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13. ABSTRACT (Maximum 200 words)

Two techniques were used to study the formation of particles from supercritical fluids. In the rapid expansion of supercritical solutions (RESS), solutes were dissolved at high pressure and precipitated by decompression across a flow restriction device. In the supercritical anti-solvent process (SAS), the supercritical fluid acted as an anti-solvent towards solutes dissolved in an organic solution. RESS experiments with poly (L-lactic acid) (L-PLA) as solute showed that decreasing the density of the solvent at the exit of the expansion device caused precipitation of small and irregular L-PLA particles to occur inside the expansion device. Larger microspheres were formed at higher exit density. Encapsulation of pyrene in L-PLA microspheres was achieved by varying the initial amounts of pyrene and L-PLA present. SAS was used to make microfibers of aromatic polyamides and protein particles in the 1-5 micron size range. The protein particles retained their biological activity upon redissolution in water. The thermodynamics of mixed solute precipitation from a supercritical solution was investigated by calculating the retrograde regions of several polynuclear aromatic compounds in a supercritical solvent. The size of the retrograde region was found to decrease as the solute's heat of sublimation increased.

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PARTICLE FORMATION DURING THE EXPANSION OF SUPERCRITICAL SOLUTIONS

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1. Objective

The objectives of this project were to investigate experimentally the formation of particles from supercritical fluids, and to understand theoretically the influence of fluid-phase nonidealities upon the process of particle formation under supercritical conditions. The experimental work placed emphasis on the practical applications of supercritical fluids for materials processing.

2. Main Findings

2.1 Rapid Expansion of Supercritical Solutions (RESS)

The solvent power of a supercritical fluid increases exponentially with density (Kumar and Johnston, 1988). Because supercritical fluids are highly compressible near the critical point, their solvent power can be adjusted between gas- and liquid-like extremes with moderate changes in pressure. In RESS, one or more solutes are dissolved in a supercritical fluid at high pressure, and precipitation occurs as a result of rapid expansion through a flow restriction device. This can lead to deposit formation from supercritical fuels, but can also be of great practical value in biomedical and pharmaceutical applications, because of the purity of the resulting solid products and, if the supercritical solvent is carbon dioxide (whose critical temperature is $31^{\circ}C$), the mildness of the operating conditions.

We investigated the precipitation of Poly (L-lactic acid) (L-PLA) and composite L-PLA - pyrene particles by rapid expansion of supercritical mixtures of CO_2 and $CHClF_2$ (Tom et al., 1994). The physical properties of the materials used in the study are listed in Table I.

Table I. Properties of Solutes and Solvents Used in RESS Studies

Compound	T_c ($^{\circ}C$)[1]	P_c (bar)[2]	T_m ($^{\circ}C$)[3]	T_g ($^{\circ}C$)[4]	MW[5]
L-PLA	-	-	162.5	62.0	ca. 10000
Pyrene	-	-	156	N/A	202.24
CO_2	31	73.8	-56.5	N/A	44.01
$CHClF_2$	96.2	49.7	-160.1	N/A	86.47

[1] Critical temperature

[2] Critical pressure

[3] Normal melting temperature

[4] Glass transition temperature

[5] Molecular weight

The most important findings that resulted from this work were:

(a) For given expansion conditions, the morphology of L-PLA particles changed from microspheres (10-50 μm) to irregular microparticles (1-10 μm) upon increasing the length-to-diameter (L/D) ratio of the expansion

device. In our work, we used two types of expansion device: orifices and capillaries. The former consisted of 0.25 mm-thick stainless steel disks, with a 25 μm laser-drilled pinhole (L/D = 9.4). Capillaries were silica tubing, with inner diameters of 30 or 50 μm. The capillaries were cut to the desired length (0.5 to 2 mm), yielding L/D ratios from 100 to 500.

(b) Microspheres were formed mainly in the free jet after exiting the expansion device. Microparticles were formed mainly inside the expansion device. The microspheres were frequently larger than the diameter of the expansion device. The microparticles were never larger than the diameter of the expansion device.

(c) Particle morphology was controlled by the density of the supercritical fluid upon exiting the expansion device. At relatively high exit densities, precipitation occurred in the free jet; at low exit densities, precipitation occurred inside the capillary expansion device. This is shown in Figures 1 and 2. Figure 1 includes all the experiments done in the study of capillary RESS of L-PLA. The calculations of Figure 2 were done with a one-dimensional fluid mechanical model of the supercritical fluid's adiabatic expansion in the capillary under choked flow conditions (Tom et al., 1994). Note that the exit density (and hence the solvent power) decrease as L/D increases.

(d) By controlling the relative amount of L-PLA and pyrene in the supercritical phase it was possible to form composite microspheres in which pyrene was encapsulated by L-PLA. This is shown in Figure 3, and suggests that RESS can be used to encapsulate solutes at mild operating conditions and without the need for solvent removal and downstream processing.

2.2 Supercritical Anti-Solvent Process

In the SAS process (Yeo et al., 1993, 1994, 1995; Winters et al., 1996a,b), a solute is dissolved in an organic liquid. A supercritical fluid having low solvent power with respect to the solute, but miscible with the liquid, is added to precipitate the solid. When the process is operated in batch mode, the supercritical fluid is dissolved in an excess of the liquid phase. This causes appreciable volumetric expansion (Figure 4), and the resulting decrease in the liquid's cohesive energy density causes the solute to precipitate. When the process is operated in continuous mode, the liquid and supercritical phases are fed continuously into a precipitator. Small, sub-millimeter droplets are created by flowing the liquid solution through a nozzle. The droplets are contacted with an excess of supercritical fluid, and, under appropriate operating conditions, the liquid evaporates isobarically and isothermally into the supercritical fluid, causing the precipitation of dry powders. This technique is of great practical value in biomedical, pharmaceutical, and polymeric materials processing applications because of the purity of the products, the mildness of the operating conditions (with CO_2 as the anti-solvent), and the high solubility of polymeric and bio-active solutes in many organic solvents.

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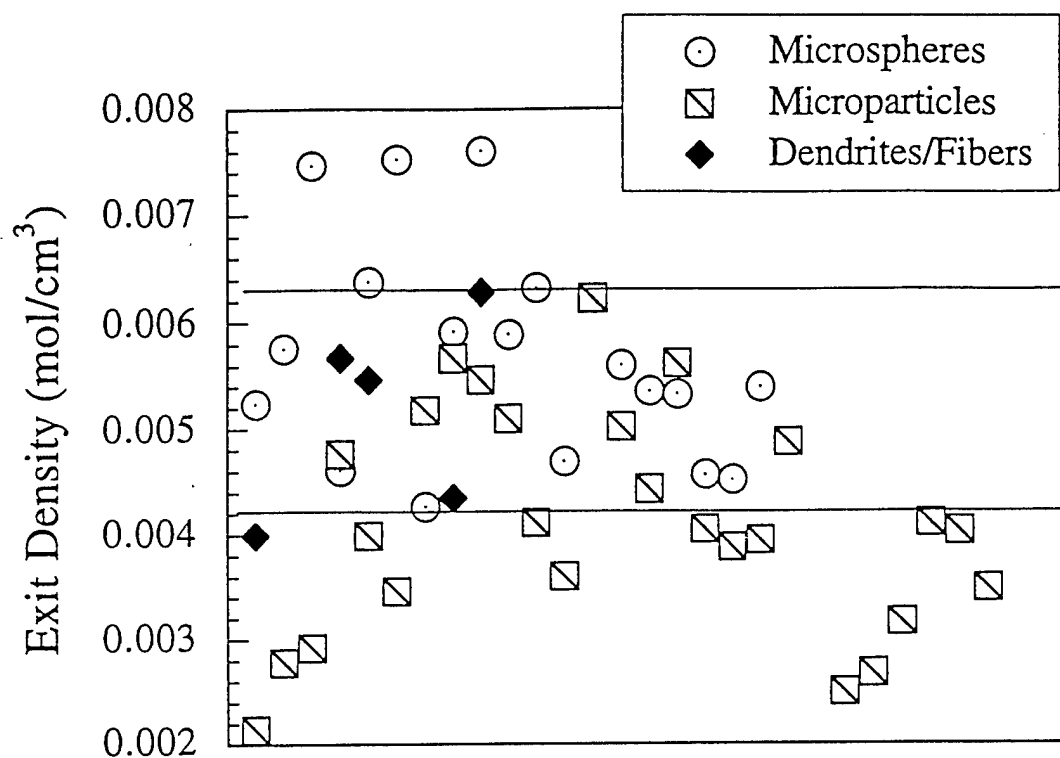
Figure 1: Influence of solvent density at the exit of the expansion device for capillary RESS of L-PLA. As the exit density increases, the morphology changes from microparticles to microspheres and the characteristic particle size increases. (Tom et al., 1994).
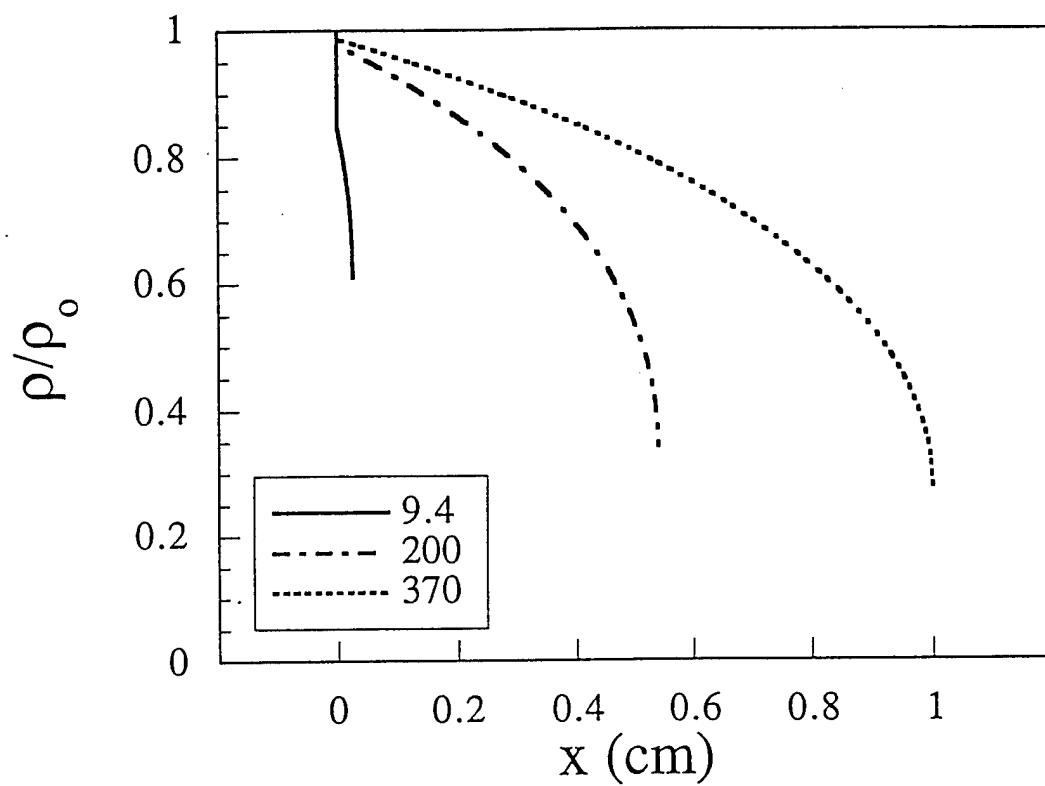
Figure 2: Calculated solvent density profile for one-dimensional expansion in capillaries of varying L/D ratios. Preexpansion conditions are 120°C and 200 bar. The solvent is a 70-30 (w) mixture of CO_2 and $CHClF_2$. (Tom et al., 1994).
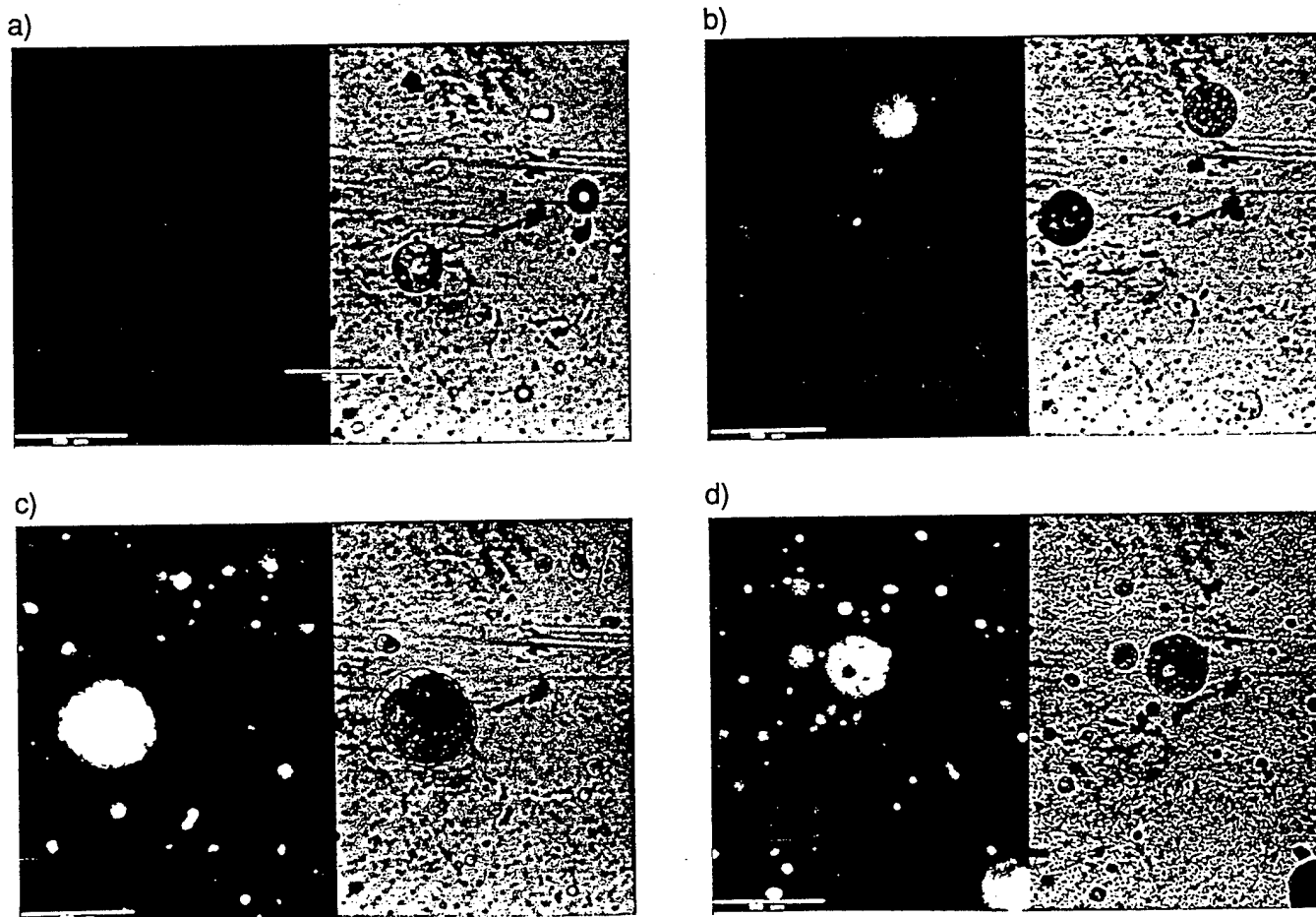
Figure 3: Fluorescence (left) and transmission (right) micrographs of L-PLA - pyrene microspheres precipitated by capillary RESS at pyrene concentrations in CO_2 of < 0.001 wt% (a), 0.0013 wt. % (b), and > 0.002 wt% (c,d). The L-PLA was extracted with 60 wt% CO_2 - 40 wt% $CHClF_2$ at 200 bar and 55°C, and pyrene was extracted with CO_2 at 200 bar and 65°C and diluted to the above concentrations. Pyrene fluoresces, L-PLA does not. Pure L-PLA spheres appear black in the fluorescence images. Note the composite microspheres of L-PLA-coated pyrene (b,c,d). The horizontal scale in the bottom left of each fluorescence image corresponds to 50 µm. (Tom et al., 1994).
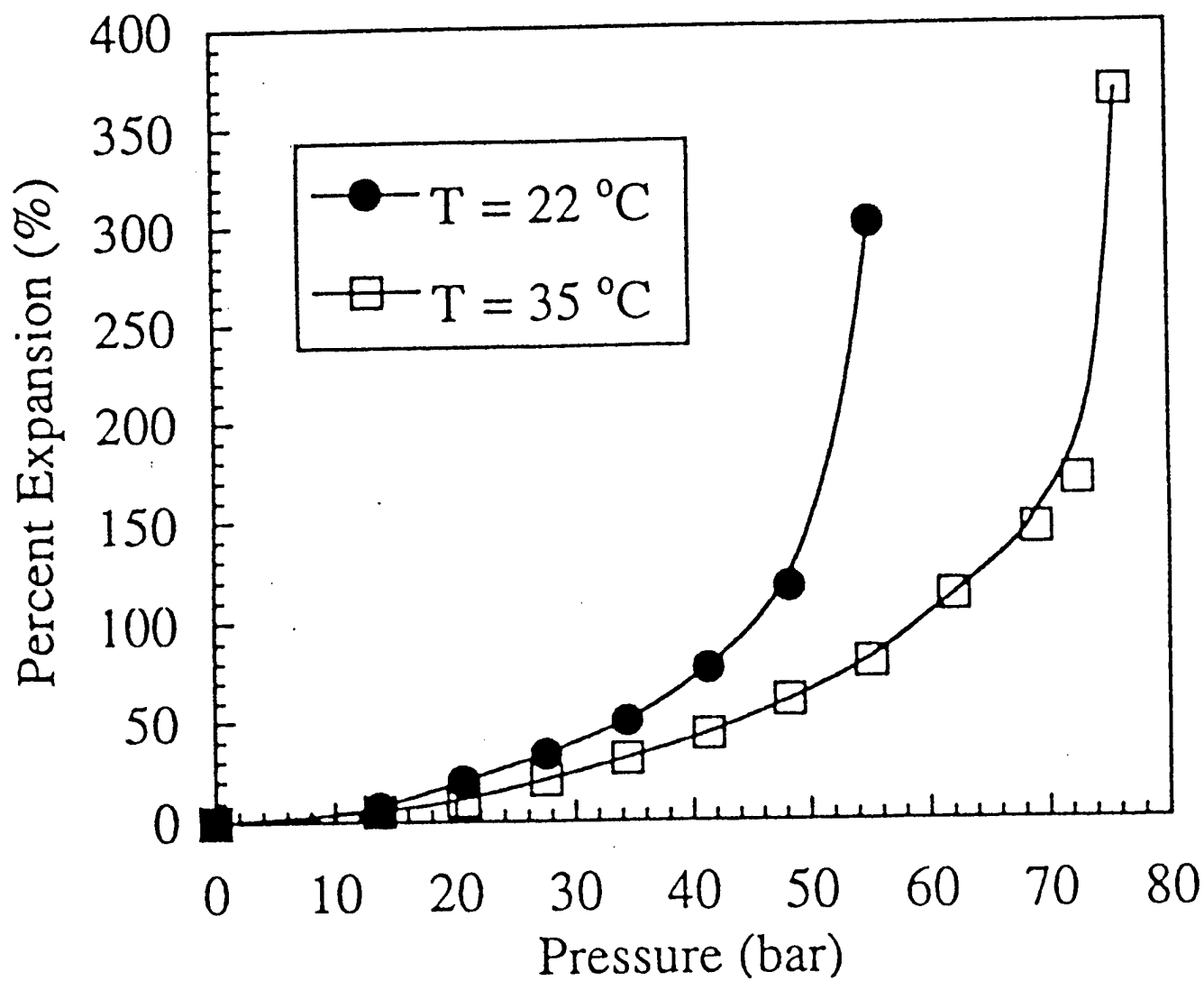
Figure 4: Volumetric expansion of liquid DMSO when contacted with CO_2 at different temperatures and pressures. (Yeo et al., 1993).

We have investigated the processing of aromatic polyamides and proteins by this technique. The most important findings that resulted from this work were:

(a) Microfibers of aromatic polyamides were formed by SAS in continuous mode, with dimethylsulfoxide (DMSO) or dimethylformamide (DMF) as organic solvents, and carbon dioxide as supercritical anti-solvent (Yeo et al., 1993). Para-linked aromatic polyamides are used as heat-resistant, high-modulus materials. Our work demonstrates for the first time that this class of compounds can be processed into useful morphologies at mild operating conditions, using the SAS process.

(b) Supercritical antisolvents can be used to control the degree of orientational anisotropy in liquid-crystalline polymeric solutions (Winters et al., 1996a). We used depolarized light spectroscopy to investigate the effect of compressed carbon dioxide on the extent of orientational order in nematic solutions of an aromatic polyamide in dimethylacetamide (DMAC). We found that dissolution of compressed carbon dioxide in DMAC caused a reversible increase in molecular ordering in the liquid-crystalline solution. Because the mechanical strength of fibers and composites wet-spun from anisotropic solutions is intimately related to the degree of molecular orientational order in solution, this finding suggests that supercritical fluids can be used to accurately control the properties of materials formed from liquid-crystalline solutions.

(c) Microparticulate powders of three proteins (insulin, trypsin, lysozyme) in the 1-5 µm size range were formed by SAS in continuous mode, with DMSO as organic solvent and carbon dioxide as supercritical anti-solvent (Yeo et al., 1994; Winters et al., 1996b). Protein particles in the 1-5 µm size range are useful for controlled release, and for direct aerosolized delivery to the lungs.

(d) The protein particles retain their biological activity upon redissolution in water. The retention of biological activity upon redissolution means that SAS is useful as a mild finishing process for the formulation of protein powders of pharmacological interest.

(e) The protein powders retain their biological activity after one year of storage at ambient temperature. This implies that SAS is also of great usefulness as a method for increasing the shelf-life of biologically active compounds.

2.3 Thermodynamics of Mixed-Solute Precipitation at Supercritical Conditions

Fluid-phase nonidealities caused by proximity to the critical point affect both the thermodynamics (i.e., the driving force) and kinetics (i.e., the rate) of solute precipitation. We have studied the former question.

The equilibrium solubility of non-volatile solutes in a supercritical solvent is a sensitive function of temperature and pressure. A distinguishing feature of the thermodynamics of

systems composed of non-volatile solutes in a supercritical solvent is retrograde behavior, whereby the equilibrium solubility of a given solute decreases with increasing temperature at constant pressure, over a broad range of thermodynamic states. Thus, if a given mixture is in the retrograde region, precipitation will occur upon heating, whereas outside the retrograde region precipitation occurs upon cooling. Consequently, the amounts and overall composition of mixed solid phases that can precipitate from a supercritical fluid depend sensitively upon whether or not retrograde regions are crossed as a result of changes in temperature and/or pressure.

We studied the retrograde regions of binary and ternary solutions of polynuclear aromatic hydrocarbons in supercritical carbon dioxide. Table II lists physical properties of the solutes used in the calculations.

Table II. Physical Properties of Solutes Used in the Study of Retrograde Behavior

Compound	T_m (°C)[1]	Δh_{sub} (kJ/mol)[2]	MW[3]
Phenanthrene	100.5	96.1	178
Anthracene	216.5	102.6	178
Pyrene	156	91.1	218

[1] Normal melting point
[2] Heat of sublimation
[3] Molecular weight

The most important conclusions that resulted from this work were:

(a) For a group of chemically similar solutes, the larger the heat of sublimation, the smaller the retrograde region in a given supercritical solvent. This is shown in Figure 5.

(b) If a supercritical solvent containing two or more dissolved solutes is expanded adiabatically, cooled isobarically, heated isobarically, or depressurized isothermally, the composition of the resulting solid phase will depend very sensitively on the location the expansion/compression path relative to retrograde regions. This points out the crucial need for understanding the underlying phase behavior in making predictions about precipitation from a supercritical fluid.

(c) For dilute mixtures composed of two or more non-volatile solutes in a supercritical solvent, the retrograde regions are insensitive to the presence of additional solutes. This means that retrograde calculations for binary mixtures can be used in the analysis of multicomponent mixtures.
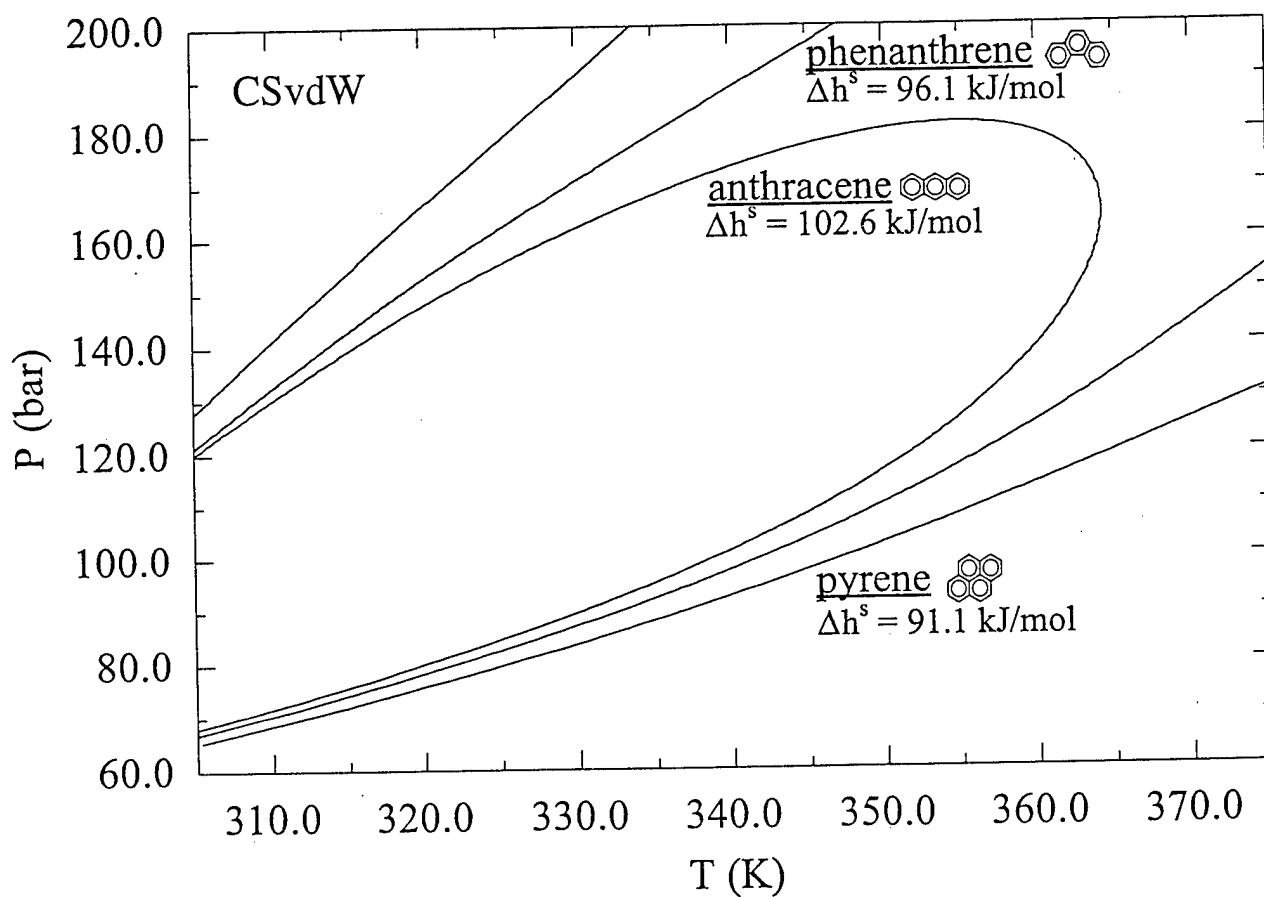
Figure 5: Calculated retrograde regions of various polycyclic aromatic compounds in supercritical carbon dioxide. Inside a substance's retrograde region the solubility decreases upon increasing the temperature isobarically. Calculations were done using the Carnahan-Starling-van der Waals equation of state.

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Winters, M.A., P.G. Debenedetti, P.D. Condo, M. Radosz, and H.-W. Schmidt. "Effects of Compressed Carbon Dioxide on the Phase Equilibrium and Molecular Order of a Lyotropic Polyamide Solution." **Macromolecules, 29**, 4904 (1996a).

Winters, M.A., B.L. Knutson, P.G. Debenedetti, H.G. Sparks, T.M. Przybycien, C.L. Stevenson, and S.J.Prestrelski. "Precipitation of Proteins in Supercritical Carbon Dioxide." **J. Pharm. Sci., 85**, 586 (1996b).

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Yeo, S.-D., P.G. Debenedetti, M. Radosz, R. Giesa, and H.-W. Schmidt. "Supercritical Antisolvent Process for a Series of Substituted Para-Linked Aromatic Polyamides." **Macromolecules, 28**, 1316 (1995).

4. Publications Describing AASERT-Sponsored Research, 8/1/93 - 7/31/96

Debenedetti, P.G. "Supercritical Fluids as Particle Formation Media." In *Supercritical Fluids. Fundamentals for Applications.* E. Kiran and J.M.H. Levelt-Sengers, eds. **NATO ASI, Ser. E, 273**, 719 (1994).

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Knutson, B.L., P.G. Debenedetti, and J.W. Tom. "Preparation of Microparticulates Using Supercritical Fluids." In *Microparticulate Systems for the Delivery of Proteins and Vaccines.* S. Cohen and H. Bernstein, eds. Marcel Dekker, New York (1996).

5. Technical Presentations on AASERT-Sponsored Research, 8/1/93 - 7/31/96

"Formation of Biologically Active Protein Powders and Polymeric Microfibers with Supercritical Anti-Solvents". 12th annual AAAR meeting, Oak Brook, Illinois, October 15, 1993.

"Rapid Expansion of Supercritical Solutions: a Comparison Between Mathematical Modeling and Experiments". AIChE annual meeting, St. Louis, November 10, 1993.

"Processing of Liquid-Crystalline Polymers Using a Supercritical Anti-Solvent: Equilibrium and Morphological Study". AIChE annual meeting, St. Louis, November 10, 1993.

"Supercritical Fluids as Particle Formation Media". Department of Chemical Engineering, Yale University, March 28, 1994.

"Supercritical Fluids as Particle Formation Media". AIChE Central Jersey Section, Princeton University, May 19, 1994.

"Supercritical Fluids as Particle Formation Media". Robert L. Mitchell Technical Center, Hoechst Celanese Corporation, Summit, New Jersey, July 14, 1994.

"Supercritical Fluids as Particle Formation Media". 3rd International Symposium on Supercritical Fluids, Strasbourg, France, October 17, 1994.

"Supercritical Fluids as Particle Formation Media". Alza Corporation, Palo Alto, California, December 9, 1994.

"Materials Processing with Supercritical Fluids". Merck Research Laboratories, Rahway, New Jersey, May 1, 1995.

"Supercritical Fluids", a four-lecture course. Astra-Hässle, Mölndal, Sweden, May 22-23, 1995.

"Materials Processing with Supercritical Fluids". Department of Chemistry and Biochemistry, University of Southern Illinois, Carbondale, Illinois, September 8, 1995.

"Materials Processing with Supercritical Fluids". Invited tutorial. American Association for Aerosol Research, 14th annual meeting. Pittsburgh, Pennsylvania, October 9, 1995.

"Theoretical and Computational Studies of Nucleation in Supercritical Fuels." AFOSR Contractors Meeting, Virginia Beach, Virginia, June 4, 1996.

"Particle Formation from Supercritical Fluids." International Fine Particle Research Institute Annual Meeting, Nancy, France, June 11, 1996.

6. Personnel

Michael Winters; graduate student

Pablo G. Dbenedetti; Professor. Principal Investigator

AUGMENTATION AWARDS FOR SCIENCE & ENGINEERING RESEARCH TRAINING (AASERT) REPORTING FORM

The Department of Defense (DoD) requires certain information to evaluate the effectiveness of the AASERT Program. By accepting this Grant which bestows the AASERT funds, the Grantee agrees to provide 1) a brief (not to exceed one page) narrative technical report of the research training activities of the AASERT-funded student(s) and 2) the information requested below. This information should be provided to the Government's technical point of contact by each annual anniversary of the AASERT award date.

1. Grantee identification data: (R&T and Grant numbers found on Page 1 of Grant)

a. __Princeton University__
   University Name

b. __F49620-93-1-0454__   c. _____
   Grant Number   R&T Number

d. __Pablo G. Debenedetti__   e. From: 8/1/93   To: 7/31/96
   P.I. Name   AASERT Reporting Period

NOTE: Grant to which AASERT award is attached is referred to hereafter as "Parent Agreement".

2. Total funding of the Parent Agreement and the number of full-time equivalent graduate students (FTEGS) supported by the Parent Agreement during the 12-month period prior to the AASERT award date.

a. Funding: $ 58,825

b. Number FTEGS: 1

3. Total funding of the Parent Agreement and the number of FTEGS supported by the Parent Agreement during the current 12-month reporting period.

a. Funding: $ 94,738

b. Number FTEGS: 1

4. Total AASERT funding and the number of FTEGS and undergraduate students (UGS) supported by AASERT funds during the current 12-month reporting period.

a. Funding: $ 141,050

b. Number FTEGS: 1

c. Number UGS: ---

VERIFICATION STATEMENT: I hereby verify that all students supported by the AASERT award are U.S. citizens.

_____   9-27-96
Principal Investigator   Date

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AASERT Research Training Activities

Mr. Michael Winters, currently a fourth-year graduate student, has been supported through the AASERT grant since August, 1993. Participation in this project has enabled Mr. Winters to gain expertise in a wide variety of experimental and theoretical areas.

In preparation for his work on protein processing under supercritical conditions, Mr. Winters visited the laboratory of Professor Todd Przybycien at the Rensselaer Polytechnic Institute's Isserman Department of Chemical Engineering to learn Raman spectroscopy. He now routinely collects and analyzes Raman spectra of protein powders obtained under supercritical conditions.

Mr. Winters has learned to used circular dichroism to investigate the conformation of proteins in solution. This was done in the laboratory of Professor Jeanette Carey, in Princeton's Chemistry Department. He has also visited the SmithKline Beecham laboratories in King of Prussia, where he was trained in the use of the Karl Fischer apparatus for measuring water content in protein samples.

For his theoretical work on the thermodynamics of mixed solute precipitation from supercritical fluids, Mr. Winters has learned to perform multicomponent phase equilibrium calculations using state-of-the-art thermodynamic perturbation theory as well as cubic equations of state. Having recently completed his study of nucleation theory, he is now investigating the rate of nucleation under supercritical conditions and its dependence on fluid-phase non-idealities. For these calculations, he will use his phase equilibrium models, coupled with fluid-mechanical calculations of expansions of supercritical fluids in nozzles and capillaries, and nucleation rate expressions.

In sum, the AASERT grant has allowed Mr. Winters to be trained in an unusually broad range of state-of-the-art experimental and theoretical methods and techniques. Though he has learned this material in the context of his doctoral work on particle formation in supercritical fluids, it is in fact broadly applicable to problems involving particle synthesis, protein formulations, industrial separations, and atmospheric nucleation.