatus (1-High pressure compressor; 2- Filter; 3-Rupture disk; 4-Molecular sieves; 5-Temperature controller; 6-Pressure transducer; 7-Stir plate; 8- Heater).

After pressurisation of the system with CO_2, it could be seen through the sapphire windows that the reagents were not completely dissolved in scCO_2. Above 190 bar we observed that the solution became homogeneous, suggestion the formation of the

microemulsion. Once stabilization of the system was achieved, the NMR tube was filled. All the NMR experiments were carried out using single-crystal sapphire high-pressure (HP) NMR tube already described [4].
High-resolution, high-pressure NMR experiments were used for characterization of the dissolved peptide. All experiments were carried out at 313 K and 400 MHz frequency on a Bruker ARX400.

RESULTS

To test the solubility of the model peptide in $scCO_2$ several experiments were performed, in the high pressure cell, without the addition of Fluorolink 7004®. By visual inspection, through the sapphire windows, it could be seen that the peptide was insoluble in the $scCO_2$.
The addition of the modified Fluorolink 7004® and D_2O allows the formation of microemulsions and the solubilisation of the peptide, that would normally be insoluble in the pure solvent. In Figure 2, the ^1H NMR spectrum of the peptide in 10% D_2O/90% H_2O (v/v) (a) and the ^1H NMR spectrum of the encapsulated Gly-Gly-Try-Arg in the 1:3 (w/w) D_2O/modified Fluorolink 7004® reverse micelles in $scCO_2$ (b) are presented. As can be seen the two spectra are very similar and the encapsulation of the peptide results only in small deviations on chemical shifts.

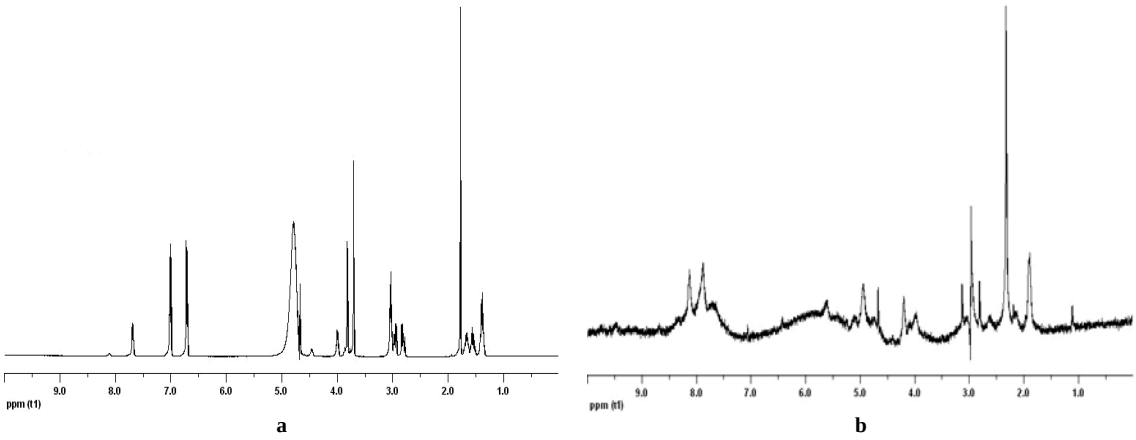

Figure 2 – ^1H NMR spectrum of the Gly-Gly-Try-Arg recorded at 400 MHz frequency, 313 K and 195 bar in: a) 10% D_2O/90% H_2O (v/v); b) water containing reverse-micelles in $scCO_2$

The use of a fluorinated surfactant, such as the modified Fluorolink 7004®, enables the formation of inverted micelles where the peptide is fully surrounded by water. The formation of water-containing reverse micelles with fluorinated surfactants is in agreement with the results obtained by other authors. [3, 6]
Our work demonstrates that it is possible to record NMR spectra of a model peptide in supercritical solvent with the addition of Fluorolink 7004®, opening the perspective of using this surfactant for the solubilisation of larger biopolymers.

At this stage we are applying high-pressure NMR techniques such as Nuclear Overhauser effect (nOe) and two dimensional HSQC to understand the molecular interactions between the solvent/surfactant/peptide and explore the use of this system to solubilise larger peptides, membrane proteins or enzymes.

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