On-Line Monitoring of Volume Expansion in Gas−Antisolvent Processes by UV−Vis Spectroscopy

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In this work UV−vis spectroscopy is used to measure in situ the volume expansion of the saturated liquid phase during gas−antisolvent (GAS) processes with compressed CO_2. The technique involves the use of two internal standards: potassium hexacyanoferrate(III) is used for polar solvents, and ferrocene for nonpolar solvents. The absorbance peaks of the reference substances are used to determine the volume expansion of the saturated liquid phase. Measurements on the dimethyl sulfoxide + CO_2, dioxane + CO_2, and dimethyl sulfoxide + water + CO_2 systems have been made in the temperature range from 298 to 313 K and for pressures up to 70 bar. The experimental results obtained compare well with available literature data.

Introduction and Background

In the past decade, supercritical fluids (SCFs) have emerged as an important alternative to organic solvents in many processes, especially for applications in the fields of pharmaceutics, nanotechnology, biomaterials, and biotechnology.[1] SCFs possess the great advantage that their chemicophysical properties, such as density, viscosity, and diffusion coefficient, may be varied continuously from liquid-like to gaslike values by simply changing pressure and temperature conditions.

In particular, it has been recognized that supercritical CO_2 offers the possibility of processing biolabile compounds at mild conditions and in a benign environment. Compressed CO_2, which has been widely employed as a solvent for extraction purposes or in organic solvent elimination/purification processes, represents an interesting and effective alternative to conventional methods and also as an antisolvent. In the gas−antisolvent (GAS) process, the CO_2 dissolution in the liquid phase causes two effects: the change of mixture composition, which finally leads to a density decrease (i.e. the volume expansion of the saturated liquid phase), and, consequently, the solute precipitation due to the sudden reduction of the solvent power. Relevant examples of GAS processes under current investigation are the recrystallization/precipitation of pharmaceuticals, the fractionation of biopolymers, and the purification of proteins.[2−4]

Unfortunately, the mechanisms governing the GAS processes are not well understood, so that it is still difficult to scale up possible applications to industrial production. Clearly, the characterization of the solvent−antisolvent−solute system behavior at high pressure is of paramount importance. In fact, the thermodynamic interaction and phase diagram between solvents and solutes, such as macromolecules, proteins, biopolymers, or colloidal particle dispersions, may be strongly related to the liquid bulk phase properties.

Two important variables in developing GAS processes are the expansion of saturated liquid-phase volume, defined as $\Delta V = (V - V_0)/V_0$, where V and V_0 are the expanded and initial total volumes of the saturated liquid phase, respectively, and the precipitation pressure, defined as the pressure at which the onset of solute precipitation occurs.

Both of these properties are primarily measured by the simple, often inaccurate, procedure of direct volumetric measurements in a windowed cell.[5] Only in a few cases has a more complex apparatus and procedure been used.[6,7]

In a previous work[8] we highlighted some interesting features concerning the key variables of GAS processes: the precipitation pressure was found to be linear with the operating temperature, the volume expansion at precipitation was shown to be independent of temperature, and solid precipitation occurs when the molar liquid density reaches a maximum.

However, more data are needed to confirm and verify these findings.

Simple spectroscopic techniques, such as UV−vis, appear to be very promising methods to analyze the concentration changes of various phases under high pressure during a GAS process. Moreover, spectroscopy allows us to obtain additional information on the solvent power and on the molecular or macromolecular environment by following the intensities and the positions of spectral peaks.

In recent years, much effort has been devoted to investigate molecular interactions of dilute solutes where supercritical media were used as solvents.[9−13] In particular, in view of enhancing the solute solubility, there has been significant exploration of the concept of local density and the clustering of solvent−solute and cosolvent−solute.[14] The evaluation of solute−solvent local composition had already been discussed by Kim and Johnston[15,16] using spectral shift features. These phenomena were recently confirmed by computer simulation techniques.[17]

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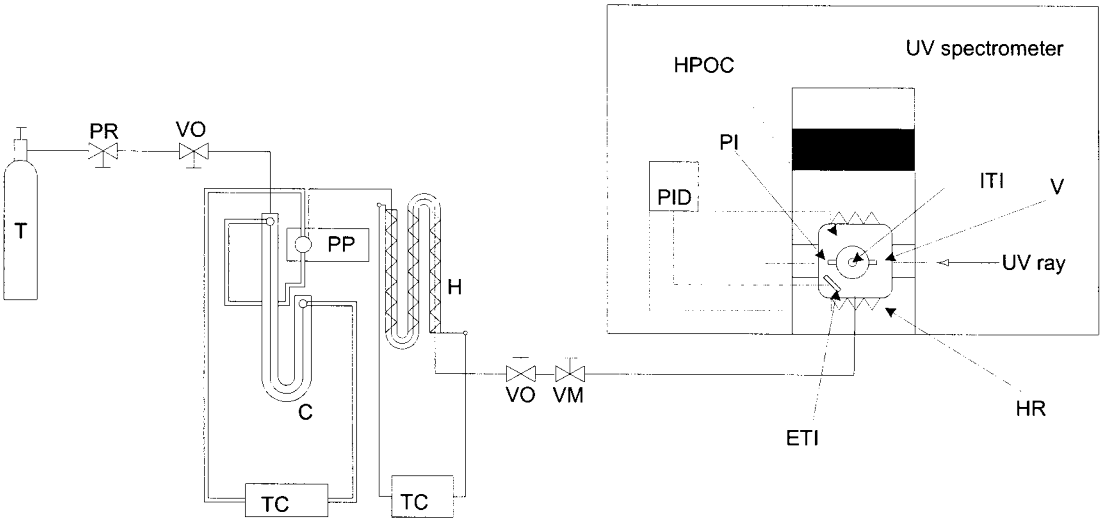

Figure 1. Scheme of the apparatus used to perform the experimental measurements: T, CO_2 tank; PR, pressure reducer; VO, on/off valve; VM, metering valve; PP, piston pump; TC, thermostat; C, cooler; H, heater; HPOC, high-pressure optical cell; PI, pressure indicator; ETI and ITI, external and internal temperature indicator; HR, heat resistance; V, vent.

Unfortunately, the process where supercritical CO_2 is used as an antisolvent has scarcely been addressed by UV−vis spectroscopy. To our knowledge, the only few works in the open literature are those by Kim and Johnston[15] and Kelley and Lemert,[18] who characterized the solvent strength and the polarity of the saturated liquid phase by the solvatochromic spectra features of a dye.

In our work, a simple technique, based on UV−vis spectroscopy with an internal standard, is used to study phenomena involved in GAS processes with compressed CO_2. In particular, the effects of pressure and temperature on the volume expansion of the saturated liquid phase are characterized for different organic solvent−CO_2 mixtures. With respect to existing techniques, the proposed method offers the advantage of collecting accurate experimental data in a relatively short time. The results, which are consistent with published literature data, are extremely valuable for theoretical understanding of the solvent influences in the solute−precipitation phenomena.

Materials and Methods

Reagents. The spectroscopy grade organic solvents dimethyl sulfoxide (DMSO) and dioxane (Aldrich, Steinheim, Germany) were used without further purification. 99.95% grade purity CO_2 was purchased from Air Liquide (Padova, Italy). As standards, ferrocene $(\pi\text{-}C_5H_5)_2Fe$, absorbing at 420 nm, was used for the nonpolar system (dioxane) and potassium hexacyanoferrate(III) ($Fe(CN)_6^{4-}$), absorbing at 440 nm, was employed for polar solvents (DMSO and DMSO + water mixture). The concentrations of the standard in the liquid solution were kept below 0.1 mass %.

Experimental Setup. In Figures 1 and 2, respectively, the schematic of the experimental setup and the optical high-pressure cell is given.

The apparatus consists of a CO_2 supply system, a high-pressure optical cell, a Perkin-Elmer (Lambda 15) UV−vis spectrometer, and a depressurizing section. The CO_2 was supplied by a reciprocating pump (model 305, Gilson, Milano, Italia) at the desired pressure, and the CO_2 flow rate was controlled by fine metering valves (model 1316G4y, Hoke, Creskill, USA). The pump cylinder was cooled with a thermostat to provide a suitable low temperature for the gas condensation.

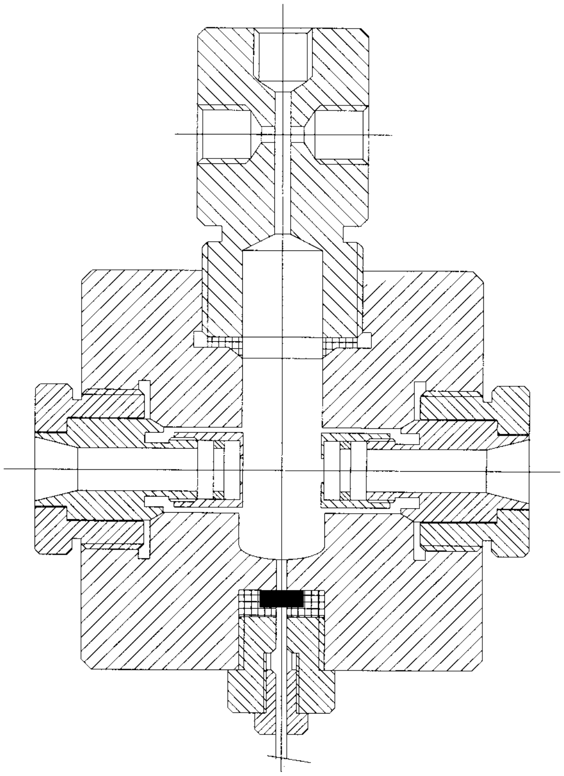

Figure 2. Sapphire windowed high-pressure cell for UV−vis spectroscopic studies.

The samples were contained in a 8 mL customized stainless steel cell (SITEC, Zurich, Switzerland) equipped with a 0.4 mm thick sapphire window. The upper cap, which had been modified to work as an expansion chamber, was connected to the pressure measurement device, the temperature probe, and the outlet line. Upon addition of CO_2, the volume of the saturated liquid phase may increase to over 1000% with respect to the initial one, and the cell should never be filled up by liquid. A 5 μm porosity metallic frit of diameter 3.5 mm (Aldrich, Steinheim, Germany) was placed at the bottom of the cell in order to ensure fine CO_2 bubbling during the pressurization step. The different components of the apparatus were connected to each other through $^1/_{16}$ in. or $^1/_8$ in. o.d. stainless steel tubing. The temperature of the cell was controlled by an electric resistance whose power was regulated using a Variac voltage regulator.

Internal temperature was measured with a Pt 100Ω resistance. The pressure was continuously monitored through a pressure transducer (model MA 01720, Data Instrument, Acton, MA) with a digital readout. Instrument calibrations were performed in the temperature range from 293.15 to 333.15 K and the pressure range from 1 to 200 bar. The optical cell temperature and pressure were typically maintained at ±0.1 K and ±0.2 bar, respectively, with respect to their set point values. The overall uncertainty of the saturated liquid-phase volume expansion measurement was estimated to be within ±2%. The repeatability of the measurement was estimated to be within ±1%.

Experimental Procedure. The cell was sealed and heated to the desired temperature and then flushed several times with CO_2 to remove any residual air. Before starting the experiment, a background spectrum of pure CO_2 at 1 bar was recorded for automatic baseline corrections of all subsequent studies. Three milliliters of solution containing the standard was then introduced into the high-pressure cell by a "sealed" system; this amount of liquid guarantees to fill the cell above the window level. The cell was then pressurized by flowing the CO_2 from the bottom through the metallic frit, to create a fine bubble dispersion within the liquid phase. To ensure equilibrium conditions at any time, the CO_2 was continuously added to the cell, causing the pressure to increase at a rate of approximately 0.1 bar/min. Similar flow rates were used in the equilibrium measurements of high-pressure gas dissolution in the liquid phase by Winter et al.[4] Prior to collecting each spectrum, all valves were closed. The measurements of the absorbance corresponding to the standard peak (λ_{max}) were repeated until the system was equilibrated and their values remained constant. In all cases, the equilibrium conditions were reached within 30 min. This procedure was repeated at all the pressure studied. Upon completion of the absorbance measurements, the optical cell was depressurized and accurately cleaned.

Results and Discussion

Volume Expansion Determination. The aim of this work is the accurate determination of the volume expansion by UV−vis spectroscopic measurements following the absorbance of a reference standard. A compound suitable for this use should fulfill the following requirements:

(a) It must be soluble in the saturated organic solvent + CO_2 mixtures but not in pure compressed CO_2. It was checked that both standards used in this work were not soluble in the CO_2 at the pressures and temperatures considered.

(b) It must not undergo precipitation itself. This latter condition was ensured by preparing solutions with low concentration (less than 0.1 mass %). Both ferrocene and potassium hexacyanoferrate(III) did not precipitate at pressures below 80 bar.

Moreover, it has been shown that the change of the solvent power and density can have a dramatic influence upon the absorbance intensity peaks of the solutes in terms of both shape and shift in the position of the peak maximum.[18] To use standards suitable for our purpose, it has been necessary that the absorption bands of the reference materials not be influenced by the chemical environment and by the other absorbing solute molecules present in the mixture.

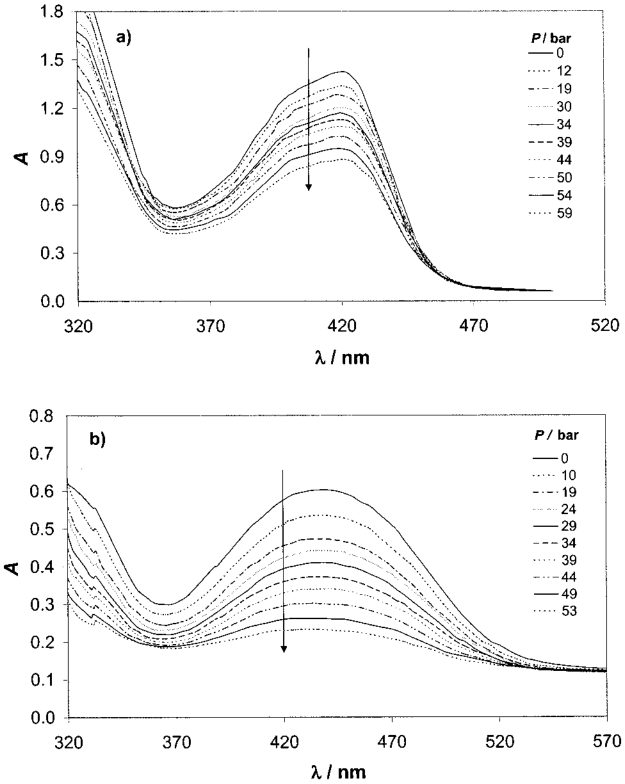

Figure 3. UV−vis spectra at various pressures for (a) hexacyanoferrate(III) in the DMSO + CO_2 system at 308 K and (b) ferrocene in the dioxane + CO_2 system at 298 K.

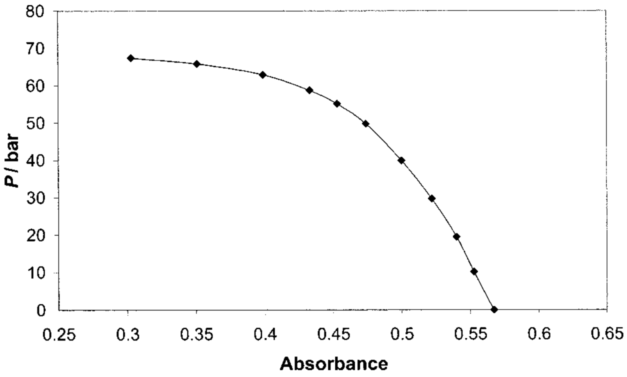

Figure 4. Effects of saturated liquid-phase volume expansion on the standard absorbance peak at different pressures of ferrocene for the dioxane + CO_2 system.

The ferrocene and the potassium hexacyanoferrate(III) are materials which fulfill these requirements. The spectra of both standards were not affected during the expansion process. As reported in Figure 3, no shape modification or shift of the position of the maximum of the potassium hexacyanoferrate(III) and ferrocene peaks was observed. An example of the typical pressure effect on the maximum peak absorbance of the internal standard for the dioxane + CO_2 system is reported in Figure 4. Note that, unfortunately, the value of the absorbance does not provide a direct measurement of the liquid density.

It is well-known that the relative absorbance variation of the standard can be related to its total concentration, c (mol L^{-1}), in the saturated liquid phase through the Lambert−Beer law of the type

$$A = Kc \tag{1}$$

where A is the molar absorbance and K is a constant

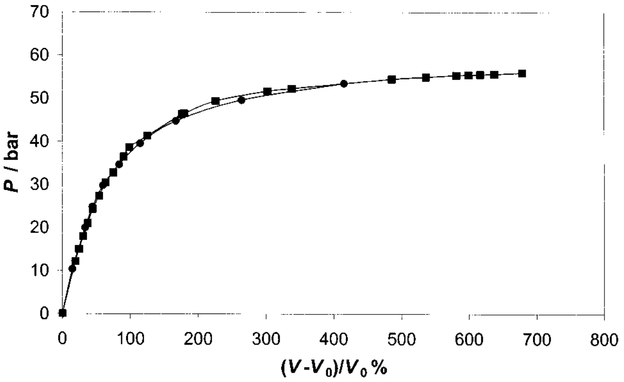

Figure 5. Comparison of saturated liquid-phase volume expansion measurements for dioxane + CO_2 at 298 K collected in this work (circles) with experimental data of Kordikowski at al.[6] (squares).

Table 1. Volume Expansions of the Saturated Liquid Phase for the DMSO + CO_2 System at 298 K, 303 K, and 307 K

$T = 298$ K		$T = 303$ K		$T = 307$ K	
P/bar	$(V - V_0)V_0$/%	P/bar	$(V - V_0)V_0$/%	P/bar	$(V - V_0)V_0$/%
9.7	5.93	10.4	6.20	12.5	7.7
19.8	12.6	19.6	13.1	20.0	12.9
24.2	16.9	29.6	21.4	30.5	22.4
29.2	22.8	36.5	28.7	34.5	26.1
30.5	24.6	43.5	38.8	39.7	31.8
33.8	29.4	49.6	50.0	44.7	38.8
35.5	31.9	54.6	63.9	50.0	47.8
40.1	41.3	59.4	86.3	54.6	58.4
44.9	53.4	62.2	113	59.2	74.5
49.6	75.3				
53.0	115				
56.4	868				
56.8	1595				

Table 2. Volume Expansions of the Saturated Liquid Phase for the Dioxane + CO_2 System at 298 K, 303 K, and 306 K

$T = 298$ K		$T = 303$ K		$T = 306$ K	
P/bar	$(V - V_0)/V_0$/%	P/bar	$(V - V_0)/V_0$/%	P/bar	$(V - V_0)/V_0$/%
10.3	14.9	9.3	13.0	9.8	13.9
19.9	33.5	20.0	31.7	19.9	32.4
24.8	44.4	24.0	40.4	25.1	43.0
29.8	59.9	28.5	52.8	29.8	54.1
34.7	83.7	36.2	84.5	36.4	76.5
39.5	114	41.9	119	42.8	103
44.7	166	49.1	193	48.8	145
49.0	263	54.0	282	55.0	221
53.5	415			59.0	327

depending on the cell geometry and on the molar adsorption coefficient, ϵ, of the internal standard. We have tested experimentally that the Lambert–Beer law holds for initial standard concentrations up to 0.1 mass %. Under these adopted conditions, the relative expansion volume of the liquid phase can be expressed in terms of absorbance as follows:

$$\frac{A_0 - A}{A} = \frac{Kc_0 - Kc}{Kc} = \frac{n/V_0 - n/V}{n/V} = \frac{V - V_0}{V_0} = \frac{\Delta V}{V_0} \tag{2}$$

where n is the moles of the internal standard and V and V_0 are the total volume of the liquid phase after and before the expansion at a given pressure, respectively. Equation 2 is only valid if the Lambert–Beer law is applicable and if the solvent used has a negligible concentration in the gas phase. These are the cases for both the components and the conditions considered in this work.

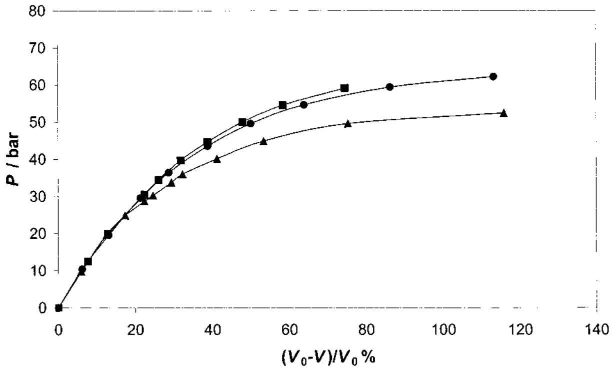

Figure 6. Experimental measurements of saturated liquid-phase volume expansions for the DMSO + CO_2 system at the temperatures 298 K (triangles), 303 K (circles), and 307 K (squares). The absorbance peak of hexacyanoferrate(III) at 440 nm was used as the reference band.

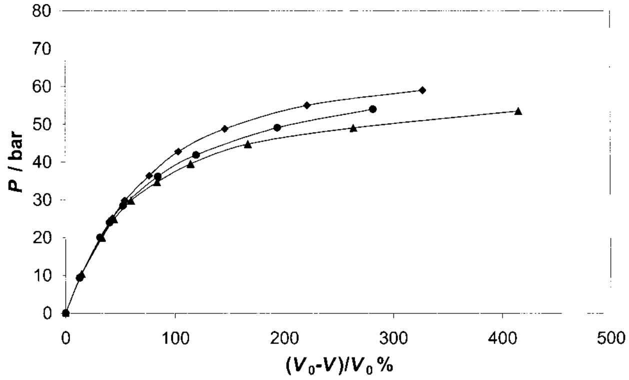

Figure 7. Experimental measurements of saturated liquid-phase volume expansions for the dioxane + CO_2 system at the temperatures 298 K (triangles), 303 K (circles), and 306 K (squares). The absorbance peak of ferrocene at 420 nm was used as the reference band.

Experimental Applications. A number of systems were investigated in order to show the versatility of the technique and to check the reliability of the method.

A comparison of the volume expansion measurements obtained by our technique with the experimental data of Kordikowski et al.[6] for the dioxane + CO_2 mixture is shown in Figure 5. The measurements are in good agreement with those reported in the literature; the relative averaged deviation between the two data sets was estimated to be within 3%.

Similar results were obtained for other solvent + CO_2 systems. Tables 1 and 2 show three isotherms of volume expansion over a pressure range from 1 to 70 bar for the DMSO + CO_2 and dioxane + CO_2 mixtures, respectively. The expansion volume data were determined using eq 2 from the standard absorbance peaks at 440 nm (potassium hexacyanoferrate(III)) and 420 nm (ferrocene) for the DMSO and dioxane systems, respectively. The results are presented in Figures 6 and 7.

Recently, the possibility of applying GAS techniques to biolabile substance processing generated problems related to the biomolecule denaturation after exposure to organic solvent and high pressure. Supercritical CO_2 was used as an antisolvent to induce microparticulate protein powder precipitation from a DMSO solution[4] or from an ethanol + water mixture system.[19] The presence of small amounts of water in the system may strongly influence the protein bioactivity. Therefore, it is interesting to study the behavior of solvents containing water. In this respect we found that

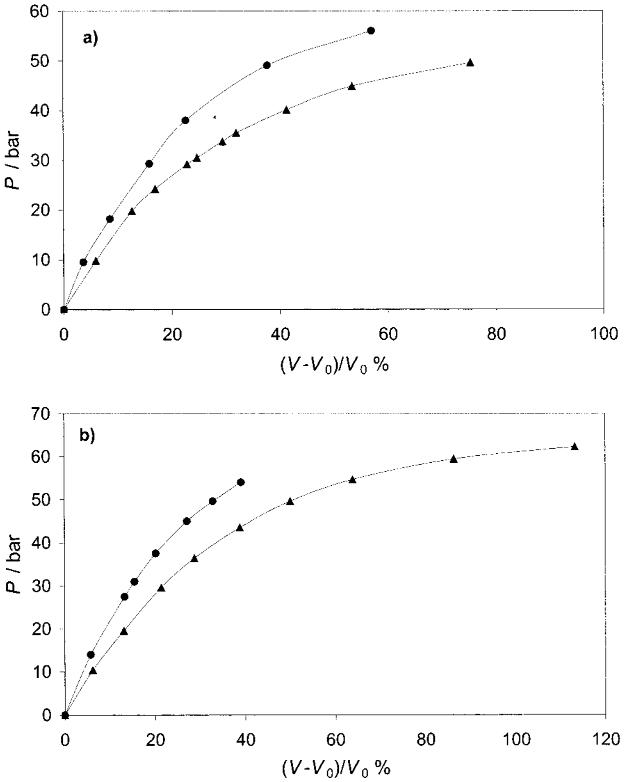

Figure 8. Comparison of saturated liquid-phase volume expansions for the DMSO + CO_2 system (circles) and for the DMSO + water + CO_2 (90/10 DMSO/water w/w) system (triangles): (a) at 298 K; (b) at 303 K.

Table 3. Volume Expansions of the Saturated Liquid Phase for the DMSO + Water + CO_2 (90/10 DMSO/Water w/w) System at 298 K and 303 K

$T = 298$ K		$T = 303$ K	
P/bar	$(V - V_0)/V_0$/%	P/bar	$(V - V_0)/V_0$/%
9.5	3.64	14.0	5.89
18.2	8.57	27.5	13.2
29.3	15.9	31.0	15.5
38.0	22.6	37.6	20.2
49.1	37.7	45.0	27.1
		49.6	32.8
		54.0	39.1

the expansion volume is strongly related to the water concentration in the saturated liquid phase, as reported in Figure 8 and Table 3 for the DMSO + water system. Note that the additional 10 mass % of water in the initial liquid mixture leads to a volume expansion reduction of approximately 30% with respect to the case of the pure solvent at the same conditions of temperature and pressure.

The simple way of measuring the volume expansion proposed in this work appears to be very promising and useful in characterizing the thermodynamic behavior and understanding the phenomena involved in GAS processes.

Conclusion

The volume expansion of the saturated liquid phase occurring in batch gas−antisolvent processes was determined by means of in-situ UV−vis spectroscopy. The use of internal standards allows the accurate determination of the volume expansion of the saturated liquid phase on using the maxima of the absorption peak of the standards.

It was shown that the proposed method is not affected by the properties of the solvents used in the volume expansion process. A number of organic solvent + CO_2 systems were measured, and in all cases the proposed technique gave accurate measurement in a relatively short time. The experimental results obtained well compare with literature data when available. The proposed simple techniques could aid the rational understanding of the phenomena involved in the GAS processes; they are also a first step for studying GAS processes of biocompatible compounds, where the effect of a small amount of water may be relevant.

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Literature Cited

(1) Teja, A. S.; Eckert, C. A. Commentary on Supercritical Fluids: Research and Applications. *Ind. Eng. Chem. Res.* **2000**, *39*, 4442.
(2) Subramaniam, B.; Rajewski, R. A.; Snavely, K. Pharmaceutical Processing with Supercritical Carbon Dioxide. *J. Pharm. Sci.* **1997**, *86*, 885.
(3) Reverchon, E. Supercritical Antisolvent Precipitation of Micro- and Nanoparticles. *J. Supercrit. Fluids* **1999**, *15*, 1.
(4) Winters, M. A.; Frankel, D. Z.; Debenedetti, P. G.; Carey, J.; Devaney, M.; Przybycien, T. M. Protein Purification with Vapor-Phase Carbon Dioxide. *Biotechnol. Bioeng.* **1999**, *62*, 247.
(5) Yeo, S. D.; Lim, G. B.; Debenedetti, P. G.; Bernstein, H. Formation of Microparticulate Protein Powders Using a Supercritical Fluid Antisolvent. *Biotechnol. Bioeng.* **1993**, *41*, 341.
(6) Kordikowski, A.; Schenk, A. P.; Van Nielen, R. M.; Peters, C. J. Volume Expansions and Vapor-Liquid Equilibria of Binary Mixtures of a Variety of Polar Solvents and Certain Near-Critical Solvents. *J. Supercrit. Fluids* **1995**, *8*, 205.
(7) de la Fuente Badilla, J. C.; Peters, C. J.; de Swaan Arons, J. Volume Expansion in Relation to the Gas-Antisolvent Process. *J. Supercrit. Fluids* **2000**, *17*, 13.
(8) Bertucco, A.; Pallado, P. Understanding Gas Antisolvent Processes of Biocompatible Polymers and Drugs with Supercritical CO_2. *High-Pressure Chemical Engineering, Proc. Int. Meeting of the GVC−FachausschuB Hochdruckverfahrenstechnek* **1999**, 239.
(9) Carrot, M. J.; Wai, C. M. UV−Visible Spectroscopy Measurement of Solubilities in Supercritical CO_2 Using High-Pressure Fiber-Optic Cells. *Anal. Chem.* **1998**, *70*, 2421.
(10) Lu, J.; Buxing, H.; Haike, Y. UV−Vis Spectroscopic Studies of Solute−Solvent and Solute-Cosolvent Interactions in Supercritical Carbon Dioxide. *Phys. Chem. Chem. Phys.* **1999**, *1*, 3269.
(11) Lu, J.; Buxing, H.; Haike, Y. Thermodynamics Studies of Chemical Equilibrium in Supercritical Carbon Dioxide-Cosolvent Solutions Using UV−Vis Spectroscopy. *J. Supercrit. Fluids* **1999**, *15*, 135.
(12) Otomo, J.; Koda, S. UV Absorption Shifts of Benzenes in Supercritical Carbon Dioxide. *J. Chem. Phys.* **1999**, *242*, 241.
(13) Draper, S. L.; Montero, G. A.; Smith, B.; Beck, K. Solubility Relationships for Disperse Dyes in Supercritical Carbon Dioxide. *Dyes Pigments* **2000**, *45*, 177.
(14) Sasaki, T.; Takeishi, H.; Yoshida, Z. Interpretation of Solubility and Solvation of Phenol-Blue in Supercritical Carbon Dioxide Based on Solute−Solvent Interaction Evaluated by Solvatochromism. *J. Supercrit. Fluids* **1999**, *15*, 23.
(15) Kim, S.; Johnston, K. P. Clustering in Supercritical Fluid Mixtures. *AIChE J.* **1987**, *33*, 1603.
(16) Kim, S.; Johnston, K. P. Molecular Interaction in Dilute Supercritical Fluid Solution. *Ind. Eng. Chem. Res.* **1987**, *26*, 1206.
(17) Liew, C. C.; Inomata, H.; Saito, S. Molecular Dynamics Study on Solvent Clustering in Supercritical Fluid Solutions Based on Particle Radial Kinetic Energy. *J. Supercrit. Fluids* **1995**, *104*, 317.
(18) Kelley, S. P.; Lemert, R. M. Solvatochromic Characterization of the Liquid Phase in Liquid-Supercritical CO_2 Mixtures. *AIChE J.* **1995**, *42*, 2047.
(19) Forbes, R. T.; Sloan, R.; Kibria, I.; Hollowood, M. E.; Humphreys, G. O.; York, P. Production of Stable Protein Particles: a Comparison of Freeze, Spray and Supercritical Drying. *World Congress on Particle Technology*, Bringhton, U.K., 7−9 July, 1998; system at 298 K and 303 K.

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