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# Supercritical Carbon Dioxide Extraction Of Cyclosporine From The Fungus Beauvaria Nivea

Bokkel Derk te

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# SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF CYCLOSPORINE FROM THE FUNGUS *BEAUVARIA NIVEA*

by

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providing the quantities of mycelia of *B. nivea* used in the supercritical extraction experiments. The personnel in the Engineering Machine Shop who provided me with help in the construction of the supercritical extraction apparatus. Special thanks go to Mrs. M. Mousseau, Department secretary of Chemical and Biochemical Engineering for her assistance in preparing the final printed version of the thesis.

Thanks are also due to my wife Joan, and my children Angelina, John, Heather and Christina for their continuous support, love and encouragement throughout the course of this research project. Their dedication and encouragement made it all more enjoyable for me despite the many long and often odd hours of hard work required for the successful completion of this thesis.

## ABSTRACT

Cyclosporine, or Cyclosporin A is an important new immunosuppressant drug, now used to prevent the immune rejection of a variety of organ transplants. Cyclosporine is produced by the fungus *Beauvaria nivea* ATCC 34921, also called *Tolypocladium inflatum* NRRL 8004. The current commercial Cyclosporine extraction process uses liquid organic solvents, with the risk of possibly hazardous residues in the final product. Governments are now aggressively regulating exposure to organic solvents. In view of this, a supercritical $CO_2$ extraction process, which results in no organic solvent residues, is very good alternative.

Cyclosporine solubility in supercritical $CO_2$ was studied using a newly designed apparatus to determine the feasibility of the extraction process. Cyclosporine solubilities up to 16-20 mg/mL of supercritical $CO_2$ could be attained easily. The pressures and temperatures used ranged from 8.2 MPa to 34.0 MPa and 308.5 K to 343 K respectively. Cyclosporine mole fraction was found to correlate linearly with reduced densities above 1.5. When a methanol co-solvent was added Cyclosporine solubility in supercritical $CO_2$ increased up to 20 times.

Cyclosporine extractions from the mycelia of the fungus *Beauvaria nivea* were done with supercritical $CO_2$ at 32.0 MPa and 314 K. The highest extraction yields, with 70 to 80% of the original Cyclosporine present in the mycelia removed, were achieved using mycelia containing 7.2 to 29.5% moisture. Completely dried mycelia had lower extraction yields. The addition of methanol showed no effect on the Cyclosporine extraction yields. Co-extracted materials observed during the extraction experiments were tentatively identified as lipids. Scanning electron micrographs were

taken of the mycelial structure and used to visualize the physical barriers to Cyclosporine removal.

The supercritical $CO_2$ extraction process was found to be feasible for Cyclosporine removal from mycelia. This work contains the first report of its kind in the literature on the supercritical $CO_2$ extraction of Cyclosporine from the mycelia of *Beauvaria nivea*, and the first data showing Cyclosporine solubility in supercritical $CO_2$. Further work remains to be done to optimize the yields and rates of supercritical $CO_2$ extraction of Cyclosporine from mycelia.

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providing the quantities of mycelia of *B. nivea* used in the supercritical extraction experiments. The personnel in the Engineering Machine Shop who provided me with help in the construction of the supercritical extraction apparatus. Special thanks go to Mrs. M. Mousseau, Department secretary of Chemical and Biochemical Engineering for her assistance in preparing the final printed version of the thesis.

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## NOMENCLATURE

| Symbol | Description | Units |
|---|---|---|
| a | Slope of equation 4.1 | |
| b | Intercept of equation 4.1 | |
| c | concentration of solute, | ($kg/m^3$) |
| C | number of components | |
| f',f",f"',... | fugacities of phases ',","',... | |
| F | number of independent variables | |
| P | number of phases | |
| P | Pressure, | (MPa) |
| $P_c$ | Critical Pressure, | (MPa) |
| T | Temperature, | (K) |
| $T_c$ | Critical Temperature, | (K) |
| $V_{CO2}$ | Volume of Carbon dioxide used, | (L at S.T.P) |
| $\rho$ | Density, | ($kg/m^3$) |
| $\rho_c$ | Critical Density, | ($kg/m^3$) |
| $\rho_r$ | Reduced Density, $\rho/\rho_c$ | |
| $Y_1, y_1$ | Mole fraction of supercritical carbon dioxide phase | |
| $Y_2, y_2$ | Cyclosporine mole fraction in the supercritical phase | |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

Supercritical fluid extraction is currently being studied as an alternative separation process by many industrial and academic research and development laboratories world wide, (McHugh and Krukonis, 1986). Extraction of materials by gases or liquids at or above their critical points was first reported by Hanny and Hogarth in 1879, and 1880. This supercritical solvent behaviour has been well studied since. McHugh and Krukonis (1986) reviewed the history of supercritical extraction research up to about 1984. Several other comprehensive reviews and collections of papers are also available and are summarized in Table 1.1.

Supercritical extractions are conducted above the critical temperature, $T_c$, and the critical pressure, $P_c$, of the solvent fluid. The critical temperature, $T_c$ is that temperature above which it is not possible to liquify the gas no matter how much pressure is applied. The corresponding critical pressure, $P_c$ is defined as the pressure required to liquify the gas at its critical temperature, $T_c$. The density of the fluid at supercritical conditions varies with temperature and pressure.

The basic controlling parameter in supercritical extraction is solvent density which is a function of temperature and pressure. It has been shown that the density of the fluid is directly proportional to its solvent power. The solvent power generally increases with density at constant temperature, and also increases with temperature at constant pressure. As the supercritical fluid approaches the density of a liquid its solvent power becomes very appreciable. Entrainers or co-solvents can also be used to increase the solvent power of the supercritical solvent.

Table 1.1 Review Literature on Supercritical Fluid Technology

| Title | Reference | Comments |
|---|---|---|
| "Extraction with supercritical fluids" | McHugh, 1986 | Review paper |
| Ber.Bunsenges.Phys.Chem. Vol. 88, 1984 | | Papers from the Symposium held at Konigstein, W. Germany |
| Journal of Fluid Phase Equilibria, Vol. 10, 1983 | | Papers from the Syposium held at Girton College, Cambridge, U.K. |
| Separation Science and Technology, Vol. 17, 1982 | | 'Special Topics Issue on Supercritical Gases in Extraction and Chromatography' |
| "Chemical Engineering at Supercritical Fluid Conditions" | Paulitis,Penninger, Gray, and Davidson, | Papers from the 1981 AIChE Meeting in New Orleans; 1983. theory, data, & applications |
| "Supercritical Fluid Extraction" | Paulitis,Krukonis, Kurnik, and Reid, 1983. | Review of theory and applications plus a large data source tabulation. |
| "Supercritical Fluids" | Johnson, 1984. | A chapter in the Encyclopedia of Chemical Technology |
| "Zum stand der Extraktion mit Komprimierten Gasen" | Brunner and Peter, 1981. | Review |
| "Extraction with Supercritical Gases" | Williams,1981. | Review |
| "Extraction with Supercritical Gases" | Schneider,Stahl, and Wilke, 1980. | Papers from the 1978 Symposium held in Essen, W. Germany; with examples of CO2 extraction |
| "Separations using Supercritical Gases" | Irani and Funk, 1977. | Literature Review with a comparison of distillation and SCF extraction. |
| "The Principles of Gas Extraction" | Paul and Wise, 1971 | Introduction to the basic concepts of SCF technology and review of SCF Chromatography |

Table 1.1 (cont.)

| Title | Reference | Comments |
|---|---|---|
| "Vapor Phase Extraction Process" | Ellis,1971 | Review paper emphasizing early applications |
| "The solubility of materials in compressed hydrocarbon gases" | Valteris, 1966 | an early review paper |
| "A review of Supercritical Fluid Extraction" | Ely and Baker, 1983 | A review of the state of SFE research and recommendations for further study |
| "Supercritical Fluids: Still seeking acceptance" Supercritical fluid | Basta and McQueen, 1985 | A review of the commercial development of processes |
| "Supercritical Fluid Extraction" | Brignole, 1986 | advantages and applications of supercritical fluids with focus on dehydration of alcohols |
| "CO2 in Solvent extraction" (Chem. Ind. 1982) | Broglie, 1982; de Filippi, 1982; Pott, 1982 | Papers presented at SCI Food Group, Food Engineering Panel, London, England,Feb. 4, 1982. Properties, fundamentals, and applications |
| "Supercritical fluids for extraction of flavors and fragrances from natural products" | Caragay, 1981 | Review |
| "Supercritical Fluid Extraction: Fundamental Principles and Modeling Methods" | Rizvi, et al., 1986 | Review |
| "Innovative Separation Process Finding Its Way into the Food Industry" | Dziezak, 1986 | Review of commercialization status in U.S.A. |
| "Extracting foodstuffs using supercritical $CO_2$" | O'Toole, et al. 1986 | Theory and applications |
| "Supercritical Fluid Chromatography" | Gere, 1983 | Theory and techniques development of systems |

Table 1.1 (cont.)

| Title | Reference | Comments |
|---|---|---|
| "The Present Status of Dense (Supercritical) Gas Extraction and Dense Gas Chromatography" | Randall, 1982 | comparison of GC, HPLC with SFC, plus an extensive summary of compounds studied |
| "Supercritical Fluid Chromatography" | Gouw and Jentoft, 1982 | Theory and techniques development of systems |
| "Process supercritical fluid chromatoghaphy" | Levy, 1986 | Theory, equipment design, and techniques |
| "Advances in supercritical fluid systems" | Levy, et al., 1987 | chromatography equipment design |
| "Capillary Supercritical Fluid Chromatography" | Novotny, et al., 1981 | review of theory and basic design |
| "Modeling Supercritical Mixtures: How Predicive Is It?" | Johnson, et al., 1989 | Discussion of deficiencies of various models and areas where further information is required |
| "Extraction of Bio-materials with compressed carbon dioxide and other solvents near critical conditions" | King, et al., 1987 | applications and physical rational for behaviour of near-critical $CO_2$ |
| "Reactions in Super-critical Fluids - A Review" | Subramaniam and McHugh, 1986 | review and discussion of the unusual phemomenon associated with these reactions |

The choice of supercritical solvents can be varied in terms of size, and polarity and the range of solvent temperatures for extraction. Some of the more popular solvents such as $CO_2$, $H_2O$, and $N_2$ are cheap, abundant, non-toxic, non-flammable, and avoid environmental problems. The low viscosity of supercritical gases allows excellent penetration into 'solid' structures such as plant materials and microorganisms.

The operating temperatures for supercritical extraction processes are usually very close to their critical temperature. When a low critical temperature supercritical solvent such as $CO_2$ is used, thermally labile compounds can be extracted without fear of degradation. Supercritical solvents can also be used for easily oxidized materials, since oxidants such as oxygen can be easily excluded from the extraction system (Ely and Baker, 1983,Broglie, 1982).

Separation of the supercritical solvent and the solute occurs very readily when the pressure or temperature are changed as in isothermal decompression or isobaric heating. The solutes can also be easily fractionated during the separation stage by using staged operations, i.e., altering the pressure and/or temperature in different stages. Once the solute has been removed the solvent gas can then be recycled and used again which can reduces separation costs considerably.

## 1.2 Current and Future Applications

There are many factors driving research in supercritical fluid solvent technology by the chemical process industry. Energy costs have been increasing and pushing up the costs of traditional energy intensive separation techniques like distillation and evaporation. Environmental legislation regulating the use of traditional organic solvents, especially

those which are chlorinated, has made the nontoxic, environmentally benign supercritical solvents such as $CO_2$ very attractive to industry. The application of supercritical solvents to waste processing is rapidly becoming an attractive alternative to traditional approaches. Tighter government control of waste treatment and discharge has been stimulating research into this application of supercritical solvents. Interest in new high performance, and high purity materials has led to the use of supercritical fluids in processes to purify or crystalize materials to purities which are difficult and/or expensive to achieve with traditional techniques. Supercritical fluids are also being used in supercritical fluid chromatography as a new analytical tool allowing easier analysis of samples which had been difficult to analyse with other techniques, Randall, 1982, Gere, 1983, Levy, 1987.

Supercritical fluids are also under investigation for application to different bioprocesses in the biotechnology and fermentation industries. There is an urgent need to develop new efficient and economic separation-purification processes for their products. These products are usually present in dilute aqueous streams which makes separation and purification a costly operation. The recovery and purification of high value biotechnology or fermentation products is thus the most expensive part of a typical process and may account for 40 to 80 % of the final production cost. Any significant reduction in separation costs will be beneficial. Supercritical carbon dioxide based processes have the potential to reduce costs and allow the generation of products free of solvent residues.

A tabulation of current commercial and possible future applications of supercritical fluid processes is summarized in Table 1.2. A brief

Table 1.2 Current and Potential Applications of Supercritical Fluids

| Application | Important Details | Reference |
|---|---|---|
| Soy Bean Oil Extraction and Soybean Flakes | $CO_2$ replaces Hexane | Anonymous, 1981<br>Eldridge et al., 1986<br>Ely and Baker, 1983 |
| | (rapeseed and sunflower) | Stahl et al., 1980 |
| Lecithin removal from Soya Oil | pilot plant scale | Peter et al., 1987 |
| Recrystallization for comminuation | fine powder formation Patented (Phasex, Inc. involved) | Anonymous, 1986<br>Basta and McQueen, 1985<br>Mohamed et al., 1989 |
| Powder, thin films and fiber formation | research | Matson et al., 1987 |
| Mevinolin extraction from Aspergillus terreus ,Efrotomycin solubility, plus steriod recrystalization | research | Larson and King, 1986 |
| Isomer separation by retrograde crystallization | 2,3- and 2-6 dimethyl-naphthalene separation from 50:50 to 79:21 ratio | Johnson et al., 1987 |
| Coal Extraction for determining maturity | Toluene or Tetrahydrofuran solvent removes alkanes, isoprenoids, and cycloalkanes | Bartle et al., 1982 |
| Residual Oil Supercritical Extraction (ROSE) | Kerr-McGee, U.S.A. commercialized | Basta and McQueen, 1985<br>Ely and Baker, 1983 |
| Hops Extraction | Hag AG, West Germany commercialized | Basta and McQueen, 1985<br>Caragay, 1981<br>Ely and Baker, 1983<br>Hubert and Vitzthum, 1980 |

Table 1.2 (cont.)

| Application | Important Details | Reference |
|---|---|---|
| Hazardous Waste Treatment | Modar, Inc. ,U.S.A. prototype testing | Basta and McQueen, 1985 |
| PCB removal from oils | research | de Filippi, 1982 |
| Absorption separation | research | Kander and Paulaitis, 1983 |
| Adsorbant cleaning (Activated Carbon, Synthetic Resins etc.) desorption | being commercialized Illiois Water Treatment Co. | de Filippi, 1982<br>Basta and McQueen, 1985<br>Ely and Baker, 1983<br>Recasens et al., 1989<br>Tan and Liou, 1988, 1989 |
| Kerogen extraction from Shale | toluene, modeling | Triday and Smith, 1988 |
| Soil Cleaning | research | Brady et al., 1987<br>Capriel et al., 1986 |
| Recovery of dilute solvents from water (ethanol, acetic acid, etc.) | Critical Fluid Systems Inc., undergoing commercialization | Basta and McQueen, 1985<br>Brignole et al, 1987<br>Briones et al, 1987<br>de Filippi and Moses, 1982<br>Ely and Baker, 1983 |
| Coal Liquifaction | Akzo Zout Chemie Nederland BV, SRI International (Menlo Park Calif.) | Basta and McQueen, 1985<br>Ely and Baker, 1983<br>Kershaw and Jezko, 1982<br>Scarrah, 1983<br>Oclay et al., 1983<br>Fong et al., 1983 |

Table 1.2 (cont.)

| Application | Important Details | Reference |
|---|---|---|
| Enhanced Oil Recovery | $CO_2$ or Propane flooding of reservoir | Ely and Baker, 1983<br>Irani and Funk, 1977 |
| Nicotine Extraction from Tobacco | multistage process | Ely and Baker, 1983<br>Gahrs, 1984<br>Hubert and Vitzthum, 1980 |
| Deodorization of Vegetable Oils | Patents , also removes free fatty acids | Ely and Baker, 1983 |
| Pyretheum extraction | Botanical Resources, U.S.A. studying commercialization | Sims, 1982<br>Basta and McQueen, 1985<br>Stahl and Schutz, 1980 |
| Decaffination of Coffee and Tea | Numerous Patents and research (Commercialized in W.Germany) | Caragay, 1981<br>Dziezak, 1986<br>Ely and Baker, 1983<br>Irani and Funk, 1977<br>Brunner, 1984<br>Zosel, 1980<br>Stahl et al., 1980<br>Gahrs, 1984<br>Ebeling and Franck, 1984 |
| Montan Wax extraction | research | Braun and Schmidt, 1984 |
| Flavor and Fragrance extraction | Review of research | Caragay, 1981<br>Ely and Baker,1983<br>Kalra et al., 1987<br>Naik et al., 1989<br>Hubert and Vitzthum, 1980<br>Stahl et al.,1984 |
| Fat Solubile Vitamins (vitamin E) | research | Chrastil, 1982<br>Ohgaki et al., 1989 |
| Steriod extraction | research | Stahl et al., 1984 |

| Application | Important Details | Reference |
|---|---|---|
| Antartic Krill Oils | research | Yamaguchi, 1986 |
| Omega-3 fatty acid extraction from fish oils | review | Sweientek, 1987 |
| Glyceride, Fatty Acid, and Lipid Extraction | research | Brunner and Peter, 1982<br>Peter and Brunner, 1980<br>Choi et al., 1987<br>Chrastil, 1982<br>de Valle and Aguilera, 1988<br>Ikushima et al., 1988,1989<br>Inomata et al., 1989<br>Ohgaki et al., 1989<br>Stahl et al, 1984 |
| Rapeseed Oil Extraction | research | King et al., 1987<br>Klein and Schulz, 1989 |
| Treatment of Wood and Wood Products and other lignocellulosic materials | research | Calimli and Olcay, 1982<br>Li and Kiran, 1988<br>McDonald et al., 1982,1983<br>Poirier et al., 1987<br>Olcay et al., 1983<br>Koll et al., 1983 |
| Oxidation Catalysis | research | Dooley and Knopf, 1987 |
| Sugar separation | research | D'Souza and Teja, 1988 |
| Oil removal from Tar Sands | research | Eisenbach et al., 1983a,b<br>Ely and Baker, 1983<br>Panzer et al., 1979<br>Stutzer et al., 1983 |

Table 1.2 (cont.)

| Application | Important Details | Reference |
|---|---|---|
| Thermal Organic Reactions | research | Metzger et al., 1983 |
| Chlorination of Alumina | supercritical carbon tetrachloride | Herrick et al., 1988 |
| Supercritical Fluid Chromatography | Analysis of polymer additives | Hirata and Okamoto, 1989 |
| | Styrene fractionation on Column Methods | Kespler, 1980<br>Yonker et al., 1984 |
| | Aromatic compounds | Schmitz et al., 1984<br>Randall, 1982, 1983 |
| Lanolin extraction from Wool | Propane or propane-propylene mixtures | Irani and Funk, 1977<br>Valteris, 1966 |
| Lipase enzyme treatment by supercritical fluid | research (no effect) | Nakamura et al., 1986 |
| Alkaline Phosphatase Catalysis in $CO_2$ | research | Randolph et al., 1985 |
| Cholesterol oxidase activity in $CO_2$ | enzymes from *Norcardia* sp., *Pseudomonas* sp., *Streptomyces* sp., and *Gloeocysticum chrysocreas* | Randolph et al., 1988 |
| Cumene oxidation | research | Suppes et al., 1989 |
| Heterogeneous catalysis in Supercritical fluids | research | Tiltscher et al, 1984 |
| Silica Aerogel preparation | research | Schmitt et al., 1983 |
| Cholesterol extraction from milk fat | review | Swientek, 1987 |
| Menhaden Oil Ethyl Ester Fractionation | research | Nisson et al., 1988 |
| Deoiling Potato Chips | research | O'Toole et al., 1986 |

Table 1.2 (cont.)

| Application | Important Details | Reference |
|---|---|---|
| Organic Chemistry in Supercritical Fluids | research | Squires et al., 1983 |
| Reactions in Supercritical Fluids | review and discussion of unusual phase behavior and other phenomenon | Subramaniam and McHugh, 1986 |

examination of Table 1.2 shows the many areas where supercritical fluid research is being conducted. The potential of this technology for improving many current processes and generating new ones is very promising.

## 1.3 Work by Previous Investigators

Most of the work published to date on solute solubilities in supercritical fluids has centred on so called model compounds. These compounds, usually aromatics, have been well studied in the past and their physical properties are well known. A brief listing of some of the more recent work on solubilities in Table 1.3 reveals about 50% of the studies used aromatic compounds. Their behaviour in supercritical solvents has formed the basis of a large body of literature devoted to modelling based on thermodynamic principles. Table 1.4 lists the large body of literature available on modelling supercritical fluid solvent behaviour.

Another large area of study in supercritical fluid behaviour is in phase equilibria of binary and ternary systems. This of course overlaps with the solubility studies a bit since the solubility study focuses on the supercritical phase alone. The phase equilibrium studies also deal with modelling from a thermodynamic perspective as well. Table 1.5 lists recent literature on phase equilibria with some comments.

A few basic types of experimental methods in supercritical extraction are used for equilibrium, solubility, extraction and mass transfer experiments. They all share the requirements of good temperature and pressure control. Most work on supercritical fluids is done in flow-through type apparatus and Table 1.6 summarizes most of the apparatus used by previous investigators. Many unique configurations have been used

Table 1.3 Recent Solubility Studies (1986 to 1989)

| Solute(s) | Solvent System(s) | Conditions | References |
|---|---|---|---|
| Triglycerides and Fatty Acids: trilaurin trimyrisitn tripalmitin lauric acid myristic acid palmitic acid | Carbon Dioxide | 313 K 8 to 30 MPa | Bamberger, et al., 1988 |
| naphthalene 1-methylnaphthalene | Carbon Dioxide | 308 K , to 8 MPa | Barker, et al., 1988 |
| n-Octadecane Phenanthrene | Propane | 390 K, 420 K 35 to 60 bar (3.5 to 6 MPa) | Dimitrelis and Prausnitz, 1989 |
| Benzoic Acid 2-Aminobenzoic Acid phthalic anhydride acridine 2-naphthol hexamethylbenzene | Carbon Dioxide with / without acetone or methanol | 9 to 35 MPa 308 K | Dobbs, et al., 1987 |
| 1-Hexadecanol Palmitic Acid | Carbon Dioxide | 318 K, 328 K, 338 K 14 to 57.5 MPa | Kramer and Thodos, 1988 |
| 1-Octadecanol Stearic Acid | Carbon Dioxide | 318 K, 328 K, 338 K 14 to 46.7 MPa | Kramer and Thodos, 1989 |
| Naphthalene Dibenzothiophene | Carbon Dioxide | 309 K, 328 K 7.5 to 27.5 MPa | Mitra et al., 1988 |
| n-Nonacosane n-Tritriacontane | Ethane | 308 to 318 K 6.47 to 20.2 MPa | Moradinia and Teja, 1988 |
| α-Tocopherol Palmitic Acid Tripalmitin | Carbon Dioxide | 298 K, 313 K 10 to 18.2 MPa | Ohgaki et al., 1989 |
| Biphenyl | Carbon Dioxide | 308.8 to 330 K up to 50.7 MPa | Paulitis et al., 1983 |

Table 1.3 (cont.)

| Solute(s) | Solvent System(s) | Conditions | References |
|---|---|---|---|
| 1,10-Decanediol<br>Benzoic Acid | Carbon Dioxide | 308 to 328 K<br>14 to 31 MPa | Pennisi and Chimowitz, 1986 |
| Monocrotaline | Carbon Dioxide-Ethanol Mixtures | 308 to 328 K<br>8.86 to 27.41 MPa | Schaeffer et al., 1988 |
| Naphthalene<br>biphenyl<br>phenanthrene<br>anthracene<br>benzoic acid<br>1,4-naphthoquinone<br>acridine<br>2-naphthol<br>2-aminoflorene | Carbon Dioxide<br>Ethane<br>Chlorotrifloromethane<br>fluoroform | 308 to 343 K<br>5.0 to 37 MPa | Schmitt and Reid, 1986a |
| Phenanthrene<br>Benzoic Acid | Carbon Dioxide or Ethane with Entrainers | 328 K<br>10 to 30 MPa | Schmitt and Reid, 1986b |
| Naphthol-isomers | Carbon Dioxide<br>9.1 to 17.2 MPa | 308 to 328 K | Tan and Weng, 1987 |
| Griseofulvin<br>Digoxin<br>Cholesterol<br>Stigmasterol<br>2-Aminobenzoic Acid | Carbon Dioxide and various cosolvents | 323 K, 24.1 MPa | Iavana et al., 1989 |
| Cholesterol<br>Stigmasterol<br>Ergosterol | Carbon Dioxide alone or with acetone, ethanol, or methanol | 308 to 333 K<br>10.2 to 35.9 MPa | Wong and Johnson, 1986 |
| Anthracene | Carbon Dioxide | 293 to 368 K<br>8.1 to 117.2 MPa | Zerda et al., 1986 |

Table 1.4 Modeling of Solubility and Extraction for Supercritical Fluids

| Title | Reference | Comments |
|---|---|---|
| Measurement and Model Prediction of Solubilites of Pure Fatty Acids, Pure Triglycerides, and Mixtures of Triglycerides in Supercritical Carbon Dioxide | Bamberger et al., 1988 | Lattice model equation used |
| Modified Carnahan-Starling-van der Waals Equation for Supercritical Fluid Extraction | Bertucco et al., 1986 | 16 fluid - liquid, 11 solid - fluid systems correlated |
| Application of the Kirkwood-Buff Theory of Solutions to Dilute Supercritical Mixtures | Cochran et al., 1987 to model | predictions are sensitive parameters used |
| An Improved Equation for Predicting the Solubility of Vegetable Oils in Supercritical CO2 | del Valle, and Aguilera, 1988 | Improved Charstil's model |
| An Analytical Carnahan-Starling-van der Waals Model for Solubility of Hydrocarbon Solids in Supercritical Fluids | Johnson and Eckert, 1981 | log solubility vs density is linear |
| Solubilities of Hydrocarbon Solids in Supercritical Fluids. The Augmented van der Waals Treatment | Johnson et al., 1982 | Hard-sphere EOS |
| Modeling Supercritical Mixtures: How Predictive Is It? | Johnson et al., 1989 | various equations discussed, and problems noted |
| Molecular Dynamics of Dilute Solutes in Supercritical Solvents | Jonah et al., 1983 | Pade EOS, and mean field approx. used for correlation |
| A Linear Correlation for Solid Solubilities in Supercritical Gases | Jonah, 1989 | useful for extrapolation and interpolation of sparse data |

Table 1.4 (cont.)

| Title | Reference | Comments |
|---|---|---|
| Prediction of Mutual Solubilites of Heavy Components with Super-Critical and Slightly Sub-Critical Solvents: The Role of Equations of State and Some Applications of a Simple Expanded Lattice Model at Subcritical Temperatures | King et al., 1984 | UNIFAC vs EOS modeling discussed |
| Solubility of Solids in Supercritical Solvents, I. General Principles | Koningsveld, et al., 1984 | mean-field lattice EOS as tool for description and prediction |
| Adaption of the Flory-Huggins Theory for Modeling Supercritical Solubilities of Solids | Kramer and Thodos, 1988b | useful for systems where solid physical properties are not available |
| Van der Waals Mixing Rules for Cubic Equations of State. Applications for Supercritical Fluid Extraction Modelling | Kwak and Mansoori, 1986 | Redlich-Kwong and Peng - Robinson equations tested |
| Solubility of Solids in Super-critical Solvents. IV. Mean-Field Lattice Gas Description for the p-T-x Space Diagram of the System Ethylene-Naphthalene | van der Haegen, et al., 1988 | EOS showed good predictive ability |

Table 1.5 Phase Equiiibrium Studies

| Title | Reference | Comments |
|---|---|---|
| New Apparatus for Measurement of Supercritical Fluid-Liquid Phase Equilibria | Adams et al., 1988 | Decane-$CO_2$ Methyllinoleate-$CO_2$ |
| Model for Phase Equilibria Correlation and Prediction. Characteristics and Application to Binary Liquid-Vapor and Binary, Ternary and Quaternary Liquid-Liquid Equilibria | Bevia et al., 1986 | Excess Gibbs energy model compared to van Laar, Wilson, UNIQUAC, and NRTL models |
| Ternary Phase Equilibria for Acetic Acid-Water Mixtures with Supercritical Carbon Dioxide | Briones et al., 1987 | selectivities and distribution coefficients reported |
| Application of a Generalized Multiproperty Apparatus to Measure Phase Equilibrium and Vapor Phase Densities of Supercritical Carbon Dioxide in n-Hexadecane systems up to 26 MPa | Charoensombut-Amon et al., 1986 | Wilson-Wegner expansion equation used to fit data |
| Vapor-Liquid Equilibrium in the System Carbon Dioxide + n-Pentane from 252 to 458 K at Pressures to 10 MPa | Cheng et al., 1989 | Modeled using Soave-Redlich-Kwong, Peng-Robinson, Kubic-Martin, and Adachi-Lu-Sugle cubic equations of state |
| High Pressure Phase Equilibria for Binary Systems Involving a Solid Phase | Cheong et al., 1986 | Correlation using van Laar equation |
| Vapor-Liquid Equilibrium in the Binary System Carbon Dioxide + n-Butane | de Fernandez, et al., 1989 | Correlation by Soave-Redlich-Kwong, and Peng-Robinson equations |
| Calculation and Prediction of Fluid Phase Equilibria from an Equation of State | Deiters, 1983 | reference for quantum corrections to equations of state |
| Density-Dependent Mixing Rules for the Calculation of Fluid Phase Equilibria at High Pressures | Deiters, 1987 | modeling equation notes |

Table 1.5 (cont.)

| Title | Reference | Comments |
|---|---|---|
| Phase Equilibria and Critical Phenomena in Fluid (n-Alkane + Water) Systems at High Pressures and Temperatures | de Loos, et al., 1983 | n-heptane and n-pentane studied |
| Fluid Phase Equilibria in Binary Ethylene + n-Alkane Systems | de Loos et al., 1984 | |
| An Experimental Study of Three- and Four-Phase Equilibria for Isopropanol-Water-Carbon Dioxide Mixtures at Elevated Pressures | Di Andreth, and Paulaitis, 1987 | phase compositions & molar volumes obtained |
| High-Pressure Phase Equilibria in the System Glucose + Fructose + Water + Ethanol + Carbon Dioxide | D'Souza and Teja, 1988 | best separation near 3-phase (L-L-V) line |
| High Pressure Phase Equilibria in the Carbon Dioxide - n-Hexadecane and Carbon Dioxide - Water Systems | D'Souza et al., 1988 | Correlation with Patel-Teja and Peng-Robinson equations |
| Vapor-Liquid Equilibria for the Carbon Dioxide-Cyclopentane system at 37.7, 45.0, and 60.0 °C | Eckert and Sandler, 1986 | Peng-Robinson and Teja-Patel equations used |
| Effect of Additive Gases on the Liquid-Liquid- Vapor Immiscibility of the Carbon Dioxide + n-Nonadecane Mixtures | Fall and Luks, 1986 | nitrogen and propane were additive gases |
| Gas-Liquid Equilibrium for Ethanol-Water-Carbon Dioxide Mixtures at Elevated Pressures | Gilbert and Paulaitis, 1986 | phase diagrams shown |
| Phase Behavior in Fluid-Solid Systems | Holder, 1986 | solids can drasticly alter phase equilibria |
| Fluid-Phase Equilibria of Binary and Ternary Mixtures of CO2 with Hexadecane, 1-Dodecanol, 1-Hexadecanol, and 2-Ethoxy-Ethanol at 333.2 and 393.2 K and at Pressures up to 33 MPa | Holscher et al., 1989 | partition coefficients, 3D phase diagrams |
| Binary Phase Diagrams from a Cubic Equation of State | Hong and Modell, 1983 | Peng-Robinson EOS |

Table 1.5 (cont.)

| Title | Reference | Comments |
|---|---|---|
| Vapor-Liquid Equilibrium Studies for the Carbon Dioxide-Methanol System | Hong and Kobayashi,1988 | K-values |
| Measurement of Vapor-Liquid Equilibria at Elevated Temperatures and Pressures using a Flow Type Apparatus | Inomata et al., 1986 | Soave-Redlich-Kwong, and an extended BWR equation were used for correlation |
| Vapor-Liquid Equilibria for Binary Mixtures of Carbon Dioxide and Fatty Acid Methyl Esters | Inomata et al., 1989 | Peng-Robinson EOS with modified van der Waals mixing rules |
| Vapor-Liquid Equilibria in the Carbon Dioxide - 1-Hexane and Carbon Dioxide - 1-Hexyne Systems | Jennings and Teja, 1989 | Patel-Teja and Trebble-Bishnoi equations used |
| Phase Equilibrium Data for Supercritical Extraction of Lemon Flavors and Palm Oils with Carbon Dioxide | Kalra et al, 1987 | visosity and density data also generated |
| Vapor Pressures of Binary Mixtures of Carbon Dioxide with Benzene, n-Hexane, and Cyclohexane up to 7 MPa | Kaminishi et al., 1987 | correlation by Barker's method and Peng-Robinson equation |
| Some Vapor/Liquid and Vapor/Solid Equilibrium Measurements of Relevance for Supercritical Extraction Operations, and Their Correlation | King et al., 1983 | Redlich-Kwong EOS, & Experimental apparatus |
| Phase Equilibria in Mixtures of Glycerides and Carbon Dioxide and Application of Continous Thermodynamics to Mixtures of Rapeseed Oil and Carbon Dioxide | Klein and Schulz, 1989 | Lattice equation of Kumar combined with Bender Equation |
| Mean-Field Lattice-Gas Description of the System $CO_2/H_2O$ | Kleintjens and Koningsveld, 1982 | solvent system encountered in supercritical extraction |
| Mean-Field Lattice-Gas Description of Fluid Phase Equilibria | Kleintjens and Koningsveld, 1983 | few experimental data needed for use |

Table 1.5 (cont.)

| Title | Reference | Comments |
|---|---|---|
| Mean-Field Lattice Gas Description of Vapor-Liquid and Supercritical Equilibria | Kleintjens, 1983 | works well in critical region |
| Supercritical Phase Equilibria Involving Solids | Koningsveld & Diepen, 1983 | mean-field lattice-gas model reviewed |
| High-Pressure Phase Studies on Fluid Mixtures of Low-Volatile Organic Substances with Supercritical Carbon Dioxide | Konrad et al., 1983 | experimental study |
| Solubility of Oxygenated Hydrocarbons in Supercritical Carbon Dioxide | Kuk and Montagna, 1983 | experimental study |
| Solid-Liquid-Gas Equilibria in Multicomponent Supercritical Fluid Systems | Lemert and Johnson, 1989 | Peng-Robinson EOS used with Regular Solution Theory |
| Phase Equilibria and Critical Curves of Binary Ammonia-Hydrocarbon Mixtures | Lentz and Franck, 1980 | proposed solvent for supercritical extraction |
| Equilibrium-Phase Properties of the Neopentane-Carbon Dioxide Binary System | Leu and Robinson, 1988 | equilibrium ratios |
| High Pressure Fluid Phase Equilibria of Alcohol-Water-Supercritical Solvent Mixtures | McHugh et al., 1983 | experimental study, Peng-Robinson EOS |
| Three-Phase Solid-Liquid-Gas Equilibria for Three Carbon Dioxide-Hydrocarbon Solid Systems, Two Ethane-Hydrocarbon Solid Systems, and Two Ethylene-Hydrocarbon Solid Systems | McHugh and Yogan, 1984 | S-L-G curve determined |
| High-Pressure Phase Behavior of Binary Mixtures of Octacosane and Carbon Dioxide | McHugh et al., 1984 | experimental study |
| Reexamination of the Multiphase Equilibria of the System Carbon Dioxide + n-Butylbenzene + n-Eicosane | Miller et al., 1989 | experimental study |

Table 1.5 (cont.)

| Title | Reference | Comments |
|---|---|---|
| High Pressure Phase Behavior in Systems Containing CO2 and Heavier Compounds with Similar Vapor Pressures | Mohamed and Holder, 1987 | Peng-Robinson EOS with VDW-1 mixing rules |
| Equation-of-State Predictions of Phase Equilibria at Elevated Pressures in Mixtures Containing Methanol | Peschel and Wenzel, 1984 | van der Waals type equation used |
| Studies on Phase Equilibria of a Multicomponent Model Mixture in Supercritical Carbon Dioxide and Trifluoromethane | Prange and Riepe, 1987 | partition coefficients, separation factors, and enhancement factors determined |
| Phase Equilibria in Fluid Systems | Schneider, 1983 | theoretical notes |
| High Pressure Phase Equilibria for Vapor Phase Extraction Processes | Stephan and Schaber, 1982 | Redlich-Kwong EOS used for modeling |
| Phase Equilibria In Fluid and Solid Mixtures at High Pressure | Street[+], 1983 | Theoretical behaviour discussed |
| Phase Behavior of the Carbon Dioxide-Styrene System | Suppes and McHugh, 1989 | Peng-Robinson EOS used for modeling |
| The Correlation and Prediction of Critical States of Mixtures Using a Corresponding States Principle | Teja and Smith, 1983 | van der Waals one fluid model used |
| High-Pressure Vapor-Liquid Equilibria with Cubic Equations of State | Tsonopoulos & Heidman, 1986 | Redlich-Kwong-Soave, Peng-Robinson, and cubic chain-of-rotators EOS tested |
| Phase Equilibria and Density Calculations for Mixtures in the Critical Range with Simple Equations of State | Vidal, 1984 | overview of usefulness and deficiencies of several EOS |
| High-Pressure Vapor-Liquid Equilibrium in Systems Containing Carbon Dioxide, 1-Hexene, andn-Hexane | Wagner and Wichterle, 1987 | Soave-Redlich-Kwong, and Peng-Robinson EOS used for correlation |

Table 1.5 (cont.)

| Title | Reference | Comments |
|---|---|---|
| Three-Phase Equilibrium and the Tricritical point | Widom, 1983 | review |
| Thermodynamic Calculation of Supercritical-Fluid Equilibria: New Mixing Rules for Equations of State | Won, 1983a | Heyen cubic EOS tested |
| Phase Equilibria of High-Boiling Organic Solutes in Compressed Supercritical Fluids - Equations of State with New Mixing Rule | Won, 1983b | Soave-Redlich-Kwong and van der Waals type EOS tested with new mixing rules |
| Phase Equilibria for Fluid Mixtures Containing Small and Large Molecules | Wu, 1988 | Boublik-Mansoori EOS used, and Soave-Redlich-Kwong with Prigogine based modifications |
| Application to Mixtures of the Peng-Robinson Equation of State with Fluid-Specific Parameters | Xu and Sandler, 1987 | vapor-liquid equil. prediction |
| A Three-Parameter Cubic Equation of State For Asymmetric Mixture Density Calculations | Yu and Lu, 1987 | volume translation of Peng-Robinson EOS to a new form |

Table 1.6 Common Experimental Methods for Solubility, Phase Equilibrium Mass Transfer and Extraction Studies

| Apparatus Description | Remarks | References |
|---|---|---|
| Sapphire pressure vessel with two recircluation loops on line sampling by loop isolation and GC analysis | Phase Equilibrium studies and solubilities (liquids and gases) | Adams et al., 1988 |
| Stainless steel column 305mm by 17mm I.D., operated as a flow through system, Column packed as alternating layers of solute and glass wool | Solubility studies (solids or semi -solids) | Bamberger et al., 1988 |
| Chromatographic retention apparatus, uses U.V. detector and solute isolation to generate pulses for retention analysis | Solubility studies (could be adapted to diffusion coefficient determination easily) | Barker et al., 1988 |
| Flow through apparatus with a view cell | Phase equilibria (liquids) | Briones et al., 1987 |
| Modified HPLC for use with Supercritical fluids | requires multiple injections (normal solids are injected dissolved in methylene chloride (enhancement factors studied) | Brown et al., 1987 |
| Two trayed columns one is the separator, the other is a regenerator column operated as a supercritical fluid distillation process | Mass transfer and separation studied | Brunner and Kreim, 1986 |
| Flow through extraction unit, HPLC column shell used as extractor | Extraction studies | Capriel et al., 1986 |
| Flow through extraction using stainless steel column or Jerguson Gage Model 17T40 | Solubilites and phase behaviour | Chang and Morreil, 1985 |
| Multiproperty apparatus: sapphire visual cell, pendent drop cell, capillary viscometer a very complex system | Phase equilibria and other properties (liquids in CO2) | Charoensombut-Amon et al., 1986 |

Table 1.6 (cont.)

| Apparatus Description | Remarks | References |
|---|---|---|
| Vapor recirculation apparatus on line GC analysis | Vapor-liquid equilibria | Cheng et al., 1989 |
| Static view cell system liquid thermostated | Solid-liquid-gas equilibrium studies | Cheong et al., 1986 |
| Magnetically mixed tubular equilibrium cell | Solubility studies | Chrastil, 1982 |
| Flow through system with specially designed horizontal extraction cell containing a rectangular channel | Mass Tranfer study | Debenedetti and Reid 1986 |
| Pilot plant scale separation system | Extraction from aqueous solutions | de Filippi and Moses, 1982 |
| Multiple apparatuses described a review on experimental methods | High pressure phase equilibria | Deiters and Schneider, 1986 |
| Piston cell system | Multiphase Equilibria | Di Andreth and Paulitis, 1987 |
| Flow through system with online GC analysis | Solubilities | Dimitrelis and Prausnitz, 1989 |
| Flow through system with microsampling technique and online densitometer | Solubility and cosolvent enhancement studies | Dobbs et al., 1986, 1987a,b |
| Two phase recirculation system with two phase sampling and view cell | Phase equilibria | D'Souza and Teja, 1988<br>D'Souza et al., 1988<br>Jennings and Teja, 1989 |
| Flow through system with receiver | Extraction system | Eldridge et al., 1986 |
| Flow through system with view cell, two feeds and two exits | Equilibrium studies with liquids and gases | Gilbert and Paulitis, 1986 |
| Microscale extraction system with sample collection for microcolumn chromatography | Extraction | Hirata and Okamoto, 1989 |

Table 1.6 (cont.)

| Apparatus Description | Remarks | References |
|---|---|---|
| Review of experimental techniques | Pressure, volume and Temperature measurements | Holste et al., 1986 |
| Liquid thermostated flow through system with separator and receiver (and online GC later) | Extraction | Ikushima et al., 1988, 1989 |
| Flow through system with supercritical fluid recycle | Vapor-liquid equilibria | Inomata et al., 1986 |
| Piston cylinder and view cell apparatus | Vapor-liquid equilibria | Inomata et al., 1989 |
| Variable volume view cell and variable volume stirred cell no flow | Phase equilibria | Kalra et al., 1987 |
| variable volume pyrex glass view cell with a mercury fluid piston | Vapor pressures, V-L equilibria | Kaminishi et al. 1987 |
| Flow through system with diffusion and mass transfer extraction cells | Diffusion coefficients and Mass transfer | Knaff and Schlunder, 1987a,b,c |
| Flow through system with liquid thermostat and cold traps | Solubilities | Kramer and Thodos, 1988 |
| Flow through system | Solubilities | Krukonis and Kurnik, 1985<br>Kurnik et al., 1981<br>Kurnik and Reid, 1982 |
| Windowed multistage contactor operated continuously | Equilibria and mass transfer | Lahiere and Fair, 1989 |
| Extraction system with on line HPLC analysis | Solubilities | Larson and King, 1986 |
| Flow through equilibrium cells with two phase sampling | Extraction | Lee and Chao, 1988 |
| Constant pressure view cell system | Solid-Liquid-Gas Equilibria | Lemert and Johnson, 1989 |

Table 1.6 (cont.)

| Apparatus Description | Remarks | References |
|---|---|---|
| Extraction system with multiple traps (separators) | Extraction | Li and Kiran, 1988 |
| Flow through extractor | Extraction | McDonald and Howard, 1982 |
| Flow through equillibrium cell | Solubilities | McHugh and Paulaitis, 1980<br>van Leer and Paulaitis, 1980 |
| Stirred view cell static | Equilibria | McHugh et al., 1984<br>Suppes and McHugh, 1989 |
| Flow through system with dual feeds and a dual outlet separator | Separation by Extraction | Mohamed and Holder, 1987 |
| Total vaporization apparatus | Solubilities by vaporization | Monge and Prausnitz, 1983 |
| Distillation style packed column for fractionation | Extractive fractionation | Nisson et al., 1988 |
| Flow through system with recirculation | Solubilities | Ohgaki et al., 1989 |
| Extraction and absorption system | Extraction with separation by absorption | Panzner et al., 1979 |
| Flow through system | Solubilities | Pennisi and Chimowitz, 1986 |
| Flow through extractor | Extraction | Poirier et al., 1987 |
| Equilibrium cell system | Multicomponent Phase Equilibria | Prange and Riepe, 1987 |
| Flow through a stirred autoclave | Extraction with entrainers | Roop and Akgerman, 1989 |
| Dual feed single pass extraction system | Solubilities | Schaeffer et al. 1988 |

Table 1.6 (cont.)

| Apparatus Description | Remarks | References |
|---|---|---|
| Flow through tubular extractor system with liquid thermostating (and sight glass) | Solubilities | Schmitt and Reid, 1986a,b |
| High temperature, high pressure view cell construction | Fluid Properties | Simon and Schmidt, 1983<br>Simon, 1983 |
| Extraction system with two separators and solvent cleaning by sorption before recycle | Extraction | Stahl et al., 1980a |
| Micro-analytical solubility determination equipment | Solubility screening | Stahl et al., 1980b |
| Taylor-Aris Dispersion Apparatus for diffusivity measurement with uv detection | Diffusion coefficients | Sun and Chen, 1985a,b,c,1986, 1987 |
| " | " | Swaid and Schneider, 1979<br>Wilsch et al., 1983 |
| Flow through extraction system with recycle of the supercritical phase | Solubility measurements | Tan and Weng, 1987 |
| Flow through system with activated carbon packed column | Extractive desorption<br>Fluid distribution<br>Fluid-Solid Mass Transfer | Tan and Liou, 1988<br>Tan and Wu, 1988<br>Tan et al., 1988 |
| Cosolvent feed system for flow through extractor | Solubility studies with co-solvents | Tavana et al., 1989 |
| High temperature extraction system | Extraction of Shale | Triday and Smith, 1988 |
| Equilibrium cell with windows and stirring mechanism | Vapor-Liquid Equilibria | Wagner and Wichterle, 1987 |
| Flow through extractor with micro-sampling valve | Solubility | Wong and Johnson, 1986 |
| FTIR cell | Solubility measurements | Zerda et al., 1986 |

Table 1.6 (cont.)

| Apparatus Description | Remarks | References |
|---|---|---|
| Photon correlation spectroscopy apparatus | Diffusion measurement | Saad and Gulari, 1984 |
| Continuous viscosity measurement apparatus | Solubility and miscibility study, and viscosity determination | Killesreiter, 1984 |
| Variable volume circulation apparatus | Fluid phase equilibria | Radosz, 1984 |
| Spectrographic apparatus | Solubility measurements | Ebeling and Franck, 1984 |
| Apparatus for measuring solubilities of solids | Solubility studies | Kwiatkowsky et al., 1984 |
| Four litre extraction system with recirculation | Extraction studies | Braun and Schmidt, 1984 |

to improve measurement accuracy, speed up data generation, or ease analysis. Several novel apparatus configurations of the non-flow-through type have been used for specialized studies. Systems involving view cells, either static, stirred or part of a flow through system have been used especially for equilibrium studies as well as solubility experiments.

Only a few mass transfer studies have appeared in the literature to date and this area will require more study in the future. Mass transfer limitations in supercritical extraction may influence process economics to a great extent. Poor mass transfer can influence both the extraction yield of a process as well as the maximum extraction rate. This area of supercritical fluid behaviour deserves further investigation.

## 1.4 Objectives of the Current Study

Cyclosporine is a relatively new immunosuppressant drug widely used for preventing the rejection of transplanted organs in patients worldwide. It is also being tested for application as a treatment for juvenile diabetes and other autoimmune diseases,(Borel, 1982) . Cyclosporine is a cyclic eleven amino acid peptide containing several unique amino acids, is highly methylated, non-polar and highly hydrophobic. Figure 1.1 shows its structure and sites of hydrogen bonding. Figure 1.2 shows a three dimensional molecular model picture of the Cyclosporine molecule. The drug was discovered by J.F. Borel from Sandoz Switzerland, and a large number of patents on Cyclosporine have been issued. Cyclosporine (A) and other types of Cyclosporins (B,C,D,E, etc.) are produced by different species of fungi, namely, *Beauvaria nivea* strains(formerly identified as *Tolypocladium inflatum* Gams), and *Cylindrocarpon lucidum* Booth. At present the *Beauvaria nivea* strain is known to be used for production of

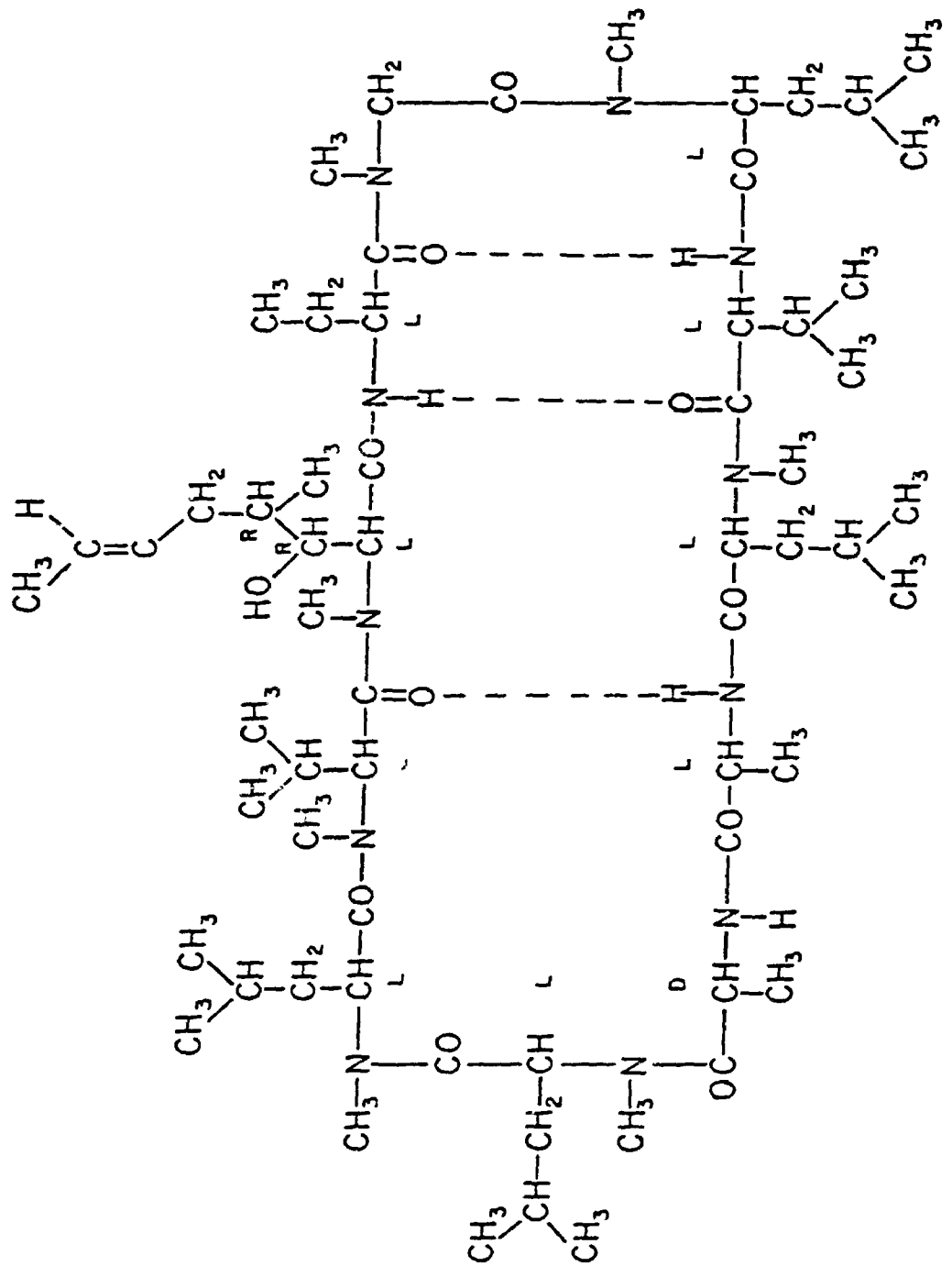


Figure 1.1 Structure of Cyclosporine (Cyclosporin A), $C_{62}H_{111}N_{11}O_{12}$ (m.w. = 1202.6)

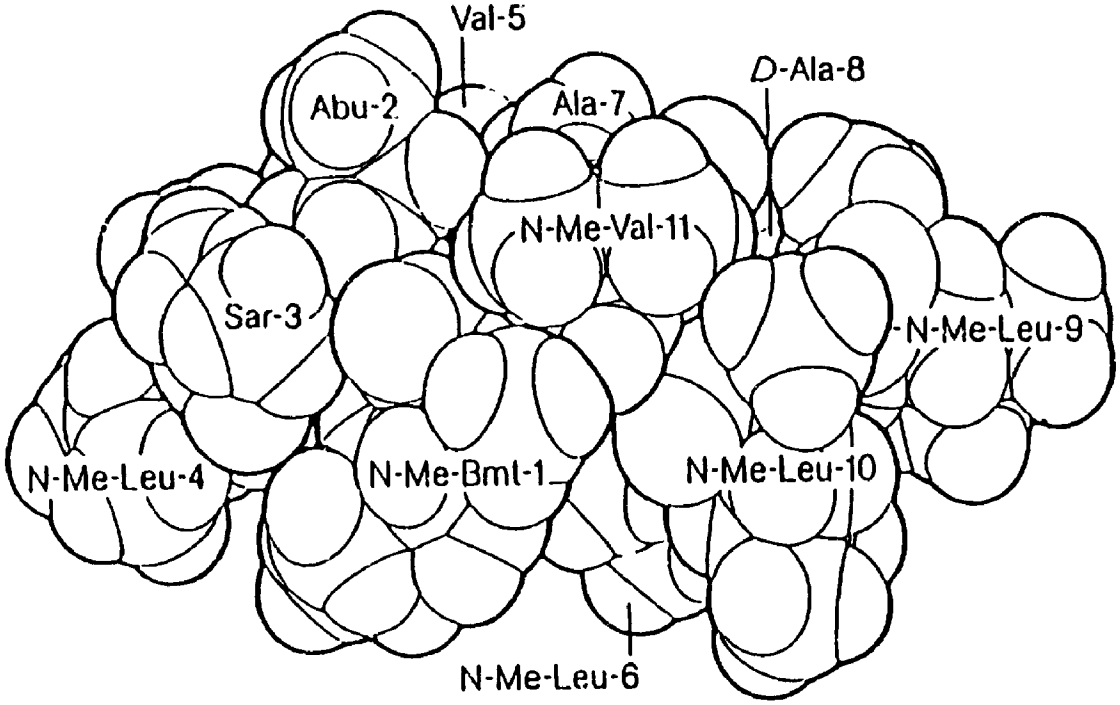



Figure 1.2 Three Dimensional Structure of Cyclosporine molecule, (Quesniaux et al., 1986)

Cyclosporine, and Sandoz of Switzerland is the only commercial producer of this drug.

The current process for Cyclosporine production uses liquid phase organic solvents at atmospheric pressure to extract the hydrophobic peptide from the fungal mycelium. There are numerous stages required to separate other compounds which are coextracted and finally the organic solvent itself has to be removed from the Cyclosporine. Ensuring that all traces of the solvent are removed adds greatly to the cost of the overall process. The current cost of the drug treatment per transplant patient per year is about $12,000.00, and therefore it is highly desirable to reduce the cost of Cyclosporine production by exploring new methods of extraction. Supercritical fluid carbon dioxide has the ability to dissolve non-polar organic compounds. In this investigation the first basic objective is to study the solubility of pure Cyclosporine in supercritical $CO_2$ solvent at different temperatures and pressures both in the absence and presence of methanol as a co-solvent. The second objective is to study the supercritical $CO_2$ extraction of Cyclosporine from the mycelia of *Beauvaria nivea* at different temperature and pressure conditions and different pretreatment methods of the mycelia.

The following detailed objectives of this investigation were formulated:

1) Design and construct the high pressure extraction apparatus with modifications as needed.
2) Determine the solubility of pure Cyclosporine in supercritical carbon dioxide at different temperatures and pressures and correlate the solubility data as a function of reduced density of the supercritical $CO_2$ solvent.

3) Determine the solubility of pure Cyclosporine in supercritical $CO_2$ in the presence of small amounts of methanol as a co-solvent and study its solubility enhancement characteristics.

4) Determine both the yields and the kinetics of supercritical carbon dioxide extraction of Cyclosporine from the mycelia of the fungus *Beauvaria nivea* at different pretreatment conditions of the mycelia.

5) Use electron microscopy to study the mycelia structure before and after supercritical extraction and using different pretreatment methods.

# CHAPTER 2

# THEORY

## 2.1 Modeling of Supercritical Fluid Equilibria and Solute Solubilities

The phenomenon of supercritical fluid solvation of solids can be explained in part by fluid phase equilibrium behavior. All phase equilibrium systems are governed by the Gibbs phase rule given by Equation 2.1

$$F = C + 2 - P \qquad \ldots 2.1$$

where, F is the number of independent variables (or degrees of freedom), C the number of components, and P the number of phases. The maximum number of independent variables gives an indication of how the phase diagram will appear in a given geometrical space. Multiphase systems of 3 or more components can be roughly described in 3D-space but more complex multi-dimensional diagrams are necessary for a full description of these systems (Streett, 1983). The simple 2D and 3D binary phase diagrams are useful for showing expected behavior in supercritical systems. Streett, 1983, reviewed the phase equilibria of binary systems and showed how they were applicable to supercritical systems. McHugh and Krukonis, 1986, expanded upon this work to point out some potential problems the experimentalist should be aware of. These included phase inversion, solid melting, the effect of the position of the solid- liquid-vapor (SLV) line and the upper critical end point (UCEP). Phase inversion occurs when the density of the supercritical phase becomes greater than that of the solute. This can lead to erronous data in flow through type experimental systems. Solid melting can also occur leading to different solubility characterisitics caused by the presence of multiple phases. Increasing the

pressure of the system may cause reduced solubility because of intersections with the SLV line. If an UCEP is present, a very large solubility enhancement may occur when conditions in the equilibrium system approach it.

The equilibrium phases of the system are also governed by the fugacity equation 2.2.

$$f_i' = f_i'' \qquad i = 1,2,3,\ldots,m \qquad 2.2$$

where two or more phases in equilibrium have the same fugacity of any phase i. Any model used to describe the phase equilibria of any system must satisfy this thermodynamic relationship. It has been noted by Prausnitz et al. (1986) that the pressure of the solvent near the critical point has a significant effect on solid solute fugacity and thus on its solubility. Very rapid changes occur at this point which could be exploited in a commercial separation to reduce energy costs.

Modeling of supercritical fluid-solute phase interactions is important for the current work and merits further discussion. Several different modeling methods have been attempted. Most rely on physical property data to calculate equation constants and the other parameters required. Simple cubic equations of state and more complex equations have all been used to model supercritical phase behaviour. Table 2.1 summarizes some of the cubic equations of state used by previous workers for modeling supercritical fluids. References specific to modeling supercritical fluid solubilities are also listed earlier in Table 1.4 with comments on the use of equations of state. The comments in Table 1.5 also indicate equation of state usage for phase equilibrium modeling. Cubic equations of state, such as the Soave-Redlich-Kwong, Peng-Robinson,

Table 2.1 Some Cubic Equations of State used for Modeling Supercritical Fluids

| Equation of State | Comments | References |
|---|---|---|
| Peng-Robinson | new mixing rules, better liquid density prediction than SRK | Kwak and Mansoori, 1986<br>Tsonopoulos and Heidman, 1986 |
| Soave-Redlich-Kwong | popular form | Tsonopoulos and Heidman, 1986 |
| Cubic-Chain of Rotators | not as good as PR or SRK | Tsonopoulos and Heidman, 1986 |
| Patel-Teja | better than PR | Jennings and Teja, 1989 |
| Carnahan-Starling-van der Waals | van der Waals form with hard sphere term, good to $\rho_r = -1.3$ | Bertucco et al., 1986<br>Johnson and Eckert, 1981 |
| Perturbed-Hard-Sphere EOS | CSvdW form variation | Oellrich et al., 1978 |
| Augmented-van der Waals | CSvdW form with square well fluid corrections good to critical point | Johnson et al., 1982 |
| Trebble-Bishnoi | better than PR | Trebble and Bishnoi, 1987, 1988<br>Trebble, 1989<br>Jennings and Teja, 1989 |
| Heyen EOS | volumetric property calculations better than PR | Won, 1983 |

Patel-Teja, Trebble-Bishnoi, van der Waals (augmented and hard-sphere forms inc.), etc. are coupled with the fugacity equation, and various mixing rules with adjustable parameters to model binary and ternary systems. They are good for correlation but unsuitable for prediction (Johnson et al. 1989). Their performance near the critical point often is poor. Some of the deficiencies of the more popular cubic equations are discussed by Trebble and Bishnoi, 1986.

The lattice models are able to account for large differences in molecular sizes. There have been several varients described, incorporating different features. Flory-Huggins terms , dual lattice types (decorated model), UNIFAC terms, and Panayiotou and Vera EOS adaptions. Each of these types are useful for modeling complex phase behaviours but some are poor near the critical region (Johnson et al. 1989). The dual lattice type has the best performance near the critical region but requires two adjustable parameters for correlation. Kirkwood-Buff solution theory uses fluctuation integrals to account for the nonideal behaviour of supercritical fluids. Expressions for the dilute solutions normally encountered in supercritical extraction involve only the solvent properties plus characterization parameters for the solute. The model is still in a crude form and requires further development to improve its accuracy (Cochran et al., 1987, Pfund et al., 1988, Johnson et al., 1989). Kramer and Thodos (1988) developed a modified expression of the Flory-Huggins theory and adapted it to model the solubilities of solids in supercritical fluids in the absence of physical property data for the solute. The solubility parameters for both the solvent and solute are required. The single binary interaction parameter used, was found to be highly dependent on the solvent solubility parameter.

A linear correlation for solid solubilities in supercritical gases was developed by Jonah (1989). It is useful for interpolation and prediction from sparse data sets. The method does not require a combining rule. The empirical parameters show only a weak temperature dependence which may make possible prediction of close isotherms from a known data set. A model developed by Chrastil (1982) was modified by del Valle and Aguilera (1988) to simplify modeling of solute solubilities in supercritical fluids. The model only required the density of the solvent and the temperature as parameters. The equation constants were generated by regression and are shown in equation 2.3.

$$\text{Ln}(c) = 40.361 - 18708/T + 2186840/T^2 + 10.724\ \text{Ln}(\rho) \quad \ldots 2.3$$

These constants were generated for vegetable oils and may require adjustment for use with other solutes.

The availablity of physical data for Cyclosporine limits the usefulness of the equations discussed to those which require minimal property data. The saturated vapor pressure of solid Cyclosporine has not been determined experimentally, as well solubility parameters are also not available for this material. This severely limits the options for correlation and modeling. Only the modified Chrastil equation can be used in addition to any empirical modeling.

## 2.2 Equations of State for Supercritical Carbon Dioxide Density Determination.

Multiple choices in equations of state are available for determining supercritical carbon dioxide fluid densities. The traditional virial form using second and third virial coefficients is only useful up to reduced

densities of 0.75 or less ( Tsonopoulos, 1974, Orbey and Vera, 1983). More often cubic equation of states are used; they are however very limited in accuracy, and some popular ones have been listed previously in Table 2.1. A more accurate approach has been to regress a pseudo-viral form equation with high order terms with experimental data. Common equations of this form include the Benedict-Webb-Rubin equation of state (Orye 1969), the Hirschfelder,Beulhler,McGee and Sutton, 1958a,b equations, the Van Huff, Houghton, and Coull set of equations, ,the Bender EOS, and several others (Huang, 1984, Huang et al., 1985). Some of these equations are either limited by their generality to multiple substances, or do not cover the critical region accurately. Huang, (1984), and Huang et al., (1985) developed a new form of the Benedict-Wedd-Rubin-Bender equation type specific to carbon dioxide. Particular attention was paid to modeling the critical region accurately and fitting to good experimental data. It is more accurate than the Bender equation in the critical region, and has an extended high pressure range. Prediction of fluid properties was found to be quite good with this equation. Density calculations are reliable to 0.1 to 0.2 % outside the critical region and accurate to 1 % in the critical region.

The IUPAC equation (Angus et al. 1976) was found to be slightly more accurate over most regions (Huang et al., 1985). However its complex functional form over the critical region makes programing for density calculations quite difficult. A new form of the IUPAC type equation of state was developed by Pitzer and Schreiber (1988) using the HGK function. This equation has at least the same accuracy as IUPAC over the whole of its range, as well it allows prediction of Cv values which the original IUPAC equation could not do accurately. As this equation appears to

Table 2.2 The Modified IUPAC Equation of State for $CO_2$ and the relevant equation constants

$$\frac{P}{\rho RT} = 1 + \omega \sum_{k=0}^{r} \sum_{j=0}^{s_k} b_{jk} \left(\frac{304.2}{T} - 1\right)^j (\omega - 1) +$$

$$(\rho/\rho_c) \sum_{i=1}^{3} g_i \delta^{l(i)-1} [l(i) - 2\alpha_i \delta^2] \exp(-\alpha_i \delta^2 - \beta_i \tau_i^2)$$

with $\omega = \rho/0.468$ g cm$^{-1}$ and T in K

$\delta = (\rho - \rho_c)/\rho_c$

$\tau_1 = \tau_2 = (T_c/T) - 1.011$

$\tau_3 = (T_c/T) - 1.009$

where:

| i | l(i) | $\alpha_i$ | $\beta_i$ | $g_i$ |
|---|---|---|---|---|
| 1 | 0 | 34 | 20000 | $-7.53 \times 10^{-4}$ |
| 2 | 2 | 40 | 20000 | $-5.73 \times 10^{-3}$ |
| 3 | 0 | 30 | 40000 | $1.84 \times 10^{-4}$ |

and the $b_{jk}$ parameters for $CO_2$ EOS are:

| k / j | 0 | 1 | 2 |
|---|---|---|---|
| 0 | -0.7255896770 | -1.669856633 | 0.4191613578 |
| 1 | 0.4481451002 | 1.269083933 | 6.057811911 |
| 2 | -0.1743673384 | -1.954404447 | -5.615197965 |
| 3 | $-4.243816093 \times 10^{-4}$ | -1.788455844 | -11.34629367 |
| 4 | 0.2668130548 | 2.718574223 | 9.462288816 |
| 5 | 0.07340283381 | 1.154789219 | 7.450988805 |
| 6 | -0.1756082074 | -2.114184586 | -6.144768702 |
| 7 | $8.844271016 \times 10^{-3}$ | 0.01488945560 | -1.445010207 |
| 8 | 0.061007749242 | 0.6239980516 | 1.194066295 |
| 9 | -0.01994277669 | -0.1666138543 | $5.923888289 \times 10^{-3}$ |

| k / j | 3 | 4 | 5 |
|---|---|---|---|
| 0 | 1.154058547 | 1.145027582 | 1.148845513 |
| 1 | 15.85978978 | 20.21837027 | 9.190077144 |
| 2 | -6.976816915 | -0.5761694929 | 3.007284937 |
| 3 | -29.10403562 | -30.02663937 | -8.361282386 |
| 4 | 10.60317379 | 0.1567993789 | -2.723216850 |
| 5 | 16.00143047 | 10.97104869 | |
| 6 | -4.667566118 | | |
| 7 | -1.997943186 | | |

| k / j | 6 |
|---|---|
| 0 | 0.7069388840 |

represent the state of the art in carbon dioxide property prediction we have chosen it for calculation of supercritical carbon dioxide densities. The equation is shown in Table 2.2 along with all the relevant constants.

## 2.3 Mycelial Structure of *Beauvaria nivea*

Cyclosporine is a non-polar cyclic eleven amino acid peptide that is produced intracellularly by the fungus *Beauvaria nivea* and is stored somewhere within the mycelia of the fungus. Due to Cyclosporine's hydrophobic nature it is thought to be associated with membrane lipids or other non-polar constituents within the fungus but the exact storage location has yet to be determined. Thus a brief review of typical mycelial structural components is necessary to indicate potential obstructions to extraction by supercritical fluids.

Figure 2.1 shows some of the major components of a typical mycelial hyphea structure. Indicated are the outer cell wall and membrane which are closely associated. The internal structures shown are the nucleus, golgi apparatus, mitochondria, endoplasmic reticulum, vesicles, etc., and their associated membranes, and the septa which are internal dividing walls. The major barrier to be breached by supercritical carbon dioxide is the cell wall. The typical fungal cell wall consists of a rigid multi-layered series of fibrous molecules. Typically these consist of an outer layer a- and b- glucans followed by a layer of thick fibers of glycoproteins, a mixed layer of glucans and proteins followed by microfibrils of chitin mixed with various proteins (Roach, 1988). This porous structure allows the transport of small molecules such as sugars and amino acids to the cell membrane where they can be selectively

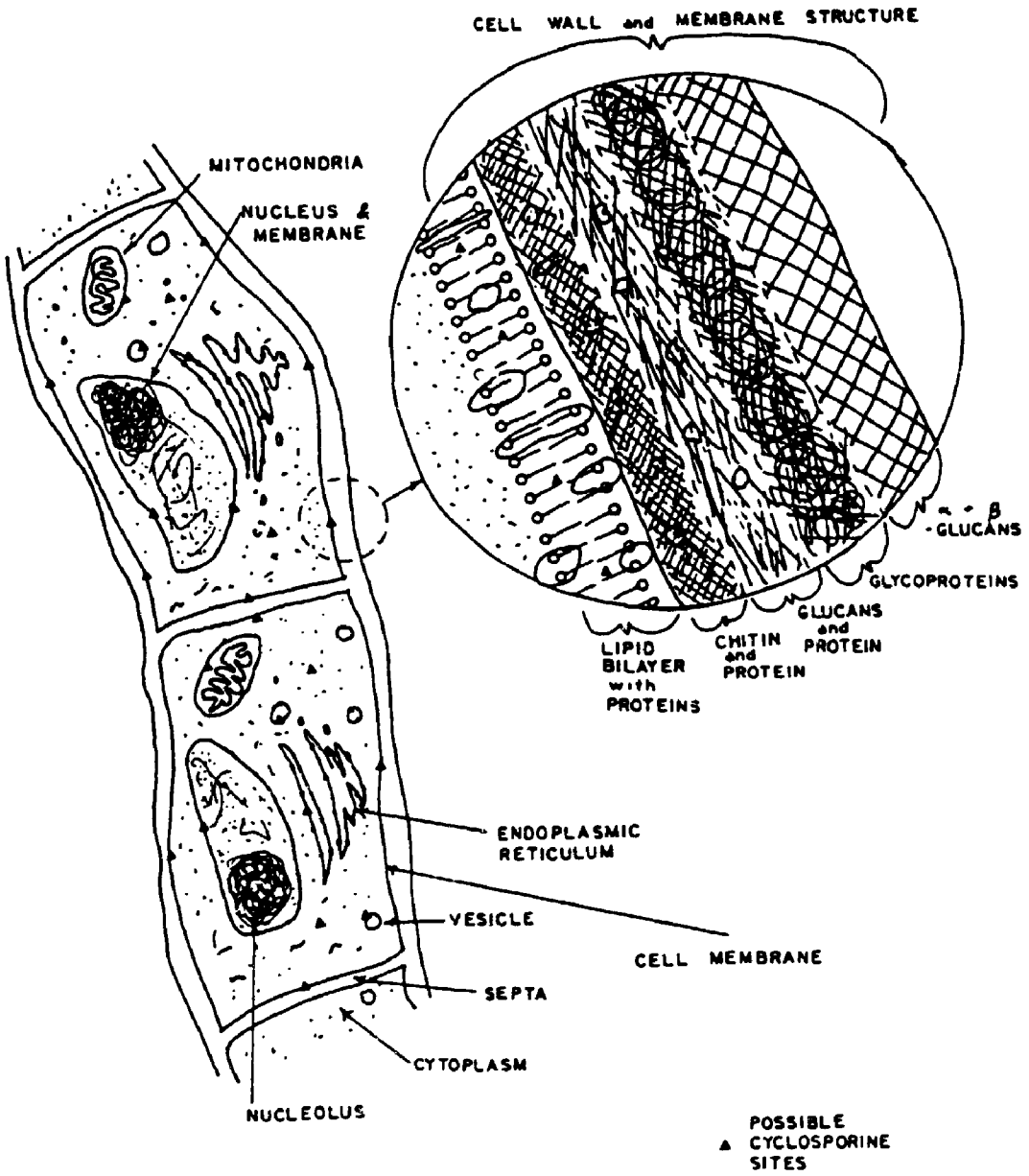



Figure 2.1 Structure of the mycelia of *Beauvaria nivea*.

absorbed by the cell. The small size of the carbon dioxide molecule would allow rapid entry of the supercritical fluid through this micro-porous structure of the cell wall. The filter-like cell wall structure does restrict the transport of larger molecules such as Cyclosporine and could be a significant barrier to their removal by supercritical fluid extraction.

The fungal cell membrane directly adjacent to the cell wall is considered to consist of the usual lipid bi-layer and associated active-transport proteins and enzymes. This membrane actively controls the entry of nutrients into and the exit of products and 'wastes' out of the cell. This lipid membrane represents one possible site for the storage of Cyclosporine within the cell.

The other components shown in Figure 2.1 are dispersed throughout the internal cytoplasmic fluids of the fungal cell. The biosynthesis of Cyclosporine in the fungal cell is probably mediated by membrane bound enzymes within the fungus. These and other internal membranes are also possible Cyclosporine storage sites.

# CHAPTER 3

# EXPERIMENTAL METHODS

## 3.1 Supercritical Extraction System: Description and Operation

The supercritical extraction system consists of several components: the liquid carbon dioxide delivery system, the extractor system, the sampling system, the separator(s) and safety systems. The safety systems include a pressure relief system and a physical enclosure to protect the operator in the event of a component failure. The apparatus was built upon the chassis of a Milton Roy supercritical extraction system. Major design changes were made to the original equipment and a large number of additions with different components were made to carry out this investigation. The solubility studies were done using the apparatus shown in Figure 3.1. It was operated as a closed loop recycle system during the solubility studies. The apparatus shown in Figure 3.2 was used for the extraction of mycelia of *Beauvaria nivea*. These systems and their operation are described below.

### 3.1.1 The Liquid Carbon Dioxide Delivery System

Commercial grade liquid carbon dioxide (99.5%) in dip tube equipped cylinders was obtained from Canox (London, Ont.). The carbon dioxide was passed through a simple shell and tube chiller. The shell side of the chiller was cooled with -15 °C polyethylene glycol antifreeze ( 50 % solution ) pumped from a 15 cu.ft. chest freezer reservoir. The liquid carbon dioxide was thus chilled to between -10 to -4 °C before entering

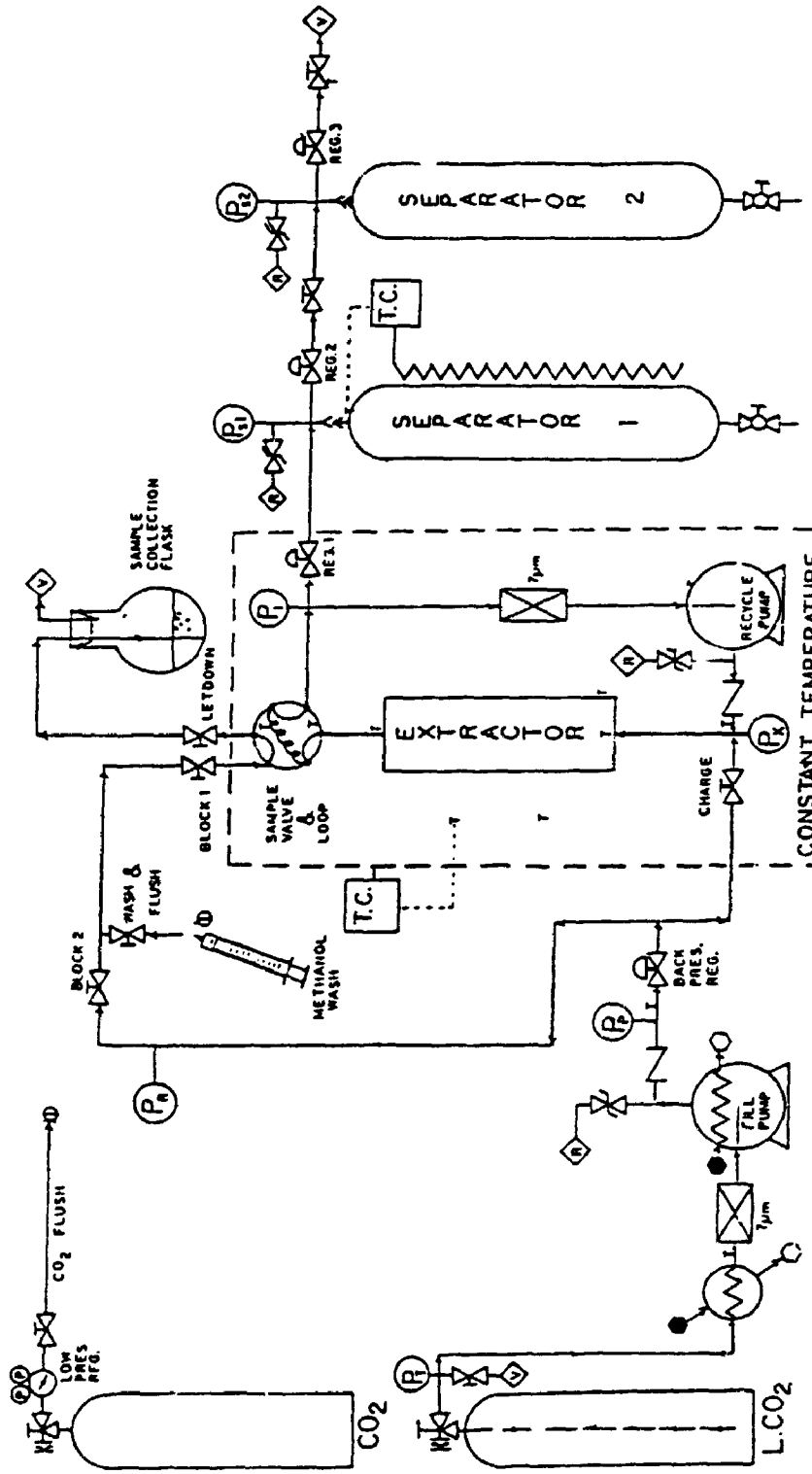



Figure 3.1 Schematic diagram of supercritical extraction apparatus used for solubility studies of pure Cyclosporine.

Table 3.1 Positioning of thermocouples in the recirculated supercritical extraction system shown in Figure 3.1

1. on recycle pump body (2)
2. at tube cross joining charge valve to system
3. at extractor inlet
4. at extractor outlet
5. on extractor body
6. on six port valve body
7. on sample loop
8. in the heater air flow path (2)

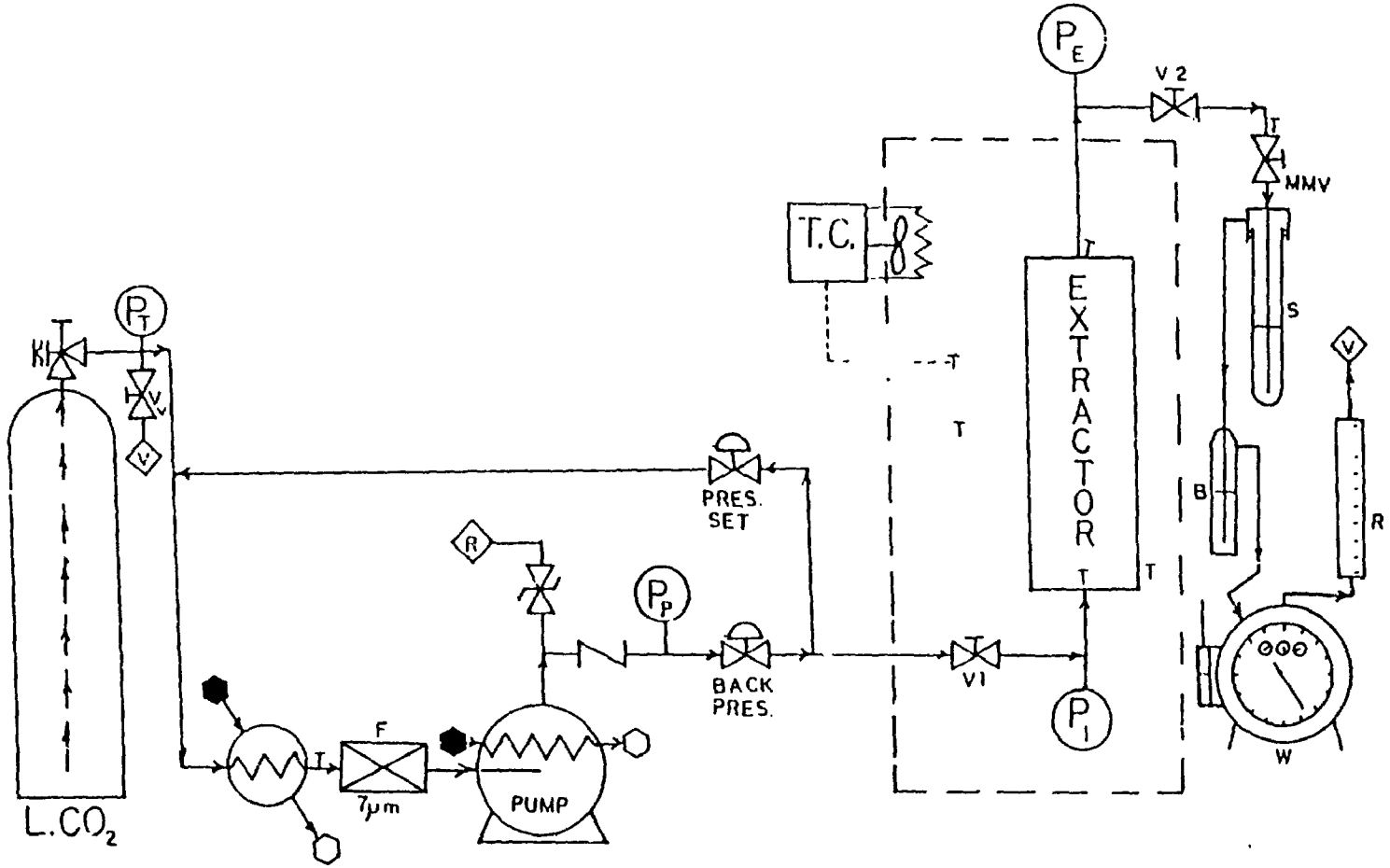



Figure 3.2 Supercritical extraction apparatus used to extract Cyclosporine from the mycelia of *Beauvaria nivea*.

Table 3.2 Positioning of the thermocouples in the continuous supercritical extraction system shown in Figure 3.2

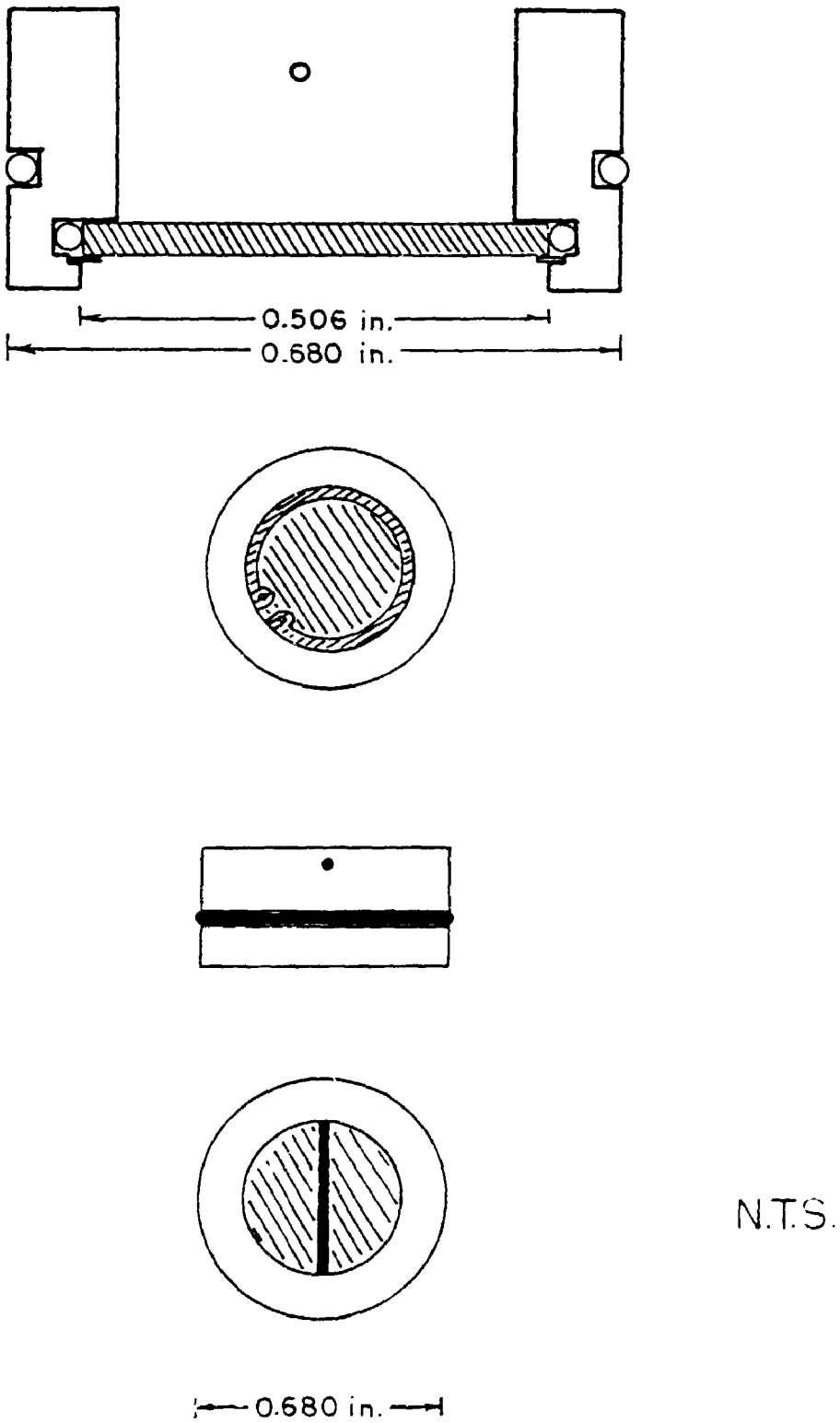
1. on the pump inlet
2. at tube cross joining V1 and Pi
3. at extractor inlet
4. at extractor outlet
5. on the extractor body
6. on the micrometering valve (MMV)
7. in the oven cavity (2)
8. in the heater air flow path (2)

the similarly chilled pump head of a Milton Roy minipump. A back pressure valve was used to hold the outlet pressure of the fill pump above 10.0 MPa. This ensured the carbon dioxide remained liquid while passing through the pump. The carbon dioxide could only attain its supercritical phase after passing through the fill pump.

For solubility studies of pure Cyclosporine the fill pump ( Figure 3.1 ) was only turned on for the initial charging of the system and refilling the sample loop with fresh carbon dioxide after taking samples. During extraction experiments the fill pump ( Figure 3.2 ) remained on during the whole experiment. Note the 'pres. set' regulator in Figure 3.2, which was used to set the extraction pressure. Any overflow was diverted back to the inlet of the chiller and recycled which allowed the Carbon dioxide flow rate to be set by the MMV micrometering valve during these experiments.

### 3.1.2 The Extractor(s) and Oven System

For most of the experiments an AE Autoclave Engineering CNLX1606 tubing nipple, and two 6F41686 adaptors were used as the basic extractor vessel. These components were rated for pressures up to 10,000 psi (69 0 MPa). The extractor was attached to the rest of the system tubing by Swagelok QF4 quick connects. Several fritted or screened inserts (Figure 3.3 ) were made to hold the extractor contents in place. The frits were supplied by Mott Metallurgical Corp part Numbers 1000-.500-0.062-0.5 ($\mu$m) or -40 ($\mu$m) or -10 ($\mu$m). The choice of frit porosity was determined by the type of experiment to be done. Experiments on pure cyclosporine used a 10 $\mu$m frit followed by a 0.5 $\mu$m frit on the outlet side of the extractor




N.T.S.

Figure 3.3 Detailed drawing of the frit holders used during supercritical extraction and solubility studies of Cyclosporine.

(Figure 3.4 ). This was done to ensure the cyclosporine crystals were not physically transported by the supercritical carbon dioxide. The mycelia extraction experiments used glass wool packing between the mycelia and 200 mesh screen at the entrance to the extractor and the 40 μm frit at the exit of the extractor. The details are shown schematically in Figure 3.5. A special small volume extractor was constructed for one of the cyclosporine solubility experiments using methanol as a cosolvent. A 30 cm. length of 0.25 inch O.D., 0.035 inch wall ASTM 213 stainless steel tubing was used as the main extraction vessel. HPLC column replacement frits (Supelco #5-9038 0.5 μm , #5-8264 2 μm ) were used at the ends of the tube. Gyrolok 4TTT-316 fittings were used at each end as closures, flow ports, and thermocouple ports. Figure 3.6 is the small extractor vessel schematic. It was used with special plumbing to reduce the recirculated system volume to 8.49 mL so a smaller quantity of cyclosporine could be used during the experiment. The large extractor based system had a volume of 103.3 mL.

An oven made from old GC components was constructed to act as a constant temperature air bath for the extractor (and the sampling valve). The temperature was maintained within 0.5 K by an Omega 4201-T RTD temperature controller. Several thermocouples ( Omega SICSS-062U-6 (or -12) ) were placed throughout the oven and extractor to determine if temperature gradients were present. These couples were connected to a Omega 410B Digicator and 405A Multipoint Selector. Typical placements are shown in the schematics of the apparatus, Figures 3.1 and 3.2 and listed in Tables 3.1 and 3.2. Temperature measurements were usually taken before every sample. Some typical measurements can be found in Appendix 1.4.

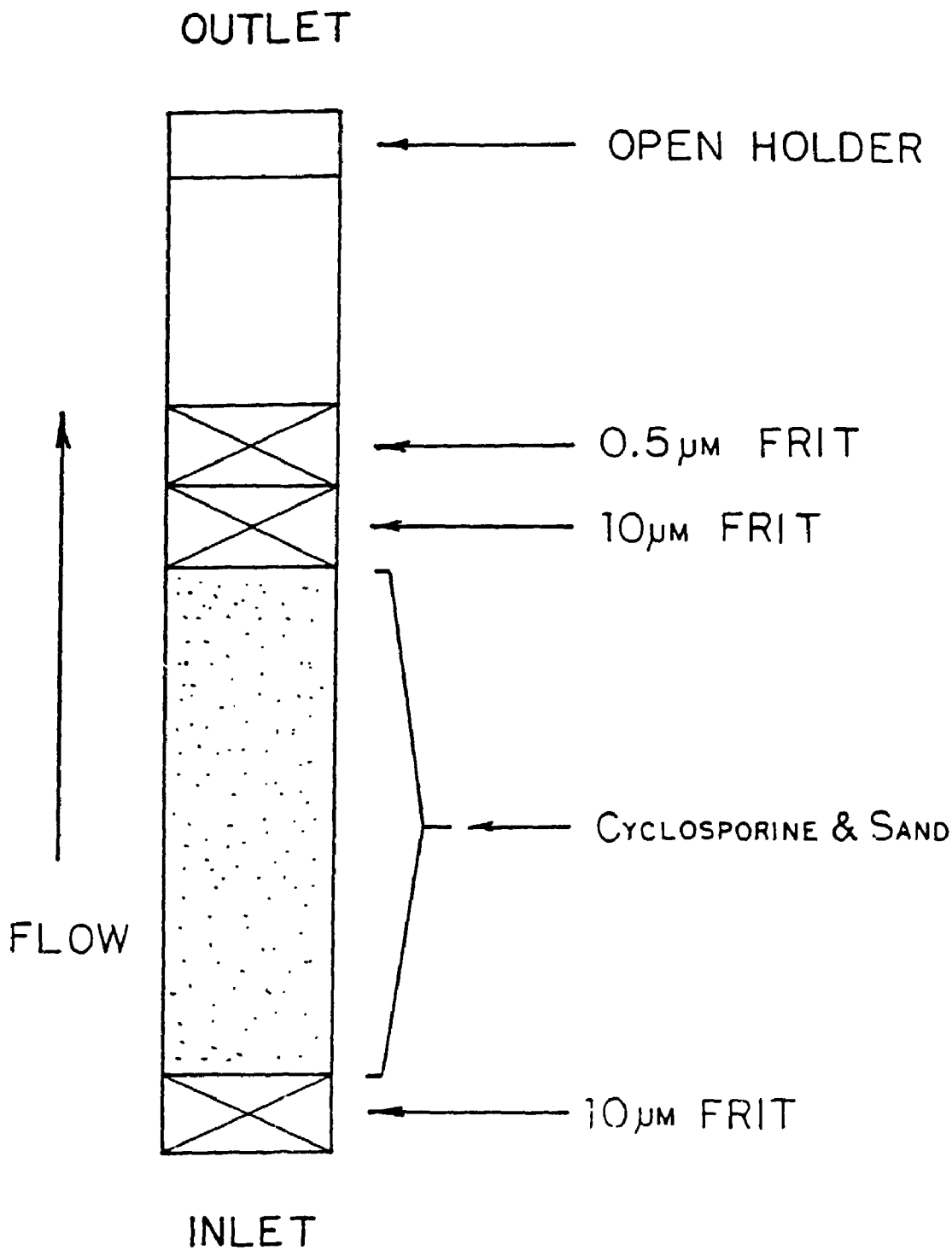



Figure 3.4 Packing arrangement and frit placement for solubility studies of pure Cyclosporine in supercritical $CO_2$.

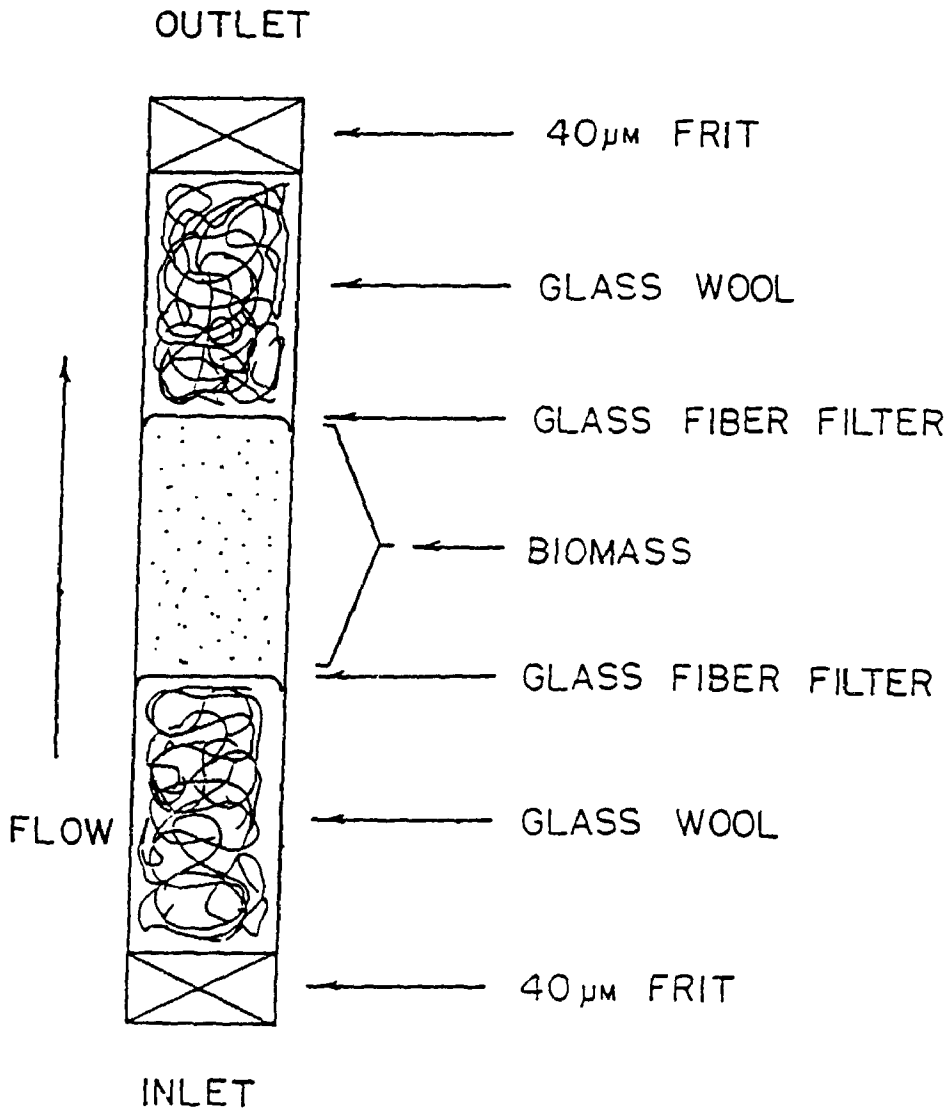



Figure 3.5 Packing arrangement and frit placement used for the supercritical extraction of Cyclosporine from the mycelia of *Beauvaria nivea*.

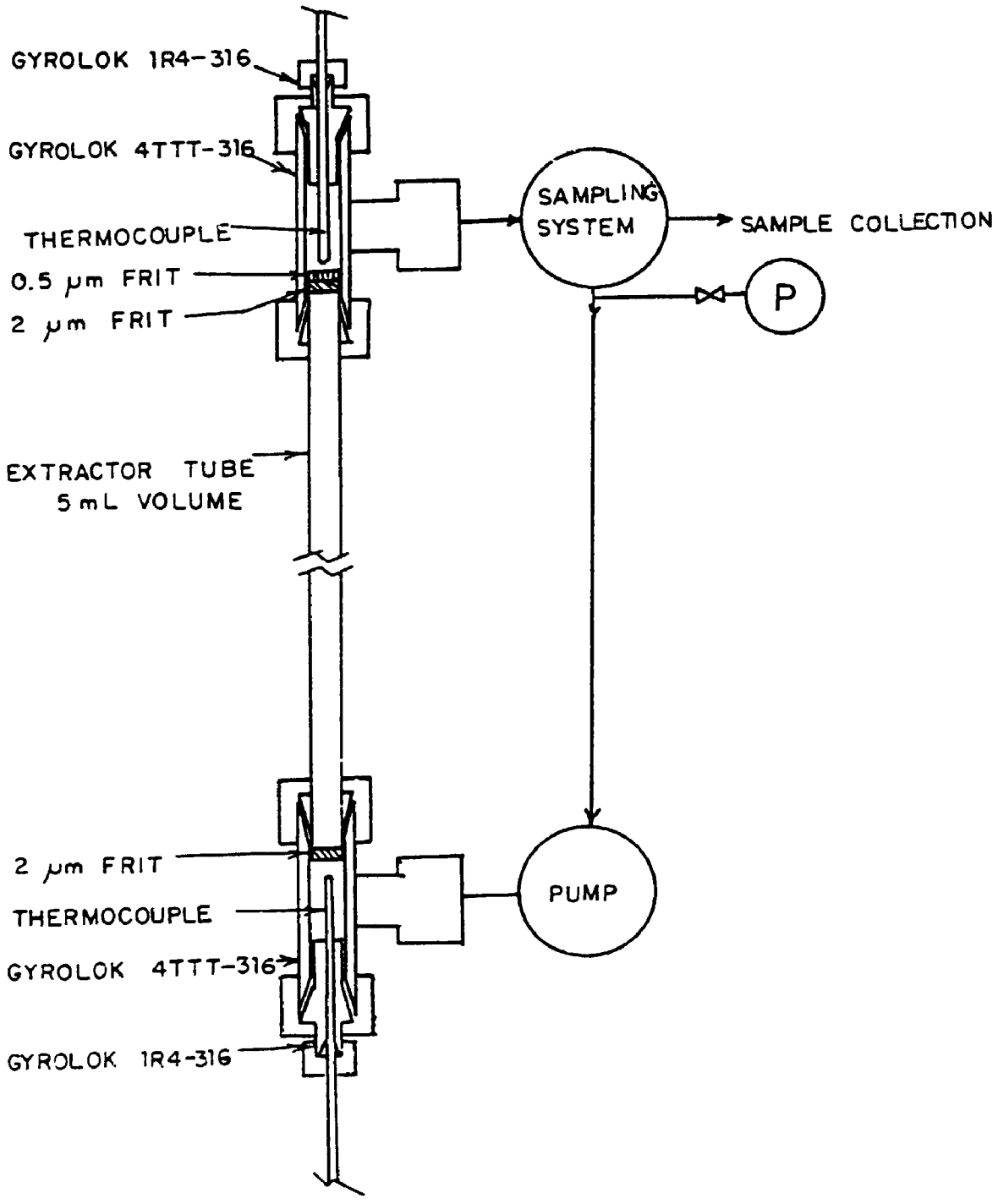



Figure 3.6 Schematic diagram of the extraction vessel and associated equipment used in supercritical extraction studies with co-solvent.

### 3.1.3 Common Operations to the Solubility Experiments

The extractor was charged with the appropriate material and closed. The temperature set point was then set and the system allowed to reach thermal equilibrium. The chiller was turned on to ensure the carbon dioxide from the supply tank remained liquid as it entered the system fill pump. The sample letdown valve, wash valve, and regulator 1 in Figure 3.1 ( or V2 and VVM in Figure 3.2 ) were closed. The charge valve,and block valves were opened while the six port sampling valve was set to leave the loop out of the main circulation circuit. The liquid $CO_2$ supply tank valve was then opened and a pre-charge of the system was made to a low pressure (approx. 1 - 2 MPa). The pre-charge was then vented through regulator 1 ( or V2 and VVM ) and the sample letdown valve to blow out any air from the system. This was done two or three times to ensure only pure $CO_2$ was present. The back pressure regulator was then adjusted to 100+ bar. The valves used for venting were closed and the system was then pressurized by turning on the pumps and setting 100% flow for the fill pump. Once the desired pressure was reached the procedure varied according to the type of experiment to be done.

### 3.1.4 Operation and Sampling System for Determination of Pure Cyclosporine Solubility in Carbon Dioxide

When pure cyclosporin A solubilities were determined the fill pump was set to zero flow and the recycle pump was set to maximum flow. The charge and block valves were shut and the three way valve was turned to the off position. A period of time was allowed for the system to reach

thermal and chemical equilibrium. This was usually about 30 to 40 minutes after which sampling commenced.

Sampling of the supercritical phase was done using a Rheodyne 7010 valve and sample loops of 0.5 ml, 20 μl , or 12 ml depending on the experiment. The sample loop was introduced into the main circulation loop for 10 minutes and then switched out. Two blocking valves prevented loop depressurization and were used to control the letdown and repressurization of the loop. The relative positions of the valves in the sampling system can be seen in Figure 3.1.

The letdown valve was used to bleed the carbon dioxide from the sample loop through a flask containing 20 milliliters of methanol. The first block valve was also opened to bleed out the carbon dioxide between it and the second block valve. Any Cyclosporine crystals formed in the letdown operation were caught in the methanol flask. A 20 milliliter wash of methanol was passed through the wash/flush valve, the first block valve, the loop, the letdown valve and into the same flask after all the carbon dioxide had been bled from the loop. This was done to ensure all the Cyclosporine isolated in the loop was in the methanol flask.

A low pressure carbon dioxide flush was used to blow out and dry any residual methanol left in the valves and the loop. This flush was typically ten minutes or longer. The residual methanol was usually gone within 2 to 3 minutes after the flush had started. This was checked a few times by disassembly of the tubing connected to the valves and checking for wetness with tissue paper. No wetting of the tissue paper was found after several minutes of flushing had taken place.

The methanol flask containing the Cyclosporine sample from the loop was removed for analysis. The methanol was evaporated using a Buchi

Rotovapor <R> vacuum evaporator and the dry flask was washed with 5 ml of HPLC grade methanol ( BDH Omnisolv methanol ) to solublize the residual material. This methanol was filtered into an HPLC vial and saved for analysis. The HPLC analysis used for Cyclosporine concentration determination is described below.

If further sampling needed to be done the wash/flush valve was closed and a short five minute bleed of high pressure carbon dioxide was passed through the second block valve through to the methanol flask. A fresh methanol flask was used for the next sample. The loop was repressurized by closing the letdown valve, opening the second block valve completely and starting the carbon dioxide feed pump. When the system pressure was reached the first and second block valves were closed and the pump stopped. The sample loop was then reintroduced into the main system by rotating the sampling valve into the correct position.

At least three samples were taken at each temperature and pressure studied to ensure equilibrium was achieved and eliminate experimental error. Several temperature measurements were done regularly before and after introducing the sample loop to the circulating supercritical phase. The sites of the thermocouples used are listed in Table 3.1.

A typical experimental run would be conducted at a fixed temperature and the pressure would be increased incrementally after every 3 to 4 samples. A wait of 30 to 40 minutes would be allowed for equilibrium to be re-established before sampling recommenced. At the end of the experimental run the system was depressurized through pressure regulators 1, 2, and 3.

Some experiments were also done from high pressure to low pressure. As well one experiment was conducted along an isopleth by starting at a

high temperature and pressure, reducing the temperature after sampling three times, waiting for thermal stability at the new set point and sampling again. This was continued to the lowest temperature attainable.

It should also be noted that the extractor was always loaded with a large excess of Cyclosporine. This ensured the true solubility was being measured as more material was present than could be dissolved in the supercritical phase.

### 3.1.5 Operation and Sampling for Determination of Solubility of Pure Cyclosporine in the Methanol - Carbon Dioxide Cosolvent System.

There were two sets of experiments done to examine Cyclosporine solubility in the methanol - carbon dioxide co-solvent system. Both were conducted as the second part of an experimental run where the same experiments were done for Cyclosporine solubility in supercritical carbon dioxide. A very large quantity of Cyclosporine was loaded into the extractor initially and the system pressurized with carbon dioxide as described above. The first part was conducted as described above. When the equilibrium at the maximum pressure had been sampled three times methanol was introduced into the main circulation loop using the sample loop. The sample loop was filled with methanol and not pressurized. Block valve 1 and the letdown valve were closed and the loop introduced into the system for 2 minutes. The loop was then switched out, depressurized and again filled with methanol. This was repeated until a specific volume of methanol had been introduced into the system. During this process the working pressure of the system also was reduced by the insertion of low pressure methanol and depressurization of the loop. This

helped conserve the Cyclosporine which was in short supply. The rest of the experiment was done the same as the first part. Specifically the temperature was not altered and the pressures used were as close as possible to those in the first part. Sampling was done in the same way except the loop was only dried for 2 minutes since any trace amounts of methanol would not significantly effect the results.

### 3.1.6 Operation and Sampling for Extraction of Cyclosporins from Mycelia of *Beauvaria nivea* ATCC 34921,(NRRL 8044) with Supercritical Carbon Dioxide

Some preliminary work was done using the system shown in Figure 3.1 but this proved to be inadequate because separator vessel samples could not be taken at time intervals without shutting down the flow and depressurizing each separator vessel individually. Then the vessels had to be washed with solvent, dried, replaced, then repressurized and thermally equilibrated. This took too long and interrupted the flow from the extractor. Thus the experimental system was altered to eliminate the problems encountered.

The extraction system used is shown in Figure 3.2. The fractionation system, sampling valve, and pressure regulators were removed. One regulator was used as part of the carbon dioxide delivery system to set the extraction pressure. An AE Autoclave Engineering 10V-2081 valve was used as a shut off valve (V2) followed by an AE Autoclave Engineering 10VRMM-2812 micrometering valve (MMV) which controls the flow rate of carbon dioxide through the system. The extractor was loaded with a known mass of material, connected into the system, and pressurized as described

above ( 3.1.1 - 3.1.3). When the system had attained the operating temperature and pressure, the valve V2 was opened. Valve VVM was then opened to the required flow rate and the time, pressure, and temperatures of the system recorded, as well readings of the rotameter, wet test meter and the setting of valve VVM were noted. These particulars were also taken whenever a new sample period was started. The flow of supercritical carbon dioxide and entrained solutes from the extractor were depressurized through the micrometering valve (MMV) and bubbled through a 70 ml threaded glass sample collection test tube containing about 20 ml of methanol. Then the flow was passed through a gas washing tube, a wet test meter, a rotameter and then vented outside the building. It was found that over 99% of the Cyclosporine removed from the mycelia were collected in the methanol of glass test tube. The wet test meter was used to measure the total flow of carbon dioxide through the extractor. The rotameter was used to determine the instantaneous flow rate at any time. The sample collection test tube was changed regularly and all the system particulars were noted at the time of each change. The volume of the contents of the sample collection tube was measured and the concentration of any cyclosporines present were made by filtering 4 ml into an HPLC vial for immediate analysis. The extraction could then be followed quite closely to determine when the apparent end poirt had been reached. When the amount of Cyclosporine extracted was significantly less than 0.02 mg per 100 normal liters of carbon dioxide passed through the sample collection tube the experiment was considered over and sample collection was terminated.

The V1 valve was shut, the pump and chiller turned off and the system depressurized through a fresh sample collection tube. The extractor

contents were weighed and a sample analysed for Cyclosporine. The tubing and valves V2 and VVM after the extractor outlet were washed with 20 ml of methanol. This methanol along with the last sample collected during depressurization, and the methanol in the gas washing bottle were also measured for volume and Cyclosporine content.

## 3.2 Production of Mycelia of *B. nivea* Used for Supercritical Extraction of Cyclosporine

The organism used in this work was *Beauvaria nivea* ATCC 34921 (NRRL 8044). It produces the endocellular cyclosporins as metabolites during fermentation. The growth medium composition used to stimulate Cyclosporine production is shown in Table 3.3. The culturing of this fungus was done as described by Margaritis and Chahal 1989,( see also Armistead 1988, Roach 1988, Tucker 1986, and Marshall 1986) .

The following steps are summarized which were used to produce sufficient quantity of the mycelia for supercritical extraction

1.) Revival of culture from freeze dried state. ( as per ATCC instructions )
2.) Transfer into and preparation of stock cultures in agar slants.
3.) Production of pre-innoculum from slant (25 to 50 mls, 4 days)
4.) Production of second pre-innoculum (2 of 100 mls, 2 days)
5.) Production of innoculum (2 of 1 L, 2 days)
6.) Fermentation in a 10 L bioreactor or fermentation in shake flasks for 6 to 8 days, followed by mycelial removal.

Table 3.3 Medium composition for Production of Cyclosporine by *Beauvaria nivea* ATCC 34921

| Component | Concentration |
|---|---|
| Fructose | 30 g/L |
| $(NH_4)_2HPO_4$ | 6 g/L |
| Yeast Extract | 5 g/L |
| $CaCl_2$ | 1 g/L |
| $MgSO_4$ | 1 g/L |
| $FeSO_4 \cdot 7H_2O$ | 15 mg/l |
| $CoCl_2$ | 15 mg/L |
| $ZnSO_4 \cdot 4H_2O$ | 10 mg/L |
| $CuSO_4$ | 2 mg/L |
| $(NH_4)_6Mo_7O_{24} \cdot 4H_{20}$ | 0.02 mg/L |
| $H_2O$ Distilled | 1 L |

The mycelia of the fungus were removed by centrifugation, either batchwise in a regular Sorval RS2 centrifuge, or continuously with a Sharples tubular centrifuge or by sieving out the mycelial pellets using a standard 1 mm mesh Tyler Sieve. The recovered mycelia (biomass) was then pretreated as required and then stored in dried form at room temperature or as wet or damp biomass in a refrigerator, or frozen before use in supercritical extraction experiments.

Several pretreatment methods of the mycelia before extraction were examined in order to determine their influence on the degree of Cyclosporine extraction from the mycelia. A total of nine different pretreatment methods were examined and summarized below.

1./ No treatment.

Fresh or refrigerated mycelia were directly loaded into the extractor after harvest.

2./ Oven drying at 80 C for 4 - 20 hours

Loaded into drying oven on aluminum foil or Whatman No 1 filter paper.

3./ Blending biomass with 1 N NaOH using a Virtis homogenizer for 15 min, incubation with 1 N NaOH for 30 min. at 60 to 80 °C, centrifuging, and drying biomass at 80 °C for 4 hours.

4./ Blending biomass using a Virtis homogenizer for 15 min, centrifuging, and drying biomass at 80 °C for 4 hours.

5./ Blending biomass with 0.1 % Sodium Tripoly Phosphate using a Virtis homogenizer for 15 min, centrifuging, and drying biomass at 80 °C for 4 hours.

6./ Air drying the biomass on glass fibers or Whatman No 1 filter paper.

7./ Autoclaving of biomass for 15 minutes on the dry cycle, then drying in an oven at 80 °C for 4 hours.

8./ French pressing the biomass at (10000 psi) twice, centrifuging the cells, and drying at 80 °C for 4 hours.

9./ Freeze-thaw treatment, mycelia were frozen for one week then thawed and refrozen for 24 hours then thawed again, centrifuged, and dried at 80 °C for 4 hours.

Virtually all the material was ground before extraction using a Thomas - Wiley Model 4 Laboratory mill. The mass of the material loaded into the extractor and left over at the end of the experiment was weighed using a Sartorius H51-**V40 'handy' balance.

## 3.3 Preparation of Biomass Samples for Cyclosporine Analysis

Two methods were used to extract Cyclosporine from mycelia prior to analysis with an HPLC system to determine the concentration of Cyclosporine in these mycelia. In the first method, 0.2 to 0.4 g of recovered mycelia was added to 10 ml of water and 50 ml of methanol and vigorously agitated for 30 minutes to extract all the cyclosporine. The mycelia were separated from the liquid by centrifugation or filtration. The methanol solution was filtered using Sartorius PTFE 13 mm dia 0.2 μm membrane filters and placed in HPLC autosampler vials.

For the second method a known amount of mycelia of 0.2 to 0.4 g was added to 10 ml of distilled water and 25 ml of ethyl acetate in a 250 ml Erlenmeyer flask. A stir bar was added and the flask sealed with an aluminum foil covered rubber stopper and Parafilm (TM.). This was stirred for 16 to 20 hours and then centrifuged to separate the phases. The ethyl acetate phase was removed from the top of the centrifuge tube with a

Pasteur pipet and placed into a round bottom flask and placed on a Buchi Rotovapor <R> for the removal of the ethyl acetate. The flask was heated to 40 °C by a water bath and vacuum of 26 " Hg was applied. Evaporation of the ethyl acetate generally occurred within 10 - 15 min. The flask was removed and cooled before adding 5 ml of methanol. The methanol was used to wash the flask and was then filtered through filters, as above, into HPLC autosampler vials.

## 3.4 HPLC Analytical Method for Measuring Cyclosporine Concentration

Two different HPLC's were used during this work and the analysis method was slightly different for each instrument. A Hewlett Packard HPLC 1084b with a variable wavelength UV detector and autosampler were used to analyze for Cyclosporine for most of the Cyclosporine solubility studies. Samples were loaded into autosampler vials by filtration through 0.2 μm PTFE filters. The mobile phase consisted of Methanol / Acetonitrile / $H_2O$ in a 20 / 44 / 36 ratio. The solvents were preheated to 32 °C and a Phenomenx Reverse Phase Spherisorb 5 C8, 150 mm by 4.6 mm I.D. column and a Waters Guard-pak precolumn with a μBondapak C18 cartridge were maintained at a temperature of 72 °C. The solvent flow rate was maintained at 1.5 ml/min and samples were eluted for 15 minutes each. The Cyclosporine detection wavelength was at 215 nm against a reference wavelength of 430 nm. A Cyclosporine elution time of 11.5 minutes was typical. Cyclosporine standards were used to determine the concentrations of Cyclosporine in the samples.

A Waters HPLC system consisting of Maxima 820 software, SIM box, an AT PC clone, two 501 pumps, 712 Wisp, 490E programmable multiwavelength

detector and a Phenomenex Reverse Phase Spherisorb 5 C8, 150 mm by 4.6 mm I.D. column and a Waters Guard-pak precolumn with a μBondapak C18 cartridge were used in later stages of the work. The mobile phase was 100 % Acetonitrile ( Omnisolve BDH) delivered by pump A and a 65:35 mixture of water : methanol (Omnisolve BDH) from pump B. The flow rate was 1.4 ml/min with 50% A and 50 % B. The Cyclosporine detection wavelength was 215 nm. Cyclosporine typicaly eluted at about 5 to 5.5 minutes for a 15 minute run. The extra run time was used to allow additional extracted material to elute from the column when supercritically or organically extracted mycelial samples were being analysed. Standards were used to establish Cyclosporine elution times and concentrations.

## 3.5 Preparation of Scanning Electron Micrographs of Pre-Dried Mycelia of *Beauvaria nivea* and Cyclosporine Crystals

Pre-dried samples of mycelia of *Beauvaria nivea* or Cyclosporine crystals were glued onto stub mounts with conductive carbon paste. A Hummer IV sputter deposition system was used to coat the mounted samples with gold. The samples were then placed in the lower stage of a ISI DS130 scanning electron microscope, examined and photographed.

# CHAPTER 4

# SOLUBILITIES OF PURE CYCLOSPORINE IN SUPERCRITICAL CARBON DIOXIDE AND IN THE CARBON DIOXIDE-METHANOL CO-SOLVENT SYSTEM

## 4.1 Experimental Procedure

Cyclosporine crystals mixed with inert acid washed sand were loaded into the extraction system at the start of each experimental run. An excess of Cyclosporine was always added to ensure the maximum solubility was always being measured. Most of the experiments were conducted at constant temperature with the pressure being raised after the solubility had been measured several times at the set conditions. Once the new pressure had been established a new equilibrium was allowed to be established for at least 15 minutes before the next sample was taken. Samples were usually taken at half hour intervals. Occasionally more time elapsed between samples. The samples were analysed by HPLC for the Cyclosporine concentration present in the sample loop. The HPLC data and the temperature and pressure conditions of the system at the time of sampling were entered into a computer program (Appendix 3.1). This program determined the mole fraction of Cyclosporine present in the sample loop from the HPLC data and the density of carbon dioxide as calculated using the modified IUPAC equation of state (Pitzer and Schreiber, 1988).

A concern was raised early on during this work that physical transport of Cyclosporine crystals could perhaps occur. To address this concern a Cyclosporine crystal population size study was undertaken using light microscopy and a ruled grid. The data were analysed with a computer program (Appendix 3.2) and the following characteristics were found. The

(a)


(b)

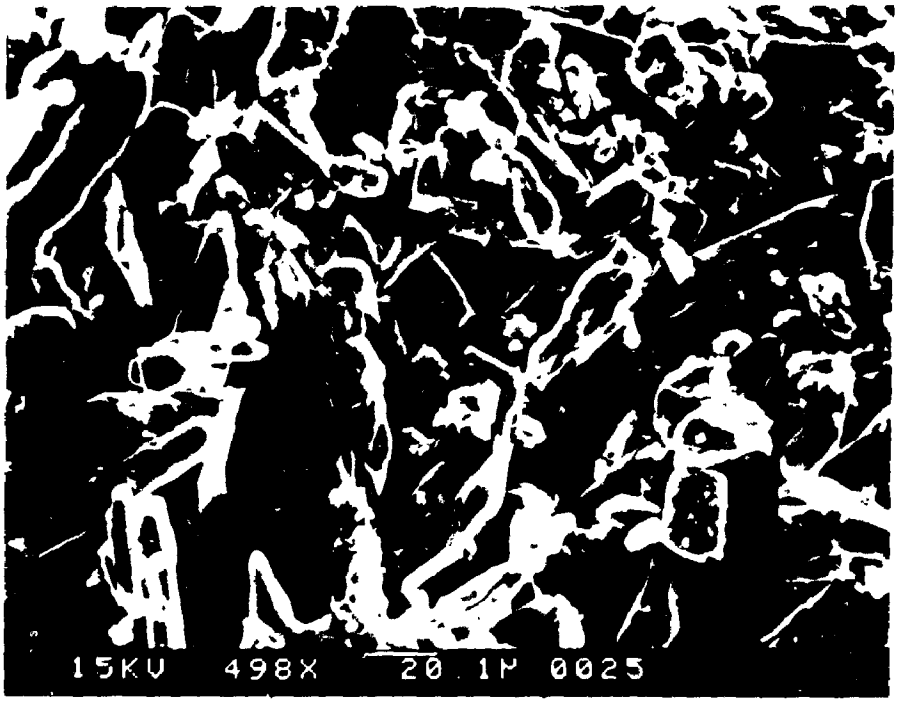



Plate 4.1 Scanning Electron micrographs of Cyclosporine Crystals.
a) 830 times magnification
b) 498 times magnification

mean size of the crystals was determined to be 1.26 μm. The volume mean size of the crystals was 21.2 μm and the Sauter mean size was 101 μm. The volume mean size shows major portion of the crystal mass was large size particles generally above 5 μm. A pair of electron micrographs of Cyclosporine crystals Plates 4.1 a,b also show that most of the particle mass was in large sized particles. The extractor outlet was thus fitted with a 0.5 μm pore sized stainless steel frit which virtually eliminated the possiblility of physical transport of the crystals through the equilibrium system.

## 4.2 Results of Cyclosporine Solubility in the Supercritical Carbon Dioxide System

Figures 4.1 to 4.7 show the experimental data of the different temperature experiments as plots of the logarithm of Cyclosporine mole fraction versus absolute pressure in megapascals (MPa). The data on the individual graphs shown are grouped into the different sets corresponding to the different experiments done at similar temperature conditions. The data belonging to a single major experimental run are marked in solid symbols on Figures 4.1 to 4.7. The other data shown represent partial experimental runs for close temperatures no more than 1 K away from the major experimental run.

A rapid decline in solubility is noticeable in each data set near the critical pressure of $CO_2$ (7.38 MPa). This can be easily seen in Figure 4.1 as the mole fraction of Cyclosporine solubilized decreases quickly below a pressure of 10 MPa. This reduced solubility at the critical point has been described by Prausnitz et al (1986). They have shown that very

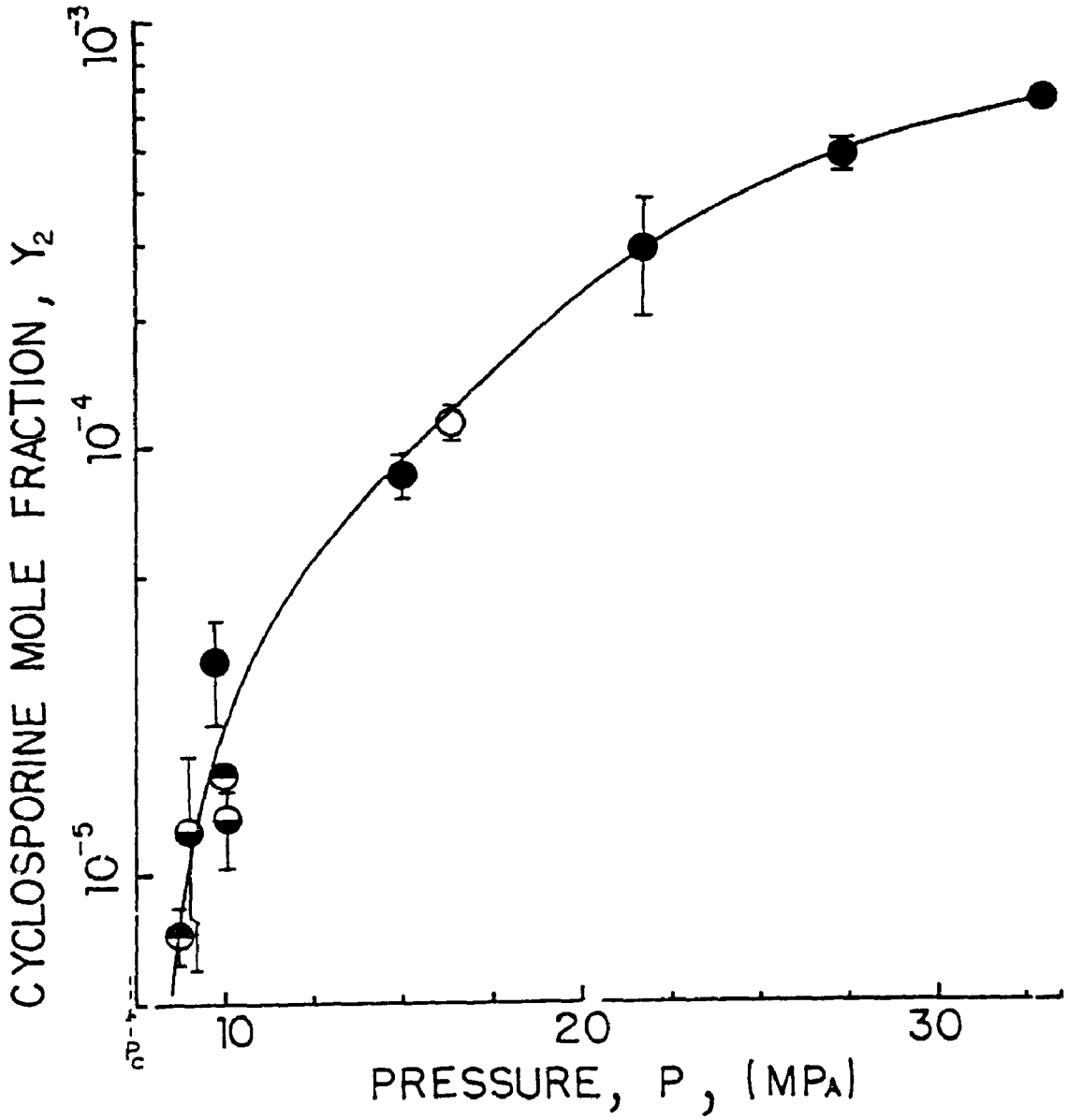



Figure 4.1 Solubility of pure Cyclosporine in supercritical carbon dioxide at 308.9 ± 0.8 K as a function of system pressure. (●) major expt. 308.5 ± 0.8 K (◒, ○, ◓) minor expts. 310.1 ± 0.1 K, 309.0 K, 308.8 K respectively.

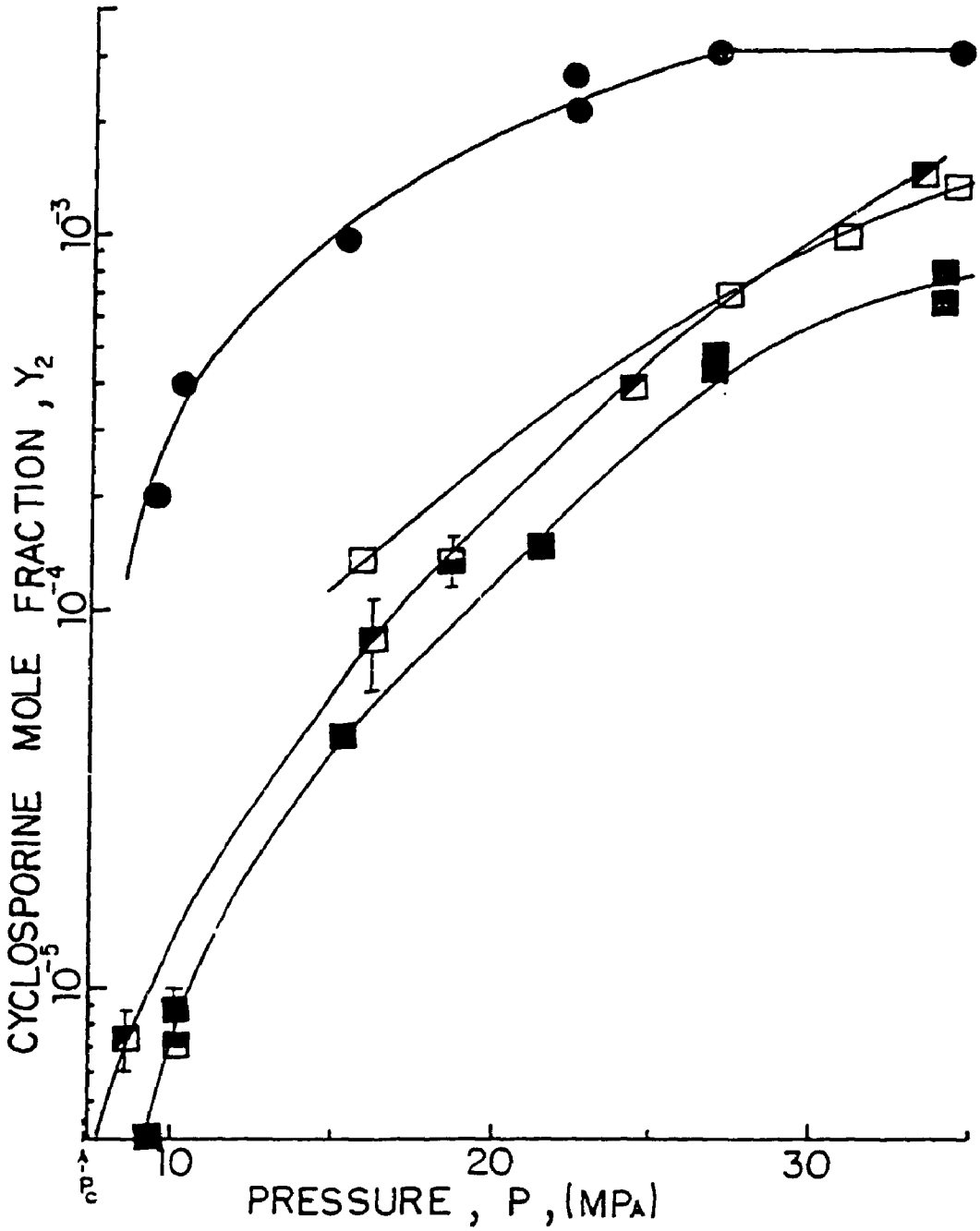



Figure 4.2 Solubility of pure Cyclosporine in supercritical carbon dioxide at 314.2 ± 0.5 K as a function of system pressure, without methanol and with 2.90 % v/v methanol added as a co-solvent. (●) with methanol added using a 0.5 mL sample loop, without methanol added: (■) major expt. using a 0.5 mL sample loop at 314.4 ± 0.3 K, minor expts.: (□, ◪, ⬓, ⬒) using a 20 μL sample loop at 313.8 ± 0.2 K, a 12 mL sample loop at 314.0 ± 0.2 K, and a 0.5 mL sample loop at 313.8 K and 314.1 K respectively.

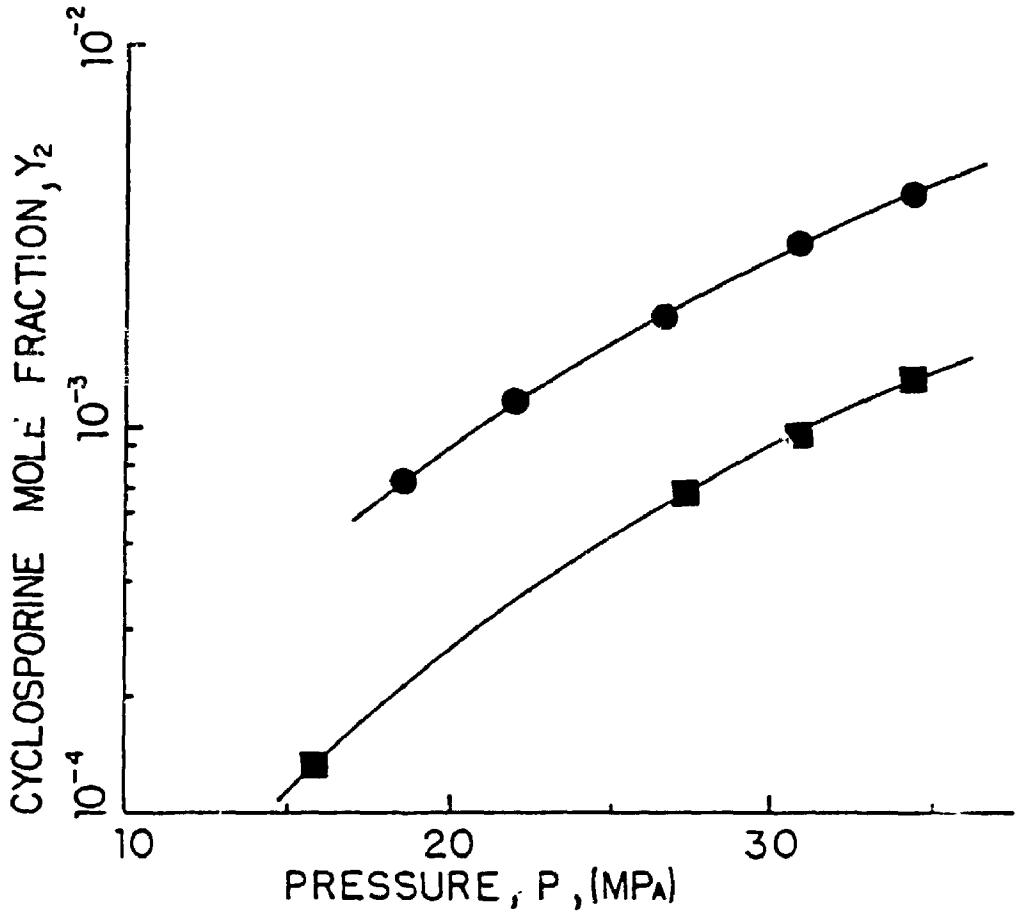



Figure 4.3 Solubility of pure Cyclospo. ne in supercritical carbon dioxide at 314.0 ± 0.5 K as a function of system pressure, without methanol (■) and with 4.71 % v/v methanol added as a co-solvent (●).

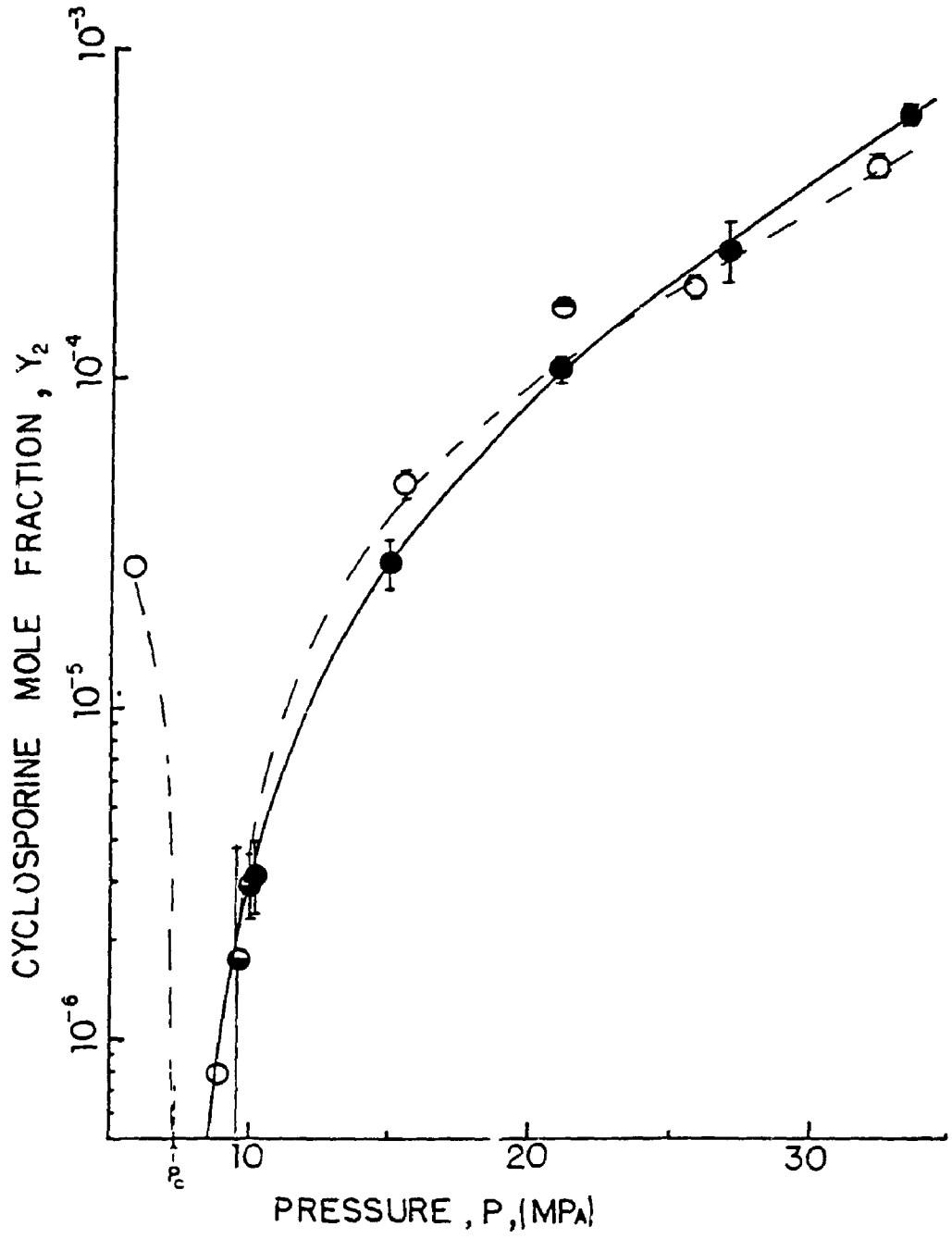



Figure 4.4 Solubility of pure Cyclosporine in supercritical carbon dioxide at 319.0 ± 0.6 K as a function of system pressure. (●) major expt. with 0.5 mL loop at 319.0 ± 0.3 K, minor expts. (○, ◒, ◓): 12 ml loop at 318.2 ± 0.5 K, 0.5 mL loop at 319.2 ± 0.1 K and 319.1 K respectively.

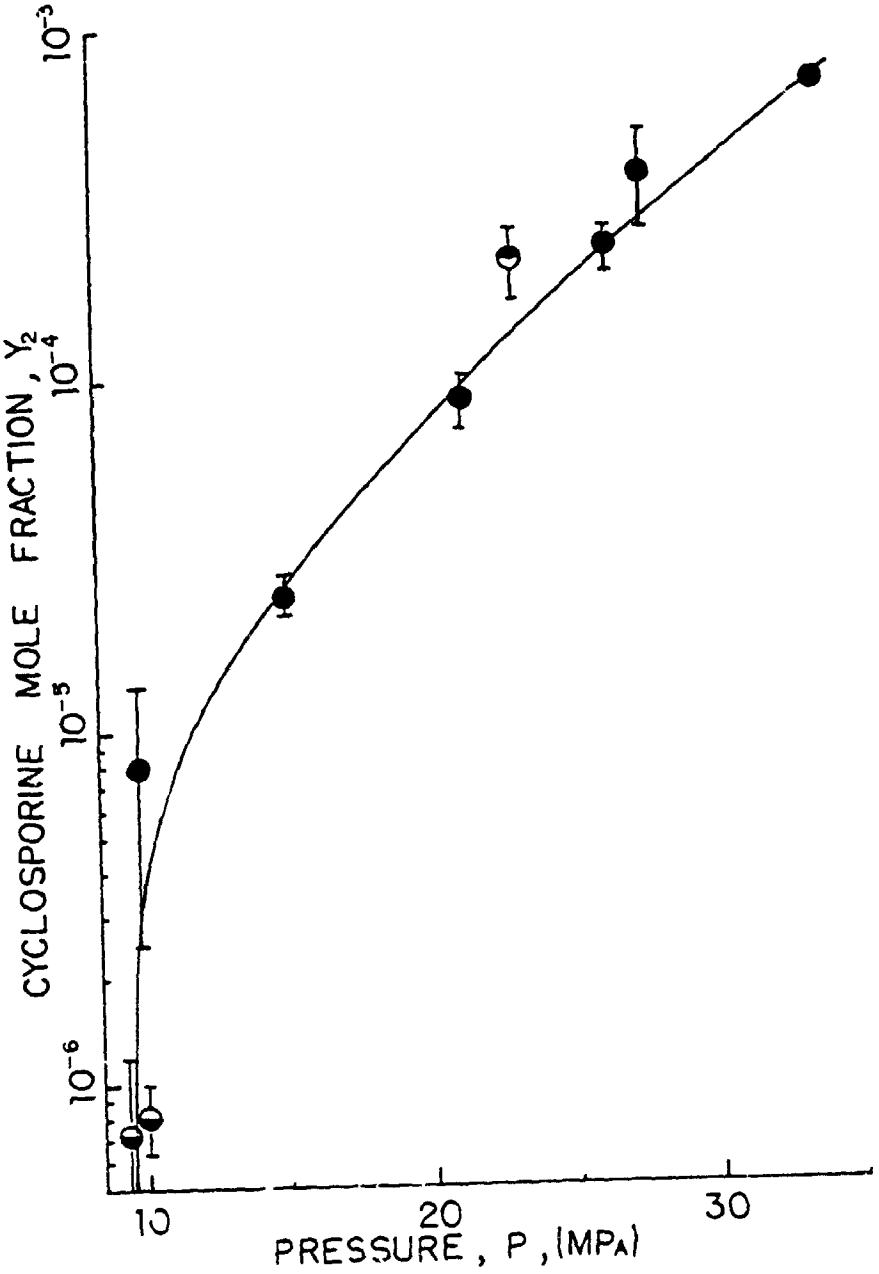



Figure 4.5 Solubility of pure Cyclosporine in supercritical carbon dioxide at 324.5 ± 0.3 K as a function of system pressure. (●) major expt. at 324.5 ± 0.3 K, (◓, ◒) minor expts. at 324.2 K and 324.3 ± 0.3 K.

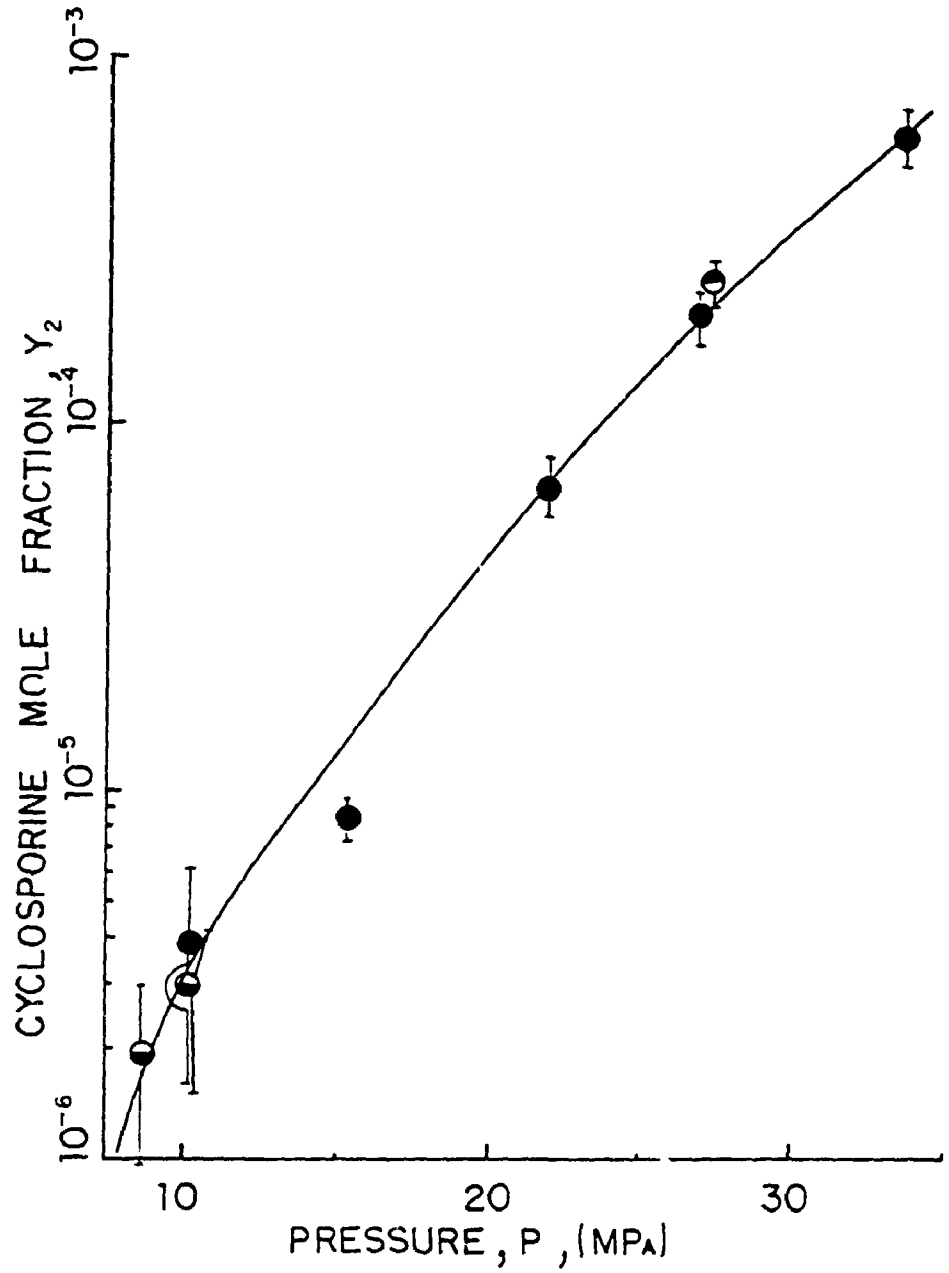



Figure 4.6 Solubility of pure Cyclosporine in supercritical carbon dioxide at 334.9 K ± 0.5 K as a function of system pressure. (●) major expt. at 334.9 ± 0.1 K, (◓, ◒) minor expts. at 334.2 and 334.4 ± 0.4 K.

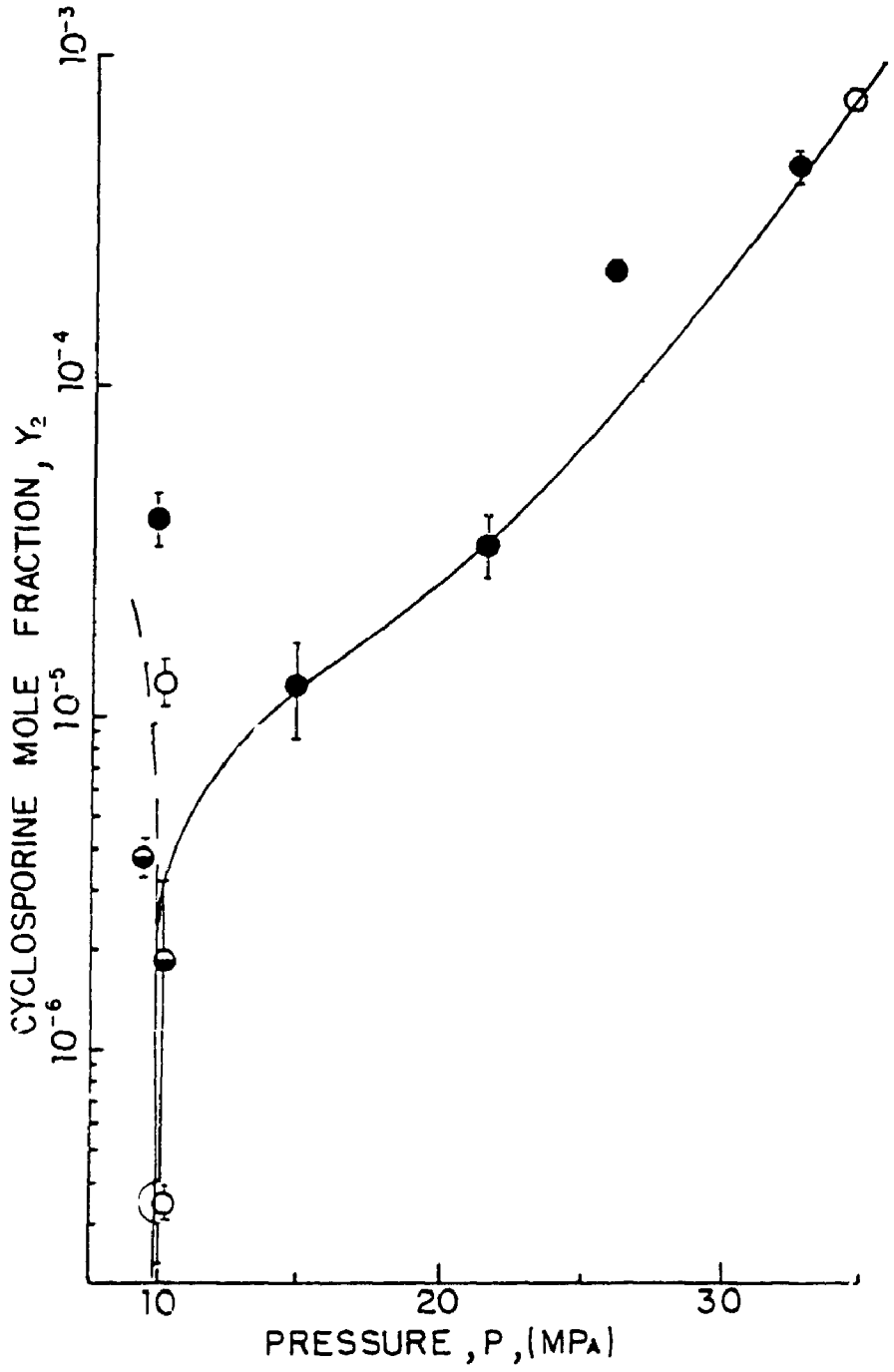



Figure 4.7 Solubility of pure Cyclosporine in supercritical carbon dioxide at 343.0 ± 0.5 K as a function of system pressure. (●) major expt. at 342.9 ± 0.4 K, (○, ◒) minor expts. at 343.2 ± 0.4 K and 343.5 ± 0.6 K.

large changes in solubility will occur near the critical point due to very rapid changes in the partial volume of the dilute solute. Figures 4.2, 4.4, 4.5, and 4.7 also show rapid decreases in solubility below 10 MPa. Figure 4.4 shows a data point below the critical pressure with moderate solubility of Cyclosporine. This also fits with the description of Prausnitz et al., 1986.

Figures 4.2 and 4.3 show the data for 314.4 K and include the solubility data of the co-solvent system. The current discussion will focus on the carbon dioxide supercritical solvent based experiments. The co-solvent data will be discussed in the next section.

Figure 4.2 shows experimental data determined using three different loop sizes and configurations. It must be noted that the largest loop did not have a constant temperature profile due to its large size. A pair of reducing unions was also required with this large loop to fit it to the sampling valve. The large loop temperature was also slightly cooler than the rest of the system. This probably resulted in the enhanced solubility shown due to increased solubility at the lower temperature in the loop. Similarly the smallest loop (20 $\mu$L) was also at a slightly lower temperature during the experiments. This 20 $\mu$L loop was part of a different system geometry. The 0.5 mL loop was always very close to the temperature at the extractor exit. Thus it is believed the data obtained with the 0.5 mL loop are the most reliable. Another contributing factor to the variation between these experiments could be the slighily different operating temperatures and different thermal gradients in the system tubing.

The data for 319 K are shown in Figure 4.4. The 12 mL loop data are closer to the 0.5 mL loop data here but show a slightly different trend. This does not appear to be significant.

The plot of the highest temperature studied is shown in Figure 4.7. The first few data points are in the low pressure region and appear at about 10 MPa. An extremely wide range of data spread occurs, suggesting the possibility that there may be two phases of about equal density. Plates 4.2 a,b, and c show the outlet of the extractor after the end of a solubility experiment. Here we see material that has apparently been liquid due to the presence of entrapped bubbles and cavities created during depressurization of the system. Since the most volatile phase in the system is circulated the distinct possibility exists that another phase could be measured rather than the supercritical phase. Another factor influencing this may be experimental variability due to numerous factors including pressure and temperature control variations, the behaviour of the solute near the critical point, and sampling error due to the small amounts of solute present at the low pressure range.

In Figure 4.7 at 26 MPa, a transition appears away from the main trend of the experimental observations. This could again be due to hidden phases or other factors, a similar trend appears in the data for 324 K as shown in Figure 4.5, where a possible transition appears at about 27 MPa.

Figure 4.8 shows the experimental data plotted as isobars for approximately constant pressure conditions as the logarithm of Cyclosporine mole fraction versus absolute temperature in degrees Kelvin. All the experiments were conducted above the carbon dioxide critical temperature of 304.21 K.

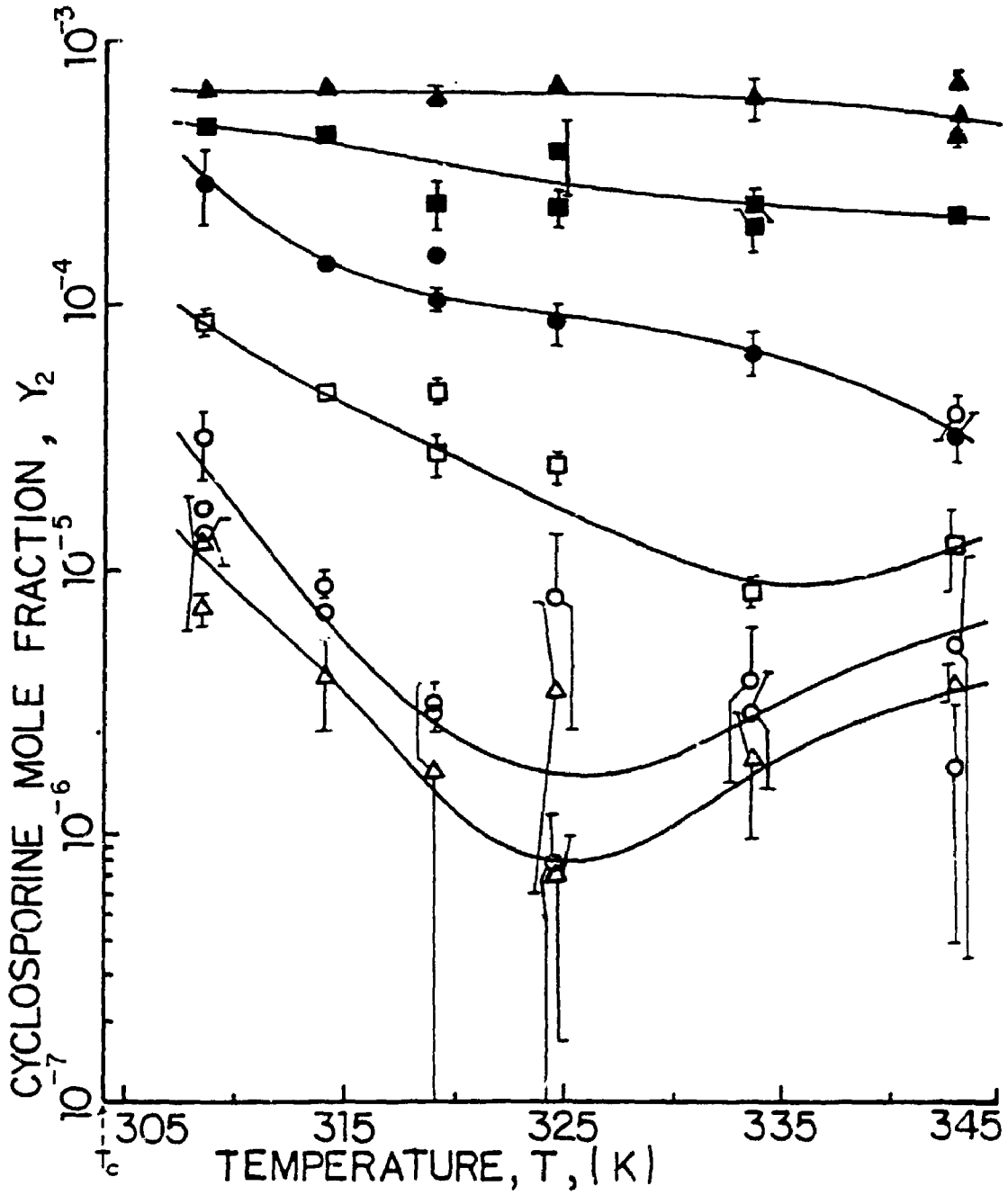
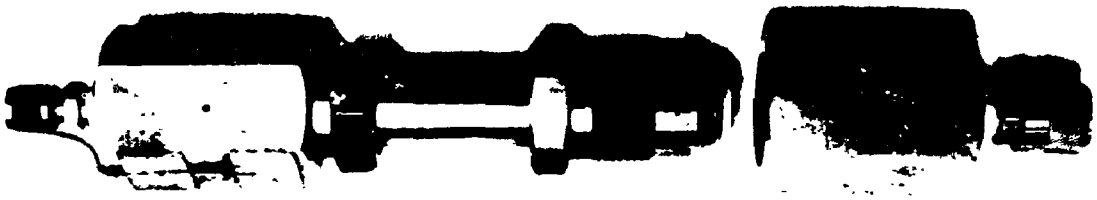
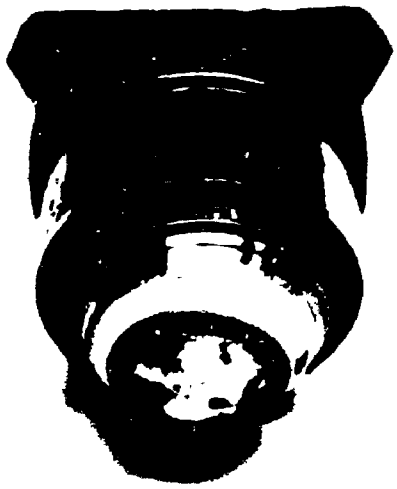



Figure 4.8 Solubility of pure Cyclosporine in supercritical carbon dioxide as a function of temperature at different constant pressures. (△) 9.0 ± 0.5 MPa, (○) 10.0 ± 0.3 MPa, (□) 15.0 ± 0.3 MPa, (●) 21.5 ± 0.5 MPa, (■) 27.0 ± 0.5 MPa, and (▲)33.5 ± 1.0 MPa.



(a)

(b)



(c)

Plate 4.2 Extraction vessel outlet view and indications of possible presence of a liquid Cyclosporine phase during solubility studies.
a) Extractor showing outlet end opened.
b) end view of extractor outlet showing "bubbly" Cyclosporine formation.
c) angle view showing the same formation as in b.

At roughly the maximum pressure studied there appears to be a point where the solubility is constant regardless of the system temperature (Figure 4.8). What the cause of this phenomenon is, is not known. The use of a view cell in future supercritical fluid solubility studies will be essential to determine if unusual phase changes occur at this point or if a phase inversion has taken place. A view cell would also allow the determination of the phase lines of the Cyclosporine - carbon dioxide system quite readily.

The experimental data at the set temperatures are plotted as the logarithm of Cyclosporine mole fraction versus the reduced density of carbon dioxide in Figures 4.9 to 4.15. The data were found to correlate linearly above a reduced density of about 1.5. The linear regions of the data were regressed by a least squares method with the computer program listed in Appendix 3.3. The constants for the linear equations were plotted as a function of temperature as shown in Figure 4.19. There appears to be a linear relationship between the constant values and the temperature over the range studied. The equation constants are listed with along with the linear equation form in Table 4.1.

An attempt was made to correlate the solubility data for Cyclosporine in carbon dioxide with the modified Chrastil equation (del Valle and Aguilera, 1988). This was a reasonably good correlation with results shown in Figure 4.20. The equation constants and the relevant statistical analysis of the data are shown in Appendix 2.4

Modeling with cubic or other equations of state was not possible since the required physical property data for Cyclosporine, critical constants, normal vapor pressure, etc. are not available in the literature.

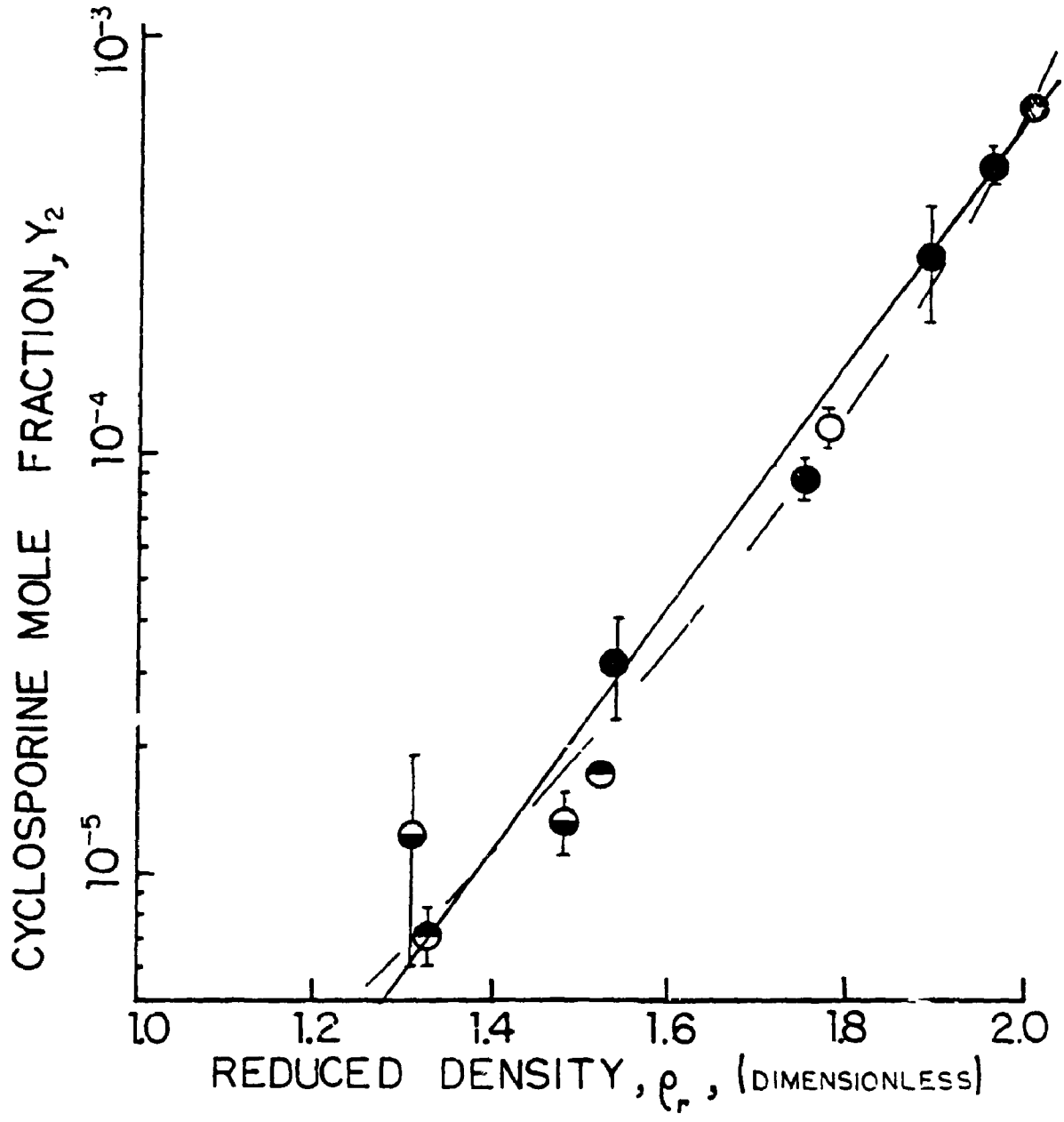



Figure 4.9 Solubility of pure Cyclosporine in supercritical carbon dioxide at 308.9 K ± 0.8 K as a function of reduced density. (●) major expt. 308.5 ± 0.8 K, (◒,○,◓) minor expts. 310.1 ± 0.1 K, 309.0 K, and 308.8 K respectively.

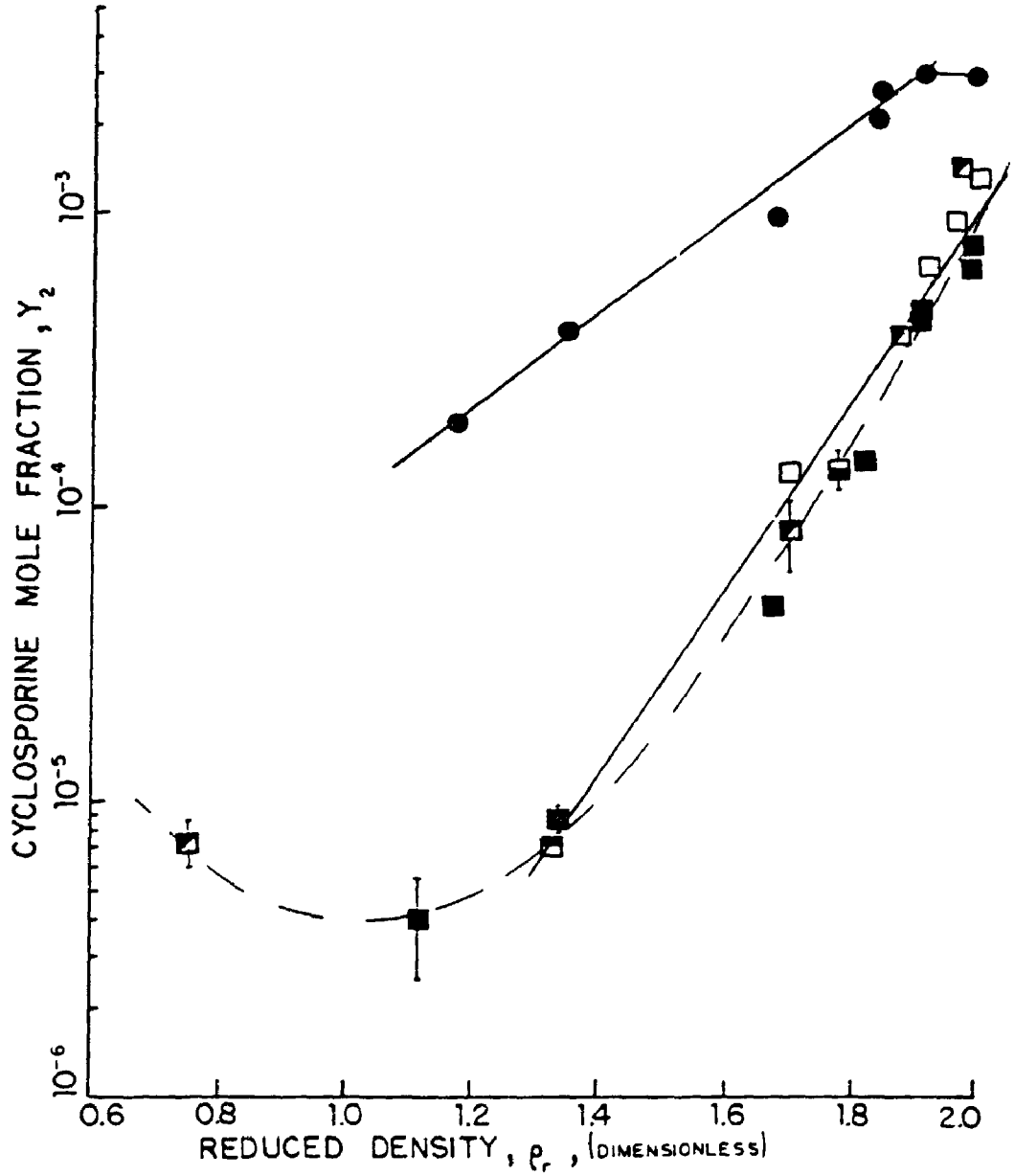



Figure 4.10 Solubility of pure Cyclosporine in supercritical carbon dioxide at 314.2 ± 0.5 K as a function of reduced density without methanol and with 2.90 % v/v methanol added as a co-solvent. (●) with methanol added and using a 0.5 mL sample loop, without methanol added: (■) major expt. using a 0.5 mL sample loop at 314.4 ± 0.3 K, minor expts.: (□, ◪, ▬, ▬) using a 20 μL sample loop at 313.8 ± 0.2 K, a 12 mL sample loop at 314.0 ± 0.2 K, and a 0.5 mL sample loop at 313.8 K and 314.1 K respectively.

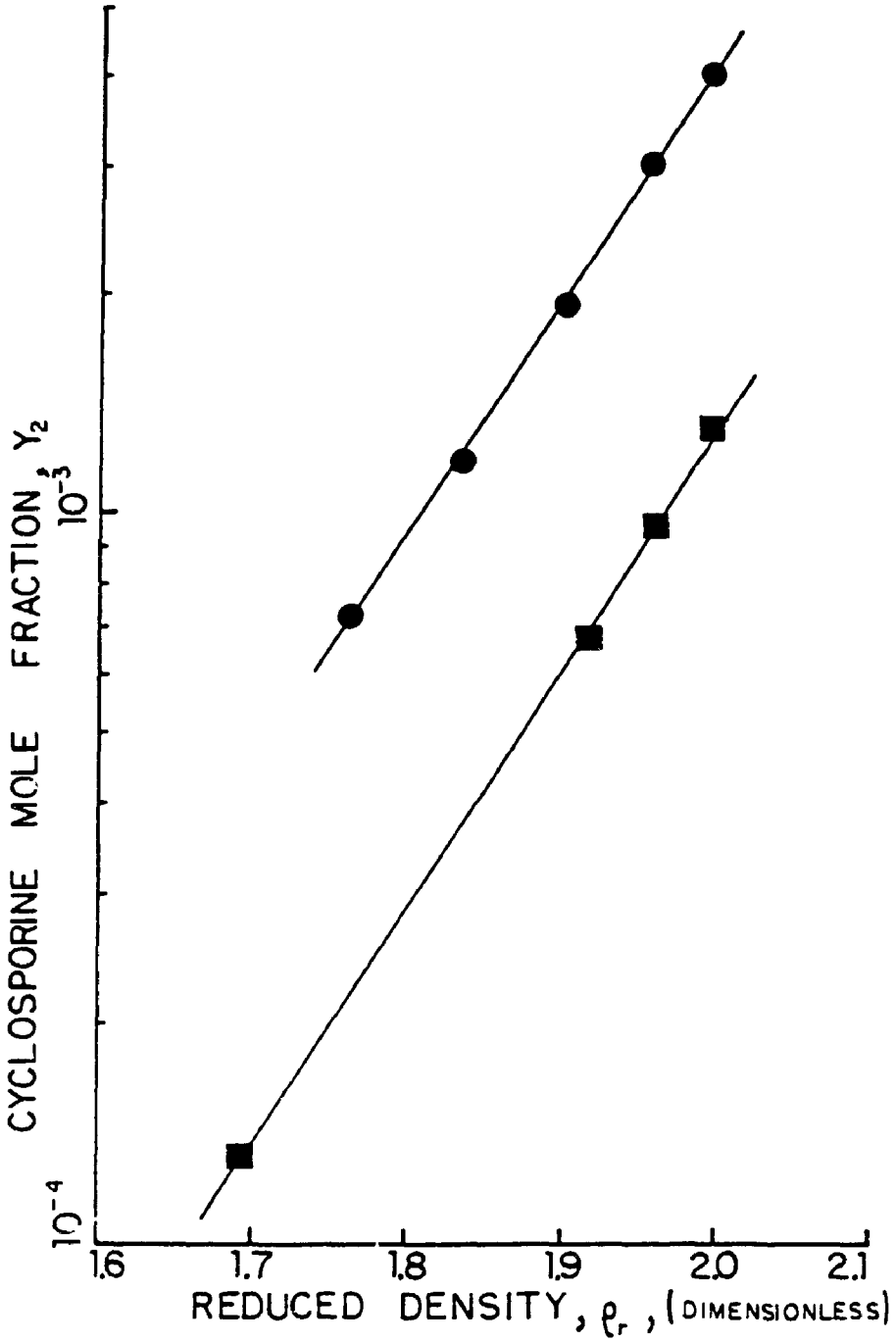



Figure 4.11 Solubility of pure Cyclosporine in supercritical carbon dioxide at 314.0 ± 0.5 K as a function of reduced density without methanol (■) and with 4.71 % v/v methanol added as a co-solvent (●).

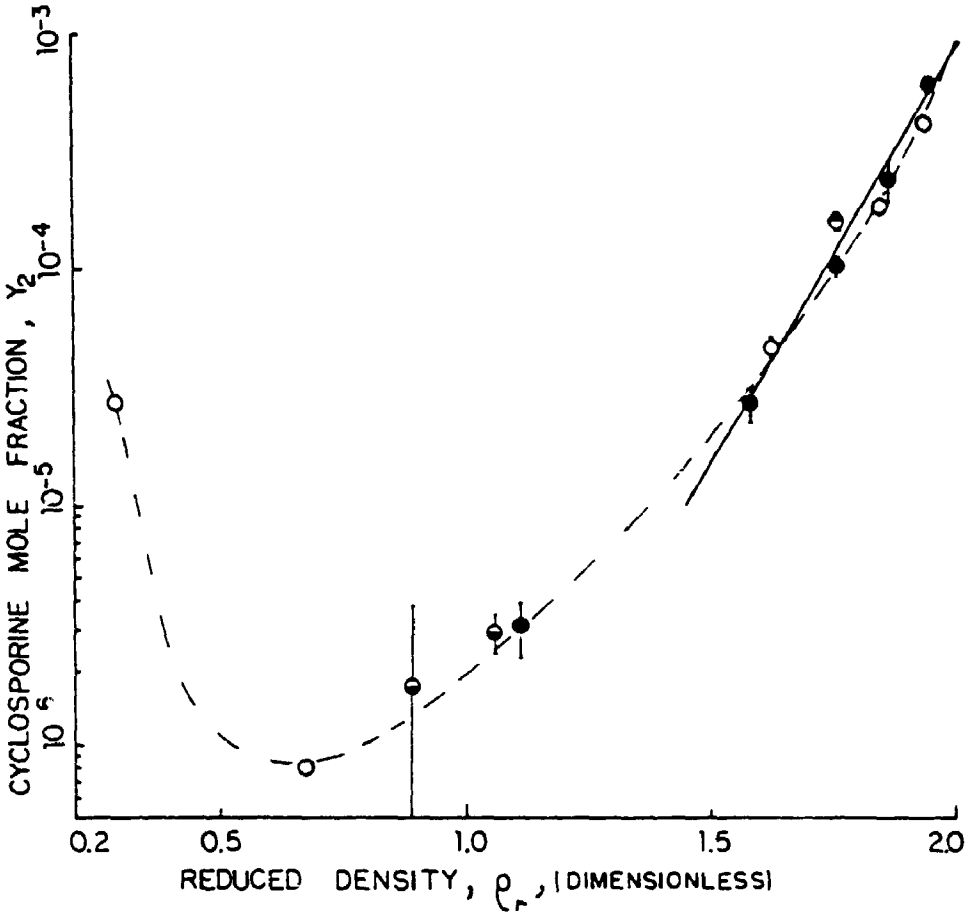



Figure 4.12 Solubility of pure Cyclosporine in supercritical carbon dioxide at 319.0 ± 0.6 K as a function of reduced density. (●) major expt. using a 0.5 mL sample loop at 319.0 ± 0.3 K, minor expts. (○, ◒, ◓): using a 12 ml sample loop at 318.2 ± 0.5 K., and a 0.5 mL sample loop at 319.2 ± 0.1 K and 319.1 K respectively.

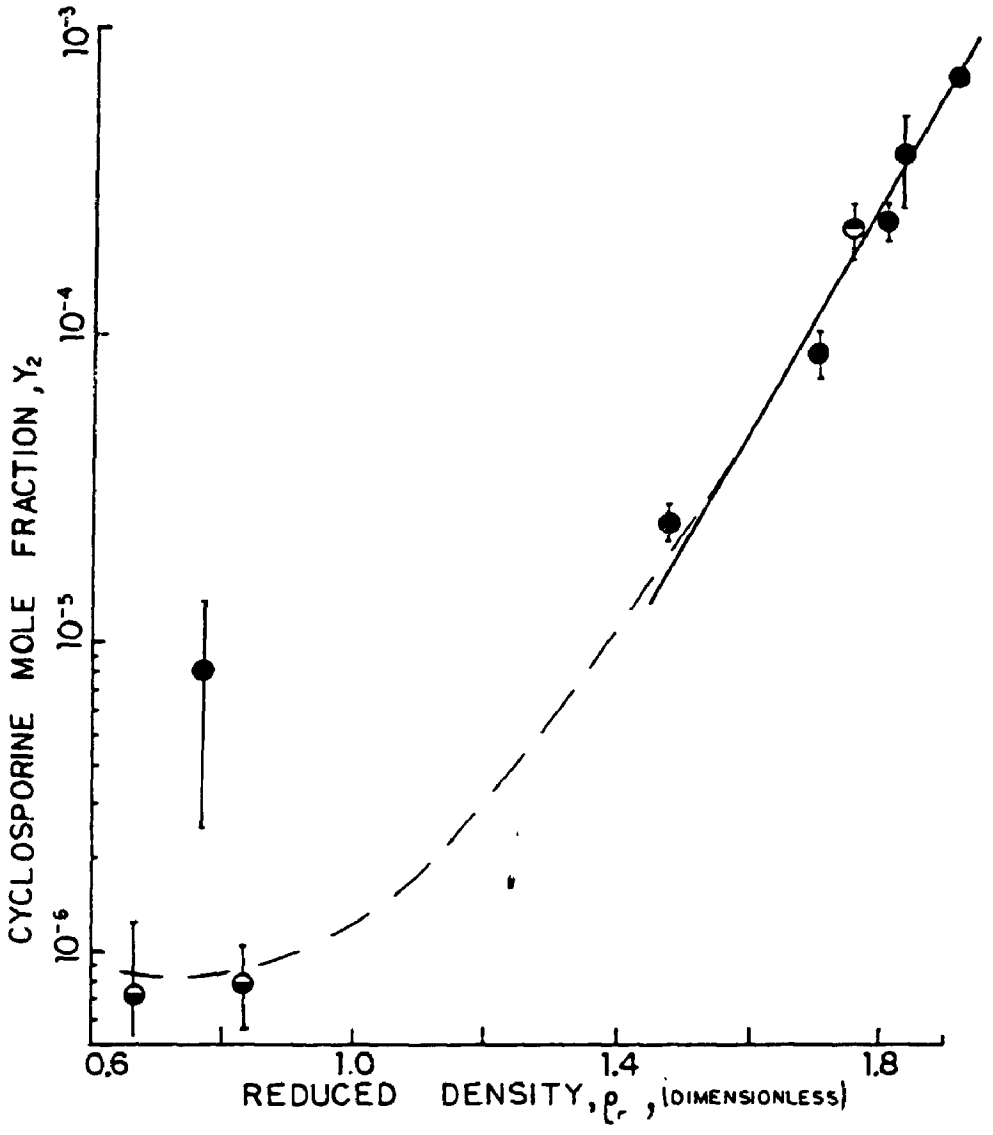



Figure 4.13 Solubility of pure Cyclosporine in supercritical carbon dioxide at 324.5 ± 0.3 K as a function of reduced density. (●) major expt. at 324.5 ± 0.3 K, (◒, ◒) minor expts. at 324.2 K and 324.3 ± 0.3 K

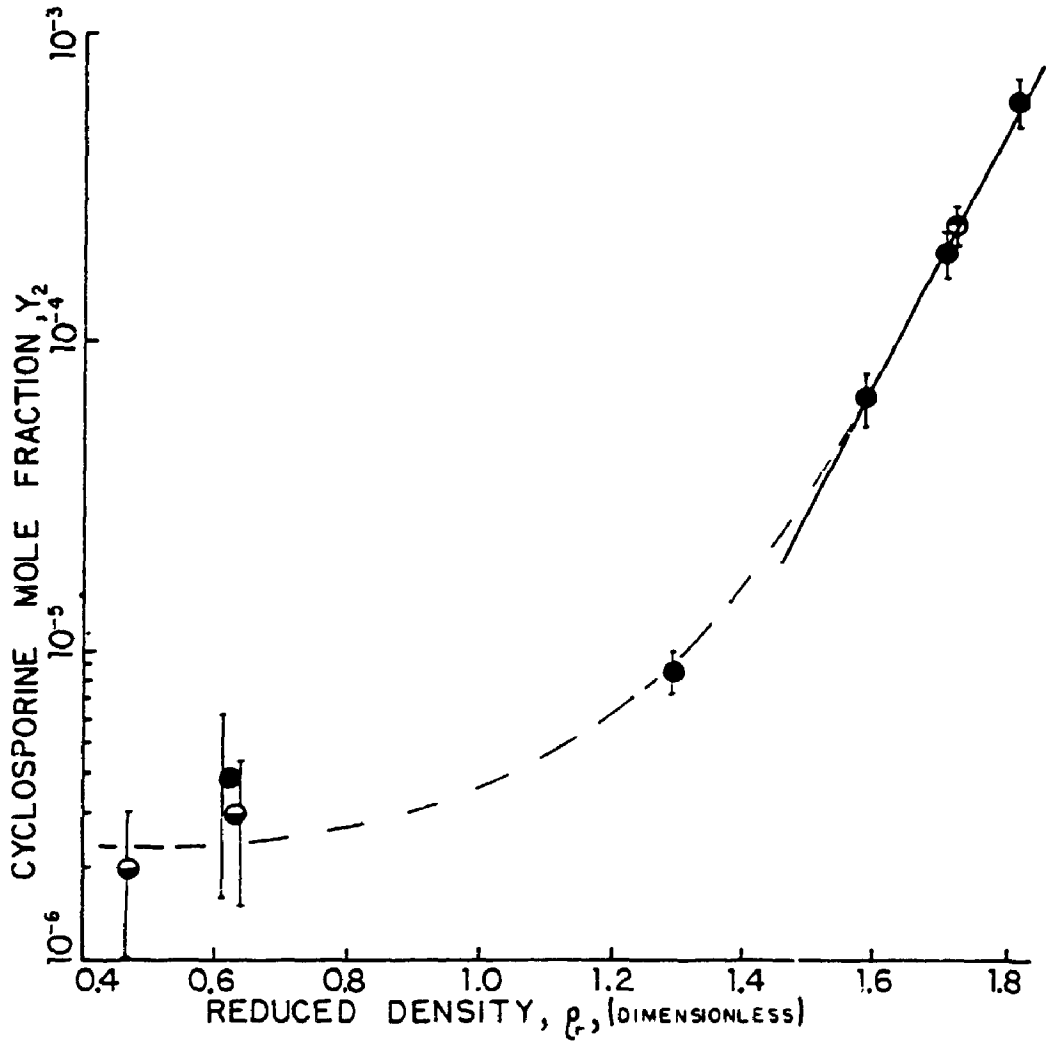



Figure 4.14 Solubility of pure Cyclosporine in supercritical carbon dioxide at 334.9 ± 0.5 K as a function of reduced density. (●) major expt. at 334.9 ± 0.1 K, (◓, ◒) minor expts. at 334.2 and 334.4 ± 0.4 K.

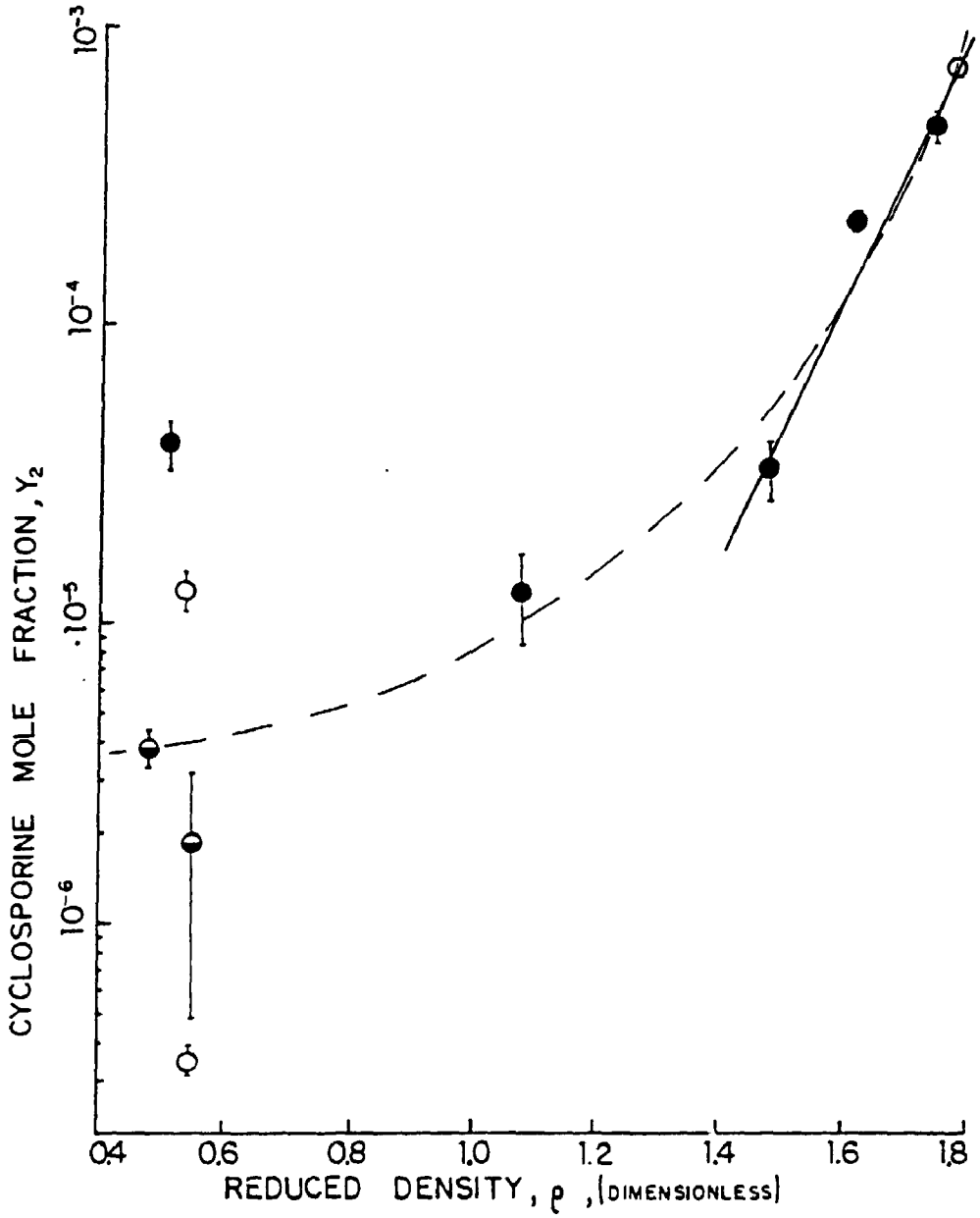



Figure 4.15 Solubility of pure Cyclosporine in supercritical carbon dioxide at 343.0 ± 0.5 K as a function of reduced density. (●) major expt. at 342.9 ± 0.4 K, (○, ◒) minor expts. at 343.2 ± 0.4 K and 343.5 ± 0.6 K.

Table 4.1 Constants from the linearization of plots of log ( mole fraction Cyclosporine) versus the reduced density of carbon dioxide.

Equation : $\log( y_2 ) = a ( \rho_r ) + b$ ... 4.1

| Temperature | a | b | reduced density lower limit |
|---|---|---|---|
| 308 | 2.845 | -8.955 | 1.3 |
| 314 | 3.229 | -9.461 | 1.3 |
| 319 | 3.492 | -10.042 | 1.4 |
| 324 | 3.703 | -10.209 | 1.5 |
| 335 | 4.267 | -10.986 | 1.4 |
| 343 | 4.257 | -10.700 | 1.4 |

Figure 4.16 shows interpolated data with pressure as the parameter plotted as logarithm Cyclosporine mole fraction versus temperature. Note the 'U' shaped nature of the curves for the lowest pressures 9 MPa and 10 MPa. This represents a region where retrograde precipitation of the solute is possible, (Chimowitz et al., 1988).

A similar plot of interpolated data using reduced density as a parameter is shown in Figure 4.17. Note the shallow 'U' shaped curves at low densities. A set of experimental data are included on this plot from an experiment where the amount of carbon dioxide in the system was held constant and the temperature varied. The system pressure varied with the temperature. When the system was stable the samples were taken. A set of data with almost constant density resulted. As seen in Figure 4.17 the data trend is similar to the data interpolated from Figures 4.9 to 4.15.

A three dimensional plot of temperature, pressure and Cyclosporine mole fraction is shown in Figure 4.18. The addition of phase line data obtained from view cell observations would greatly enhance the usefulness of this figure in the future. The phase data would also allow irregularities in the current data to be more readily explained.

Appreciable data scattering seems to occur at low pressures and between experiment repeats. This might be due to the fact that the very tight temperature control required to achieve stable equilibria at low pressures was not possible. It has been shown (Prausnitz et al., 1986) that very large changes in solute solubility occur close to the critical point. Slight changes in temperature or pressure can markedly effect the solubility there. As the system was operated as a closed loop the pressure did not change during the course of sampling at the fixed set point. However, the temperature did fluctuate slightly during experiments and

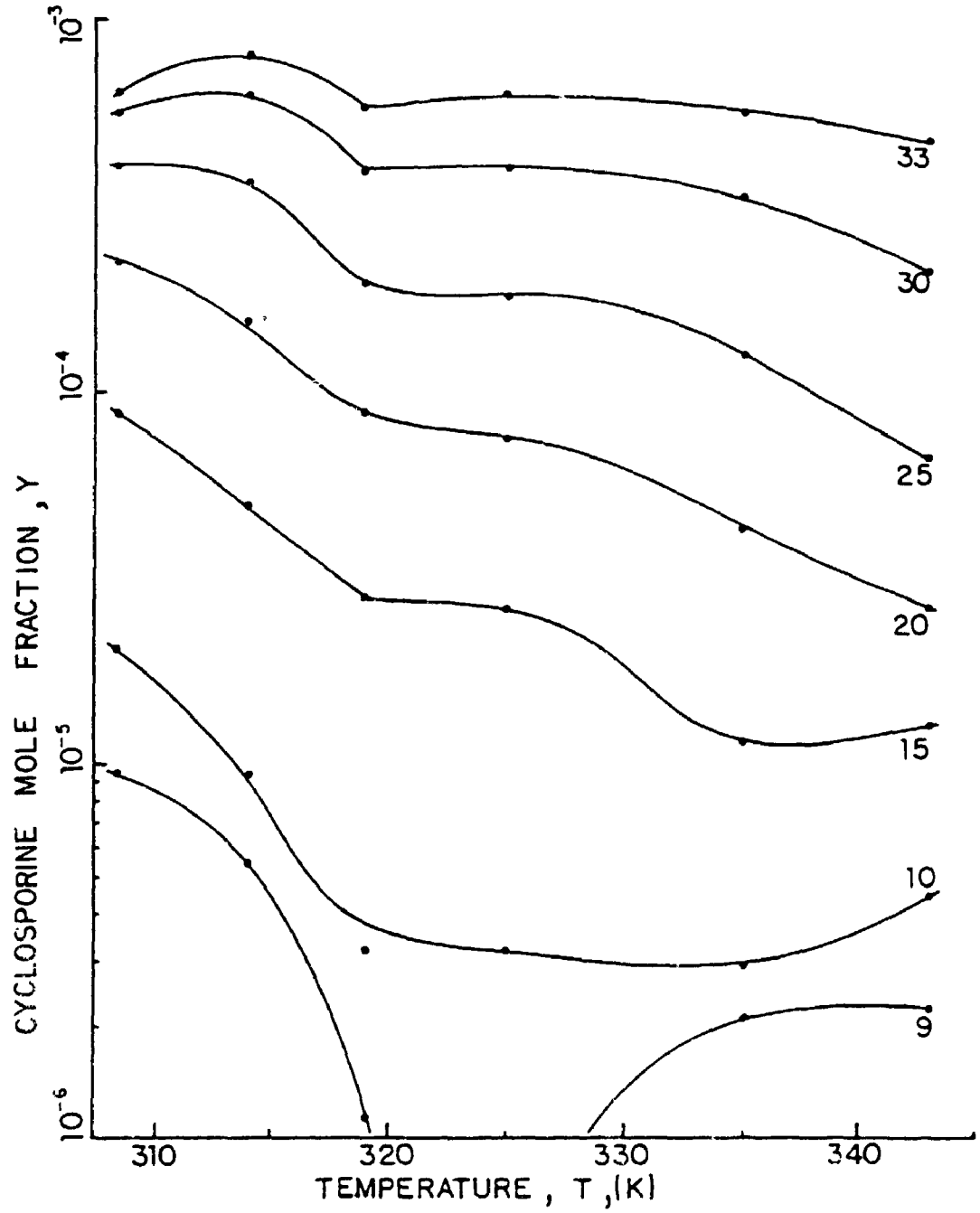



Figure 4.16 Solubility of pure Cyclosporine in supercritical carbon dioxide as a function of temperature with pressure as a parameter. Based on interpolated data from experimental results.

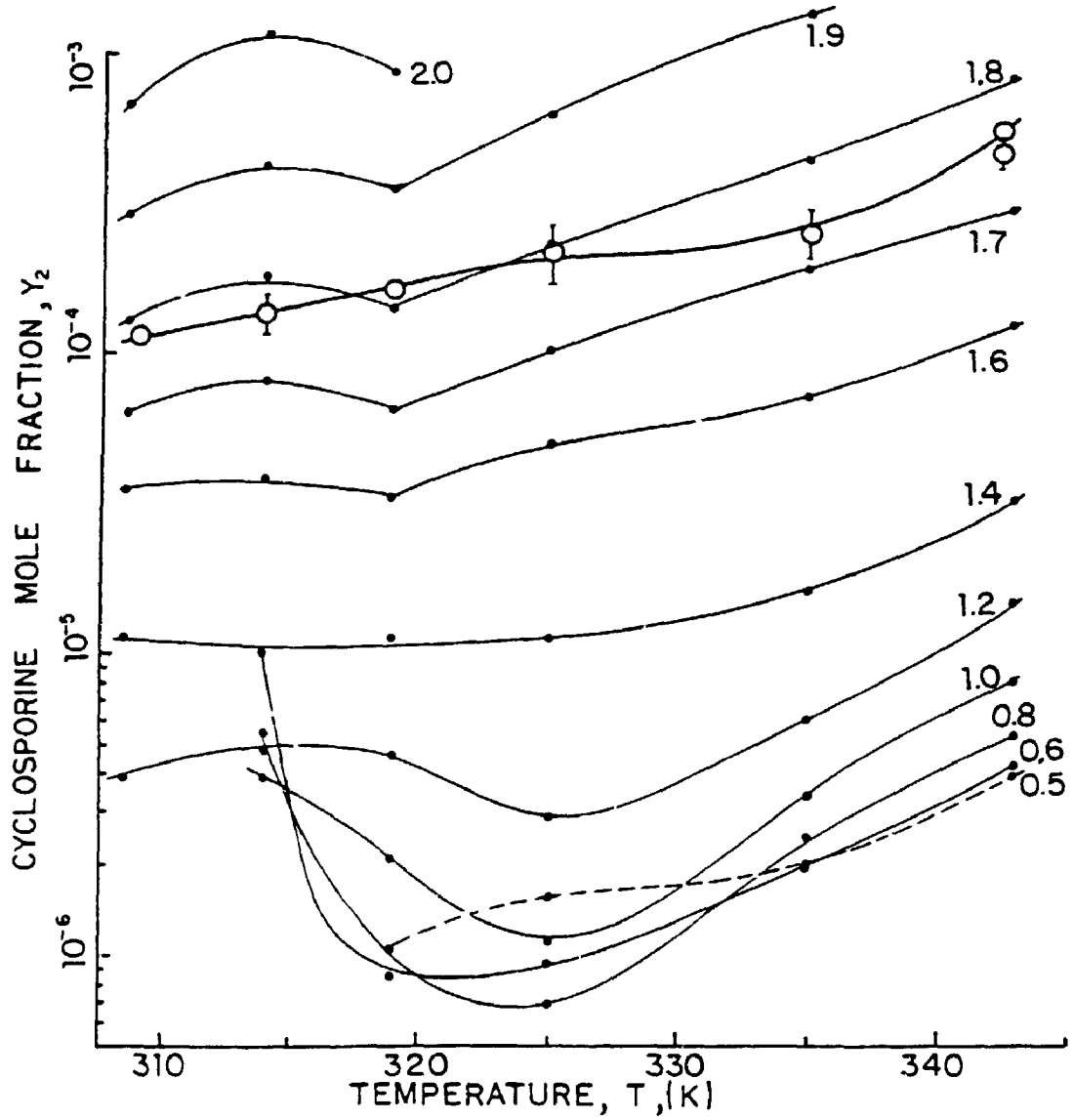



Figure 4.17 Solubility of pure Cyclosporine in supercritical carbon dioxide as a function of temperature with reduced density as a parameter. Based on interpolated data from experimental results.

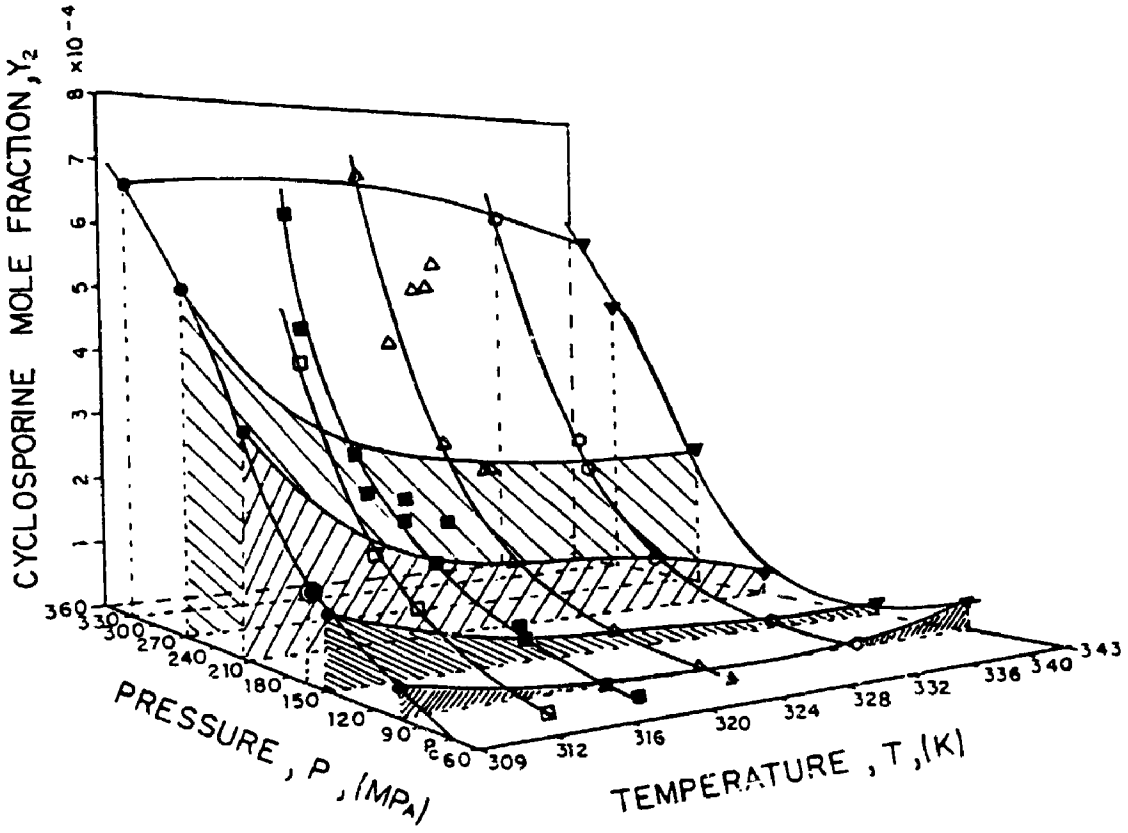



Figure 4.18 Three dimensional plot of pure Cyclosporine solubility data in supercritical carbon dioxide as a function of both temperature and pressure. (●) 308.9 K, (□) 314.0 K, (■) 319.0 K, (△) 324.5 K, (○) 334.9 K, and (▼) 342.9 K.

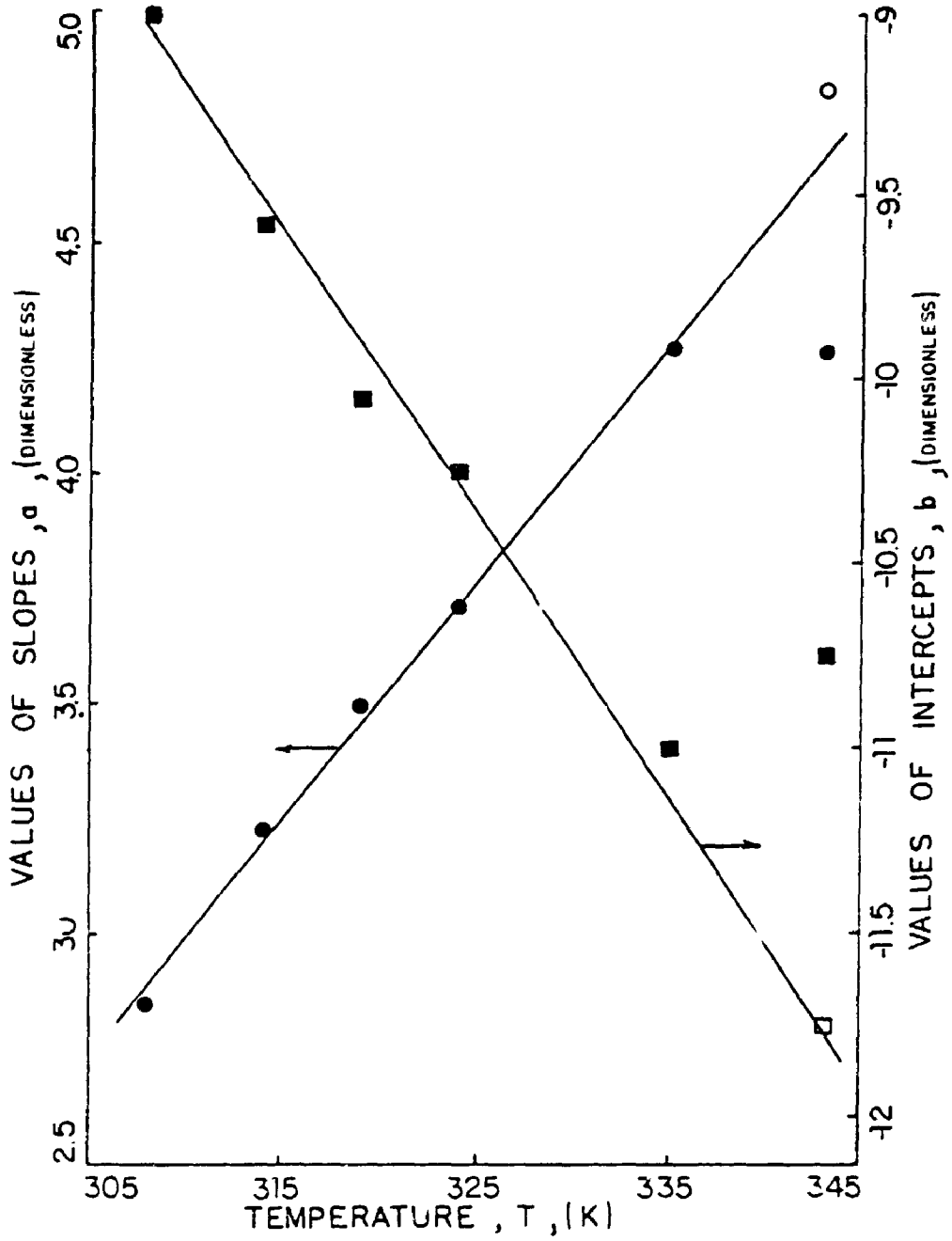



Figure 4.19 Parameters of the linear correlation equations for pure Cyclosporine solubility data in supercritical carbon dioxide plotted as a function of extraction temperature. (●) a, and (■) b.

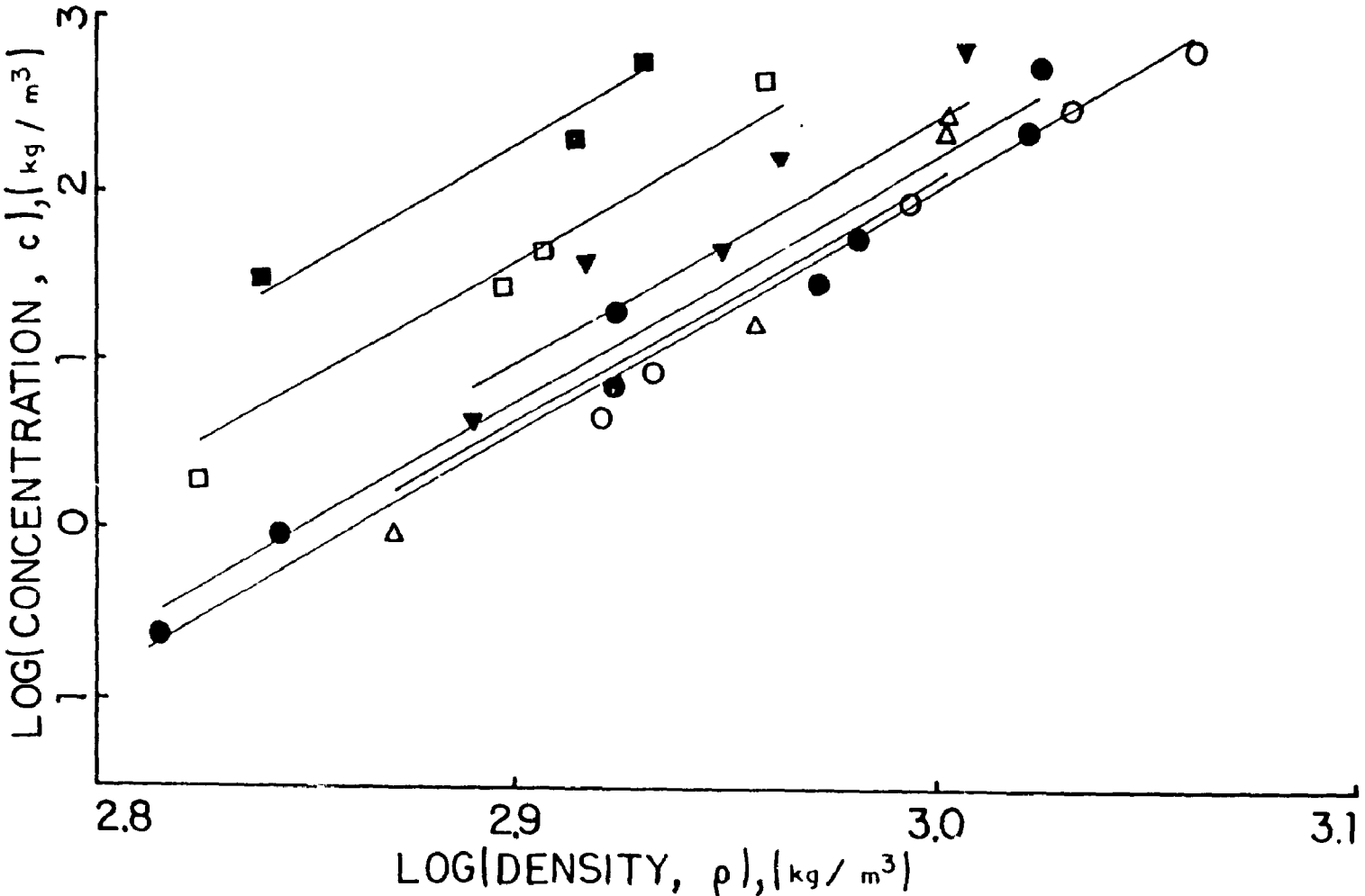



Figure 4.20 Correlation of pure Cyclosporine solubility data in supercritical carbon dioxide according to the modified Chrastil equation proposed by Del Valle and Aguilera, 1988. Log of Cyclosporine concentration plotted against log of supercritical $CO_2$ density at different isotherms. (○) 308.9 K, (△) 314 K, (●) 319 K, (▼) 324.5 K, (□) 334.9 K, and (■) 342.9 K. (——) isotherm temperatures increase from bottom to top and have same temperature values as the data shown.

between experiments as an exact temperature profile could not be repeated. A better heat transfer fluid than air should be used in future experiments to achieve better temperature control. It will also ensure that variation in temperature between parts of the apparatus is mimimal. In some early experiments temperature gradients of about 2 to 4 K occurred between components in the system. A second improved oven geometry reduced these gradients to less than 1 K, which greatly improved the accuracy of temperature control.

## 4.3 Results of Pure Cyclosporine Solubility in the Supercritical Carbon Dioxide - Methanol Co-Solvent System

Figures 4.2, 4.3, 4.10 and 4.11 show experimental data for Cyclosporine solubility in carbon dioxide with methanol added as a co-solvent. Figures 4.2 and 4.10 show the results of an experiment conducted in the 103.3 mL volume extraction system with 3 mL of methanol which was added through the sample loop. The methanol concentration in the supercritical extraction system was 2.90 % v/v. The same experiment without methanol addition is also shown in the same Figures. Several grams of Cyclosporine mixed with sand along with material reclaimed from earlier experiments was loaded into the extraction vessel. The initial methanol co-solvent data showed an increase in the solubility of Cyclosporine of twerty times greater than that obtained in the absence of methanol (supercritical $CO_2$ only). This increase in solubility may be explained by the fact that Cyclosporine is highly soluble in methanol. The addition of only 2.90 % v/v methanol proved very promising in terms of significant increases of Cyclosporine solubility in the supercritical extraction

system. The data show a distinct solubility plateau after the pressure had reached 25 MPa. Which may be due to depletion of all Cyclosporine available for extraction at that time.

Figure 3.6 shows a new low volume extraction vessel and system designed to reduce the amount of cyclosporine required for determining the solubility in carbon dioxide and in the carbon dioxide - methanol co-solvent system. This new system had a recycled volume of 8.49 mL with the pressure gauge volume of 33 mL isolated from the system by a valve when it was not in use. A 2.8 gram quantity of Cyclosporine was loaded into the extraction vessel which would allow a maximum solubility of 330 mg/mL to be attained without reaching a solubility plateau. A 20 $\mu$L sample loop was used for this experiment to minimize Cyclosporine removal by sampling. First a solubility study using just supercritical carbon dioxide was done as a control. These data are shown in Figure 4.3 along with the data for the carbon dioxide - methanol co-solvent system. A smaller volume of methanol 0.4 mL was added through the sample loop giving a methanol concentration of 4.71 % v/v . The cosolvent system attained solubilities which were about three times those attained by the control. No plateau was present as plenty of excess Cyclosporine was available. The maximum solubility achieved by the cosolvent system was 103 mg Cyclosporine / mL of system volume.

The cosolvent solubility data were also plotted as log of mole fraction of Cyclosporine versus reduced density of supercritical $CO_2$ in Figures 4.10 and 4.11. A linear plot resulted for these data above a reduced density of about 1.2. Constants for these data were determined and are shown in Table 4.2.

Table 4.2 Constants from the linearization of Plots of log ( mole fraction Cyclosporine) versus reduced density in the cosolvent system.

Equation : $\log( y_2 ) = a ( \rho_r ) + b$ 4.1

| | | |
|---|---|---|
| 2.90 % v/v methanol | a = 1.6153 | b = -5.6293 |
| 4.71 % v/v methanol | a = 3.2291 | b = -8.8409 |

# CHAPTER 5

# EXTRACTION OF CYCLOSPORINE FROM THE MYCELIA OF *Beauvaria nivea* ATCC 34921, (NRRL 8004).

## 5.1 Experimental Procedure

Fermentor and shake flask culture fluids containing the mycelia of the fungi imperfecti *Beauvaria nivea* ATCC 34921 (NRRL 8004) were obtained from fellow graduate student P.S.Chahal 1990,( Ph.D. thesis in progress). The mycelia were separated from the culture fluids by sieving or centrifugation. The mycelia were loaded into the extraction vessel, which was placed into the rest of the supercritical extraction system. The supercritical system was first allowed to equilibrate at the required constant temperature and then pressurized to the required pressure with carbon dioxide. The experiment commenced when the flow of carbon dioxide was started by slowly turning open the main flow control valve in early experiments. Later a micrometering valve was used for this purpose. This allowed the carbon dioxide to flow through the extractor at pressure and then be depressurized through the main flow control valve.

The carbon dioxide then passed through the separator vessel, which vessel was changed regularly. The carbon dioxide flow rate was monitored using a rotameter and a wet test meter to measure the total volume of carbon dioxide used during the course of supercritical extraction. These data were recorded along with the system temperature and pressure whenever the separator vessel was changed. Each separator vessel at atmospheric pressure was filled with methanol to trap the Cyclosporine as it came out from the extraction vessel and allowed for the continuous removal of

carbon dioxide gas. This methanol was sampled for Cyclosporine content and immediately analysed by HPLC. Thus the experiment could be allowed to continue until sampling indicated almost no more Cyclosporine removal from the mycelia over a significant time period. The amount of Cyclosporine removed was thus obtained as a function of extraction time with continuous supply of supercritical extracting solvent $CO_2$. These measurements formed the basis for studying the kinetics of Cyclosporine removal. The sampling rate between and during experiments was varied depending on the rates of Cyclosporine removal. In some experiments the sampling was done very rapidly during the initial stages of extraction when the rates were relatively fast.

## 5.2 Supercritical Extraction of Cyclosporine from Mycelia of *B. nivea*. Experiments No. 35 to No. 57.

The main focus of the research on the supercritical extraction of Cyclosporine from the mycelia of *Beauvaria nivea* concentrated on improving the yield of the extraction process. Table 5.1 summarizes the research on the effect of various pretreatments, mycelial states, and extraction conditions on the overall extraction yield of Cyclosporine obtained. Most of the research focused on the high pressure range 30 to 35 MPa and temperatures of 314 K and 330 K.

One experiment conducted at 15.2 MPa and 314 K, Run #35 showed good results but at much slower extraction rates. The mycelia used in Run #35 were partially oven dried. A comparison of the results of experiment #35 with experiment #38 (conducted at 32.5 MPa and 314.8 K) is shown in Figures 5.1 and 5.2. Figure 5.1 shows the yield of Cyclosporine

Table 5.1 Summary of Mycelial Extraction Experiments

NOTE: Yield is defined as (mg of Cyclosporine removed) / (mg of Cyclosporine originally present in the mycelia)

| Expt. No. | Pressure (MPa) | Temperature (K) | Yield | Comments |
|---|---|---|---|---|
| 35 | 15.2 | 314 | 0.6645 | semi-dry |
| 37 | 32.6 | 314.5 | 0.4747 | pre-frozen oven dried |
| 38 | 32.5 | 314.8 | 0.6311 | " oven dried |
| 39 | 32.2 | 314.1 | 0.5729 | " air dried (355 - 850 μm) |
| 40 | 32.0 | 314.8 | 0.6155 | " air dried ( 0 - 355 μm) |
| 41-44 | 32.0 | 314 | 0.175 | remains of 39 + 40 -> (0 - 125 μm) |
| 39-44 | 32.0 | 314 | 0.6648 | combined results |
| 45 | 32.2 | 314 | 0.6596 | 29.5 % moisture |
| 46 | 32.0 | 333 | 0.4760 | remains of 45 |
| 45-46 | 32.0 | 314-333 | 0.8216 | combined results |
| 47 | 32.0 | 332.5 | 0.4001 | 80.1 % moisture |
| 48 | 32.0 | 332.4 | 0.5804 | dry material |
| 49 | 34.7 | 310.9 | 0.4098 | mixed material |
| 50 | 34.3 | 311 | 0.3671 | oven dried (212 - 355 μm) |
| 51 | 32.9 | 313.8 | 0.0 | dense packed wet biomass bed, 76.9% moisture |
| 52 | 32.6 | 302 -> 313.6 | 0.3878 | surface growth mycelia |
| 53 | 32.8 | 313.2 | 0.2370 | oven dried (212 - 355 μm) |
| 54 | 32.9 | 313.7 | 0.2522 | #53 remains, methanol treated |
| 53-54 | 32.9 | 313.7 | 0.4293 | combined results |
| 55 | 32.9 | 314.1 | 0.5951 | 11.7 % moisture & methanol treated |
| 56 | 32.9 | 313.9 | 0.6884 | 7.2 % moisture |
| 57 | 32.9 | 314 -> 327.3 | 0.6930 | 14.7 % moisture ( 2mm ) |
| 58 | 32.9 | 313.8 | 0.3541 | air dried (7.5 day old culture) |
| 59 | 32.9 | 313.1 | 0.2260 | oven dried (6.5 day old culture) |

Table 5.1 (cont.)

| Expt. No. | Pressure (MPa) | Temperature (K) | Yield | Comments |
|---|---|---|---|---|
| 60 | 32.9 | 314.4 | 0.2974 | blended, oven dried (0.5 mm) |
| 61 | 33.1 | 314.7 | 0.2970 | blended, oven dried (0.5 mm) |
| 60-61x | 32.7 | 314.7 | 0.3115 | remains of 60 + 61 |
| 66 | 33.2 | 314.7 | 0.5102 | remains of 60 + 61 NaOH treated |
| 62 | 33.1 | 315.6 | 0.4921 | air dried (0.5 mm) |
| 63 | 33.0 | 314.4 | 0.387 | autoclaved, air dried (0.5 mm) |
| 64 | 32.9 | 314.3 | 0.1992 | freeze - thaw, and oven dried (0.5 mm) |
| 65 | 30.9 | 314.5 | 0.0918 | french pressed, 80 % spores (0.5 mm) |
| 67 | 33.4 | 314.1 | 0.2916 | NaOH treat. (8 day old culture), dried |
| 68 | 33.5 -> 31.1 | 314.2 | 0.1500 | oven dried, (0.5 mm) |
| 69 | 33.7 -> 29.3 | 314.3 | 0.3403 | air dried, (0.5 mm) |
| 70 | 33.6 | 314.1 | 0.1717 | methanol treat., oven dried (0.5 mm) |
| 71 | 33.3 -> 31.1 | 314.2 | 0.2676 | freeze dried (2 mm) |
| 72 | 31.4 | 314.1 -> 327.6 | 0.2344 | freeze dried (10 mm) |
| 73 | 31.5 | 313.9 | 0.4803 | air dried ( 90 $\mu$m ) |

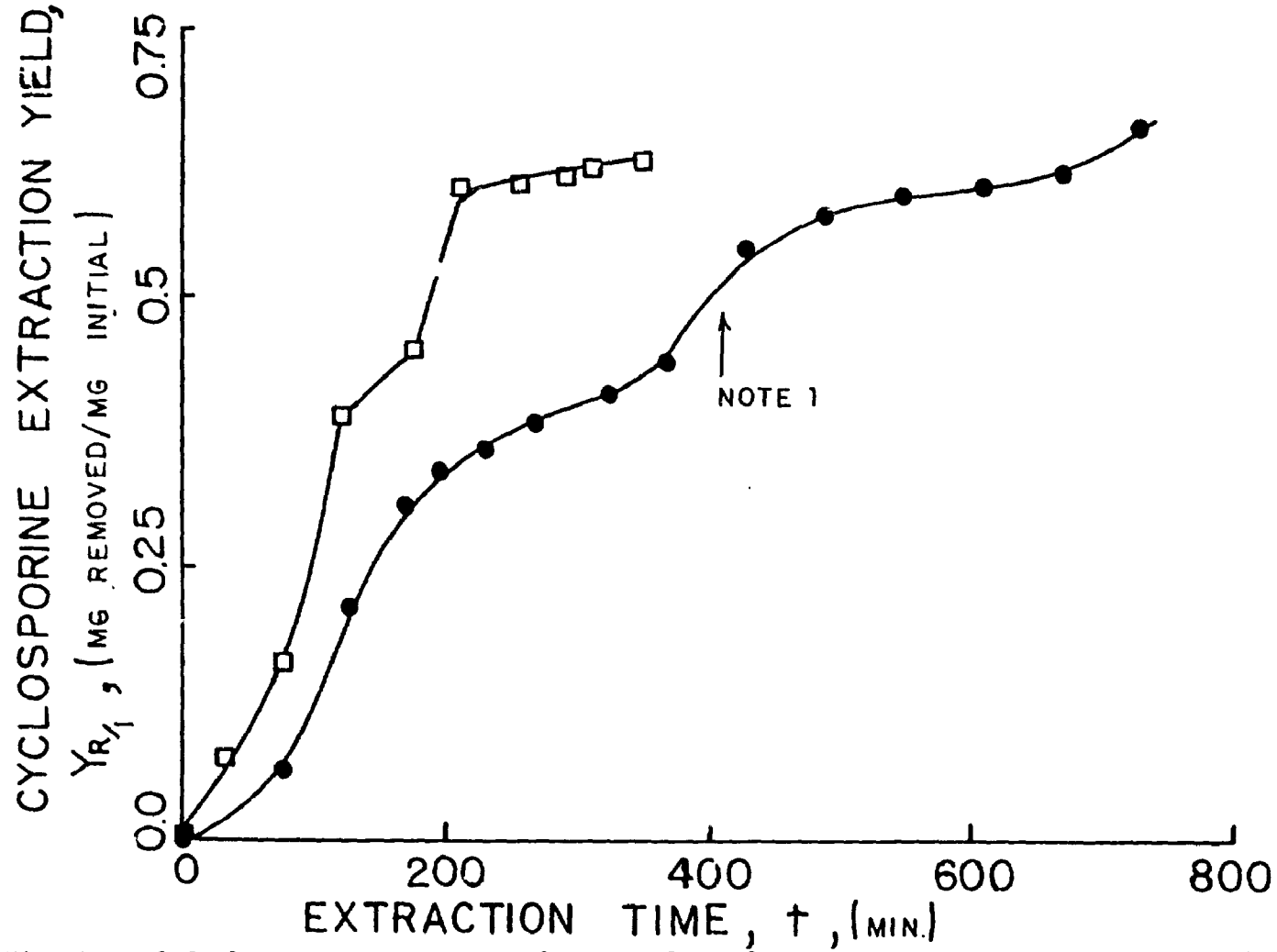



Figure 5.1 Kinetics of Cyclosporine extraction from mycelia of *Beauvaria nivea*, at 15.2 MPa and 314 K (Experiment #35, ● ) and at 32.5 MPa and 314.8 K (Experiment #38, □ ). Extraction yield of cyclosporine versus extraction time. Note 1: Carbon dioxide flow was stopped overnight. Collected materials melted from valve into sampling tube while flow was stopped.

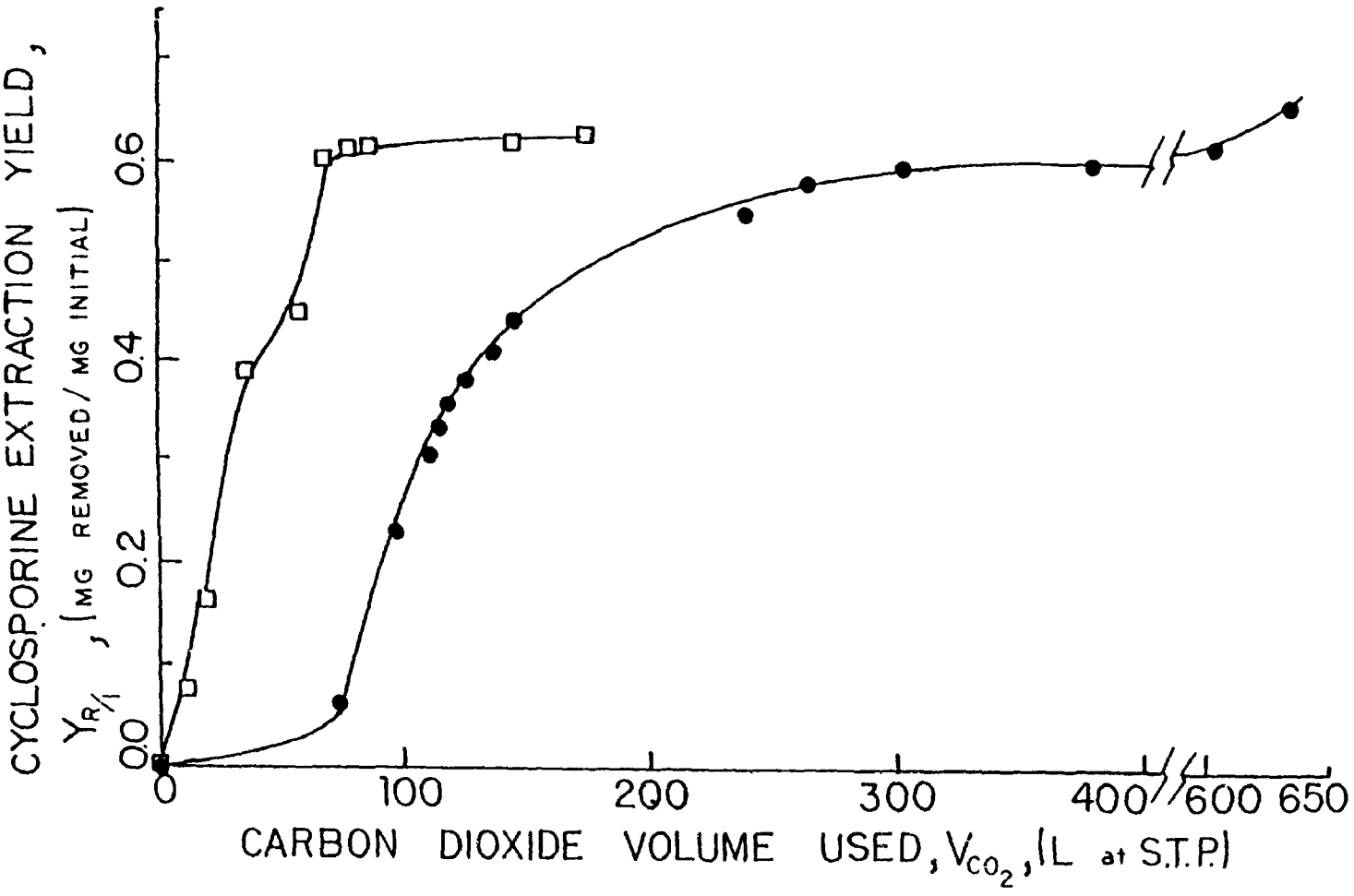



Figure 5.2 Kinetics of Cyclosporine extraction from mycelia of *Beauvaria nivea*, at 15.2 MPa and 314 K (Experiment #35, ●) and at 32.5 MPa and 314.8 K (Experiment #38, □). Extraction yield of cyclosporine versus carbon dioxide cummulative volume used.

extraction from the mycelia expressed as mg Cyclosporine removed per mg of original total Cyclosporine present in the mycelia, plotted as a function of extraction time. Both experiments had similar final yields but experiment #35 took much longer to reach the apparent maximum yield. However the flow rates were not the same for these two experiments. In order to compare these results properly another independent variable was used. The convention of plotting the yield results as a function of carbon dioxide usage was adopted to eliminate the influence of flow rate variations between experiments.

Figure 5.2 shows the yield of Cyclosporine extraction from mycelia as a function of the total cummulative volume of carbon dioxide used (at S.T.P.) in experiments #35 and #38. The early lag in experiment #35 was due to the extractor bypass valve being left open. Ignoring this first portion of the curve we readily observe that at least three to four times the amount of carbon dioxide was required in experiment #35 to obtain the same yield as in experiment #38. The supercritical $CO_2$ extraction yields for Runs #35 and #38 were found to be 0.665 (66.5 % of original Cyclosporine removed) and 0.631 respectively. Although these yields are lower than those obtained using liquid organic solvents, such as methanol at atmospheric pressure, the use of supercritical $CO_2$ is more selective than organic solvents and fewer components are co-extracted from the mycelia thus resulting in a cleaner product.

Two experiments were conducted at 32 MPa and 314.5 K to determine if particle size was a limiting factor. Material which came from the same fermentation was air dried, ground and separated into two fractions. The first fraction extracted had particle sizes ranging from 355 $\mu$m to 850 $\mu$m, and the second fraction had particles sizes less than 355 $\mu$m. The results

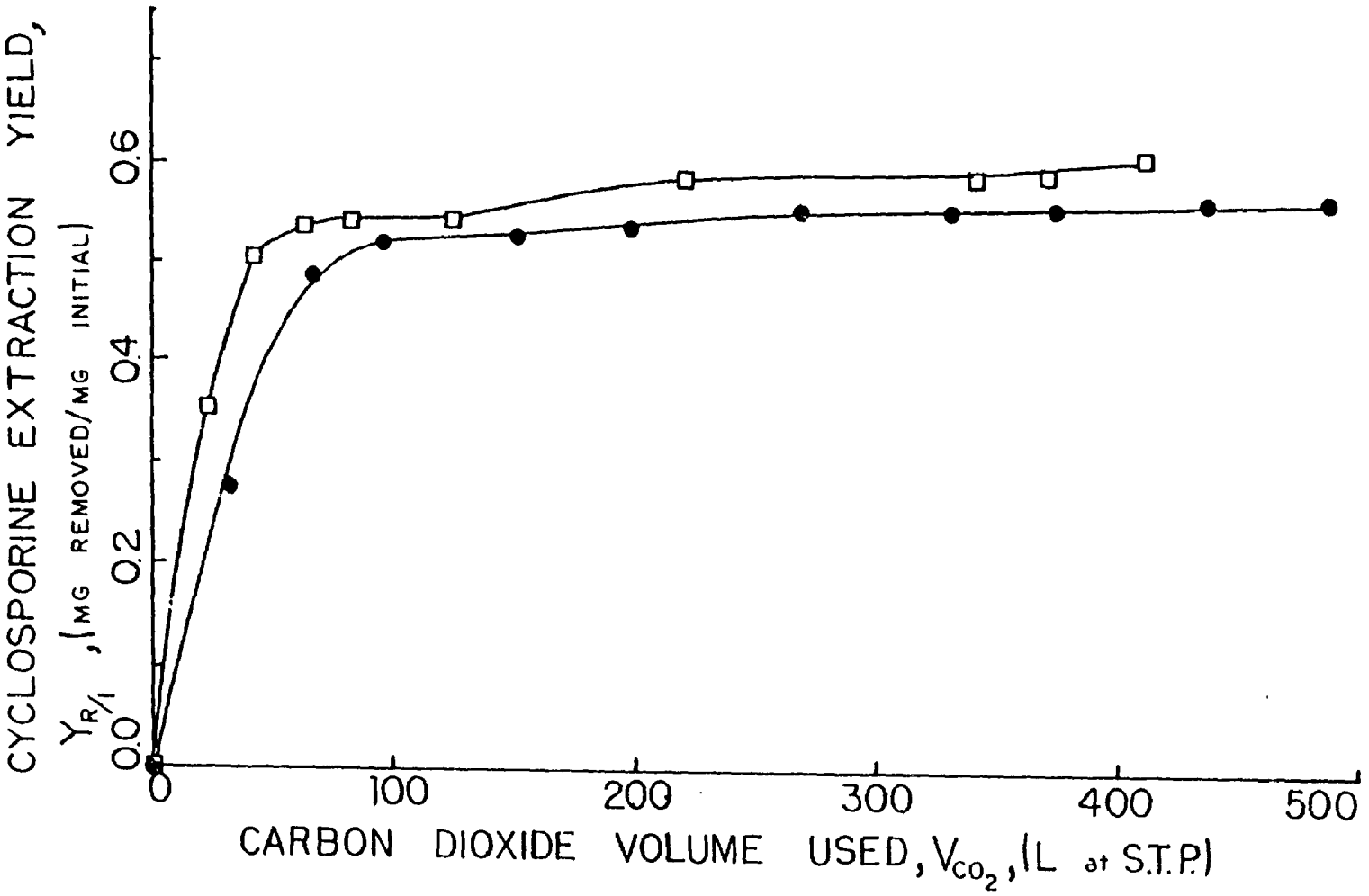



Figure 5.3 Kinetics of Cyclosporine extraction from mycelia of *Beauvaria nivea*, at 32.5 MPa and 314.8 K, using 355 to 855 μm particles (Experiment #39, ●) and using particles less than 355 μm (Experiment #40, □). Extraction yield of Cyclosporine versus carbon dioxide cummulative volume used.

of these experiments, runs #39 and #40 respectively, are shown in Figure 5.3. The yields of Cyclosporine extracted from the mycelia in these experiments were fairly close. The error in Cyclosporine standard determination by HPLC is about ±2.5%. Based on this estimate of the error there is no significant difference between the final yields of Cyclosporine extraction from the two different sized particle fractions studied. However, Figure 5.3 shows that the rate of supercritical extraction from the smaller sized particle fraction was about two times higher than those for the larger particle size fraction. This suggests the particle size does limit the mass transfer rate of the extraction. It is possible, however, to find much larger improvements in the rates of supercritical extraction of Cyclosporine as the size of the mycelia particles is reduced further.

The mycelia leftover from experiments #39 and #40 were combined and re-extracted in series of extractions in runs #41 to #44 conducted at the same conditions used previously (32 MPa and 314.5 K). Experimental run #41 was an extraction of the combined material from experiments #39 and #40 without any further treatment and removed a further 0.03 fraction ( 3 %) of the Cyclosporine remaining in the mycelia. The mycelial remains of experiment #41 were ground to consist of particles less than 125 $\mu$m in size and reloaded into the extraction vessel for experimental runs #42 and #43. These two experiments (#42 and #43) removed another 0.144 fraction (14.4 %) of the remaining Cyclosporine from the mycelia. One milliliter of water was then added to the extraction vessel inlet just before experimental run #44. The addition of this water did not result in any major increase in Cyclosporine removal from the mycelia during run #44. Experiments #41 to #44 inclusive increased the overall yield of

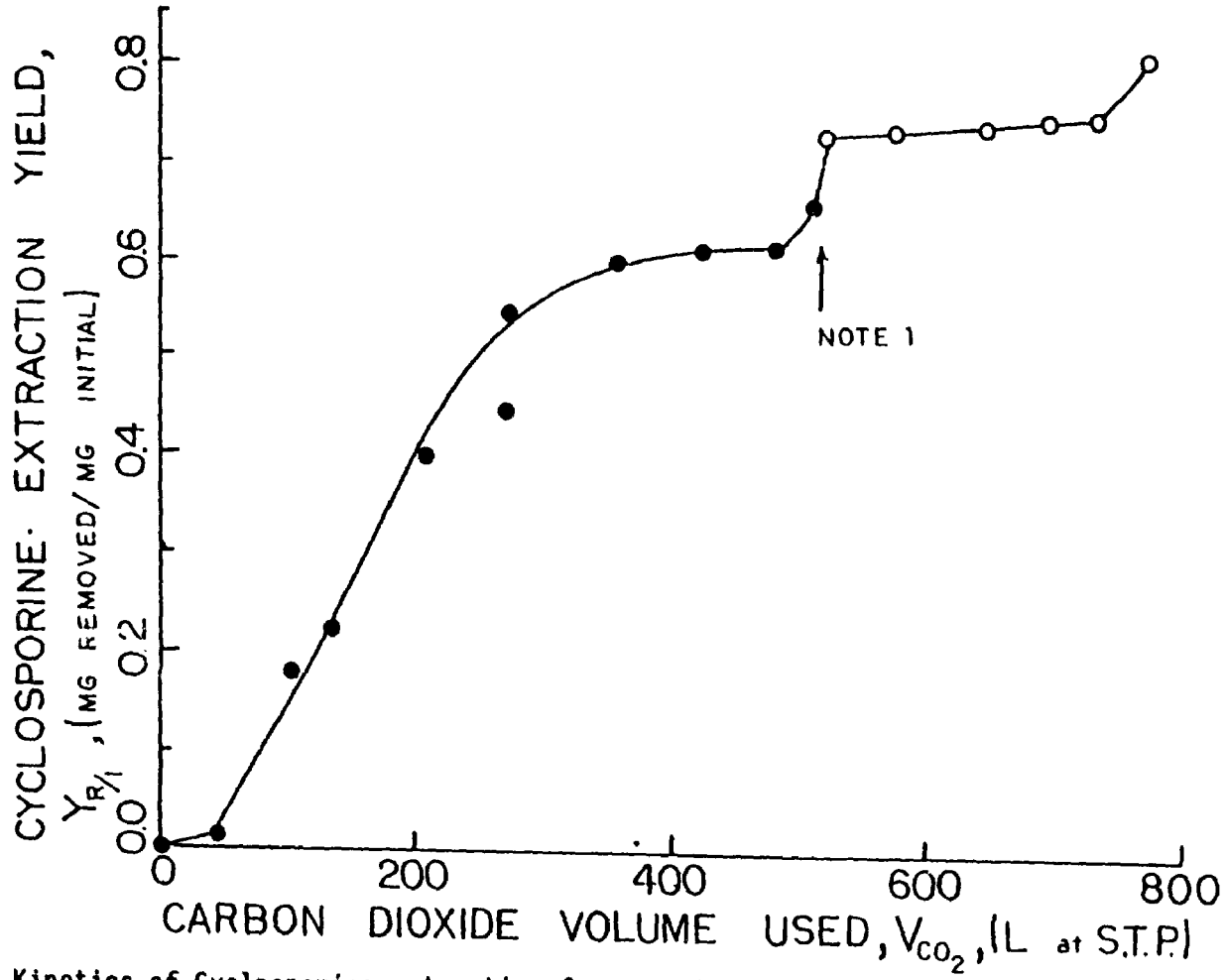



Figure 5.4 Kinetics of Cyclosporine extraction from mycelia of *Beauvaria nivea* initially containing 29.5 % moisture, at 32 MPa and 314 K (Experiment #45, ●) and at 32 MPa and 333 K (Experiment #46, O). Extraction yield of Cyclosporine versus carbon dioxide cummulative volume used. Note 1: Extraction system was depressurized between experiments. Melting of material frozen to valve occurred resulting in an apparent increase in concentration while the $CO_2$ flow was stopped.

Cyclosporine extracted from the original mycelia by 0.071 (7.1 %) from a yield of 0.594 (averaged) for experiments #39 and #40 to 0.665 at the end of experiment #44. NOTE: The total original amount of Cyclosporine in the mycelia before supercritical extraction was determined by using liquid phase methanol and/or ethyl acetate total extraction until all Cyclosporine was removed.

In experiments #45 and #46 an extraction yield of 0.82 (82 % removal of original total Cyclosporine in mycelia) was achieved with supercritical carbon dioxide. This extraction yield of 0.82 is the highest achieved in this research work. The yield of Cyclosporine extracted from mycelia as a function of carbon dioxide volume uses for experiments #45 and #46 is shown in Figure 5.4. A yield of 66 % was attained in the first extraction stage (expt. #45). The mycelial material loaded into the extraction vessel at the start of experiment #45 contained 29.5% moisture. It was partially dried by the end of experiment #45. A gradient of moisture content was found when the remaining material was segregated into top (outlet) , middle , and bottom (inlet) portions and tested for moisture content. The moisture contents were 29 % , 18 % ,and 11 % moisture for the top, middle and bottom of the extraction vessel contents as determined by weighing before and after drying in an oven at 80 °C. This dried material was then reloaded into the extractor for experiment #46 to determine if further Cyclosporine could be removed. The remaining dried mycelial material was then extracted initially at about 32 MPa and 333 K. The temperature was later shifted down to 309 K in an attempt to further improve the yield. A glance at the numerical results for experiment #46 shown in Appendix 2.5, indicates a slight recovery in the extraction rate after shifting the set point to 309 K.

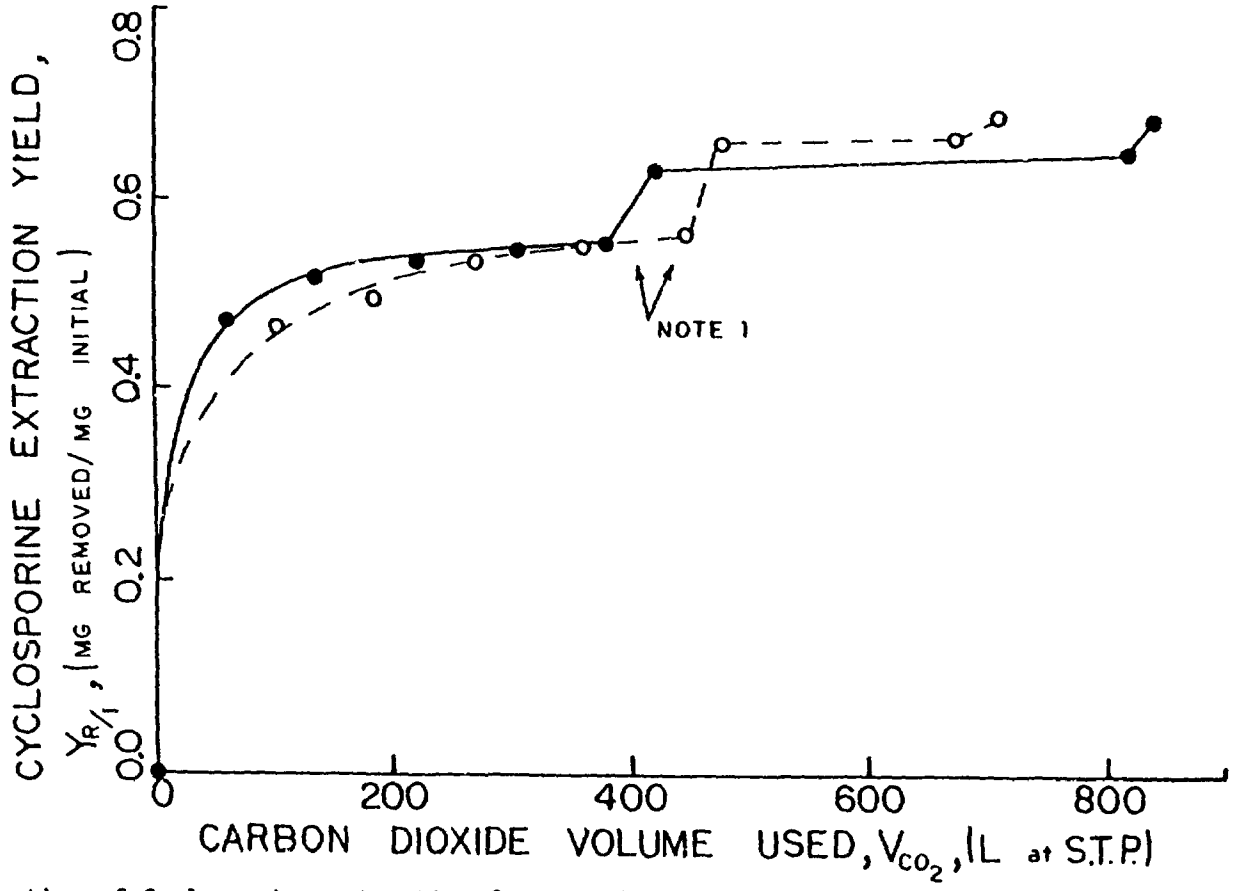



Figure 5.5 Kinetics of Cyclosporine extraction from mycelia of *Beauvaria nivea* containing 7.2 % moisture (Experiment #56, ●) and from mycelia containing 14.7 % moisture (Experiment #57, O) conducted at 32 MPa and 314 K. After ~400 L S.T.P. of cummulative carbon dioxide volume used the temperature was shifted to 327 K. Extraction yield of Cyclosporine versus carbon dioxide cummulative volume used. Note 1: Extraction system was depressurized between temperature changes. Melting of material frozen to valve occurred resulting in an apparent increase in concentration while the $CO_2$ flow was stopped.

The majority of supercritical $CO_2$ extraction yields were about 70% removal of the original total amount of Cyclosporine present in the mycelia of *B. nivea*. Examination of the experimental results shown in Table 5.1 suggested the importance of moisture content of the mycelia. Most of the higher yielding experiments had moisture present in the initial mycelia loaded into the extraction vessel. Moisture contents of 7.2, and 14.7 for experiments #56 and #57, gave equally good results. The results of these experiments are shown in Figure 5.5. The Cyclosporine extraction yields for these two experiments were quite close. It was noticed that slightly slower extraction occurred at the higher moisture content. Comparison with the extraction rate of experiment #45 shows that the higher moisture content severely decreased the extraction rates.

Experiments #56 and #57 were also conducted in a similar manner to combined experiments #45 and #46. At the apparent end of extraction at 314 K the system was depressurized to 0.1 MPa and then repressurized to 32 MPa at 327 K. Extraction then continued at the new conditions until an additional three or four hundred liters of carbon dioxide had passed through the extractor. The Cyclosporine extracted over this period was measured. The experiments were then terminated since little additional Cyclosporine appeared to have been extracted. Washing the extraction system outlet tubing and letdown valves with 20 milliliters of methanol recovered some additional Cyclosporine which originated from the mycelia and subsequently was deposited on the inside walls of the outlet tubing and valves during the extraction process. The Cyclosporine recovered in the methanol wash represented 5.6 % and 3.0 % of the total yield of Cyclosporine extracted in experiments #56 and #57 respectively.

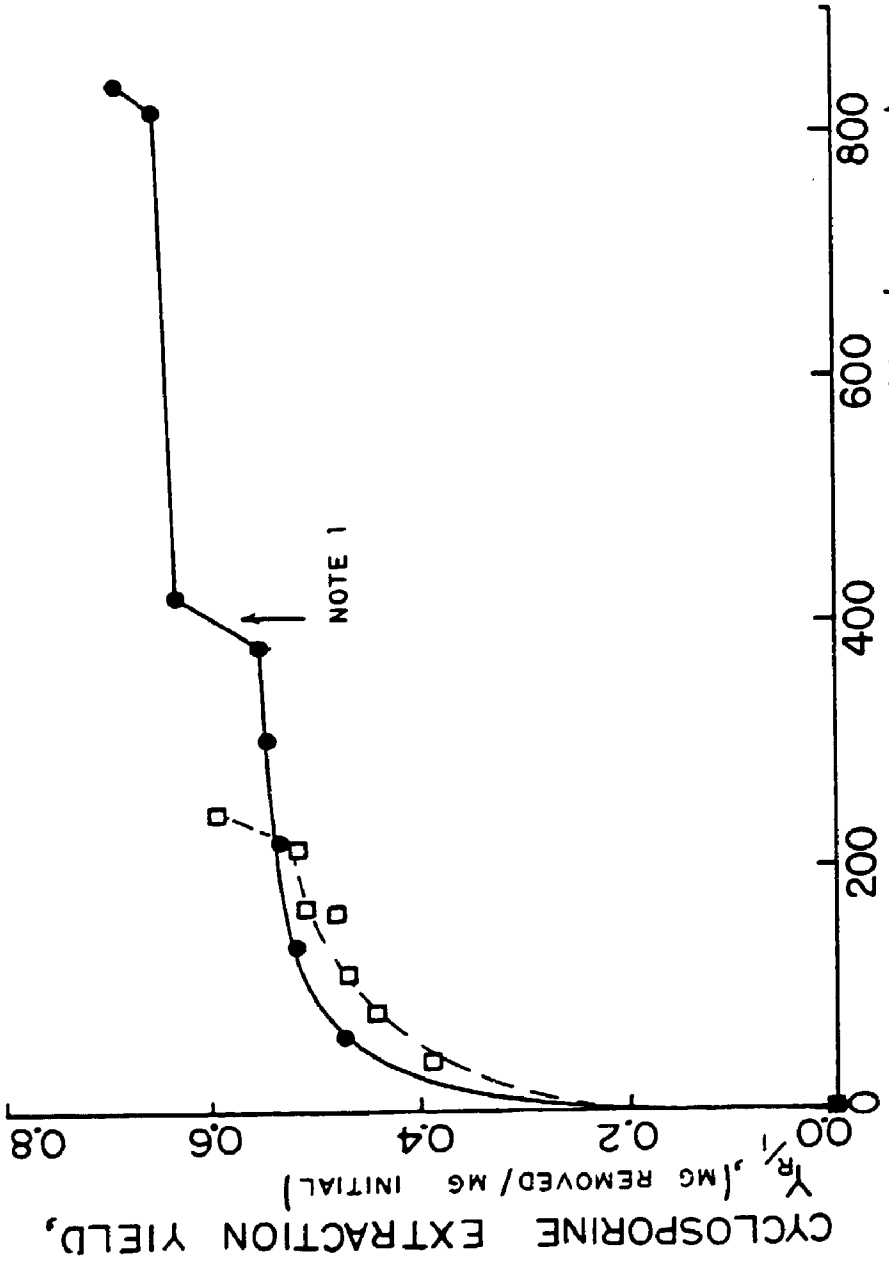



Figure 5.6 Kinetics of Cyclosporine extraction from mycelia of *Beauvaria nivea* containing 11.7 % moisture and treated with methanol (Experiment #55,□) and from non-methanol treated mycelia containing 7.2 % moisture (Experiment #56,●). The experiments were conducted at 32.5 MPa and 314.8 K. Extraction yield of Cyclosporine versus carbon dioxide cummulative volume used. Note 1: Extraction system was depressurized between temperature changes. Melting of material frozen to valve occurred resulting in an apparent increase in concentration while the $CO_2$ flow was stopped.

Experiment #55 was also done to test the effect of methanol pretreatment (30 minutes of exposure to 100% methanol followed by drying) of mycelia on the extraction of Cyclosporine. Figure 5.6 shows the results of experiment #55 compared with the results of experiment #56. The mycelia in experiment #55 had an 11.7% moisture content, while in experiment #56 they had a 7.2 % moisture. Both experiments used material from the same fermentation. The methanol treated material was not any easier to extract than the non-treated material. This can be seen by the similar yield attained by both experiments just before the time experiment #55 was terminated. Again the influence of moisture content on extraction rate should be noted.

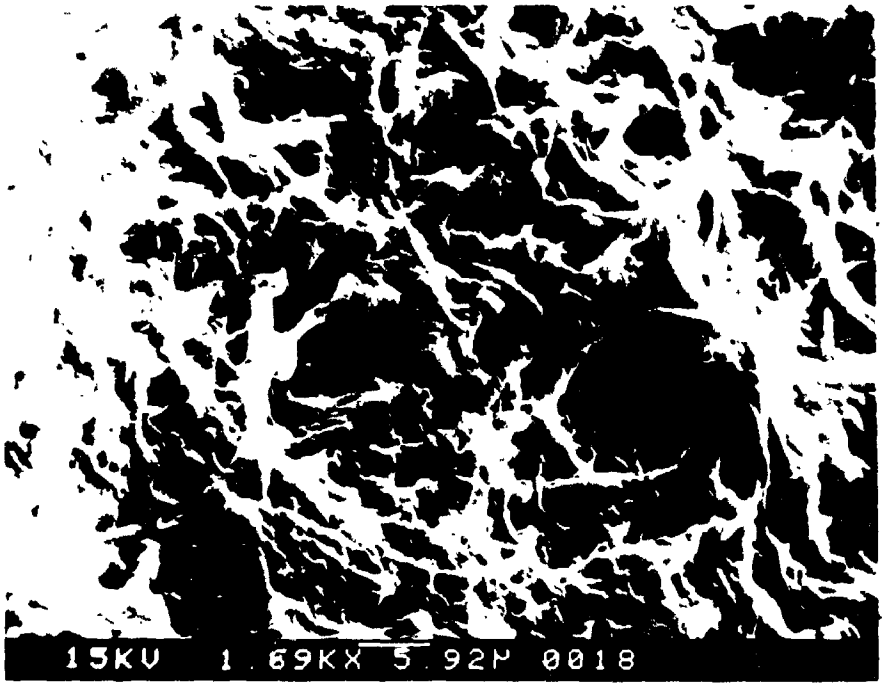
## 5.3 Supercritical Extraction of Cyclosporine Using Dried Mycelia. Experiments No. 58 to No. 73.

Completely dried mycelia were used for all the experiments reported in this section, namely, experiments #58 to #73. The much poorer extraction results of these experiments summarized in Table 5.1, show that complete drying of the mycelia biomass was counter productive. Some different drying methods of the mycelia were used in order to improve the yields of these extractions and also some pretreatment methods of the mycelia were used. Three different drying methods were used, namely, oven drying, air drying, and freeze drying of the harvested mycelia. Before drying some mycelia were autoclaved, some were freeze-thaw treated, some french pressed, and some were homogenized with a laboratory blender. A sodium hydroxide treatment was also used with limited success and methanol treatment was not very successful. Of the drying methods used, the air

drying was the most successful with Cyclosporine extraction yields of 34 % to 48 % from the mycelia.

Scanning Electron Microscopy was used in order to observe the structural changes on the mycelia due to different drying and pretreatment methods before and after extraction. The scanning electron micrographs shown in Plates 5.1 to 5.8 indicate some of the effects of the drying processes on the mycelia of *Beauvaria nivea*. Most obvious is the difference between oven dried mycelia and air or freeze dried mycelia. Plate 5.1a shows the outer exposed surface of oven dried material before grinding. The ground material is shown in Plate 5.1b. Grinding of the mycelia was done using a Wiley Mill mounted with a 0.5 mm pore screen. There are few distinct visible mycelia, and minimal porosity. The methanol-treated and oven dried material shown in Plates 5.5a,b is even more dense in appearance than the material which was just oven dried. This contrasts sharply with the appearance of the air and freeze dried mycelia outer surfaces shown in Plates 5.3a and 5.7a respectively. These Plates show individual hyphea and even conidia are visible still attached to the hyphea. There is much open area between the mycelial hyphea and the material appears much less dense than the oven dried mycelia. The ground air dried and freeze dried materials shown in Plates 5.3b and 5.7b shows many broken and open mycelia hyphea on the outer surfaces. The inner surfaces are believed to be more intact and less open. The visible contrast between oven dried mycelia and air or freeze dried mycelia offers a physical explaination for the distinct differences seen in supercritical extraction yields of these materials. The results in experiments #68 and #70 show low extraction yields which correspond to the observed dense tight structure of oven dried mycelia. It appears that this tight dense

(a)




(b)

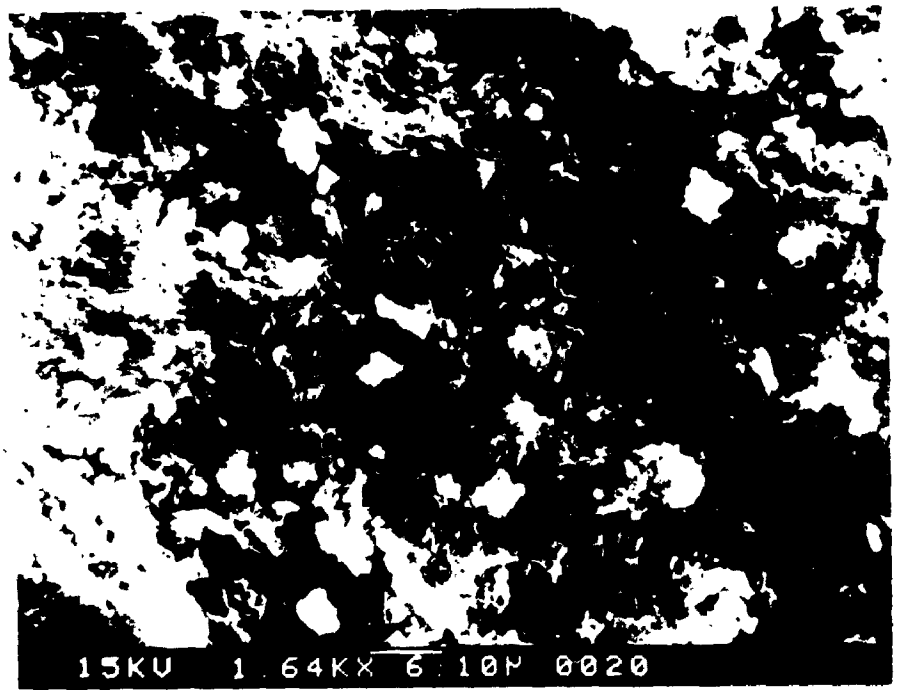
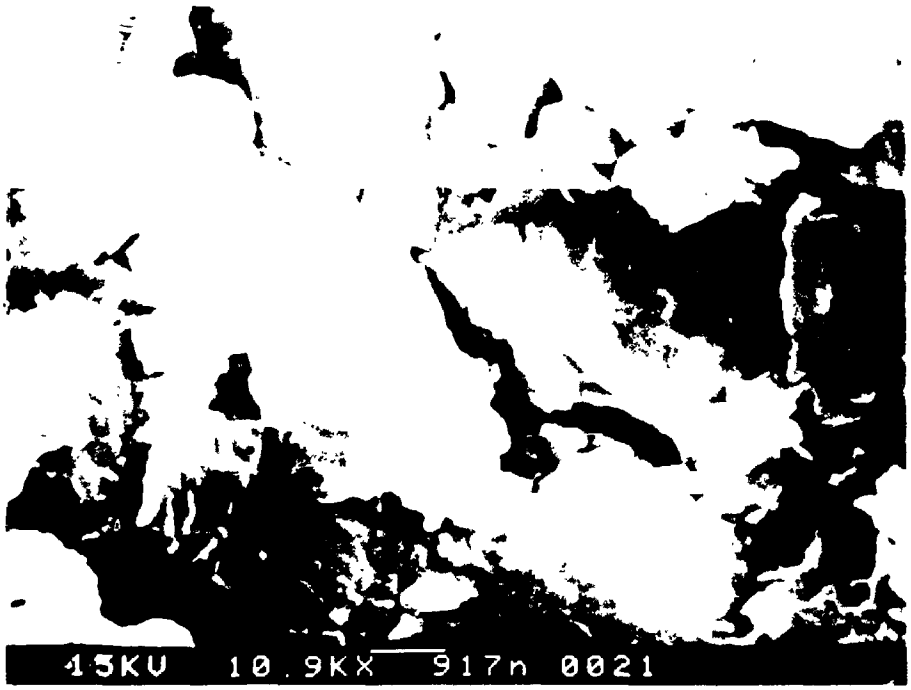



Plate 5.1 Scanning Electron Micrograph of oven dried mycelia of *Beauvaria nivea* at 1600 times magnification. a) before grinding, b) after grinding with Wiley mill ,(0.5 mm pore screen).

(a)




(b)

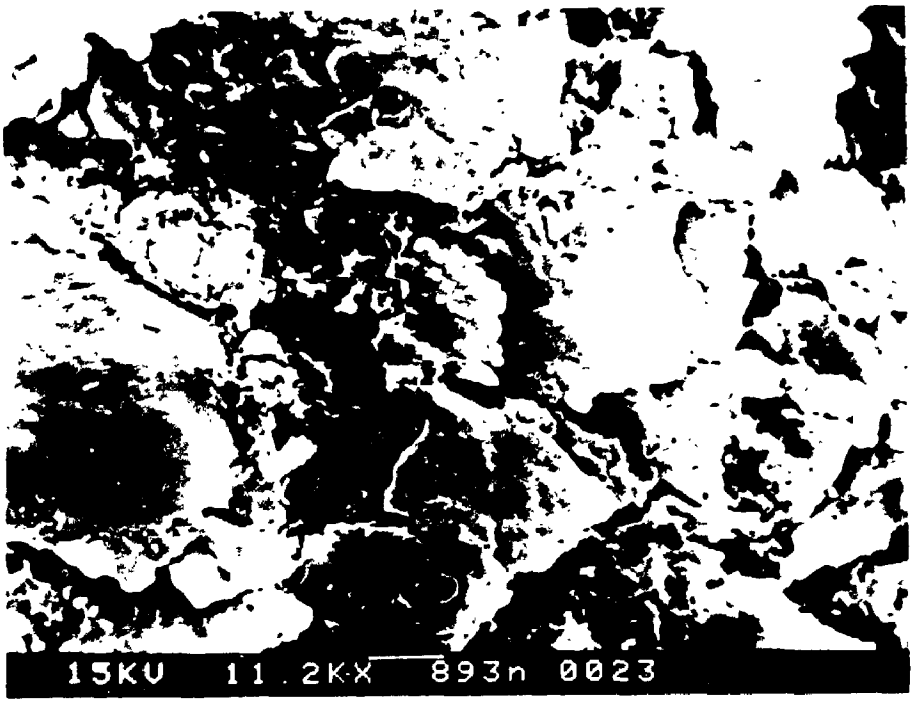
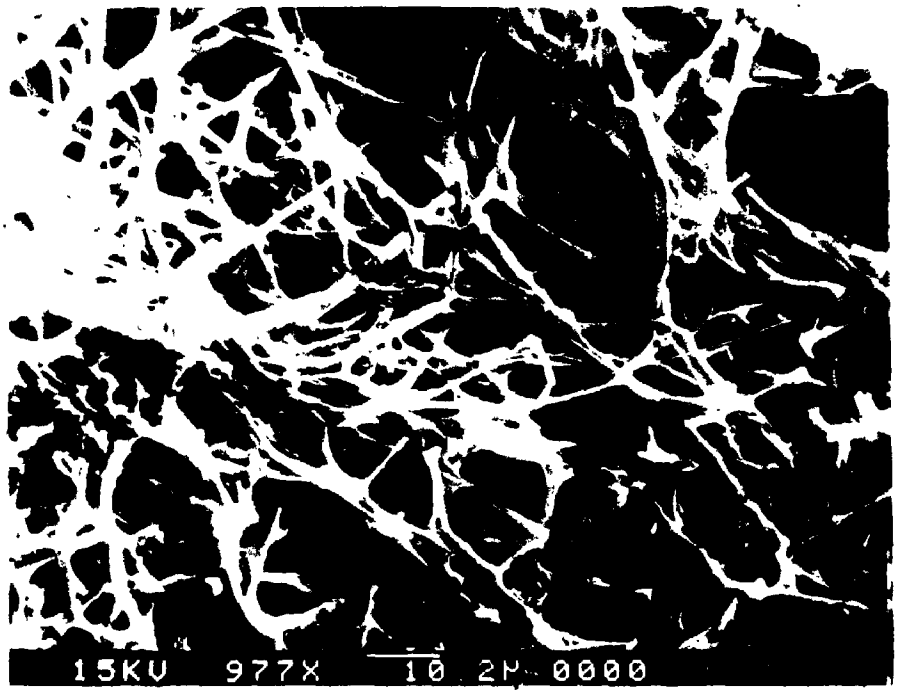



Plate 5.2 Scanning Electron Micrograph of oven dried mycelia of *B. nivea* at 11000 times magnification.
a) before extraction, b) after extraction with supercritical carbon dioxide.

(a)




(b)

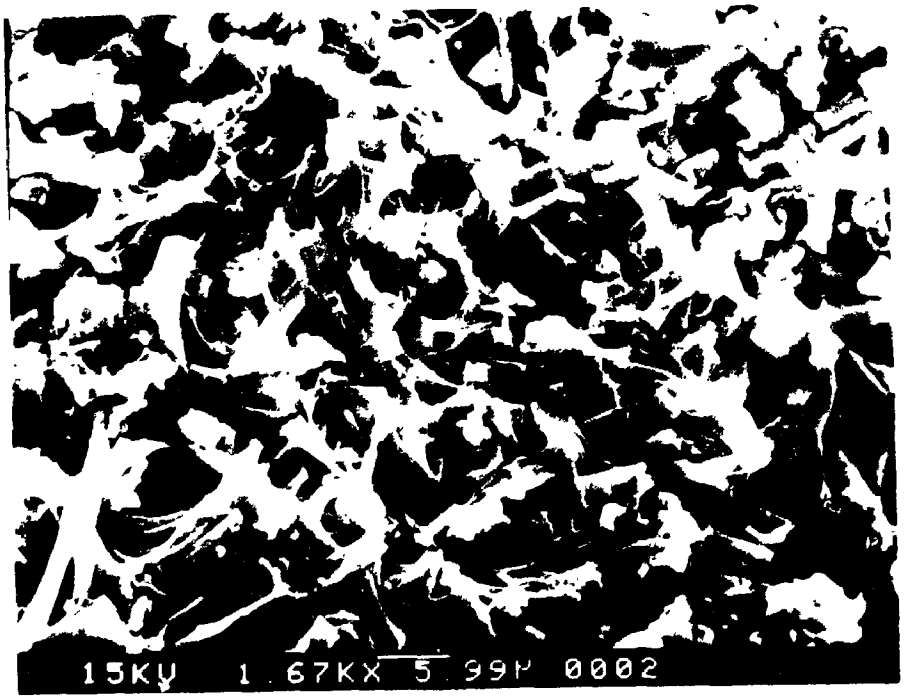


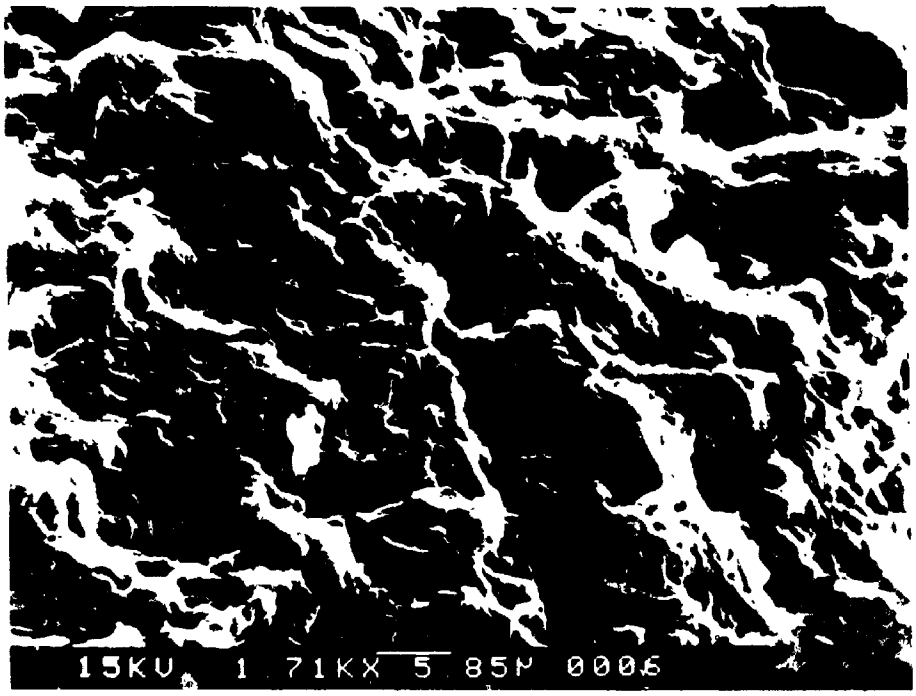

Plate 5.3 Scanning Electron Micrograph of air dried mycelia of *B. nivea*.
a) at 977 times magnification, before grinding
b) at 1600 times magnification, after grinding with Wiley mill, (0.5 mm pore screen)

(a)


(b)


Plate 5.4 Scanning Electron Micrograph of air dried mycelia of *B. nivea* at 11000 times magnification.
a) before extraction, b) after extraction with supercritical carbon dioxide

(a)




(b)

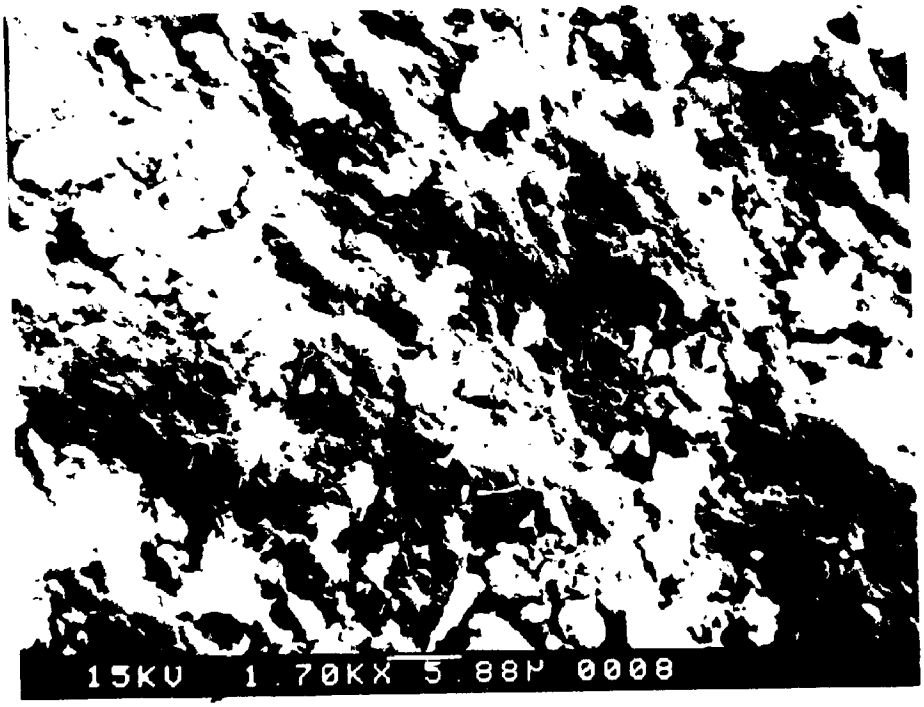
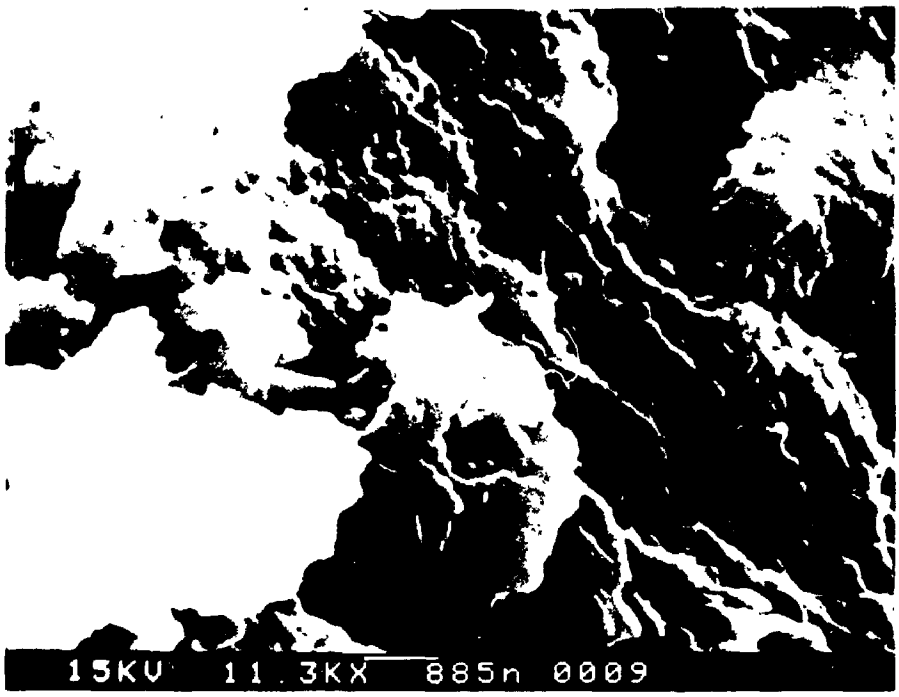



Plate 5.5 Scanning Electron Micrograph of methanol treated and oven dried mycelia of *Beauvaria nivea* at 1700 times magnification.
a) before grinding, b) after grinding with Wiley mill, (0.5 mm pore screen)

(a)




(b)

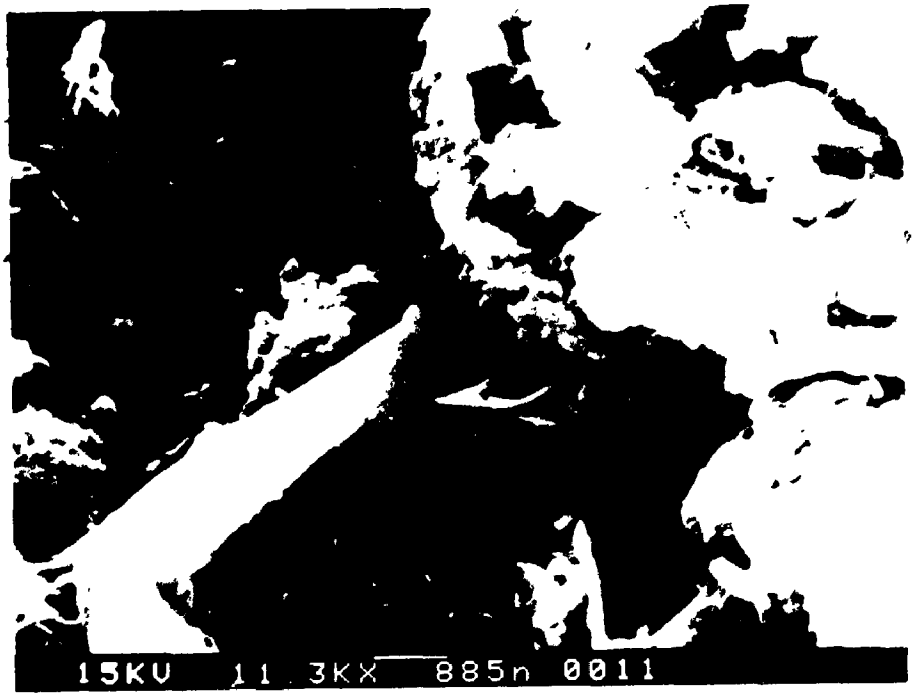
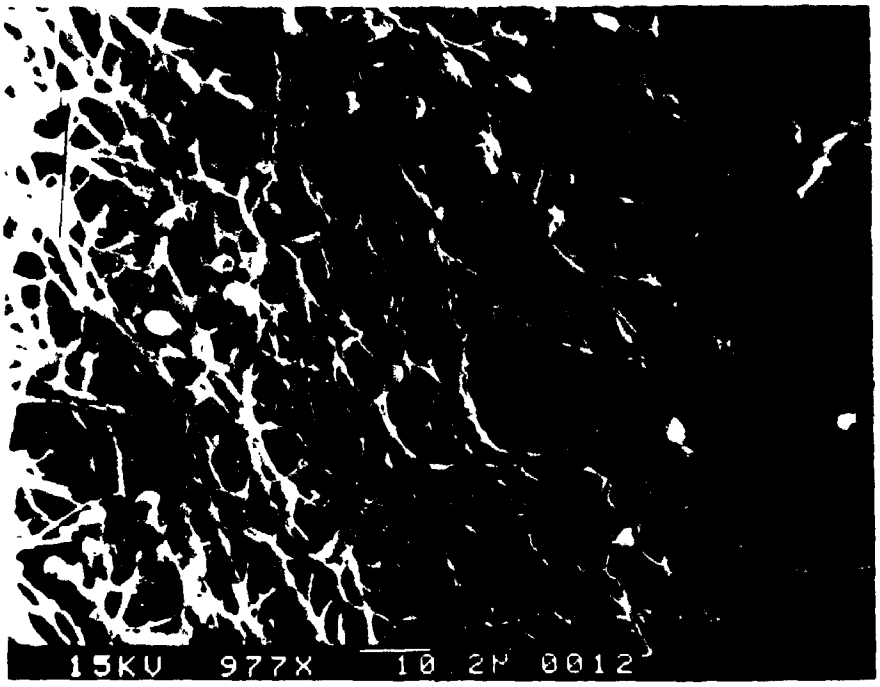



Plate 5.6 Scanning Electron Micrograph of methanol treated and oven dried mycelia of *Beauvaria nivea* at 11300 times magnification.
a) before extraction, b) after extraction with supercritical carbon dioxide

(a)




(b)

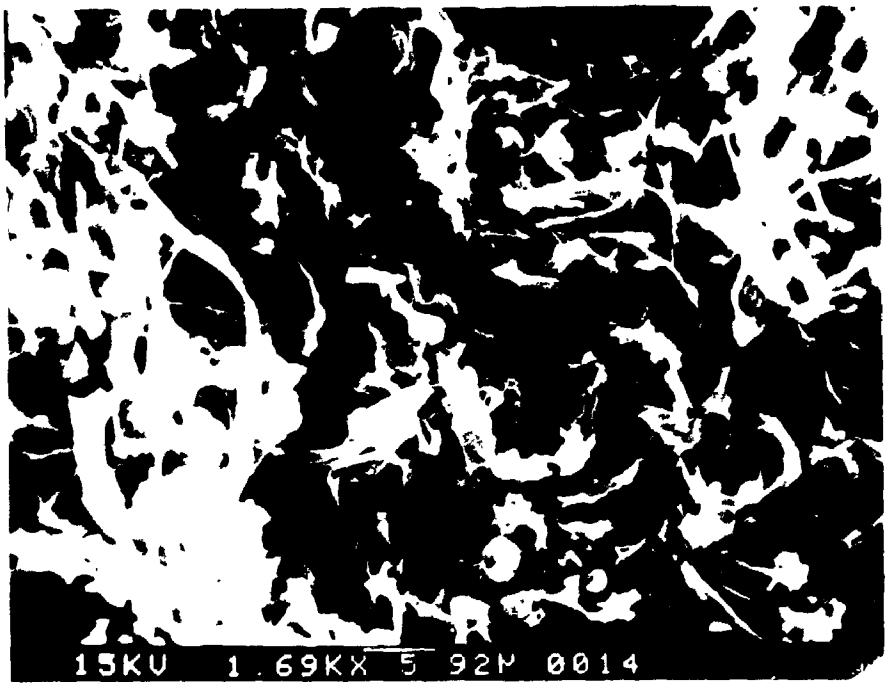



Plate 5.7 Scanning Electron Micrograph of freeze dried mycelia of *Beauvaria nivea*.
a) at 977 times magnification, before grinding
b) at 1690 times magnification, after grinding with with Wiley mill, (0.5 mm pore screen)

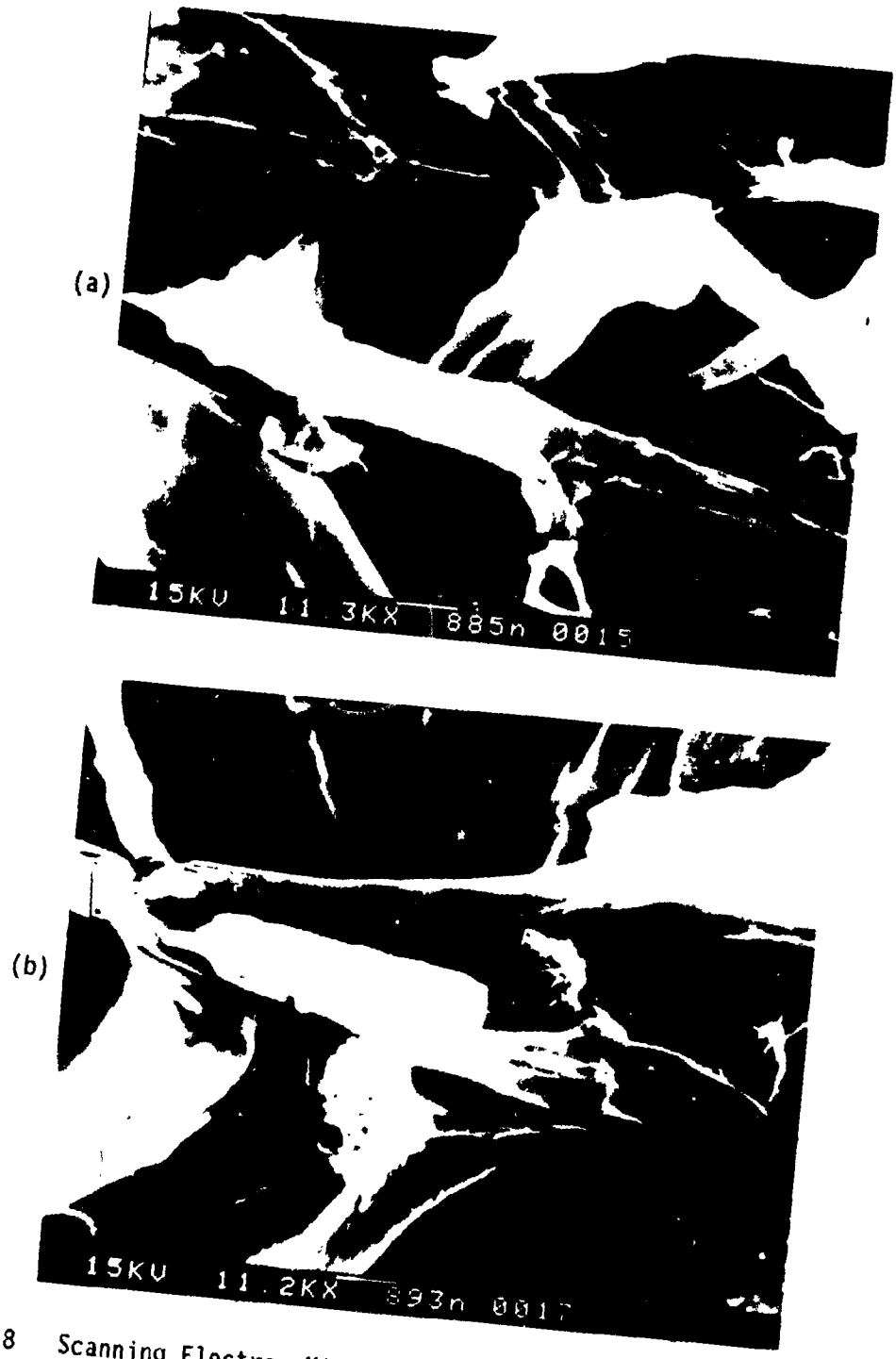



Plate 5.8 Scanning Electron Micrograph of freeze dried mycelia of *B. nivea* at 11000 times magnification. a) before extraction, b) after extraction with supercritical carbon dioxide

mycelia structure might prevent the access of supercritical $CO_2$ solvent and/or the removal of Cyclosporine from the solid mycelia. In comparison the more open and visibly broken mycelial structure of the air dried mycelia gave the highest extraction yields of Cyclosporine which is shown in the results of experiments #69 and #73. The finest grind of less than 90 $\mu$m in extraction experiment #73 using the air dried mycelia had a better yield than the course ground of 0.5 mm or less used in experiment #69. Comparison of Plates 5.3b (0.5 mm air dried material) and 5.7b (2.0 mm freeze dried material) showed the freeze dried mycelia did not have as many broken mycelial hyphea as the air dried mycelia. This could explain the poorer yields of the freeze dried extractions done in experiments #71 and #72. These extractions also used more coarsely ground mycelia due to handling difficulties. This coarsely ground material would also be expected to have fewer hypheal breaks.

Comparison of mycelia both before and after extraction with supercritical carbon dioxide was also made. Plates 5.2a and b, 5.4a and b, 5.6a and b, and 5.8a and b show the mycelia for oven dried, air dried, methanol treated - oven dried, and freeze dried, respectively. The 'a' Plate shows mycelia before supercritical extraction and the 'b' Plate shows mycelia after extraction. At the magnification level examined and treatment methods used, the scanning electron micrographs shown in Plates 5.2a and b, 5.4 a and b, 5.6a and b, and 5.8 a and b reveal no significant surface structural changes of the mycelia before and after supercritical $CO_2$ extraction of Cyclosporine.

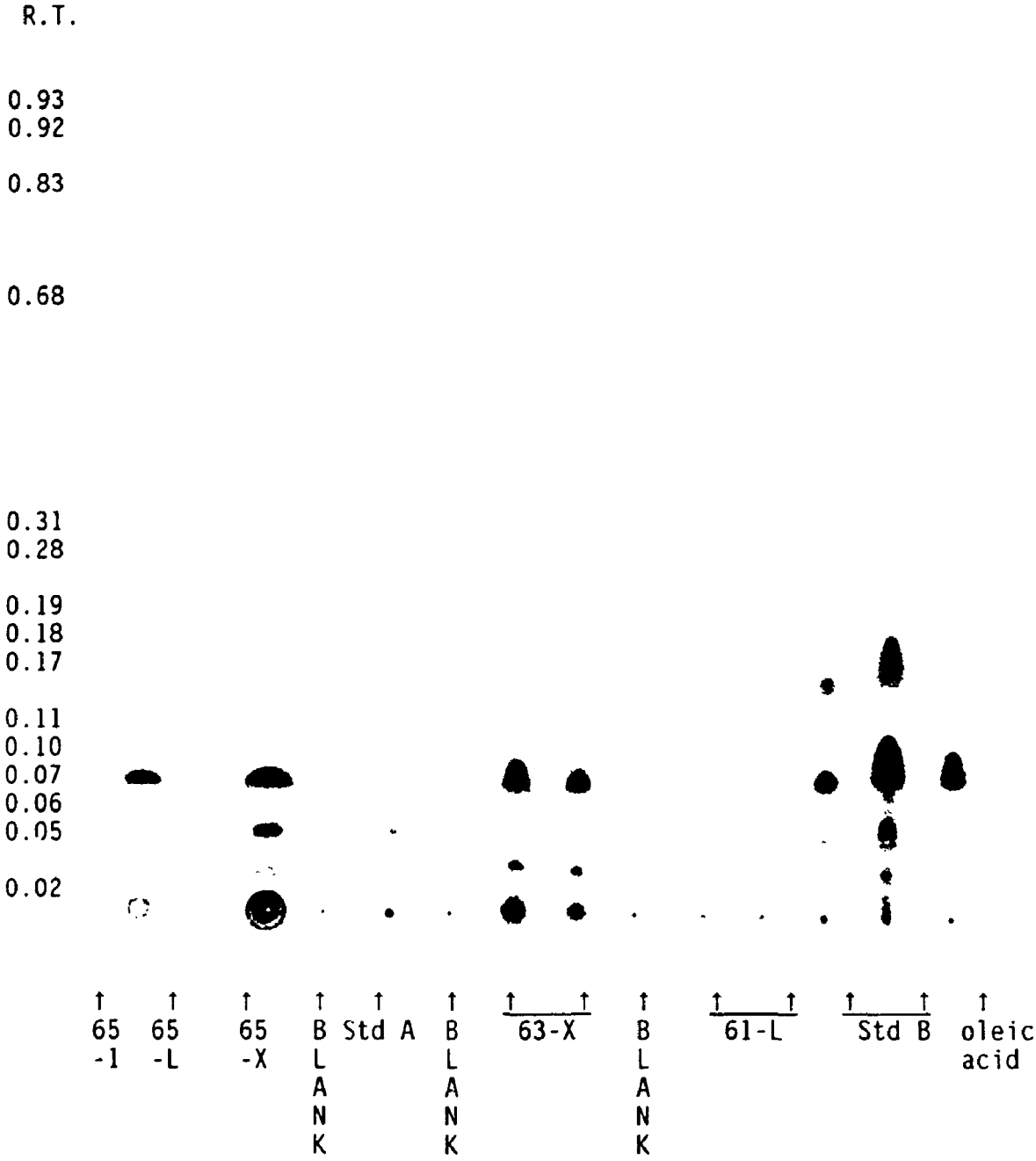



Plate 5.9 Thin Layer Chromatography plate of materials co-extracted during supercritical $CO_2$ extractions of mycelia of *B. nivea*.

Std. A
- 0.934 - Cholesterol palmitate
- 0.279 - tripalmitate
- 0.176 - palmitic acid
- 0.103 - Cholesterol

Std. B
- 0.919 - Cholesterol oleate
- 0.684 - methyl oleate
- 0.309-0.279 - triolein
- 0.165 - oleic acid
- 0.110 - Cholesterol

## 5.4 Other Materials Co-Extracted with Cyclosporine from Mycelia of *B. nivea*

During the course of extractions it was observed that a somewhat "viscous" material was also precipitated in the methanol of the separator vessel as the supercritical $CO_2$ phase bubbled through. The amounts of these precipitated materials varied between experiments. Some of the separation experiments had significant amounts of these "viscous" precipitated materials present, about 1 to 2 mL. It was suspected from their appearence and previous literature reports (Choi et al. 1987) that these might be lipids that were co-extracted from the mycelia of *B. nivea*. Thin-layer chromatography (TLC) was done in an attempt to start identifying these materials. A solvent system appropriate for lipids (Kates, 1986) was used consisting of 90 volumes petroleum ether, 10 volumes diethyl ether and 1 volume glacial acetic acid. Cyclosporine does not migrate on the plate when this solvent system is used (data not shown). The silica gel TLC plate was stained with iodine vapor.

Plate 5.9 shows typical results of a TLC plate obtained. Two standard lipid mixtures 'A' and 'B' were obtained from Supelco Ltd. The 'A' lipid mixture in elution order consisted of Cholesterol-palmitate, tripalmitate, palmitic acid, and Cholesterol. The 'B' mixture in elution order consisted of Cholesterol-oleate, methyl oleate, triolein, oleic acid, and Cholesterol. Oleic acid was also used as a standard. It is apparent that some of the material seen in the various extraction samples elutes in similar positions to those of the lipids in the standards. This is highly suggestive of lipid co-extraction with Cyclosporine. It was

felt that once the factors controling yields were known and high yields were readily attained then further study of co-extracted materials would be more worthwhile.

# CHAPTER 6

## CONCLUSIONS AND RECOMMENDATIONS

The experimental results described in this thesis have shown that it is feasible to extract Cyclosporine (Cyclosporin A) from the mycelia of *Beauvaria nivea* using supercritical carbon dioxide at different temperatures and pressures. Solubilities of pure Cyclosporine in pure supercritical carbon dioxide and in the presence of methanol as a co-solvent were also studied. Solubilities of pure Cyclosporine in the range of 16 - 20 g Cyclosporine/L supercritical $CO_2$ were achieved at temperatures 308.5 K to 343 K and pressures ranging from 8.2 MPa to 34.0 MPa. The maximum extraction yield achieved was 70 % to 80 % removal of the original amount of Cyclosporine present in the mycelia of *Beauvaria nivea* having a moisture content of 7.2 % w/w to 29.5 % w/w.

The highest rates of supercritical extraction of Cyclosporine were obtained in experiment #38 at 314.8 K and 32.5 MPa which gave a carbon dioxide density of 21.02 kmol/cubic meter. Samples taken during solubility experiments at approximately the same conditions had Cyclosporine concentrations of 18 mg CyA / mL of supercritical fluid volume at the system conditions.

The method of mycelia pretreatment before supercritical extraction was found to have an important effect on yields and rates of Cyclosporine extraction. Oven dried mycelia have shown the worst yields when extracted. Physical examination by SEM showed this material to have a completely solid non-porous surface. On the other hand the air dried material which was quite open and had many broken mycelia was more easily extracted. The best yielding material however was minimally dried having a moisture

content of 29.5 %, and it also had one of the poorest extraction rates. These data suggest that several factors may be interacting with each other to restrict the removal of some of the Cyclosporine from the mycelia microstructure as shown in Figure 2.1. In the fully oven dried material all the cell structures have collapsed together forming a dense virtually impermeable matrix which might prevent the supercritical fluid from reaching the Cyclosporine sites and/or removal of the Cyclosporine from the mycelial microstructure. The air dried material has only partially collapsed together, and the individual mycelia were not packed together. This means more area for supercritical fluid penetration to the Cyclosporine sites was available. However the collapse of internal structures interfered with complete Cyclosporine removal. When air dried material was more finely ground, resulting in more broken and open mycelia better extraction yields and rates were achieved. SEM pictures of the air dried mycelia showed few natural pores in the dried mycelial hyphea. The moist mycelia were closer to their natural state and thus all the internal structures were basically intact. The supercritical fluid was thus able to enter more areas of the mycelial hyphea than it could with fully dried material. However the presence of water decreased the rate of extraction appreciably due to the hydrophobic nature of the Cyclosporine molecule.

The following possible avenues could be studied to improve the extraction yields. Improved grinding of the air dried material to an extremely fine state could be attempted to show if a physical mass transfer barrier is present. Chemical treatment of moist mycelia could be done to open up its structure more fully to improve accessibility. A different extractor configuration such as a stirred tank could be used to attempt extraction of completely wet material. This should only be done

after view cell experiments on the carbon dioxide - water - Cyclosporine ternary system have been done to determine solubilities and the position of the phase lines. View cell experiments for the carbon dioxide - Cyclosporine system are also necessary. The carbon dioxide- water system has been studied previously by others and the relavent data are available (D'Souza et al., 1988 , Patel and Eubank, 1988).

The modeling of Cyclosporine solubility was not entirely satisfactory. Most systems in the literature have been modeled using equations of state to predict the behaviour of the solutes in the supercritical solvent. This was simply not possible here due to the absence of suitable critical constants or vapor pressure data for Cyclosporine. The large complex nature of the Cyclosporine molecule and its amino acid structure precluded using group contribution methods as most of these were designed for relatively simple hydrocarbon based molecules. Two methods to determine the critical temperature were attempted from Reid et al. (1987). Fedor's method and Joback's method but the values were far too large, well above 1000 K, and the Joback value was more than twice the Fedor value. This lack of agreement and in view of the 140 to 150 °C Cyclosporine decomposition temperature, this approach was deemed to be too uncertain for reliable results.

The only equation suitable for correlating the solubility data for pure Cyclosporine in supercritical carbon dioxide was the modified Chrastil equation suggested by del Valle and Aguilera, (1988). This equation was found to be quite good for the prediction of vegetable oil and fatty acid solubility in supercritical carbon dioxide. This correlation was found to be reasonably good for the Cyclosporine solubility data produced in this study. A simple linearization of the

solubility data over a limited range versus the reduced density of the supercritical solvent was successful, and the constants found for this linearization could also be predicted using a linear equation form.

In conclusion, the supercritical extraction of Cyclosporine from the mycelia of *Beauvaria nivea* is certainly feasible. However much work needs to be done to improve the yields, and extraction rates of this new process. The solubility of Cyclosporine in supercritical carbon dioxide over a wide range of conditions has been demonstrated. The addition of methanol as a co-solvent has also been shown to enhance the solubility of Cyclosporine in supercritical carbon dioxide.

# APPENDIX 1

## PHYSICAL DATA PERTAINING TO THE SUPERCRITICAL EXTRACTION SYSTEM

# Appendix 1.1 Sample Loop Calibrations

## Appendix 1.1 Sample Loop Calibrations

1. 20 uL loop

   a) using Merium Instruments Fluid no. 3

   empty loop was weighed to be 6.6977 g

   three times the 20 uL loop was filled with Merium Instruments Fluid No 3, S.G.= 2.95 and weighed.

   6.7591 g, 6.7616 g , 6.7708 g -> ave = 6.7638 g
   - 6.6977 g tare
   ---
   0.0661 g fluid

   $$\frac{0.0661 \text{ g fluid}}{2.95 \text{ s.g.} \times 0.9982 \text{ g/mL}} = 0.0224 \text{ mL or } 1.1224 \times 20 \text{ uL}$$

   b) using methanol

   twice the loop was filled with methanol and weighed

   6.7187 g, 6.7207 g -> ave = 6.7197 g
   - 6.6977 g tare
   ---
   0.0220 g methanol

   $$\frac{0.0220 \text{ g methanol}}{0.7917 \text{ g/mL methanol}} = 0.0278 \text{ mL or } 1.3894 \times 20 \text{ uL}$$

   c) 0.020 inch I.D. tubing was used, length required for 20 uL

   is $$\frac{0.020 \text{ mL} \times 4}{\pi \left( 0.020 \text{ in I.D.} \times 2.54 \text{ cm/in} \right)^2} = 9.868 \text{ cm}$$

   measured length was 9.6 cm or 0.9728 x 9.868 cm -> 19.46 $\mu$L

The variation in calibration was such that the nominal value of 20 $\mu$L was used for the 0.5 %v/v methanol co-solvent experiment and the corresponding experiment without co-solvent only.

2. 0.5 mL loop calibration

a) using Merium Instruments Fluid No 3

Three times the 0.5 mL loop was filled with fluid, S.G. = 2.95 and weighed.

20.5776 g, 20.6003 g, 20.5542 g -> ave = 20.5774 g
- 19.0990 g tare
---
1.4784 g fluid

$$\frac{1.4784 \text{ g fluid}}{2.95 \times 0.9982} = 0.502 \text{ mL or } 1.004 \times 0.5 \text{ mL}$$

b) using methanol

twice the loop was filled with methanol and weighed

19.5038 g, 19.4957 g -> ave = 19.4998 g
- 19.0990 g tare
---
0.4008 g methanol

$$\frac{0.4008 \text{ g methanol}}{0.7917 \text{ g/mL}} = 0.5062 \text{ mL or } 1.012 \times 0.5 \text{ mL}$$

c) The 0.5 mL loop was constructed of 0.030 inch I.D. Stainless Steel tubing 109.6 cm long (with an error of 0.1 cm) This gave a calculated volume of 0.4999 ± 0.0004 mL The nominal volume of 0.5 mL was considered acceptable and was used for solubility studies at all temperatures and pressures.

# Appendix 1.2 Pressure and Temperature Measurement Accuracy

## Appendix 1.2 Pressure and Temperature Measurement Accuracy.

The main pressure gauge used was obtained from Manobourdon model VV22716 with ± 0.5 % accuracy @ 20 °C. The guage measurement range was 0 to 10,000 psig.

The experimental temperature was measured using an Omega 410B Digicator reading in degrees Fahrenheit combined with SIGSS-062U-12 thermocouples.
The digital read out gave temperatures ± 0.1 °F these were converted to degrees Kelvin later. The quoted instrument accuracy was ± 0.6 °F.

## Appendix 1.3 Calculation of Internal Volumes of the Supercritical Extraction Systems

Appendix 1.3 Calculation of Internal Volumes of the Supercritical Extraction Systems

a./ Large Volume Solubility System:

0.25 inch O.D. by 0.035 inch wall tubing lengths (cm.),

4.0, 4.0, 2.54, 6.0, 9.0, 9.0, 14.0, 5.5, and five of 3.0 cm lengths

Total = 69.04 cm

0.25 in. - 2 x 0.035 in. = 0.180 in. I.D. x 2.54 cm/in. = 0.4572 cm

length tubing required for 1 mL of volume is,

$$1.0\ cm^3 / ((\pi \times 0.4572^2) / 4.0) = 6.0911\ cm$$

therefore

69.04/6.0911 = 11.335 mL of volume in 0.25 inch tubing.

0.125 inch O.D. by 0.035 inch wall tubing lengths (cm)
3.5, 18.5, and 91.44 cm , Total = 113.44 cm

0.125 in. - 2 X 0.035 in. = 0.055 in I.D. x 2.54 cm/in. = 0.1397 cm length tubing required for 1 mL of volume is,

$$1.0\ cm^3 / (( \pi \times 0.1397^2) / 4.0 ) = 65.2405\ cm$$

therefore

113.44/ 65.2405 = 1.7388 mL in 0.125 inch O.D. tubing

Extractor tube, 0.688 inch I.D. by 6 inches long

$$( \pi \times (0.688 \times 2.54)^2 / 4.0 ) \times 6.0 \times 2.54 = 36.553\ mL$$

Extractor end fitting volumes including quick connect fittings, were determined experimentally with methanol.

The volumes were: 6.5 mL for the inlet, and 3.5 mL for the outlet of the extractor

The following commercial fittings were also used:

| | | internal volume (mL) | subtotal |
|---|---|---|---|
| from Gyrolok: | 2 x 4C-316 | 0.679 | 1.357 |
| | 2 x 4RU1-316 | 0.015 | 0.030 |
| | 2 x 4RU2-316 | 0.051 | 0.102 |
| | 1R4-316 | 0.330 | 0.330 |
| | 3 x 4TTT-316 | 0.509 | 1.527 |
| | 2TTT-316 | 0.138 | 0.138 |
| | 4A8-316 | 0.500 | 0.500 |
| from Swagelok: | 2 x QF4-S-400 | 0.362 | 0.724 |
| | SS-5354 | 4.054 | 4.054 |
| | | | 8.733 |

| | Totals |
|---|---|
| 0.25 inch tubing | 11.335 |
| 0.125 inch tubing | 1.7388 |
| extractor tube | 36.553 |
| extractor fittings top | 3.50 |
| extractor fittings bottom | 6.40 |
| commercial fittings | 8.733 |
| pressure gauge | 33.0 |
| Large Extractor System Volume = | 103.3 mL |

b./ Small Extractor System Volume

Extractor tube => 12 inches of 0.25 inch O.D., 0.035 inch wall, Stainless Steel ATSM 213 tubing

(12 in x 2.54 cm/in)/6.0911cm/mL = 5.004 mL

Total length of 0.125 inch O.D., 0.035 inch wall tubing = 37.5 inches

(37.5 x 2.54) cm / 65.2405 cm/mL = 1.46 mL

Total length of 0.0625 inch O.D., 0.030 I.D. stainless tubing = 22 inches

$(22 \times 2.54)\ \text{cm} \times \left( (\pi \times (0.030 \times 2.54)^2) / 4 \right)$
$= 0.255\ \text{mL}$

The following commercial fittings were used,

| | | internal volume (mL) | subtotal |
|---|---|---|---|
| from Gyrolok: | 2 x 4TTT-316 | 0.509 | 1.018 |
| | 2TTT-316 | 0.138 | 0.138 |
| | 1TTT-316 | 0.050 | 0.050 |
| | 4RU2-316 | 0.051 | 0.051 |
| | 4RU1-316 | 0.015 | 0.015 |
| | | | 1.272 mL |

Pressure gauge shut off valve inlet volume = 0.5 mL

| Totals |
|---|
| 5.004 |
| 1.460 |
| 0.255 |
| 1.272 |
| 0.500 |
| 8.49 mL |

Appendix 1.4 Sample Extractor Oven Temperature Profiles

Appendix 1.4 Sample Extractor Oven Temperature Profiles

Experiment # 26 (partial) (solubility study)

| sample | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9a | T9b |
|---|---|---|---|---|---|---|---|---|---|
| 26-22 | 146.6 | 151.5 | 158.8 | 158.8 | 160.7 | 161.2 | 154.1 | 161.4 | 159.9 |
| 26-23 | 152.9 | 151.9 | 158.4 | 158.6 | 160.3 | 161.4 | 155.6 | 161.4 | 159.6 |
| 26-24 | 153.4 | 152.1 | 158.5 | 158.8 | 160.4 | 161.4 | 155.6 | 161.5 | 159.6 |
| 26-25 | 153.6 | 152.2 | 158.6 | 158.9 | 160.4 | 161.6 | 155.4 | 161.5 | 159.6 |
| 26-26 | 153.9 | 152.3 | 158.6 | 158.8 | 160.4 | 161.6 | 155.2 | 161.6 | 159.9 |

where the temperatures are in degrees Farhenheit

T2 pump body

T3 six way valve outlet

T4 extractor inlet

T5 extractor body

T6 extractor outlet

T7 loop surface

T8 six way valve body

T9a heater outlet T.C.

T9b heater outlet R.T.D.

Appendix 1.4 Sample Extractor Oven Temperatu Profiles (cont.)

Experiment # 38 (early extractions, Six way valve in place )

| sample | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9a | T9b | Tlet |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 38-1 | 21.6 | 106.0 | 107.6 | 106.7 | 106.8 | 107.6 | 107.7 | 107.4 | 108.1 | 105.4 | 122 |
| 38-2 | 21.5 | 106.3 | 107.8 | 107.0 | 106.9 | 107.8 | 107.9 | 107.4 | 108.4 | 105.7 | 122 |
| 38-3 | 22.5 | 106.4 | 107.9 | 107.2 | 107 1 | 107.9 | 108.1 | 107.9 | 108.4 | 105.7 | 121 |
| 38-4 | 23.0 | 106.5 | 108.0 | 107.2 | 107.3 | 108.0 | 108.1 | 107.9 | 108.3 | 105.7 | 121 |
| 38-5 | 23.4 | 106.5 | 108.0 | 107.3 | 107.1 | 108.1 | 108.4 | 108.1 | 108.6 | 105.8 | 122 |
| 38-6 | 24.0 | 106.4 | 108.0 | 107.3 | 107.2 | 108.1 | 108.3 | 108.0 | 108.4 | 105.6 | 122 |
| 38-7 | 24.5 | 106.2 | 107.8 | 107.2 | 107.1 | 108.0 | 108.0 | 107.9 | 108.4 | 105.6 | 122 |
| 38-8 | 28.0 | 105.4 | 107.3 | 107.2 | 107.1 | 107.8 | 107.9 | 107.8 | 108.3 | 105.5 | 100 |

where the temperatures are in degrees Farhe eit

T1 pump inlet

T2 pump outlet

T3 six way valve outlet

T4 extractor inlet

T5 extractor body

T6 extractor outlet

T7 loop surface

T8 six way valve body

T9a heater outlet T.C.

T9b heater outlet R.T.D.

Tlet letdown valve

Appendix 1.4 Sample Extractor Oven Temperature Profiles (cont.)

Experiment # 54 (with streamlined piping without six way valve )

| sample | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9a | T9b | Tlet |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 54-1 | 33.8 | 102.8 | 104.9 | 104.5 | 104.5 | 105.6 | 105.2 | 104.7 | 106.0 | 103.3 | 79 |
| 54-2 | 34.8 | 103.4 | 105.8 | 105.3 | 105.2 | 106.0 | 106.5 | 105.4 | 106.7 | 104.1 | 89 |
| 54-3 | 35.2 | 104.3 | 106.1 | 105.5 | 105.4 | 106.2 | 106.7 | 105.7 | 106.9 | 104.3 | 95 |
| 54-4 | 35.4 | 104.5 | 106.1 | 105.6 | 105.4 | 106.5 | 106.8 | 105.8 | 107.0 | 104.3 | 98 |
| 54-5 | 35.4 | 104.6 | 106.2 | 105.6 | 105.5 | 106.5 | 106.8 | 105.8 | 106.8 | 104.4 | 100 |

where the temperatures are in degrees Farhenheit

T1 pump inlet
T2 pump outlet
T3 lower oven
T4 extractor inlet
T5 extractor body
T6 extractor outlet
T7 upper oven
T8 mid oven
T9a heater outlet T.C.
T9b heater outlet R.T.D.
Tlet letdown valve

# Appendix 1.5 Calculation of Cyclosporine Crystal Packed Volume

## Appendix 1.5 Calculation of Cyclosporine Crystal Packed Volume

0.1724 g of Cyclosporine crystals were packed into a 9 mm deep by 6 mm diameter cavity (0.25447 mL volume). This gave a packed density of 0.6775 g Cyclosporine / mL.

## APPENDIX 2

## EXPERIMENTAL DATA

# Appendix 2.1 Cyclosporine Solubility Data Summaries

Appendix 2.1 Cyclosporine Solubility Data Summaries

Data summary for 308.9 K CyA solubility studies

source files:

a)data2021 c)data2801 d)data2326 e)data2622

Data:

| file | temp | CO2 pres | CO2 red.den. | CyA conc. | CyA mole frac. |
|---|---|---|---|---|---|
| a | 307.44 | 9.84-0.14 | 1.539 | 0.609-0.166 | 3.11-0.85e-5 |
| a | 308.09 | 15.03-0.05 | 1.752 | 1.91-0.21 | 8.58-0.95e-5 |
| a | 308.5 | 21.68-0.06 | 1.884 | 6.94-2.21 | 2.89-0.92e-4 |
| a | 309.04 | 27.34-0.0 | 1.957 | 11.89-1.07 | 4.77-0.43e-4 |
| a | 309.37 | 32.97-0.20 | 2.016 | 16.63-0.17 | 6.47-0.07e-4 |
| c | 310.17 | 9.00-0.0 | 1.309 | 0.210-0.108 | 1.26-0.65e-5 |
| c | 310.13 | 10.12-0.04 | 1.481 | 0.247-0.043 | 1.31-0.23e-5 |
| d | 309.04 | 16.30-0.08 | 1.772 | 2.52-0.25 | 1.12-0.11e-4 |
| e | 308.84 | 8.72-0.0 | 1.327 | 0.120-0.018 | 7.12-1.06e-6 |
| e | 308.82 | 10.13-0.04 | 1.523 | 0.329-0.019 | 1.70-0.10e-5 |

Appendix 2.1 Cyclosporine Solubility Data Summaries

Data summary for 314.0 K CyA solubility studies

source files:

a)data2801 b)data7_14 d)data2622 e)data2326 (f)data3100)
g)data284w

Data:

| file | temp | CO2 pres | CO2 red.den. | CyA conc. | CyA mole frac. |
|---|---|---|---|---|---|
| a | 314.08 | 9.32-0.02 | 1.114 | 0.0559-0.0211 | 3.94-1.49e-6 |
| a | 314.11 | 10.17-0.0 | 1.336 | 0.150-0.016 | 8.81-0.92e-6 |
| a | 314.28 | 15.27-0.0 | 1.668 | 0.971-0.030 | 4.57-0.14e-5 |
| a | 314.5 | 21.43-0.07 | 1.816 | 3.35-0.07 | 1.45-0.03e-4 |
| a | 314.56 | 26.81-0.16 | 1.901 | 10.44-0.54 | 4.31-0.22e-4 |
| g | 314.56 | 26.81-0.16 | 1.901 | 11.35-0.39 | 4.68-0.16e-4 |
| a | 314.69 | 33.92-0.20 | 1.985 | 16.36-0.24 | 6.47-0.10e-4 |
| g | 314.68 | 33.94-0.20 | 1.985 | 19.76-0.73 | 7.81-0.29e-4 |
| b | 315.91 | 33.31-0.20 | 1.969 | 36.48-1.12 | 1.45-0.04e-3 |
| b | 313.82 | 24.21-0.27 | 1.870 | 9.07-0.32 | 3.81-0.13e-4 |
| b | 314.28 | 16.20-0.15 | 1.697 | 1.76-0.47 | 8.15-2.16e-5 |
| b | 314.00 | 8.565-0.06 | 0.748 | 0.0698-0.0122 | 7.33-1.28e-6 |
| d | 314.09 | 10.13-0.04 | 1.331 | 0.119-0.008 | 7.05-0.49e-6 |
| e | 313.79 | 18.58-0.08 | 1.766 | 3.03-0.45 | 1.35-0.20e-4 |
| f | 313.54 | 15.79-0.0 | 1.696 | 2.84-0.16 | 1.32-0.08e-4 |
| f | 313.87 | 27.34 | 1.915 | 16.22 | 6.65e-4 |
| f | 313.96 | 30.92-0.0 | 1.958 | 23.77-0.23 | 9.53-0.09e-4 |
| f | 313.98 | 34.44-0.0 | 1.995 | 33.30-1.43 | 1.31-0.06e-3 |

Appendix 2.1 Cyclosporine Solubility Data Summaries

Data summary for 314.0 K CyA solubility studies with methanol

source files:

a)data280w 2.90% methanol b)data3111 4.71% methanol
c)data2851
d)data2849

Data:

| file | temp | CO2 pres | CO2 red.den. | CyA conc. | CyA mole frac. |
|---|---|---|---|---|---|
| a | 314.09 | 9.478-0.0 | 1.174 | 5.57-0.68 | 3.73-0.45e-4 |
| c | 314.09 | 9.478-0.0 | 1.174 | 5.58 | 3.72e-4 |
| d | 314.12 | 9.478-0.0 | 1.172 | 2.85-0.09 | 1.91-0.06e-4 |
| c | 314.04 | 10.23-0.02 | 1.348 | 6.75-0.35 | 3.93-0.20e-4 |
| a | 314.09 | 15.27-0.0 | 1.671 | 20.26-0.13 | 9.51-0.06e-4 |
| c | 314.11 | 15.29-0.04 | 1.671 | 20.39-1.08 | 9.57-0.50e-4 |
| a | 314.08 | 22.41-0.08 | 1.838 | 49.07-1.19 | 2.09-0.05e-3 |
| c | 314.08 | 22.41-0.08 | 1.838 | 61.55-2.90 | 2.62-0.12e-3 |
| a | 314.15 | 26.95-0.04 | 1.907 | 74.76-5.50 | 3.07-0.23e-3 |
| c | 314.15 | 26.95-0.04 | 1.907 | 72.45-1.99 | 2.97-0.08e-3 |
| a | 314.26 | 34.51-0.0 | 1.994 | 75.01-1.25 | 2.95-0.05e-3 |
| c | 314.26 | 34.51-0.0 | 1.994 | 75.36-2.07 | 2.96-0.08e-3 |
| b | 314.02 | 18.56-0.02 | 1.762 | 16.23-0.28 | 7.23-0.12e-4 |
| b | 314.09 | 22.07-0.08 | 1.832 | 27.37-0.98 | 1.17-0.04e-3 |
| b | 314.15 | 26.60-0.04 | 1.902 | 46.75-0.74 | 1.93-0.03e-3 |
| b | 314.22 | 30.89-0.06 | 1.955 | 74.52-1.04 | 2.98-0.04e-3 |
| b | 314.21 | 34.38-0.09 | 1.993 | 102.5-1.0 | 4.02-0.04e-3 |

Appendix 2.1 Cyclosporine Solubility Data Summaries

Data summary for 319.0 K CyA solubility studies

source files:
a)data2021 c)data2801 e)data2326 f)data7_14

Data:

| file | temp | CO2 pres | CO2 red.den. | CyA conc. | CyA mole frac. |
|---|---|---|---|---|---|
| a | 318.50 | 10.19-0.04 | 1.110 | 0.0442-0.0115 | 3.13-0.81e-6 |
| a | 319.04 | 14.96-0.12 | 1.577 | 0.541-0.090 | 2.69-0.45e-5 |
| a | 319.11 | 20.98-0.14 | 1.756 | 2.30-0.21 | 1.03-0.10e-4 |
| a | 319.11 | 26.92-0.21 | 1.861 | 5.63-1.22 | 2.37-0.51e-4 |
| a | 319.22 | 33.37-0.30 | 1.943 | 15.07-1.17 | 6.08-0.47e-4 |
| c | 319.16 | 9.62-0.0 | 0.881 | 0.0195-0.023 | 1.74-2.04e-6 |
| c | 319.24 | 10.17-0.0 | 1.061 | 0.0398-0.0074 | 2.94-0.55e-6 |
| e | 319.09 | 20.91-0.06 | 1.755 | 3.57-0.27 | 1.60-0.12e-4 |
| f | 318.04 | 5.858 | 0.293 | 0.102 | 2.72e-5 |
| f | 318.04 | 8.789 | 0.673 | 0.00685 | 7.98e-7 |
| f | 317.77 | 15.48-0.09 | 1.620 | 0.961-0.092 | 4.66-0.44e-5 |
| f | 319.02 | 25.67-0.26 | 1.843 | 4.31-0.37 | 1.83-0.16e-4 |
| f | 318.16 | 32.17-0.16 | 1.938 | 10.35-0.84 | 4.19-0.34e-4 |

Appendix 2.1 Cyclosporine Solubility Data Summaries

Data summary for 324.5 K CyA solubility studies

source files:
a) data2221 f)data2801
g) data2326

Data:

| file | temp | CO2 pres | CO2 red.den. | CyA conc. | CyA mole frac. |
|---|---|---|---|---|---|
| a | 324.13 | 27.61-0.0 | 1.826 | 8.70-2.74 | 3.74-1.18e-4 |
| a | 324.24 | 33.75-0.11 | 1.908 | 16.23-1.02 | 6.67-0.42e-4 |
| a | 324.34 | 9.89-0.0 | 0.764 | 0.078-0.054 | 8.04-5.54e-6 |
| a | 324.72 | 14.98-0.09 | 1.473 | 0.452-0.059 | 2.41-0.32e-5 |
| a | 324.69 | 21.30-0.15 | 1.699 | 1.84-0.32 | 8.51-1.48e-5 |
| a | 324.67 | 26.46-0.14 | 1.802 | 5.24-0.76 | 2.28-0.33e-4 |
| f | 324.44 | 9.41-0.0 | 0.663 | 0.0061-0.0043 | 7.26-5.17e-7 |
| f | 324.11 | 10.17-0.0 | 0.836 | 0.0084-0.0024 | 7.93-2.29e-7 |
| g | 324.22 | 23.10-0.06 | 1.744 | 4.76-0.96 | 2.14-0.43e-4 |

Appendix 2.1 Cyclosporine Solubility Data Summaries

Data summary for 335.0 K CyA solubility studies

source files:
a)data2122 c)data2326 d)data2801

Data:

| file | temp | CO2 pres | CO2 red.den. | CyA conc. | CyA mole frac. |
|---|---|---|---|---|---|
| a | 334.72 | 10.17-0.0 | 0.625 | 0.0309-0.0182 | 3.89-2.29e-6 |
| a | 334.61 | 15.27-0.0 | 1.290 | 0.136-0.018 | 8.27-1.12e-6 |
| a | 334.80 | 21.82-0.0 | 1.591 | 1.33-0.25 | 6.57-1.24e-5 |
| a | 334.91 | 26.85-0.06 | 1.710 | 4.16-0.70 | 1.91-0.32e-4 |
| a | 334.93 | 33.61-0.11 | 1.820 | 13.88-2.50 | 5.[illegible] 1.08e-4 |
| c | 334.15 | 27.34-0.0 | 1.726 | 5.23-0.75 | 2.38-0.34e-4 |
| d | 334.51 | 8.69-0.04 | 0.465 | 0.0116-0.0059 | 1.96-1.00e-6 |
| d | 334.37 | 10.17-0.0 | 0.629 | 0.0236-0.0111 | 2.95-1.39e-6 |

Appendix 2.1 Cyclosporine Solubility Data Summaries

Data summary for 343.0 K CyA solubility studies

source files:
a) data2326 e)data2622 f)data2801

Data:

| file | temp | CO2 pres | CO2 red.den. | CyA conc. | CyA mole frac. |
|---|---|---|---|---|---|
| a | 343.27 | 9.80-0.16 | 0.513 | 0.259-0.048 | 3.97-0.73e-5 |
| a | 342.93 | 14.86-0.06 | 1.079 | 0.172-0.058 | 1.25-0.42e-5 |
| a | 343.11 | 21.52-0.26 | 1.477 | 0.605-0.134 | 3.22-0.71e-5 |
| a | 342.33 | 25.96-0.0 | 1.615 | 4.43-0.34 | 2.15-0.17e-4 |
| a | 342.63 | 32.51-0.0 | 1.740 | 9.92-1.21 | 4.48-0.54e-4 |
| e | 342.87 | 34.53-0.04 | 1.769 | 15.83-0.16 | 7.02-0.07e-4 |
| e | 343.43 | 10.17-0.0 | 0.545 | 0.0024-0.0003 | 3.44-0.38e-7 |
| e | 343.48 | 10.10 | 0.538 | 0.088-0.014 | 1.29-0.20e-5 |
| f | 343.11 | 9.41-0.0 | 0.480 | 0.0233-0.0032 | 3.80-0.52e-6 |
| f | 342.99 | 10.17 | 0.548 | 0.0129-0.0096 | 1.85-1.37e-6 |

Appendix 2.2 Cyclosporine Solubility Data Sources

Appendix 2.2 (cont.)  Data7_14  file

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | injection vol. (ul) | SCE loop vol. (ml) |
|---|---|---|---|
| 04/08/88 | 1.3690e-08 | 20.0 | 1.0 |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 13-10 | 07/28/88 | 20:24 | 4800 | 108.70 | 5171000 | 10.00 |
| 13-9 | 07/28/88 | 19:48 | 4800 | 109.00 | 5322000 | 10.00 |
| 13-8 | 07/28/88 | 19:10 | 4850 | 109.20 | 5497000 | 10.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 33.311 | 315.91 | 0.047963 | 20.849 | 36.483850 | 1.4530e-03 |
| 0.199 | 0.14 | (red.den.= 1.96877) | | 1.116742 | 4.4474e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 13-7 | 07/28/88 | 12:09 | 3460 | 105.00 | 1266000 | 10.00 |
| 13-6 | 07/28/88 | 11:31 | 3475 | 105.10 | 1319500 | 10.00 |
| 13-5 | 07/28/88 | 10:54 | 3500 | 105.50 | 1335000 | 10.00 |
| 13-4 | 07/28/88 | 10:10 | 3550 | 105.20 | 1379000 | 10.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 24.207 | 313.82 | 0.050486 | 19.807 | 9.068769 | 3.8057e-04 |
| 0.272 | 0.12 | (red.den.= 1.87038) | | 0.319283 | 1.3399e-05 |

Appendix 2.2 (cont.) Data7_14 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 13-3 | 07/28/88 | 9:32 | 2310 | 105.50 | 254200 | 10.00 |
| 13-2 | 07/27/88 | 8:52 | 2345 | 106.10 | 269500 | 10.00 |
| 13-1 | 07/27/88 | 8:11 | 2350 | 106.50 | 402800 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/ml CO2) | molefrac |
|---|---|---|---|---|---|
| 16.201 | 314.28 | 0.055636 | 17.974 | 1.761637 | 8.1493e-05 |
| 0.150 | 0.28 | (red.den.= 1.69725) | | 0.466237 | 2.1568e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 12-7 | 07/22/88 | 14:22 | 1240 | 105.80 | 9000 | 10.00 |
| 12-6 | 07/22/88 | 13:15 | 1220 | 105.60 | 12760 | 10.00 |
| 12-5 | 07/22/88 | 12:27 | 1225 | 105.50 | 10040 | 10.00 |
| 12-4 | 07/22/88 | 11:27 | 1225 | 105.20 | 9000 | 10.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/ml CO2) | molefrac |
|---|---|---|---|---|---|
| 8.565 | 314.00 | 0.126268 | 7.920 | 0.069819 | 7.3307e-06 |
| 0.060 | 0.14 | (red.den.= 0.74784) | | 0.012155 | 1.2762e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 10-6 | 07/21/88 | 9:18 | 1850 | 104.30 | 12450 | 10.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/ml CO2) | molefrac |
|---|---|---|---|---|---|
| 12.857 | 313.32 | 0.059523 | 16.800 | 0.085220 | 4.2180e-06 |
| 0.000 | 0.00 | (red.den.= 1.58642) | | 0.000000 | 0.0000e+00 |

Appendix 2.2 (cont.) Data7_14 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 9-3 | 07/19/88 | 12:18 | 4400 | 122.10 | 1907000 | 5.00 |
| 9-2 | 06/09/88 | 9:27 | 4500 | 123.20 | 1942000 | 5.00 |
| 9-1 | 06/09/88 | 14:46 | 4475 | 123.10 | 6792000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 30.840 | 323.59 | 0.050312 | 19.876 | 12.139608 | 5.0762e-04 |
| 0.359 | 0.34 | (red.den.= 1.87685) | | 9.618275 | 4.0219e-04 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 14-1 | 08/03/88 | 10:23 | 835 | 112.80 | 74220 | 2.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 5.858 | 318.04 | 0.321777 | 3.108 | 0.101607 | 2.7186e-05 |
| 0.000 | 0.00 | (red.den.= 0.29346) | | 0.000000 | 0.0000e+00 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 14-7 | 08/03/88 | 21:17 | 1260 | 112.80 | 5000 | 2.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 8.789 | 318.04 | 0.140229 | 7.131 | 0.006845 | 7.9816e-07 |
| 0.000 | 0.00 | (red.den.= 0.67339) | | 0.000000 | 0.0000e+00 |

Appendix 2.2 (cont.)  Data7_14  file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 14-9 | 08/04/88 | 11:05 | 2220 | 112.80 | 123900 | 10.00 |
| 14-10 | 08/04/88 | 11:47 | 2250 | 112.40 | 135000 | 10.00 |
| 14-11 | 12:33 | 08/04 | 2225 | 112.10 | 150900 | 10.00 |
| 14-12 | 08/04/88 | 1:08 | 2225 | 112.00 | 151700 | 10.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 15.477 | 317.77 | 0.058305 | 17.151 | 0.960867 | 4.6583e-05 |
| 0.093 | 0.20 | (red.den.= 1.61958) | | 0.091780 | 4.4495e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 14-13 | 08/04/88 | 23:20 | 3750 | 114.90 | 669600 | 10.00 |
| 14-14 | 08/04/88 | 12:07 | 3700 | 114.60 | 568300 | 10.00 |
| 14-15 | 08/05/88 | 00:34 | 3675 | 114.20 | 649400 | 10.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 25.669 | 319.02 | 0.051233 | 19.519 | 4.306190 | 1.8342e-04 |
| 0.263 | 0.20 | (red.den.= 1.84311) | | 0.366990 | 1.5632e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 14-16 | 08/05/88 | 10:47 | 4680 | 112.80 | 1644000 | 10.00 |
| 14-17 | 08/05/88 | 11:38 | 4625 | 112.90 | 1352000 | 10.00 |
| 14-18 | 08/05/88 | 12:08 | 4650 | 113.10 | 1502000 | 10.00 |
| 14-19 | 08/05/88 | 13:07 | 4650 | 113.30 | 1553000 | 10.00 |

Appendix 2.2 (cont.)  Data7_14  file

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 32.170 | 318.16 | 0.048722 | 20.525 | 10.354774 | 4.1933e-04 |
| 0.155 | 0.12 | (red.den.= 1.93813) | | 0.836498 | 3.3875e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 7-1 | 06/06/88 | 12:12 | 3900 | 120.70 | 2560000 | 2.00 |
| 7-2 | 06/06/88 | 14:21 | 3975 | 121.00 | 2198000 | 2.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 27.249 | 322.51 | 0.051460 | 19.433 | 3.256851 | 1.3934e-04 |
| 0.366 | 0.12 | (red.den.= 1.83500) | | 0.350427 | 1.4993e-05 |

Appendix 2.2 Data1517 file

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | injection vol. (ul) | SCE loop vol. (ml) |
|---|---|---|---|
| 08/18/88 | 8.2004e-09 | 50.0 | 1.0 |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 15-1 | 8/10/88 | 9:05 | 1200 | 124.70 | 61420 | 5.00 |
| 15-2 | 8/10/88 | 9:56 | 1190 | 124.00 | 18470 | 5.00 |
| 15-3 | 8/10/88 | 10:34 | 1175 | 124.30 | 4000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 8.295 | 324.45 | 0.190751 | 5.242 | 0.022931 | 3.6372e-06 |
| 0.087 | 0.20 | (red.den.= 0.49504) | | 0.024490 | 3.8844e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 15-5 | 8/10/88 | 22:14 | 1660 | 124.80 | 68900 | 5.00 |
| 15-6 | 8/10/88 | 22:52 | 1675 | 125.30 | 9190 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 11.598 | 324.84 | 0.083277 | 12.008 | 0.032018 | 2.2172e-06 |
| 0.073 | 0.20 | (red.den.= 1.13391) | | 0.034623 | 2.3976e-06 |

Appendix 2.2 Data1517 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 16-0 | 8/12/88 | 8:55 | 3675 | 128.50 | 2908000 | 10.00 |
| 16-1 | 8/12/88 | 9:39 | 3600 | 128.20 | 2210000 | 10.00 |
| 16-2 | 8/12/88 | 10:22 | 3550 | 128.00 | 1871000 | 10.00 |
| 16-2 | 8/12/88 | 10:22 | 3550 | 128.00 | 1791000 | 10.00 |
| 16-3 | 8/12/88 | 11:07 | 3525 | 128.00 | 5133500 | 10.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 24.784 | 326.56 | 0.053865 | 18.565 | 4.563851 | 2.0438e-04 |
| 0.412 | 0.12 | (red.den.= 1.75306) | | 2.273244 | 1.0180e-04 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 17-2 | 8/15/88 | 21:47 | 2120 | 127.80 | 35630 | 10.00 |
| 17-2 | 8/15/88 | 21:47 | 2120 | 127.80 | 35460 | 10.00 |
| 17-3 | 8/15/88 | 22:31 | 2075 | 127.50 | 45080 | 10.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 14.615 | 326.32 | 0.066589 | 15.018 | 0.064056 | 3.5468e-06 |
| 0.179 | 0.10 | (red.den.= 1.41808) | | 0.008609 | 4.7668e-07 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 17-4 | 8/16/88 | 9:33 | 4500 | 127.90 | 7022000 | 10.00 |
| 17-5 | 8/16/88 | 10:41 | 4400 | 127.60 | 6831000 | 10.00 |
| 17-6 | 8/16/88 | 11:26 | 4375 | 127.50 | 6908000 | 10.00 |

Calculated results:

Appendix 2.2 Data1517 file

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 30.611 | 326.30 | 0.051023 | 19.599 | 11.349900 | 4.8131e-04 |
| 0.456 | 0.12 | (red.den.= 1.85072) | | 0.157604 | 6.6834e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 17-9 | 8/16/88 | 22:58 | 3475 | 117.50 | 2511000 | 10.00 |
| 17-10 | 8/16/88 | 23:35 | 3470 | 117.40 | 2556000 | 10.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 24.043 | 320.62 | 0.052462 | 19.061 | 4.155143 | 1.8123e-04 |
| 0.024 | 0.04 | (red.den.= 1.79994) | | 0.052187 | 2.2762e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 17-1 | 8/15/88 | 21:05 | 2150 | 128.00 | 100700 | 10.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 14.925 | 326.48 | 0.065825 | 15.192 | 0.165156 | 9.0399e-06 |
| 0.000 | 0.00 | (red.den.= 1.43453) | | 0.000000 | 0.0000e+00 |

Appendix 2.2 Data2021 file

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | injection vol. (ul) | SCE loop vol (ml) |
|---|---|---|---|
| 13/09/88 | 8.1662e-09 | 50.0 | 0.5 |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 21-1 | 9/12/88 | 9:33 | 1430 | 93.70 | 221500 | 5.00 |
| 21-1 | 9/12/88 | 9:58 | 1430 | 93.60 | 402000 | 5.00 |
| 21-2 | 9-12-88 | 9:58 | 1400 | 93.60 | 433400 | 5.00 |
| 21-3 | 9/12/88 | 10:24 | 1390 | 94.00 | 434000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 9.840 | 307.44 | 0.061343 | 16.302 | 0.608747 | 3.1050e-05 |
| 0.142 | 0.11 | (red.den.= 1.53935) | | 0.166457 | 8.4905e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 21-4 | 9/12/88 | 10:59 | 2160 | 94.80 | 1081000 | 5.00 |
| 21-6 | 9/12/88 | 12:45 | 2170 | 95.00 | 1264000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 15.028 | 308.09 | 0.053893 | 18.555 | 1.914967 | 8.5810e-05 |
| 0.049 | 0.08 | (red.den.= 1.75215) | | 0.211341 | 9.4702e-06 |

Appendix 2.2 Data2021 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 21-7 | 9/12/88 | 1:27 | 3140 | 95.50 | 3098000 | 5.00 |
| 21-8 | 9/12/88 | 1:56 | 3125 | 95.50 | 3905000 | 5.00 |
| 21-9 | 9/12/88 | 2:28 | 3125 | 95.90 | 5744000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/ml CO2) | molefrac |
|---|---|---|---|---|---|
| 21.682 | 308.50 | 0.050132 | 19.947 | 6.939611 | 2.8920e-04 |
| 0.060 | 0.15 | (red.den.= 1.88361) | | 2.214873 | 9.2303e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 21-10 | 9/12/88 | 3:07 | 3950 | 96.60 | 7280000 | 5.00 |
| 21-11 | 9/12/88 | 3:36 | 3950 | 96.60 | 7936000 | 5.00 |
| 21-12 | 9/12/88 | 4:02 | 3950 | 96.60 | 6624000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/ml CO2) | molefrac |
|---|---|---|---|---|---|
| 27.336 | 309.04 | 0.048252 | 20.725 | 11.889944 | 4.7683e-04 |
| 0.000 | 0.00 | (red.den.= 1.95701) | | 1.071402 | 4.2967e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 21-13 | 9/12/88 | 4:36 | 4800 | 97.10 | 10220000 | 5.00 |
| 21-14 | 9/12/88 | 4:58 | 4750 | 97.10 | 10060000 | 5.00 |
| 21-15 | 9/12/88 | 5:27 | 4750 | 97.40 | 10260000 | 5.00 |

Appendix 2.2 Data2021 file

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 32.966 | 309.37 | 0.046839 | 21.350 | 10.626322 | 6.4715e-04 |
| 0.199 | 0.10 | (red.den.= 2.01602) | | 0.172845 | 6.7277e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 20-1 | 9/07/88 | 10:07 | 1470 | 113.40 | 35150 | 5.00 |
| 20-2 | 9/07/88 | 10:32 | 1460 | 113.60 | 22160 | 5.00 |
| 20-3 | 9/07/88 | 11:05 | 1460 | 113.90 | 23970 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 10.191 | 318.50 | 0.085082 | 11.753 | 0.044250 | 3.1306e-06 |
| 0.040 | 0.14 | (red.den.= 1.10986) | | 0.011491 | 8.1296e-07 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 20-4 | 9/07/88 | 11:42 | 2175 | 114.90 | 280000 | 5.00 |
| 20-5 | 9/07/88 | 12:07 | 2150 | 114.50 | 324100 | 5.00 |
| 20-6 | 9/07/88 | 12:34 | 2140 | 114.40 | 389400 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 14.959 | 319.04 | 0.059871 | 16.703 | 0.540873 | 2.6926e-05 |
| 0.124 | 0.15 | (red.den.= 1.57721) | | 0.089895 | 4.4753e-06 |

Appendix 2.2 Data2021 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 20-7 | 9/07/88 | 1:06 | 3050 | 115.00 | 1271000 | 5.00 |
| 20-8 | 9/07/88 | 1:33 | 3025 | 114.70 | 1416000 | 5.00 |
| 20-9 | 9/07/88 | 2:03 | 3010 | 114.50 | 1531000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 20.981 | 319.11 | 0.053773 | 18.597 | 2.296327 | 1.0267e-04 |
| 0.139 | 0.14 | (red.den.= 1.75605) | | 0.212791 | 9.5138e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 20-10 | 9/07/88 | 2:44 | 3925 | 114.80 | 4308000 | 5.00 |
| 20-11 | 9/07/88 | 3:15 | 3875 | 114.60 | 3030000 | 5.00 |
| 20-12 | 9/07/88 | 3:45 | 3870 | 114.80 | 3001000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 26.922 | 319.11 | 0.050730 | 19.712 | 5.628669 | 2.3738e-04 |
| 0.210 | 0.06 | (red.den.= 1.86140) | | 1.218990 | 5.1409e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 20-13 | 9/07/88 | 4:16 | 4875 | 115.10 | 8460000 | 5.00 |
| 20-14 | 9/07/88 | 4:44 | 4800 | 114.80 | 9332000 | 5.00 |
| 20-15 | 9/07/88 | 5:10 | 4800 | 114.90 | 9882000 | 5.00 |

Appendix 2.2 Data2021 file

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 33.368 | 319.22 | 0.048590 | 20.580 | 15.066039 | 6.0836e-04 |
| 0.299 | 0.08 | (red.den.= 1.94337) | | 1.171111 | 4.7289e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 18-1 | 8/23/88 | 8:44 | 1400 | 114.80 | 148700 | 5.00 |
| 18-2 | 8/23/88 | 9:09 | 1400 | 114.80 | 38550 | 5.00 |
| 18-3 | 8/23/88 | 10:00 | 1400 | 114.80 | 251100 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 9.754 | 319.15 | 0.101609 | 9.842 | 0.238643 | 2.0163e-05 |
| 0.000 | 0.00 | (red.den.= 0.92933) | | 0.173610 | 1.4668e-05 |

Appendix 2.2 Data2122 file

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | injection vol. (ul) | SCE loop vol. (ml) |
|---|---|---|---|
| 20/09/88 | 8.3529e-09 | 50.0 | 0.5 |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 22-1 | 9/19/88 | 9:51 | 1460 | 142.70 | 8062 | 5.00 |
| 22-1 | 9/19/88 | 9:51 | 1460 | 142.70 | 14460 | 5.00 |
| 22-2 | 9/19/88 | 10:17 | 1460 | 142.90 | 10250 | 5.00 |
| 22-2 | 9/19/88 | 10:17 | 1460 | 142.90 | 13820 | 5.00 |
| 22-3 | 9/19/88 | 10:47 | 1460 | 142.90 | 33460 | 5.00 |
| 22-3 | 19/09/88 | 10:47 | 1460 | 142.90 | 31060 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 10.138 | 334.72 | 0.151141 | 6.616 | 0.030937 | 3.8881e-06 |
| 0.000 | 0.06 | (red.den.= 0.62477) | | 0.018251 | 2.2938e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 22-4 | 09/19/88 | 11-28 | 2200 | 142.70 | 97980 | 5.00 |
| 22-4 | 09/19/88 | 11/28 | 2200 | 142.70 | 88800 | 5.00 |
| 22-5 | 09/19/88 | 11-56 | 2200 | 142.60 | 78360 | 5.00 |
| 22-5 | 09/19/88 | 11-56 | 2200 | 142.60 | 82080 | 5.00 |
| 22-6 | 09/19/88 | 12:24 | 2200 | 142.60 | 66860 | 5.00 |
| 22-6 | 09/19/88 | 12-24 | 2200 | 142.60 | 73820 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 15.270 | 334.61 | 0.073214 | 13.659 | 0.135846 | 8.2702e-06 |
| 0.000 | 0.03 | (red.den.= 1.28976) | | 0.018425 | 1.1217e-06 |

Appendix 2.2 Data2122 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 22-7 | 09/19/88 | 1-17 | 3150 | 143.00 | 761400 | 5.00 |
| 22-7 | 09/19/88 | 1-17 | 3150 | 143.00 | 907200 | 5.00 |
| 22-8 | 09/19/88 | 1-45 | 3150 | 143.10 | 615400 | 5.00 |
| 22-8 | 09/19/88 | 1-45 | 3150 | 143.10 | 671600 | 5.00 |
| 22-9 | 09/19/88 | 2-10 | 3150 | 142.80 | 803800 | 5.00 |
| 22-9 | 09/19/88 | 2-10 | 3150 | 142.80 | 1023000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 21.820 | 334.80 | 0.059359 | 16.847 | 1.331564 | 6.5720e-05 |
| 0.000 | 0.08 | (red.den.= 1.59082) | | 0.251407 | 1.2408e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 22-10 | 09/19/88 | 2-45 | 3890 | 143.30 | 2026000 | 5.00 |
| 22-10 | 09/19/88 | 2-45 | 3890 | 143.30 | 2077000 | 5.00 |
| 22-11 | 09/19/88 | 3-08 | 3880 | 143.20 | 2223000 | 5.00 |
| 22-11 | 09/19/88 | 3-08 | 3880 | 143.20 | 2832000 | 5.00 |
| 22-12 | 09/19/88 | 3-32 | 3870 | 143.00 | 2887000 | 5.00 |
| 22-12 | 09/19/88 | 3-32 | 3870 | 143.00 | 2888000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 26.853 | 334.91 | 0.055235 | 18.105 | 4.160579 | 1.9106e-04 |
| 0.062 | 0.08 | (red.den.= 1.70959) | | 0.701226 | 3.2201e-05 |


3
1.0
1.1
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1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
MicroD

Appendix 2.2 Data2122 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 22-13 | 09/19/88 | 4-10 | 4880 | 143.30 | 6345000 | 5.00 |
| 22-13 | 09/19/88 | 4-10 | 4880 | 143.30 | 6550000 | 5.00 |
| 22-14 | 09/19/88 | 4-35 | 4850 | 143.10 | 8916000 | 5.00 |
| 22-14 | 09/19/88 | 4-35 | 4850 | 143.10 | 8726000 | 5.00 |
| 22-15 | 09/19/88 | 5-01 | 4850 | 143.20 | 9804000 | 5.00 |
| 22-15 | 09/19/88 | 5-01 | 4850 | 143.20 | 9516000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 33.610 | 334.93 | 0.051870 | 19.279 | 13.881685 | 5.9839e-04 |
| 0.107 | 0.05 | (red.den.= 1.82047) | | 2.498686 | 1.0771e-04 |

Appendix 2.2 Data2221 file

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | injection vol. (ul) | SCE loop vol. (ml) |
|---|---|---|---|
| 09/19/88 | 8 3529e-09 | 50.0 | 0.5 |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 22-16 | 09/20/88 | 10-37 | 3990 | 123.80 | 3128000 | 5.00 |
| 22-16 | 09/20/88 | 10-37 | 3990 | 123.80 | 3132000 | 5.00 |
| 22-17 | 09/20/88 | 11-03 | 3990 | 123.70 | 5803000 | 5.00 |
| 22-17 | 09/20/88 | 11-03 | 3990 | 123.70 | 5949000 | 5.00 |
| 22-18 | 09/20/88 | 11-29 | 3990 | 123.80 | 6562000 | 5.00 |
| 22-18 | 09/20/88 | 11-29 | 3990 | 123.80 | 6662000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 27.611 | 324.13 | 0.051723 | 19.334 | 8.697039 | 3.7391e-04 |
| 0.000 | 0.03 | (red.den.= 1.82566) | | 2.743690 | 1.1796e-04 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 22-19 | 09/20/88 | 12-06 | 4900 | 124.00 | 8992000 | 5.00 |
| 22-19 | 09/20/88 | 12-06 | 4900 | 124.00 | 8962000 | 5.00 |
| 22-20 | 09/20/88 | 12-41 | 4870 | 123.90 | 9898000 | 5.00 |
| 22-20 | 09/20/88 | 12-41 | 4870 | 123.90 | 9794000 | 5.00 |
| 22-21 | 09/20/88 | 1-15 | 4870 | 124.00 | 10420000 | 5.00 |
| 22-21 | 09/20/88 | 1-15 | 4870 | 124.00 | 10210000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 33.748 | 324.24 | 0.049489 | 20.207 | 16.225787 | 6.6727e-04 |
| 0.107 | 0.03 | (red.den.= 1.90808) | | 1.022058 | 4.2031e-05 |

Appendix 2.2 Data2221 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 21-16 | 09/14/88 | 9-33 | 1420 | 124.10 | 87460 | 5.00 |
| 21-16 | 09/14/88 | 9-33 | 1420 | 124.10 | 84460 | 5.00 |
| 21-17 | 09/14/88 | 10-03 | 1420 | 124.00 | 40000 | 5.00 |
| 21-17 | 09/14/88 | 10-03 | 1420 | 124.00 | 38950 | 5.00 |
| 21-18 | 09/14/88 | 10:30 | 1420 | 124.30 | 1f110 | 5.00 |
| 21-18 | 09/14/88 | 10-30 | 1420 | 124.30 | 12060 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 9.892 | 324.34 | 0.123606 | 8.090 | 0.078250 | 8.0427e-06 |
| 0.000 | 0.08 | (red.den.= 0.76395) | | 0.053923 | 5.5423e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 21-19 | 09/14/88 | 11-08 | 2175 | 125.30 | 316300 | 5.00 |
| 21-19 | 09/14/88 | 11-08 | 2175 | 125.30 | 313800 | 5.00 |
| 21-20 | 09/14/88 | 11-34 | 2150 | 124.60 | 255200 | 5.00 |
| 21-20 | 09/14/88 | 11-34 | 2150 | 124.60 | 233700 | 5.00 |
| 21-21 | 09/14/88 | 12-00 | 2150 | 124.60 | 252400 | 5.00 |
| 21-21 | 09/14/88 | 12-00 | 2150 | 124.60 | 250900 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 14.982 | 324.72 | 0.064109 | 15.598 | 0.451697 | 2.4079e-05 |
| 0.089 | 0.20 | (red.den.= 1.47294) | | 0.059170 | 3.1542e-06 |

Raw experimental data:

Appendix 2.2 Data2221 file

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 21-22 | 09/14/88 | 12-34 | 3100 | 125.10 | 1334000 | 5.00 |
| 21-22 | 09/14/88 | 12-34 | 3100 | 125.10 | 1335000 | 5.00 |
| 21-23 | 09/14/88 | 1-02 | 3075 | 124.60 | 1067000 | 5.00 |
| 21-23 | 09/14/88 | 1-02 | 3075 | 124.60 | 1048000 | 5.00 |
| 21-24 | 09/14/88 | 1-34 | 3050 | 124.60 | 912600 | 5.00 |
| 21-24 | 09/14/88 | 1-34 | 3050 | 124.60 | 914000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 21.303 | 324.69 | 0.055589 | 17.989 | 1.840589 | 8.5073e-05 |
| 0.154 | 0.14 | (red.den.= 1.69868) | | 0.320011 | 1.4791e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 21-25 | 09/14/88 | 2-08 | 3850 | 125.00 | 2554000 | 5.00 |
| 21-25 | 09/14/88 | 2-08 | 3850 | 125.00 | 2571000 | 5.00 |
| 21-26 | 09/14/88 | 2-38 | 3810 | 124.60 | 3567000 | 5.00 |
| 21-26 | 09/14/88 | 2-38 | 3810 | 124.60 | 3445000 | 5.00 |
| 21-27 | 09/14/88 | 3-07 | 3810 | 124.60 | 3346000 | 5.00 |
| 21-27 | 09/14/88 | 3-07 | 3810 | 124.60 | 3351000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 26.462 | 324.67 | 0.052402 | 19.083 | 5.243951 | 2.2845e-04 |
| 0.142 | 0.11 | (red.den.= 1.80200) | | 0.758032 | 3.3023e-05 |

Appendix 2.2 (cont.) Data2326 file

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | injection vol. (ul) | SCE loop vol. (ml) |
|---|---|---|---|
| 11/22/88 | 1.1808e-08 | 50.0 | 0.5 |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 23-1 | 11/14/88 | 10:56 | 1370 | 158.20 | 90640 | 5.00 |
| 23-1 | 11/14/88 | 10:56 | 1370 | 158.20 | 86640 | 5.00 |
| 24-1 | 11/16/88 | 9:00 | 1425 | 158.20 | 80720 | 5.00 |
| 24-1 | 11/16/88 | 9:00 | 1425 | 158.20 | 130600 | 5.00 |
| 24-2 | 11/16/88 | 9:29 | 1420 | 158.30 | 123200 | 5.00 |
| 24-2 | 11/16/88 | 9:29 | 1420 | 158.30 | 127300 | 5.00 |
| 24-3 | 11/16/88 | 9:58 | 1410 | 158.20 | 116600 | 5.00 |
| 24-3 | 11/16/88 | 9:58 | 1410 | 158.20 | 121100 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 9.797 | 343.27 | 0.184238 | 5.428 | 0.258831 | 3.9651e-05 |
| 0.159 | 0.03 | (red.den.= 0.51254) | | 0.047578 | 7.2887e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 24-4 | 11/16/88 | 10:52 | 2150 | 157.60 | 72940 | 2.70 |
| 24-4 | 11/16/88 | 10:52 | 2150 | 157.60 | 76180 | 2.70 |
| 24-5 | 11/16/88 | 11:19 | 2140 | 157.80 | 114000 | 5.00 |
| 24-5 | 11/16/88 | 11:19 | 2140 | 157.80 | 129500 | 5.00 |
| 24-6 | 11/16/88 | 11:46 | 2130 | 157.40 | 54200 | 5.00 |
| 24-6 | 11/16/88 | 11:46 | 2130 | 157.40 | 69460 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 14.856 | 342.93 | 0.087539 | 11.424 | 0.172049 | 1.2524e-05 |
| 0.062 | 0.10 | (red.den.= 1.07871) | | 0.058144 | 4.2323e-06 |

Appendix 2.2 (cont.)  Data2326  file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 24-7 | 11/16/88 | 12:37 | 3140 | 158.10 | 296200 | 5.00 |
| 24-7 | 11/16/88 | 12:37 | 3140 | 158.10 | 309800 | 5.00 |
| 24-8 | 11/16/88 | 1:04 | 3120 | 157.80 | 288100 | 5.00 |
| 24-8 | 11/16/88 | 1:04 | 3120 | 157.80 | 271900 | 5.00 |
| 25-1 | 11/16/88 | 4:01 | 3060 | 157.90 | 172500 | 5.00 |
| 25-1 | 11/16/88 | 4:01 | 3060 | 157.90 | 198100 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 21.521 | 343.11 | 0.063947 | 15.638 | 0.604806 | 3.2159e-05 |
| 0.257 | 0.08 | (red.den.= 1.47566) | | 0.134085 | 7.1297e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-1 | 11/18/88 | 10:25 | 3750 | 156.30 | 1635000 | 5.00 |
| 26-1 | 11/18/88 | 10:25 | 3750 | 156.30 | 1762000 | 5.00 |
| 26-2 | 11/18/88 | 10:53 | 3750 | 156.50 | 1995000 | 5.00 |
| 26-2 | 11/18/88 | 10:53 | 3750 | 156.50 | 1969000 | 5.00 |
| 26-3 | 11/18/88 | 11:20 | 3750 | 156.75 | 1917000 | 5.00 |
| 26-3 | 11/18/88 | 11:20 | 3750 | 156.75 | 1978000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 25.957 | 342.33 | 0.058467 | 17.104 | 4.430362 | 2.1534e-04 |
| 0.000 | 0.11 | (red.den.= 1.61509) | | 0.343809 | 1.6711e-05 |

Appendix 2.2 (cont.) Data2326 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-4 | 11/18/88 | 12:10 | 4700 | 157.00 | 3504000 | 5.00 |
| 26-4 | 11/18/88 | 12:10 | 4700 | 157.00 | 3741000 | 5.00 |
| 26-5 | 11/18/88 | 12:35 | 4700 | 157.00 | 4676000 | 5.00 |
| 26-5 | 11/18/88 | 12:35 | 4700 | 157.00 | 4697000 | 5.00 |
| 26-6 | 11/18/88 | 1:09 | 4700 | 157.20 | 4539000 | 5.00 |
| 26-6 | 11/18/88 | 1:09 | 4700 | 157.20 | 4057000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 32.507 | 342.63 | 0.054268 | 18.427 | 9.924230 | 4.4764e-04 |
| 0.000 | 0.06 | (red.den.= 1.74004) | | 1.206045 | 5.4399e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-7 | 11/21/88 | 9:15 | 3950 | 141.80 | 1970000 | 5.00 |
| 26-7 | 11/21/88 | 9:15 | 3950 | 141.80 | 1915000 | 5.00 |
| 26-8 | 11/21/88 | 9:49 | 3950 | 141.80 | 2508000 | 5.00 |
| 26-8 | 11/21/88 | 9:49 | 3950 | 141.80 | 2467000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 27.336 | 334.15 | 0.054700 | 18.282 | 5.230944 | 2.3787e-04 |
| 0.000 | 0.00 | (red.den.= 1.72631) | | 0.746029 | 3.3925e-05 |

Appendix 2.2 (cont.)  Data2326  file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-9 | 11/21/88 | 10:40 | 3350 | 124.30 | 1431000 | 5.00 |
| 26-9 | 11/21/88 | 10:40 | 3350 | 124.30 | 1437000 | 5.00 |
| 26-10 | 11/21/88 | 11:08 | 3330 | 123.80 | 2464000 | 5.00 |
| 26-10 | 11/21/88 | 11:08 | 3330 | 123.80 | 2506000 | 5.00 |
| 26-11 | 11/21/88 | 11:50 | 3330 | 123.80 | 2156000 | 5.00 |
| 26-11 | 11/21/88 | 11:50 | 3330 | 123.80 | 2102000 | 5.00 |
| 26-12 | 11/21/88 | 12:30 | 3330 | 123.80 | 2020000 | 5.00 |
| 26-12 | 11/21/88 | 12:30 | 3330 | 123.80 | 2018000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 23.095 | 324.22 | 0.054147 | 18.468 | 4.762757 | 2.1440e-04 |
| 0.064 | 0.13 | (red.den.= 1.74394) | | 0.955242 | 4.3000e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-13 | 11/21/88 | 1:25 | 3025 | 114.80 | 1423000 | 5.00 |
| 26-13 | 11/21/88 | 1:25 | 3025 | 114.80 | 1410000 | 5.00 |
| 26-14 | 11/21/88 | 1:52 | 3010 | 114.60 | 1646000 | 5.00 |
| 26-14 | 11/21/88 | 1:52 | 3010 | 114.60 | 1576000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 20.906 | 319.09 | 0.053816 | 18.582 | 3.574872 | 1.5995e-04 |
| 0.060 | 0.06 | (red.den.= 1.75465) | | 0.273935 | 1.2257e-05 |

Appendix 2.2 (cont.) Data2326 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-15 | 11/21/88 | 2:32 | 2690 | 105.50 | 1126000 | 5.00 |
| 26-15 | 11/21/88 | 2:32 | 2690 | 105.50 | 1112000 | 5.00 |
| 26-16 | 11/21/88 | 3:02 | 2670 | 104.80 | 1475000 | 5.00 |
| 26-16 | 11/21/88 | 3:02 | 2670 | 104.80 | 1419000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 18.579 | 313.79 | 0.053479 | 18.699 | 3.029933 | 1.3472e-04 |
| 0.080 | 0.22 | (red.den.= 1.76572) | | 0.450668 | 2.0038e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-17 | 11/21/88 | 4:01 | 2360 | 97.60 | 958200 | 5.00 |
| 26-17 | 11/21/88 | 4:01 | 2360 | 97.60 | 1030000 | 5.00 |
| 26-18 | 11/21/88 | 4:25 | 2340 | 95.60 | 1075000 | 5.00 |
| 26-18 | 11/21/88 | 4:25 | 2340 | 95.60 | 1206000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 16.304 | 309.04 | 0.053283 | 18.768 | 2.520536 | 1.1166e-04 |
| 0.080 | 0.64 | (red.den.= 1.77220) | | 0.246147 | 1.0905e-05 |

Appendix 2.2 (cont.)  Datw2619  file

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | injection vol. (ul) | SCE loop vol. (ml) |
|---|---|---|---|
| 01/13/88 | 1.0430e-09 | 20.0 | 0.5 |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-19 | 11/24/88 | 2:38 | 4990 | 157.40 | 7525066 | 20.00 |
| 26-19 | 11/24/88 | 2:38 | 4990 | 157.40 | 7519291 | 20.00 |
| 26-20 | 11/24/88 | 3:08 | 4990 | 157.50 | 6950831 | 20.00 |
| 26-20 | 11/24/88 | 3:08 | 4990 | 157.50 | 6951377 | 20.00 |
| 26-21 | 11/24/88 | 3:36 | 5000 | 157.60 | 8982271 | 20.00 |
| 26-21 | 11/24/88 | 3:36 | 5000 | 157.60 | 8968441 | 20.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 34.529 | 342.87 | 0.053370 | 18.737 | 16.304620 | 7.2306e-04 |
| 0.036 | 0.05 | (red.den.= 1.76932) | | 1.947273 | 8.6355e-05 |

Appendix 2.2 (cont.)  Data2619  file

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | injection vol. (ul) | SCE loop vol. (ml) |
|---|---|---|---|
| 11/22/88 | 1.1808e-08 | 50.0 | 0.5 |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-19 | 11/24/88 | 2:38 | 4990 | 157.40 | 5264000 | 5.00 |
| 26-19 | 11/24/88 | 2:38 | 4990 | 157.40 | 5994000 | 5.00 |
| 26-20 | 11/24/88 | 3:08 | 4990 | 157.50 | 5235000 | 5.00 |
| 26-20 | 11/24/88 | 3:08 | 4990 | 157.50 | 4963000 | 5.00 |
| 26-20 | 11/24/88 | 3:08 | 4990 | 157.50 | 5381000 | 5.00 |
| 26-21 | 11/24/88 | 3:36 | 5000 | 157.60 | 5009000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 34.518 | 342.86 | 0.053373 | 18.736 | 12.534586 | 5.5599e-04 |
| 0.028 | 0.04 | (red.den.= 1.76922) | | 0.878322 | 3.8959e-05 |

Appendix 2.2 (cont.) Data2622 file

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | injection vol. (ul) | SCE loop vol. (ml) |
|---|---|---|---|
| 12/02/88 | 8.9700e-09 | 50.0 | 0.5 |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-19 | 11/24/88 | 2:38 | 4990 | 157.40 | 8888000 | 5.00 |
| 26-19 | 11/24/88 | 2:38 | 4990 | 157.40 | 8884000 | 5.00 |
| 26-19 | 11/24/88 | 2:38 | 4990 | 157.40 | 8874000 | 5.00 |
| 26-19 | 11/24/88 | 2:38 | 4990 | 157.40 | 8774000 | 5.00 |
| 26-20 | 11/24/88 | 3:08 | 4990 | 157.50 | 8626000 | 5.00 |
| 26-20 | 11/24/88 | 3:08 | 4990 | 157.50 | 8798000 | 5.00 |
| 26-21 | 11/24/88 | 3:36 | 5000 | 157.60 | 8856000 | 5.00 |
| 26-21 | 11/24/88 | 3:36 | 5000 | 157.60 | 8874000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 34.523 | 342.86 | 0.053369 | 18.737 | 15.826220 | 7.0185e-04 |
| 0.032 | 0.05 | (red.den.= 1.76935) | | 0.160545 | 7.1197e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-23 | 11/28/88 | 10:01 | 1460 | 158.50 | 1403 | 5.00 |
| 26-24 | 11/28/88 | 10:29 | 1460 | 158.50 | 1162 | 5.00 |
| 26-24 | 11/28/88 | 10:29 | 1460 | 158.50 | 1434 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 10.168 | 343.43 | 0.173217 | 5.773 | 0.002391 | 3.4445e-07 |
| 0.000 | 0.00 | (red.den.= 0.54515) | | 0.000267 | 3.8476e-08 |

Appendix 2.2 (cont.) Data2622 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-29 | 11/24/88 | 2:20 | 1460 | 142.40 | 444 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/ml CO2) | molefrac |
|---|---|---|---|---|---|
| 10.168 | 334.48 | 0.150449 | 6.647 | 0.000797 | 9.9649e-08 |
| 0.000 | 0.00 | (red.den.= 0.62765) | | 0.000000 | 0.0000e+00 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-38 | 11/30/88 | 2:31 | 1450 | 114.90 | 3574 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/ml CO2) | molefrac |
|---|---|---|---|---|---|
| 10.099 | 319.21 | 0.090670 | 11.029 | 0.006412 | 4.8342e-07 |
| 0.000 | 0.00 | (red.den.= 1.04145) | | 0.000000 | 0.0000e+00 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-22 | 11/28/88 | 9:28 | 1450 | 158.60 | 54510 | 5.00 |
| 26-22 | 11/28/88 | 9:28 | 1450 | 158.60 | 43740 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/ml CO2) | molefrac |
|---|---|---|---|---|---|
| 10.099 | 343.48 | 0.175396 | 5.701 | 0.088130 | 1.2853e-05 |
| 0.000 | 0.00 | (red.den.= 0.53837) | | 0.013662 | 1.9926e-06 |

Appendix 2.2 (cont.) Data2622 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-39 | 11/30/88 | 3:20 | 1325 | 105.70 | 4611 | 5.00 |
| 26-40 | 11/30/88 | 3:53 | 1320 | 105.40 | 3232 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 9.220 | 314.01 | 0.087770 | 11.393 | 0.007035 | 5.1345e-07 |
| 0.024 | 0.12 | (red.den.= 1.07586) | | 0.001749 | 1.2767e-07 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol (ml) |
|---|---|---|---|---|---|---|
| 26-41 | 11/30/88 | 4:15 | 1460 | 105.70 | 73360 | 5.00 |
| 26-41 | 11/30/88 | 4:15 | 1460 | 105.70 | 63490 | 5.00 |
| 26-42 | 11/30/88 | 5:02 | 1450 | 105.70 | 64000 | 5.00 |
| 26-42 | 11/30/88 | 5:02 | 1450 | 105.70 | 65440 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 10.133 | 314.09 | 0.070955 | 14.093 | 0.119431 | 7.0466e-06 |
| 0.040 | 0.00 | (red.den.= 1.33082) | | 0.008252 | 4.8687e-07 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-43 | 11/30/88 | 6:45 | 1250 | 96.10 | 76160 | 5.00 |
| 26-43 | 11/30/88 | 6:45 | 1250 | 96.10 | 75280 | 5.00 |
| 26-44 | 11/30/88 | 7:12 | 1250 | 96.40 | 59080 | 5.00 |
| 26-44 | 11/30/88 | 7:12 | 1250 | 96.40 | 57750 | 5.00 |

Appendix 2.2 (cont.)  Data2622  file

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 8.720 | 308.84 | 0.071166 | 14.052 | 0.120319 | 7.1200e-06 |
| 0.000 | 0.10 | (red.den.= 1.32 9) | | 0.017962 | 1.0629e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 26-45 | 11/30/88 | 7:50 | 1460 | 96.40 | 193800 | 5.00 |
| 26-45 | 11/30/88 | 7:50 | 1460 | 96.40 | 191100 | 5.00 |
| 26-46 | 11/30/88 | 8:15 | 1450 | 96.00 | 176000 | 5.00 |
| 26-46 | 11/30/88 | 8:15 | 1450 | 96.00 | 172700 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 10.133 | 308.82 | 0.062007 | 16.127 | 0.329020 | 1.6964e-05 |
| 0.040 | 0.13 | (red.den.= 1.52288) | | 0.019006 | 9.7993e-07 |

Appendix 2.2 (cont.) Data2801 file

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | vol. (ul) | injection (ml) | SCE loop vol. |
|---|---|---|---|---|
| 010689 | 7.2309e-09 | 50.0 | 0.5 | |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-1 | 12/22/88 | 9:59 | 1350 | 158.10 | 18417 | 5.00 |
| 28-1 | 12/22/88 | 9:59 | 1350 | 158.10 | 16519 | 5.00 |
| 28-2 | 12/22/88 | 10:39 | 1350 | 158.00 | 17910 | 5.00 |
| 28-3 | 12/22/88 | 11:09 | 1350 | 157.70 | 13647 | 5.00 |
| 28-3 | 12/22/88 | 11:09 | 1350 | 157.70 | 13930 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 9.409 | 343.11 | 0.196682 | 5.084 | 0.023261 | 3.8043e-06 |
| 0.000 | 0.11 | (red.den.= 0.48011) | | 0.003197 | 5.2282e-07 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-4 | 12/22/88 | 12:10 | 1460 | 157.70 | 2931 | 5.00 |
| 28-4 | 12/22/88 | 12:10 | 1460 | 157.70 | 1458 | 5.00 |
| 28-5 | 12/22/88 | 12:57 | 1460 | 157.80 | 13740 | 5.00 |
| 28-5 | 12/22/88 | 1:22 | 1460 | 157.70 | 14980 | 5.00 |
| 28-6 | 1:22 | 1:22 | 1460 | 157.70 | 4465 | 5.00 |
| 28-6 | 12/22/88 | 1:22 | 1460 | 157.70 | 15900 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 10.168 | 342.99 | 0.172222 | 5.806 | 0.012889 | 1.8458e-06 |
| 0.000 | 0.02 | (red.den.= 0.54830) | | 0.009594 | 1.3740e-06 |

Appendix 2.2 (cont.) Data2801 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-7 | 12/28/88 | 8:59 | 1250 | 142.40 | 13253 | 5.00 |
| 28-7 | 12/28/88 | 8:59 | 1250 | 142.40 | 9304 | 5.00 |
| 28-8 | 12/28/88 | 9:30 | 1240 | 142.50 | 4135 | 5.00 |
| 28-8 | 12/28/88 | 9:30 | 1240 | 142.50 | 5450 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 8.685 | 334.51 | 0.203040 | 4.925 | 0.011621 | 1.9620e-06 |
| 0.040 | 0.03 | (red.den.= 0.46507) | | 0.005947 | 1.0040e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-10 | 12/28/88 | 10:47 | 1460 | 142.20 | 24940 | 5.00 |
| 28-10 | 12/28/88 | 10:47 | 1460 | 142.20 | 22950 | 5.00 |
| 28-11 | 12/28/88 | 11:10 | 1460 | 142.20 | 20465 | 5.00 |
| 28-11 | 12/28/88 | 11:10 | 1460 | 142.20 | 14354 | 5.00 |
| 28-12 | 12/28/88 | 11:47 | 1460 | 142.20 | 9358 | 5.00 |
| 28-12 | 12/28/88 | 11:47 | 1460 | 142.20 | 5997 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 10.168 | 334.37 | 0.150127 | 6.661 | 0.023636 | 2.9507e-06 |
| 0.000 | 0.00 | (red.den.= 0.62899) | | 0.011096 | 1.3852e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-13 | 12/28/88 | 12:40 | 1350 | 124.50 | 4141 | 5.00 |
| 28-13 | 12/28/88 | 12:40 | 1350 | 124.50 | 9224 | 5.00 |
| 28-14 | 12/28/88 | 1:12 | 1350 | 124.20 | 4090 | 5.00 |
| 28-14 | 12/28/88 | 1:12 | 1350 | 124.20 | 1537 | 5.00 |
| 28-15 | 12/28/88 | 1:37 | 1350 | 124.20 | 2187 | 5.00 |

Appendix 2.2 (cont.)  Data2801  file

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 9.409 | 324.44 | 0.142518 | 7.017 | 0.006126 | 7.2595e-07 |
| 0.000 | 0.09 | (red.den.= 0.66258) | | 0.004362 | 5.1695e-07 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-16 | 12/28/88 | 2:30 | 1460 | 123.80 | 4953 | 5.00 |
| 28-16 | 12/28/88 | 2:30 | 1460 | 123.80 | 4213 | 5.00 |
| 28-17 | 12/28/88 | 3:04 | 1460 | 123.70 | 4416 | 5.00 |
| 28-17 | 12/28/88 | 3:04 | 1460 | 123.70 | 5762 | 5.00 |
| 28-18 | 12/28/88 | 3:36 | 1460 | 123.70 | 8440 | 5.00 |
| 28-18 | 12/28/88 | 3:36 | 1460 | 123.70 | 7262 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 10.168 | 324.11 | 0.112962 | 8.853 | 0.008447 | 7.9345e-07 |
| 0.000 | 0.03 | (red.den.= 0.83593) | | 0.002441 | 2.2933e-07 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-19 | 12/28/88 | 4:21 | 1380 | 114.90 | 938 | 5.00 |
| 28-19 | 12/28/88 | 4:21 | 1380 | 114.90 | 2960 | 5.00 |
| 28-20 | 12/28/88 | 4:50 | 1380 | 114.80 | 13840 | 5.00 |
| 28-20 | 12/28/88 | 4:50 | 1380 | 114.80 | 9460 | 5.00 |
| 28-21 | 12/28/88 | 5:15 | 1380 | 114.70 | 40340 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 9.616 | 319.16 | 0.107164 | 9.331 | 0.019534 | 1.7407e-06 |
| 0.000 | 0.05 | (red.den.= 0.88116) | | 0.022931 | 2.0434e-06 |

Appendix 2.2 (cont.) Data2801 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-22 | 12/28/88 | 6:17 | 1460 | 114.90 | 20490 | 5.00 |
| 28-22 | 12/28/88 | 6:17 | 1460 | 114.90 | 36390 | 5.00 |
| 28-23 | 12/28/88 | 7:12 | 1460 | 115.00 | 27720 | 5.00 |
| 28-23 | 12/28/88 | 7:12 | 1460 | 115.00 | 26300 | 5.00 |
| 28-24 | 12/28/88 | 7:39 | 1460 | 115.00 | 26400 | 5.00 |
| 28-24 | 12/28/88 | 7:39 | 1460 | 115.00 | 27700 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 10.168 | 319.24 | 0.089035 | 11.232 | 0.039770 | 2.9444e-06 |
| 0.000 | 0.03 | (red.den.= 1.06058) | | 0.007400 | 5.4785e-07 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-25 | 12/28/88 | 8:25 | 1340 | 105.80 | 22050 | 5.00 |
| 28-25 | 12/28/88 | 8:25 | 1340 | 105.80 | 31660 | 5.00 |
| 28-26 | 12/28/88 | 8:53 | 1335 | 105.60 | 43220 | 5.00 |
| 28-26 | 12/28/88 | 8:53 | 1335 | 105.60 | 64520 | 5.00 |
| 28-27 | 12/28/88 | 9:23 | 1335 | 105.60 | 31730 | 5.00 |
| 28-27 | 12/28/88 | 9:23 | 1335 | 105.60 | 38760 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 9.317 | 314.08 | 0.084774 | 11.796 | 0.055904 | 3.9408e-06 |
| 0.018 | 0.06 | (red.den.= 1.11389) | | 0.021081 | 1.4861e-06 |

Appendix 2.2 (cont.)  Data2801  file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-28 | 12/28/88 | 10:05 | 1460 | 105.80 | 88940 | 5.00 |
| 28-28 | 12/28/88 | 10:05 | 1460 | 105.80 | 104200 | 5.00 |
| 28-29 | 12/28/88 | 10:38 | 1460 | 105.70 | 103400 | 5.00 |
| 28-29 | 12/28/88 | 10:38 | 1460 | 105.70 | 111600 | 5.00 |
| 28-30 | 12/28/88 | 11:05 | 1460 | 105.70 | 94860 | 5.00 |
| 28-30 | 12/28/88 | 11:05 | 1460 | 105.70 | 118900 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 10.168 | 314.11 | 0.070694 | 14.145 | 0.149897 | 8.8115e-06 |
| 0.000 | 0.03 | (red.den.= 1.33574) | | 0.015707 | 9.2329e-07 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-31 | 12/29/88 | 10:25 | 1290 | 98.50 | 225900 | 5.00 |
| 28-31 | 12/29/88 | 10:25 | 1290 | 98.50 | 255700 | 5.00 |
| 28-32 | 12/29/88 | 10:55 | 1290 | 98.80 | 95200 | 5.00 |
| 28-32 | 12/29/88 | 10:55 | 1290 | 98.80 | 93060 | 5.00 |
| 28-33 | 12/29/88 | 11:26 | 1290 | 98.60 | 101400 | 5.00 |
| 28-33 | 12/29/88 | 11:26 | 1290 | 98.60 | 99840 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 8.996 | 310.17 | 0.072135 | 13.863 | 0.209961 | 1.2594e-05 |
| 0.000 | 0.08 | (red.den.= 1.30905) | | 0.108062 | 6.4818e-06 |

Appendix 2.2 (cont.) Data2801 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-34 | 12/29/88 | 12:16 | 1460 | 99.00 | 169700 | 5.00 |
| 28-34 | 12/29/88 | 12:16 | 1460 | 99.00 | 197100 | 5.00 |
| 28-35 | 12/29/88 | 12:42 | 1450 | 98.30 | 126800 | 5.00 |
| 28-35 | 12/29/88 | 12:42 | 1450 | 98.30 | 142600 | 5.00 |
| 28-36 | 12/29/88 | 1:14 | 1450 | 98.40 | 188200 | 5.00 |
| 28-36 | 12/29/88 | 1:14 | 1450 | 98.40 | 198300 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 10.122 | 310.13 | 0.063743 | 15.688 | 0.246501 | 1.3065e-05 |
| 0.036 | 0.19 | (red.den.= 1.48140) | | 0.043301 | 2.2951e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-37 | 12/29/88 | 2:19 | 2200 | 106.00 | 663600 | 5.00 |
| 28-37 | 12/29/88 | 2:19 | 2200 | 106.00 | 683600 | 5.00 |
| 28-38 | 12/29/88 | 2:48 | 2200 | 106.00 | 639800 | 5.00 |
| 28-38 | 12/29/88 | 2:48 | 2200 | 106.00 | 701800 | 5.00 |
| 28-39 | 12/29/88 | 3:18 | 2200 | 106.10 | 665200 | 5.00 |
| 28-39 | 12/29/88 | 3:18 | 2200 | 106.10 | 673400 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 15.270 | 314.28 | 0.056623 | 17.661 | 0.970724 | 4.5703e-05 |
| 0.000 | 0.03 | (red.den.= 1.66769) | | 0.030160 | 1.4200e-06 |

Appendix 2.2 (cont.) Data2801 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-40 | 12/29/88 | 4:21 | 3100 | 106.40 | 2291000 | 5.00 |
| 28-40 | 12/29/88 | 4:21 | 3100 | 106.40 | 2419000 | 5.00 |
| 28-41 | 12/29/88 | 4:48 | 3100 | 106.40 | 2294000 | 5.00 |
| 28-41 | 12/29/88 | 4:48 | 3100 | 106.40 | 2279000 | 5.00 |
| 28-42 | 12/29/88 | 5:20 | 3080 | 106.50 | 2310000 | 5.00 |
| 28-42 | 12/29/88 | 5:20 | 3080 | 106.50 | 2313000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 21.429 | 314.50 | 0.051990 | 19.234 | 3.351763 | 1.4488e-04 |
| 0.071 | 0.03 | (red.den.= 1.81628) | | 0.074067 | 3.2016e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-43 | 12/29/88 | 5:55 | 3900 | 106.50 | 6840000 | 5.00 |
| 28-43 | 12/29/88 | 5:55 | 3900 | 106.50 | 6670000 | 5.00 |
| 28-44 | 12/29/88 | 6:22 | 3870 | 106.50 | 7406000 | 5.00 |
| 28-44 | 12/29/88 | 6:22 | 3870 | 106.50 | 7592000 | 5.00 |
| 28-45 | 12/29/88 | 7.00 | 3850 | 106.60 | 7318000 | 5.00 |
| 28-45 | 12/29/88 | 7:00 | 3850 | 106.60 | 7496000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 26.807 | 314.56 | 0.049665 | 20.135 | 10.441902 | 4.3105e-04 |
| 0.155 | 0.03 | (red.den.= 1.90131) | | 0.543294 | 2.2427e-05 |

Appendix 2.2 (cont.) Data2801 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-46 | 12/29/88 | 7:42 | 4940 | 106.80 | 11260000 | 5.00 |
| 28-46 | 12/29/88 | 7:42 | 4940 | 106.80 | 11150000 | 5.00 |
| 28-47 | 12/29/88 | 8:11 | 4900 | 106.80 | 11280000 | 5.00 |
| 28-47 | 12/29/88 | 8:11 | 4900 | 106.80 | 11160000 | 5.00 |
| 28-48 | 12/29/88 | 8:39 | 4875 | 106.70 | 11450000 | 5.00 |
| 28-48 | 12/29/88 | 8:39 | 4875 | 106.70 | 11570000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 33.920 | 314.69 | 0.047579 | 21.018 | 16.358706 | 6.4678e-04 |
| 0.202 | 0.03 | (red.den.= 1.98469) | | 0.240852 | 9.5227e-06 |

Appendix 2.2 (cont.) Data2849 file

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | injection vol. (ul) | SCE loop vol. (ml) |
|---|---|---|---|
| 01/02/89 | 7.2309e-09 | 50.0 | 0.5 |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-49 | 01/02/89 | 12:18 | 1360 | 105.90 | 1900000 | 5.00 |
| 28-49 | 01/02/89 | 12:18 | 1360 | 105.90 | 1935000 | 5.00 |
| 28-50 | 01/02/89 | 12:39 | 1360 | 105.60 | 2027000 | 5.00 |
| 28-50 | 01/02/89 | 12:39 | 1360 | 105.60 | 2017000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 9.478 | 314.12 | 0.080581 | 12.410 | 2.848613 | 1.9084e-04 |
| 0.000 | 0.10 | (red.den.= 1.17185) | | 0.089860 | 6.0200e-06 |

Appendix 2.2 (cont.)  Data284w  file

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | injection vol. (ul) | SCE loop vol. (ml) |
|---|---|---|---|
| 010689 | 1.0430e-09 | 20.0 | 0.5 |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-43 | 12/29/88 | 5:55 | 3900 | 106.50 | 5216116 | 20.00 |
| 28-43 | 12/29/88 | 5:55 | 3900 | 106.50 | 5187020 | 20.00 |
| 28-44 | 12/29/88 | 6:22 | 3870 | 106.50 | 5559858 | 20.00 |
| 28-44 | 12/29/88 | 6:22 | 3870 | 106.50 | 5576815 | 20.00 |
| 28-45 | 12/29/88 | 7.00 | 3850 | 106.60 | 5563006 | 20.00 |
| 28-45 | 12/29/88 | 7:00 | 3850 | 106.60 | 5536877 | 20.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 26.807 | 314.56 | 0.0[illegible]0665 | 20.135 | 11.347733 | 4.6842e-04 |
| 0.155 | 0.03 | (red.den.= 1.90131) | | 0.386583 | 1.5958e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-46 | 12/29/88 | 7:42 | 4940 | 106.80 | 9162026 | 20.00 |
| 28-46 | 12/29/88 | 7:42 | 4940 | 106.80 | 9178573 | 20.00 |
| 28-48 | 12/29/88 | 8:39 | 4875 | 106.70 | 9775052 | 20.00 |
| 28-48 | 12/29/88 | 8:39 | 4875 | 106.70 | 9774081 | 20.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 33.937 | 314.68 | 0.047573 | 21.020 | 19.759495 | 7.8104e-04 |
| 0.259 | 0.03 | (red.den.= 1.98494) | | 0.727887 | 2.8771e-05 |

Appendix 2.2 (cont.) Data2851 le

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | injection vol. (ul) | SCE loop vol. (ml) |
|---|---|---|---|
| 01/06/89 | 1.1417e-08 | 20.0 | 0.5 |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-51 | 01/02/89 | 1:02 | 1360 | 105.60 | 977800 | 5.00 |
| 28-51 | 01/02/89 | 1:02 | 1360 | 105.60 | 977800 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 9.478 | 314.04 | 0.080159 | 12.475 | 5.581771 | 3.7191e-04 |
| 0.000 | 0.00 | (red.den.= 1.17802) | | 0.000000 | 0.0000e+00 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-52 | 01/02/89 | 1:28 | 1470 | 105.60 | 1264000 | 5.00 |
| 28-52 | 01/02/89 | 1:28 | 1470 | 105.60 | 1256000 | 5.00 |
| 28-53 | 01/02/89 | 2:07 | 1470 | 105.60 | 1129000 | 5.00 |
| 28-53 | 01/02/89 | 2:07 | 1470 | 105.60 | 1162000 | 5.00 |
| 28-54 | 01/02/89 | 2:24 | 1465 | 105.60 | 1140000 | 5.00 |
| 28-54 | 01/02/89 | 2:24 | 1465 | 105.60 | 1147000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 10.225 | 314.04 | 0.070041 | 14.277 | 6.753156 | 3.9316e-04 |
| 0.018 | 0.00 | (red.den.= 1.34819) | | 0.346219 | 2.0156e-05 |

Appendix 2.2 (cont.) Data2851 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-55 | 01/02/89 | 2:56 | 2210 | 105.80 | 3229000 | 5.00 |
| 28-55 | 01/02/89 | 2:56 | 2210 | 105.80 | 3510000 | 5.00 |
| 28-57 | 01/02/89 | 3:37 | 2200 | 105.70 | 3694000 | 5.00 |
| 28-56 | 01/02/89 | 3:17 | 2200 | 105.70 | 3683000 | 5.00 |
| 28-56 | 01/02/89 | 3:17 | 2200 | 105.70 | 3746000 | 5.00 |
| 28-57 | 01/02/89 | 3:37 | 2200 | 105.70 | 3567000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 15.293 | 314.11 | 0.056509 | 17.696 | 20.387908 | 9.5709e-04 |
| 0.036 | 0.03 | (red.den.= 1.67104) | | 1.080091 | 5.0704e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-58 | 01/02/89 | 4:09 | 3250 | 105.70 | 10290000 | 5.00 |
| 28-58 | 01/02/89 | 4:09 | 3250 | 105.70 | 11380000 | 5.00 |
| 28-59 | 01/02/89 | 4:32 | 3230 | 105.70 | 10810000 | 5.00 |
| 28-59 | 01/02/89 | 4:32 | 3230 | 105.70 | 11360000 | 5.00 |
| 28-60 | 01/02/89 | 4:50 | 3225 | 105.60 | 10650000 | 5.00 |
| 28-60 | 01/02/89 | 4:50 | 3225 | 105.60 | 10200000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 22.406 | 314.08 | 0.051367 | 19.468 | 61.547144 | 2.6220e-03 |
| 0.082 | 0.03 | (red.den.= 1.83833) | | 2.900326 | 1.2356e-04 |

Appendix 2.2 (cont.) Data2851 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-61 | 01/02/89 | 5:21 | 3900 | 105.80 | 12560000 | 5.00 |
| 28-61 | 01/02/89 | 5:21 | 3900 | 105.80 | 12850000 | 5.00 |
| 28-62 | 01/02/89 | 5:42 | 3890 | 105.80 | 12260000 | 5.00 |
| 28-62 | 01/02/89 | 5:42 | 3890 | 105.80 | 12560000 | 5.00 |
| 28-63 | 01/02/89 | 6:03 | 3890 | 105.80 | 12630000 | 5.00 |
| 28-63 | 01/02/89 | 6:03 | 3890 | 105.80 | 13290000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 26.945 | 314.15 | 0.049521 | 20.194 | 72.450379 | 2.9745e-03 |
| 0.036 | 0.00 | (red.den.= 1.90686) | | 1.990431 | 8.1718e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 28-64 | 01/02/89 | 7:26 | 4990 | 105.90 | 13550000 | 5.00 |
| 28-64 | 01/02/89 | 7:26 | 4990 | 105.90 | 13290000 | 5.00 |
| 28-65 | 01/02/89 | 7:44 | 4990 | 106.00 | 13590000 | 5.00 |
| 28-65 | 01/02/89 | 7:44 | 4990 | 106.00 | 12700000 | 5.00 |
| 28-66 | 01/02/89 | 8:07 | 4990 | 106.10 | 13230000 | 5.00 |
| 28-66 | 01/02/89 | 8:07 | 4990 | 106.10 | 12850000 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 34.506 | 314.26 | 0.047358 | 21.116 | 75.361714 | 2.9589e-03 |
| 0.000 | 0.05 | (red.den.= 1.99395) | | 2.067838 | 8.1189e-05 |

Appendix 2.2 (cont.) Data3100 file

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | injection vol. (ul) | SCE loop vol. (ml) |
|---|---|---|---|
| 02/10/89 | 9.1500e-10 | 20.0 | 0.0 |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 31-1 | 02/07/89 | 9:27 | 2275 | 104.40 | 238356 | 5.00 |
| 31-2 | 02/07/89 | 9:53 | 2275 | 105.00 | 258604 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 15.787 | 313.54 | 0.055694 | 17.955 | 2.841990 | 1.3160e-04 |
| 0.000 | 0.24 | (red.den.= 1.69550) | | 0.163756 | 7.5827e-06 |

Raw experimental data:

| sample code | date | time | pres psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 31-3 | 02/07/89 | 10:23 | 3150 | 105.20 | 5153212 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 21.820 | 313.82 | 0.051580 | 19.387 | 58.939862 | 2.5216e-03 |
| 0.000 | 0.00 | (red.den.= 1.83073) | | 0.000000 | 0.000[illegible]+00 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 31-5 | 02/07/89 | 11:22 | 3950 | 105.30 | 1418291 | 5.00 |

Appendix 2.2 (cont.)  Data3100  file

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 27.336 | 313.87 | 0.049321 | 20.275 | 16.221703 | 6.6484e-04 |
| 0.000 | 0.00 | (red.den.= 1.91457) | | 0.000000 | 0.0000e+00 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 31-4 | 02/07/89 | 10:57 | 3150 | 105.20 | 1772926 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 21.820 | 313.82 | 0.051580 | 19.387 | 20.277841 | 8.6896e-04 |
| 0.000 | 0.00 | (red.den.= 1.83073) | | 0.000000 | 0.0000e+00 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 31-6 | 02/07/89 | 11:50 | 3940 | 105.20 | 3201841 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 27.267 | 313.82 | 0.049332 | 20.271 | 36.621056 | 1.5000e-03 |
| 0.000 | 0.00 | (red.den.= 1.91415) | | 0.000000 | 0.0000e+00 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 31-7 | 02/07/89 | 12:20 | 4470 | 105.40 | 2093402 | 5.00 |
| 31-8 | 02/07/89 | 12:50 | 4470 | 105.50 | 2064371 | 5.00 |

Appendix 2.2 (cont.)  Data3100  file

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 30.921 | 313.96 | 0.048230 | 20.734 | 23.777264 | 9.5268e-04 |
| 0.000 | 0.04 | (red.den.= 1.95788) | | 0.234789 | 9.4072e-06 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 31-9 | 02/07/89 | 1:32 | 4980 | 105.50 | 2999882 | 5.00 |
| 31-10 | 02/07/89 | 2:15 | 4980 | 105.50 | 2823094 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 34.437 | 313.98 | 0.047324 | 21.131 | 33.300144 | 1.3087e-03 |
| 0.000 | 0.00 | (red.den.= 1.99537) | | 1.429779 | 5.6190e-05 |

Appendix 2.2 (cont.)  Data3111  file

HPLC analysis standard and other common information

| date | cyclosporine std. (mg/AREA) | injection vol. (ul) | SCE loop vol. (ml) |
|---|---|---|---|
| 02/10/89 | 9.1500e-10 | 20.0 | 0.0 |

standard deviations are shown as the second line of results

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 31-11 | 02/07/89 | 4:00 | 2675 | 105.60 | 1443390 | 5.00 |
| 31-12 | 02/07/89 | 4:56 | 2675 | 105.50 | 1394985 | 5.00 |
| 31-13 | 02/07/89 | 5:15 | 2680 | 105.60 | 1419659 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 18.556 | 314.02 | 0.053581 | 18.663 | 16.233755 | 7.2276e-04 |
| 0.020 | 0.03 | (red.den.= 1.76235) | | 0.276834 | 1.2325e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 31-14 | 02/07/89 | 5:35 | 3200 | 105.70 | 2367455 | 5.00 |
| 31-15 | 02/07/89 | 6:00 | 3180 | 105.70 | 2488845 | 5.00 |
| 31-16 | 02/07/89 | 6:18 | 3180 | 105.70 | 2322997 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 22.073 | 314.09 | 0.051536 | 19.404 | 27.371070 | 1.1716e-03 |
| 0.080 | 0.00 | (red.den.= 1.83230) | | 0.981868 | 4.2027e-05 |

Appendix 2.2 (cont.) Data3111 file

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 31-17 | 02/07/89 | 6:38 | 3850 | 105.80 | 4102589 | 5.00 |
| 31-18 | 02/07/89 | 7:01 | 3840 | 105.80 | 4142972 | 5.00 |
| 31-19 | 02/07/89 | 7:24 | 3840 | 105.80 | 4016471 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 26.600 | 314.15 | 0.049643 | 20.144 | 46.748997 | 1.9261e-03 |
| 0.040 | 0.00 | (red.den.= 1.90215) | | 0.739019 | 3.0448e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 31-20 | 02/07/89 | 7:52 | 4475 | 105.80 | 6410427 | 5.00 |
| 31-21 | 02/07/89 | 8:16 | 4460 | 106.00 | 6560090 | 5.00 |
| 31-22 | 02/07/89 | 8:51 | 4460 | 106.00 | 6574637 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 30.886 | 314.22 | 0.048294 | 20.707 | 74.515900 | 2.9835e-03 |
| 0.060 | 0.06 | (red.den.= 1.95529) | | 1.039655 | 4.1626e-05 |

Raw experimental data:

| sample code | date | time | pres (psi) | temp (F) | HPLC peak area | solvent vol. (ml) |
|---|---|---|---|---|---|---|
| 31-23 | 02/07/89 | 9:17 | 4985 | 106.00 | 8943908 | 5.00 |
| 31-24 | 02/07/89 | 9:40 | 4970 | 105.90 | 9054490 | 5.00 |
| 31-25 | 02/07/89 | 10:03 | 4960 | 105.80 | 8879882 | 5.00 |

Calculated results:

| pressure (MPa) | temperature (K) | CO2vol. (m^3/kmol) | density (kmol/m^3) | loop conc (mg/mlCO2) | molefrac |
|---|---|---|---|---|---|
| 34.380 | 314.21 | 0.047378 | 21.107 | 102.473442 | 4.0208e-03 |
| 0.087 | 0.06 | (red.den.= 1.99309) | | 1.010302 | 3.9642e-05 |

Appendix 2.3 Cyclosporine Solubility versus Reduced Density Linear Regression Results

Appendix 2.3 Linear regression results

Results for 308.9 K

| CO2 reduced density | CyA mole fraction | standard deviation |
|---|---|---|
| 1.54 | 3.110e-05 | 8.500e-06 |
| 1.75 | 8.580e-05 | 9.500e-06 |
| 1.88 | 2.890e-04 | 9.200e-05 |
| 1.96 | 4.770e-04 | 4.300e-05 |
| 2.02 | 6.470e-04 | 7.000e-06 |
| 1.31 | 1.260e-05 | 6.500e-06 |
| 1.48 | 1.310e-05 | 2.300e-06 |
| 1.77 | 1.120e-04 | 1.100e-05 |
| 1.33 | 7.120e-06 | 1.060e-06 |
| 1.33 | 9.840e-06 | 1.470e-06 |
| 1.52 | 1.700e-05 | 1.000e-06 |
| 1.52 | 2.340e-05 | 1.400e-06 |

Reduced density low end cutoff = 1.32

count = 11

red den. = 1.539000 log10(molefrac) = -4.507240 (std.dev.)/molefrac = 0.273312
red den. = 1.752000 log10(molefrac) = -4.066513 (std.dev.)/molefrac = 0.110723
red den. = 1.884000 log10(molefrac) = -3.539102 (std.dev.)/molefrac = 0.318339
red den. = 1.957000 log10(molefrac) = -3.321482 (std.dev.)/molefrac = 0.090147
red den. = 2.016000 log10(molefrac) = -3.189096 (std.dev.)/molefrac = 0.010819
red den. = 1.481000 log10(molefrac) = -4.882729 (std.dev.)/molefrac = 0.175573
red den. = 1.772000 log10(molefrac) = -3.950782 (std.dev.)/molefrac = 0.098214
red den. = 1.327000 log10(molefrac) = -5.147520 (std.dev.)/molefrac = 0.148876
red den. = 1.327000 log10(molefrac) = -5.007005 (std.dev.)/molefrac = 0.149390
red den. = 1.523000 log10(molefrac) = -4.769551 (std.dev.)/molefrac = 0.058824
red den. = 1.523000 log10(molefrac) = -4.630784 (std.dev.)/molefrac = 0.059829

equation of line is -8.955192 + 2.844887 x = y
adev =0.223917 bdev= 0.134747

Appendix 2.3 Linear regression results

chi -squared = 0.096384 q= 1.000000

line trials table using fixed densities 1.4 1.6 1.8 2.0
0.000011 0.000040 0.000146 0.000543

fit using ( std.dev.)/molefrac.

equation of line is -9.247679 + 3.004926 x = y
adev =0.144541 bdev= 0.073115
chi -squared = 8.041734 q= 0.529946

line trials table using fixed densities 1.4 1.6 1.8 2.0
0.000009 0.000036 0.000145 0.000578

Appendix 2.3 Linear regression results

Results for 314.0 K

| CO2 reduced density | CyA mole fraction | standard deviation |
|---|---|---|
| 1.11 | 3.940e-06 | 1.490e-06 |
| 1.34 | 8.810e-06 | 9.200e-07 |
| 1.67 | 4.570e-05 | 1.400e-06 |
| 1.82 | 1.450e-04 | 3.000e-06 |
| 1.90 | 4.310e-04 | 2.200e-05 |
| 1.90 | 4.680e-04 | 1.600e-05 |
| 1.99 | 6.470e-04 | 1.000e-05 |
| 1.99 | 7.810e-04 | 2.900e-05 |
| 1.97 | 1.450e-03 | 4.000e-05 |
| 1.87 | 3.810e-04 | 1.300e-05 |
| 1.70 | 8.150e-05 | 2.160e-05 |
| 0.75 | 7.330e-06 | 1.280e-06 |
| 1.33 | 9.730e-06 | 6.700e-07 |
| 1.33 | 7.050e-06 | 4.900e-07 |
| 1.77 | 1.350e-04 | 2.000e-05 |
| 1.70 | 1.320e-04 | 8.000e-06 |
| 1.91 | 6.650e-04 | 1.000e-08 |
| 1.96 | 9.530e-04 | 9.000e-06 |
| 2.00 | 1.310e-03 | 6.000e-05 |

Reduced density low end cutoff = 1.20

count = 17

red den. = 1.336000 log10(molefrac) = -5.055024 log10(std. dev.) = -6.036212
red den. = 1.668000 log10(molefrac) = -4.340084 log10(std. dev.) = -5.853872
red den. = 1.816000 log10(molefrac) = -3.838632 log10(std. dev.) = -5.522879
red den. = 1.901000 log10(molefrac) = -3.365523 log10(std. dev.) = -4.657578
red den. = 1.901000 log10(molefrac) = -3.329754 log10(std. dev.) = -4.795880
red den. = 1.985000 log10(molefrac) = -3.189096 log10(std. dev.) = -5.000000
red den. = 1.985000 log10(molefrac) = -3.107349 log10(std. dev.) = -4.537602
red den. = 1.969000 log10(molefrac) = -2.838632 log10(std. dev.) = -4.397940
red den. = 1.870000 log10(molefrac) = -3.419075 log10(std. dev.) = -4.886056
red den. = 1.697000 log10(molefr c) = -4.088842 log10(std. dev.) =

Appendix 2.3 Linear regression results

```
-4.665546
 red den. = 1.331000 log10(molefrac) = -5.011887 log10(std. dev.) =
-6.173925
 red den. = 1.331000 log10(molefrac) = -5.151811 log10(std. dev.) =
-6.309804
 red den. = 1.766000 log10(molefrac) = -3.869666 log10(std. dev.) =
-4.698970
 red den. = 1.696000 log10(molefrac) = -3.879426 log10(std. dev.) =
-5.096910
 red den. = 1.915000 log10(molefrac) = -3.177178 log10(std. dev.) =
-8.000000
 red den. = 1.958000 log10(molefrac) = -3.020907 log10(std. dev.) =
-5.045757
 red den. = 1.995000 log10(molefrac) = -2.882729 log10(std. dev.) =
-4.221849


 equation of line is -9.414391 + 3.203155 x   = y
   adev =0.282739  bdev= 0.158280
chi -squared = 0.330864  q= 1.000000

line trials table using fixed densities 1.4 1.6 1.8 2.0
   0.000012        0.000051        0.000225        0.000982

  fit using log ( std.dev.)

 equation of line is -9.518815 + 3.262979 x   = y
    adev =10.961293  bdev= 6.021887
chi -squared = 0.012632  q= 1.000000

line trials table using fixed densities 1.4 1.6 1.8 2.0
   0.000011      0.000050      0.000226      0.001017
```

Appendix 2.3 Linear regression results

Results for 319.0 K

| CO2 reduced density | CyA mole fraction | standard deviation |
|---|---|---|
| 1.58 | 2.690e-05 | 4.500e-06 |
| 1.76 | 1.030e-04 | 1.000e-05 |
| 1.86 | 2.370e-04 | 5.100e-05 |
| 1.86 | 3.370e-04 | 4.200e-05 |
| 1.94 | 6.080e-04 | 4.700e-05 |
| 1.94 | 7.430e-04 | 8.500e-05 |
| 0.88 | 1.740e-06 | 2.040e-06 |
| 1.06 | 2.940e-06 | 5.500e-07 |
| 1.80 | 1.810e-04 | 2.000e-06 |
| 1.04 | 6.680e-07 | 1.000e-08 |
| 1.04 | 4.830e-07 | 1.000e-08 |
| 1.75 | 1.600e-04 | 1.200e-05 |
| 0.29 | 2.720e-05 | 1.000e-08 |
| 0.67 | 7.980e-07 | 1.000e-08 |
| 1.62 | 4.660e-05 | 4.400e-06 |
| 1.84 | 1.830e-04 | 1.600e-05 |
| 1.94 | 4.190e-04 | 3.400e-05 |

Reduced density low end cutoff = 1.40

count = 11

red den. = 1.577000 log10(molefrac) = -4.570248 (std.dev.)/molefrac = 0.167286
red den. = 1.756000 log10(molefrac) = -3.987163 (std.dev.)/molefrac = 0.097087
red den. = 1.861000 log10(molefrac) = -3.625252 (std.dev.)/molefrac = 0.215190
red den. = 1.861000 log10(molefrac) = -3.472370 (std.dev.)/molefrac = 0.124629
red den. = 1.943000 log10(molefrac) = -3.216096 (std.dev.)/molefrac = 0.077303
red den. = 1.943000 log10(molefrac) = -3.129011 (std.dev.)/molefrac = 0.114401
red den. = 1.800000 log10(molefrac) = -3.742321 (std.dev.)/molefrac = 0.011050
red den. = 1.755000 log10(molefrac) = -3.795880 (std.dev.)/molefrac = 0.075000
red den. = 1.620000 log10(molefrac) = -4.331614 (std.dev.)/molefrac = 0.094421
red den. = 1.843000 log10(molefrac) = -3.737549 (std.dev.)/molefrac = 0.087432
red den. = 1.938000 log10(molefrac) = -3.377786 (std.dev.)/molefrac = 0.081146

Appendix 2.3 Linear regression results

```
 equation of line is -10.042013 + 3.491826 x   = y
   adev =0.439679  bdev= 0.242555
chi -squared = 0.081790  q= 1.000000

line trials table using fixed densities 1.4 1.6 1.8 2.0
   0.000007        0.000035        0.000175        0.000874

  fit using  ( std.dev.)/molefrac.

 equation of line is -9.770345 + 3.348483 x   = y
    adev =0.476141  bdev= 0.264049
chi -squared = 9.116577  q= 0.426583

line trials table using fixed densities 1.4 1.6 1.8 2.0
   0.000008      0.000039      0.000181      0.000845
```

Appendix 2.3 Linear regression results

Results for 324.5 K

| CO2 reduced density | CyA mole fraction | standard deviation |
|---|---|---|
| 1.83 | 3.740e-04 | 1.180e-04 |
| 1.83 | 4.240e-04 | 8.000e-05 |
| 1.91 | 6.670e-04 | 4.200e-05 |
| 1.91 | 7.070e-04 | 2.000e-05 |
| 0.76 | 8.040e-06 | 5.540e-06 |
| 1.47 | 2.410e-05 | 3.200e-06 |
| 1.70 | 8.510e-05 | 1.480e-05 |
| 1.80 | 2.280e-04 | 3.300e-05 |
| 1.80 | 3.700e-04 | 5.600e-05 |
| 0.50 | 3.640e-06 | 3.880e-06 |
| 1.13 | 2.220e-06 | 2.400e-06 |
| 1.42 | 3.550e-06 | 4.800e-07 |
| 1.43 | 9.040e-06 | 1.000e-08 |
| 1.75 | 2.040e-04 | 1.020e-04 |
| 1.85 | 4.810e-04 | 7.000e-06 |
| 1.82 | 4.730e-04 | 3.000e-05 |
| 1.87 | 3.910e-04 | 1.620e-04 |
| 1.88 | 5.070e-04 | 4.020e-04 |
| 0.66 | 7.260e-07 | 5.170e-07 |
| 0.84 | 7.930e-07 | 2.290e-07 |
| 1.74 | 2.140e-04 | 4.300e-05 |

Reduced density low end cutoff = 1.50

count = 1

red den. = 1.826000 log10(molefrac) = -3.427128 log10(std. dev.) = -3.928118
red den. = 1.826000 log10(molefrac) = -3.372634 log10(std. dev.) = -4.096910
red den. = 1.908000 log10(molefrac) = -3.175874 log10(std. dev.) = -4.376751
red den. = 1.908000 log10(molefrac) = -3.150581 log10(std. dev.) = -4.698970
red den. = 1.699000 log10(molefrac) = -4.070070 log10(std. dev.) = -4.829738
red den. = 1.802000 log10(molefrac) = -3.642065 log10(std. dev.) = -4.481486
red den. = 1.802000 log10(molefrac) = -3.431798 log10(std. dev.) = -4.251812
red den. = 1.753000 log10(molefrac) = -3.690370 log10(std. dev.) = -3.991400
red den. = 1.851000 log10(molefrac) = -3.317855 log10(std. dev.) = -5.154902

Appendix 2.3 Linear regression results

red den. = 1.824000 log10(molefrac) = -3.325139 log10(std. dev.) = -4.522879
red den. = 1.873000 log10(molefrac) = -3.407823 log10(std. dev.) = -3.790485
red den. = 1.877000 log10(molefrac) = -3.294992 log10(std. dev.) = -3.395774
red den. = 1.744000 log10(molefrac) = -3.669586 log10(std. dev.) = -4.366531

equation of line is -10.209376 + 3.703456 x = y
adev =0.782595 bdev= 0.429159
chi -squared = 0.097378 q= 1.000000

line trials table using fixed densities 1.4 1.6 1.8 2.0
0.000009 0.000052 0.000286 0.001576

fit using log ( std.dev.)

equation of line is -9.917643 + 3.542576 x = y
adev =36.547798 bdev= 20.009726
chi -squared = 0.005148 q= 1.000000

line trials table using fixed densities 1.4 1.6 1.8 2.0
0.000011 0.000056 0.000288 0.001471

Appendix 2.3 Linear regression results

Results for 335.0 K

| CO2 reduced density | CyA mole fraction | standard deviation |
|---|---|---|
| 0.63 | 3.890e-06 | 2.290e-06 |
| 1.29 | 8.270e-06 | 1.120e-06 |
| 1.59 | 6.570e-05 | 1.240e-05 |
| 1.71 | 1.910e-04 | 3.200e-05 |
| 1.82 | 5.980e-04 | 1.080e-04 |
| 1.82 | 6.300e-04 | 8.100e-05 |
| 1.73 | 2.380e-04 | 3.400e-05 |
| 0.63 | 9.960e-08 | 1.000e-08 |
| 0.63 | 1.380e-07 | 1.000e-08 |
| 0.47 | 1.960e-06 | 1.000e-06 |
| 0.63 | 2.950e-06 | 1.390e-06 |

Reduced density low end cutoff = 1.40

count = 5

red den. = 1.591000 log10(molefrac) = -4.182435 (std.dev.)/molefrac = 0.188737
red den. = 1.710000 log10(molefrac) = -3.718967 (std.dev.)/molefrac = 0.167539
red den. = 1.820000 log10(molefrac) = -3.223299 (std.dev.)/molefrac = 0.180602
red den. = 1.820000 log10(molefrac) = -3.200660 (std.dev.)/molefrac = 0.128571
red den. = 1.726000 log10(molefrac) = -3.623423 (std.dev.)/molefrac = 0.142857

equation of line is -10.986472 + 4.267171 x = y
adev =0.203664 bdev= 0.117354
chi -squared = 0.001482 q= 1.000000

line trials table using fixed densities 1.4 1.6 1.8 2.0
0.000010 0.000069 0.000495 0.003531

fit using ( std.dev.)/molefrac.

equation of line is -11.061948 + 4.311397 x = y
adev =1.570545 bdev= 0.898283
chi -squared = 0.057573 q= 0.996389

line trials table using fixed densities 1.4 1.6 1.8 2.0
0.000009 0.000069 0.000500 0.003638

Appendix 2.3 Linear regression results

Results for 343.0 K

| CO2 reduced density | CyA mole fraction | standard deviation |
|---|---|---|
| 0.51 | 3.970e-05 | 7.300e-06 |
| 1.08 | 1.250e-05 | 4.200e-06 |
| 1.48 | 3.220e-05 | 7.100e-06 |
| 1.62 | 2.150e-04 | 1.700e-05 |
| 1.74 | 4.480e-04 | 5.400e-05 |
| 1.74 | 5.300e-04 | 3.200e-05 |
| 1.77 | 7.230e-04 | 8.600e-05 |
| 1.77 | 5.560e-04 | 3.900e-05 |
| 1.77 | 7.020e-04 | 7.000e-06 |
| 0.55 | 3.440e-07 | 3.800e-08 |
| 0.54 | 1.290e-05 | 2.000e-06 |
| 0.48 | 3.800e-06 | 5.200e-07 |
| 0.55 | 1.850e-06 | 1.370e-06 |

Reduced density low end cutoff = 1.40

count = 7

red den. = 1.477000 log10(molefrac) = -4.492144 (std.dev.)/molefrac = 0.220497
red den. = 1.615000 log10(molefrac) = -3.667562 (std.dev.)/molefrac = 0.079070
red den. = 1.740000 log10(molefrac) = -3.348722 (std.dev.)/molefrac = 0.120536
red den. = 1.740000 log10(molefrac) = -3.275724 (std.dev.)/molefrac = 0.060377
red den. = 1.769000 log10(molefrac) = -3.140862 (std.dev.)/molefrac = 0.118949
red den. = 1.769000 log10(molefrac) = -3.254925 (std.dev.)/molefrac = 0.070144
red den. = 1.769000 log10(molefrac) = -3.153663 (std.dev.)/molefrac = 0.009972

equation of line is -10.700196 + 4.256905 x = y
adev =0.577603 bdev= 0.339741
chi -squared = 0.042923 q= 1.000000

line trials table using fixed densities 1.4 1.6 1.8 2.0
0.000018 0.000129 0.000917 0.006510

fit using ( std.dev.)/molefrac.

equation of line is -9.816064 + 3.764972 x = y

Appendix 2.3 Linear regression results

```
    adev =0.740497  bdev= 0.419448
chi -squared = 4.468596  q= 0.484096

line trials table using fixed densities 1.4 1.6 1.8 2.0
   0.000029      0.000161      0.000914      0.005175
```

Appendix 2.4 Cyclosporine Data Multiple Regression Results

## Appendix 2.4 Statistical data for multiple regression done to generate Figure 4.20.

(Program used BSTAT by R.W.Wilson (c) 1989 Shareware)

CORRELATION TABLE

| VARIABLE | -1/T | 1/T^2 | log den. | log conc. |
|---|---|---|---|---|
| 1-1/T | 1.0000 | -0.9999 | -0.4517 | 0.1229 |
| 21/T^2 | -0.9999 | 1.0000 | 0.4528 | -0.1193 |
| 3log den. | -0.4517 | 0.4528 | 1.0000 | 0.0049 |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |
| 11 | | | | |
| 12 | | | | |
| 13 | | | | |
| 14 | | | | |
| 15 | | | | |
| 16 | | | | |
| 17 | | | | |
| 18 | | | | |
| 19 | | | | |

Multiple Regression Analysis
Dependent Variable log conc.

| Variable | Mean | Std Err | Coefficient | Std Err | T | Beta |
|---|---|---|---|---|---|---|
| CONSTANT | 1.000 | 0.000 | 91.69769 | 34.9720 | 2.622 | 0.00000 |
| -1/T | -0.003 | 0.000 | 82789.43073 | 22623.7484 | 3.659 | 0.70907 |
| 1/T^2 | 0.000 | 0.000 | 2511489.38736 | 673923.2124 | 3.405 | 0.18432 |
| log den. | 2.940 | 0.063 | 15.71698 | 0.6401 | 24.554 | 1.00904 |

Multiple Regression Analysis

Standard error 0.19

R-SQUARED 0.9625 Adjusted R-Squared 0.9588

F VALUE 214.8257

Degrees of freedom: Numerator 3 Denominator 25

Percent chance of a higher value; if no relation 0.00

Durbin-Watson statistic 1.68
Squared deviations due regression 24.289
Squared deviations due residuals 0.946
Estimated Cochran value for next iteration 1.0567
Farrar Glauber Prob of insignificant multicolinearity 0.00
Condition index of data matrix is 133.42
# of runs 14 Probability of randomness NA
Estimate of ridge parameter 0.0000

Appendix 2.5 (cont.)

Experiment #39 done May 4 to 5, 1989 (pre-micrometering valve)

mycelial extraction, harvested March frozen till Apr. 19, biomass recovered by sieving, air dried 24 hr then oven dried 72 hr, ground and sieved to be 355 um to 855 um

| Extraction conditions | | letdown valve |
|---|---|---|
| 32.3 - 32.2 MPa | 314.0 - 314.3 K | 316 - 321 K |
| 4675 - 4650 psig | 105.6 - 106.2 F | 110 - 118 F |

CyA Std = 1.188 mg/mL -> HPLC area
23659759, 23911773
23514857, 22549989
23731012, 24105242
23573158, 22872947
24046855, 24471593
23852869, 23210120
22707340, 22739909
22716132, 23672243

ave = 23457862.38 ~ 587927.55 (2.51 % variation)

Biomass loaded, 1.0992 g extracted, 5 mL, 1.81e7 -> 4.5833 mg -> 4.1697 mg/g

8.9928 g -> 37.497 mg CyA loaded (estimate)

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed |
|---|---|---|---|---|---|---|
| 39-1 | 45 | 32.7 | 20 | 9.79e6 | 9.916 | 0.2768 |
| 39-2 | 90 | 67 | 20 | 7.51e6 | 7.607 | 0.4892 |
| 39-3 | 135 | 95.7 | 20 | 1.27e6 | 1.286 | 0.5251 |
| 39-4 | 185 | 152 | 20 | 230195 | 0.233 | 0.5316 |
| 39-5 | 225 | 199 | 20 | 325807 | 0.330 | 0.5408 |
| flow off for 15 min | | | | | | |
| 39-6 | 255 | 269 | 20 | 597305 | 0.605 | 0.5577 |
| 39-7 | 300 | 332 | 20 | 42979 | 0.044 | 0.5589 |
| 39-L1 | 330 | 373.1 | 20 | 43406 | 0.044 | 0.5602 |
| repressurized to 31.8 MPa | | | | | | |
| 39-8 | 450 | 438.5 | 20 | 335028 | 0.339 | 0.5696 |
| 39-L2 | 480 | 487 | 20 | 31750 | 0.032 | 0.5705 |
| 39-J | --- | --- | 25 | 68155 | 0.086 | 0.5729 |

20.522 mg removed

Appendix 2.5 (cont.)

Experiment #35 done Apr. 6 - 7, 1989 (pre-micrometering valve)

mycelial extraction, semi-dry biomass harvested in Feb. 1989

| Extraction conditions | | letdown valve |
|---|---|---|
| 15.2 MPa | 313.5 - 314.8 K | 310 - 323 K |
| 2190 psig | 104.7 - 107.0 F | 99 - 121 F |

Biomass loaded CyA Std = 1.188 mg/mL -> area = 23457862

8.8324 g

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed |
|---|---|---|---|---|---|---|
| 35-1 | 75 | 74 | 11.7 | 3401236 | 2.015 | 0.0662 |
| 35-2 | 125 | 97.3 | 15 | 6654090 | 5.055 | 0.2324 |
| 35-3 | 170 | 111 | 17 | 2.71e6 | 2.333 | 0.3091 |
| 35-4 | 196 | 113 | 19.2 | 929905 | 0.904 | 0.3388 |
| 35-5 | 230 | 118 | 19 | 722409 | 0.695 | 0.3617 |
| 35-6 | 268 | 126.7 | 18.5 | 705563 | 0.661 | 0.3834 |
| 35-7 | 325 | 137.2 | 17.8 | 993607 | 0.896 | 0.4129 |
| 35-8 | 367 | 144 | 18.6 | 1.10e6 | 1.036 | 0.4469 |
| flow stopped overnight | | | | | | |
| 35-9 | 427 | 238 | 11 | 5.77e6 | 3.214 | 0.5526 |
| 35-10 | 487 | 265 | 15.3 | 1.12e6 | 0.868 | 0.5811 |
| 35-11 | 547 | 303 | 14.3 | 787242 | 0.570 | 0.5999 |
| pressure drop to 10.4 MPa followed by recovery to 15.2 MPa | | | | | | |
| 35-12 | 607 | 380.4 | 11.5 | 471928 | 0.275 | 0.6089 |
| 35-13 | 667 | 605.2 | 9.0 | 794964 | 0.362 | 0.6208 |
| 35-L | 727 | 635.2 | 14.2 | 1847921 | 1.329 | 0.6645 |

20.213 mg removed

35-E (total extraction), 5 mL, 4.03e7 -> 10.205 mg left
20.213 mg removed
---
30.418 mg initially

Appendix 2.5 (cont.)

Experiment #37 done Apr. 20, 1989 (pre-micrometering valve)

mycelial extraction, harvested March frozen till Apr. 19,
biomass recovered by sieving, oven dried 24 hr on aluminium foil,
flaked off foil and used as large flakes

wet mycelia 2.0567g -> 0.2477 g -> (0.1204 dry/wet)
wet mycelia ethyl acetate extraction 3.0468 g, 5 mL, 8.11e6 ->
2.1042 mg

5.736 mg/g dry mycelia

| Extraction conditions | | letdown valve |
|---|---|---|
| 33.2 - 32.0 MPa | 314.1 - 314.8 K | 320 - 324 K |
| 4800 - 4625 psig | 105.7 - 107.0 F | 117 - 124 F |

Biomass loaded CyA Std = 1.188 mg/mL -> area = 23074666, 22714055
ave = 22894360

10.88 g -> 62.408 mg CyA loaded

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed |
|---|---|---|---|---|---|---|
| 37-1 | 30 | 19 | 20 | 1.62e7 | 15.813 | 0.2694 |
| 37-2 | 60 | 39 | 20 | 7.08e6 | 7.348 | 0.3871 |
| 37-3 | 90 | 50 | 20 | 1.20e6 | 1.245 | 0.4071 |
| 37-4 | 120 | 60 | 20 | 896195 | 0.930 | 0.4220 |
| 37-5 | 180 | 86 | 20 | 807831 | 0.838 | 0.4354 |
| 37-6 | 240 | 117 | 20 | 380350 | 0.395 | 0.4418 |
| 37-L | 300 | 147 | 20 | 1.98e6 | 2.055 | 0.4747 |
| 37-J | --- | --- | 43 | 0 | 0 | |

29.624 mg removed

37-E (total extract ->20 mL lost part of sample
~0.5 to 1.0 mL remaining),

5 mL, 5.22e6 -> 1.3543 mg/0.5 mL x 20 mL = 54.174 mg left
(estimate)
or
-> 1.3543 mg/mL x 20 mL = 27.087 mg left (estimate)

27.087 mg left ( estimate )
29.624 mg removed
---
56.711 mg initially

62.408 mg load estimated (90.9 %)

Appendix 2.5 (cont.)

Experiment #38 done Apr. 25, 1989 (pre-micrometering valve)

mycelial extraction, harvested March frozen till Apr. 19,
biomass recovered by sieving, oven dried 24 hr on aluminium foil,
flaked off foil and used as large flakes

wet mycelia 2.0567g -> 0.2477 g -> (0.1204 dry/wet)
wet mycelia ethyl acetate extraction 3.0468 g, 5 mL, 8.11e6 ->
2.1042 mg

5.736 mg/g dry mycelia

Extraction conditions letdown valve

| | | |
|---|---|---|
| 33.1 - 32.0 MPa | 314.6 - 314.9 K | 323 K |
| 4650 - 4625 psig | 106.7 - 107.2 F | 122 F |

Biomass loaded CyA Std = 1.188 mg/mL -> area = 23733803, 23885041, 22563885, 23699767, 23843059
ave = 23545111

9.4682 g -> 54.310 mg Cya loaded

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed |
|---|---|---|---|---|---|---|
| 38-1 | 30 | 12 | 20 | 3.93e6 | 3.966 | 0.0774 |
| 38-2 | 75 | 17 | 20 | 4.47e6 | 4.511 | 0.1655 |
| 38-3 | 120 | 36.5 | 20 | 1.14e7 | 11.504 | 0.3901 |
| 38-4 | 175 | 57.5 | 20 | 3.19e6 | 3.219 | 0.4529 |
| 38-5 | 210 | 67 | 20 | 7.81e6 | 7.881 | 0.6068 |
| 38-6 | 255 | 77.5 | 20 | 453983 | 0.458 | 0.6157 |
| 38-7 | 290 | 86.5 | 20 | 175844 | 0.177 | 0.6192 |
| 38-8 | 310 | 144 | 20 | 267635 | 0.270 | 0.6244 |
| 38-L | 350 | 174 | 20 | 338241 | 0.341 | 0.6311 |
| 38-J | --- | --- | 20 | 0 | 0 | |

32.327 mg removed

38-E (total extract), 5 mL, 7.49e7 -> 18.896 mg left
32.327 mg removed
---
51.223 mg initially

(94.3 % of 54.310 mg CyA load estimate)

Appendix 2.5 (cont.)

Experiment #39 done May 4 to 5, 1989 (pre-micrometering valve)

mycelial extraction, harvested March frozen till Apr. 19, biomass recovered by sieving, air dried 24 hr then oven dried 72 hr, ground and sieved to be 355 um to 855 um

| Extraction conditions | | letdown valve |
|---|---|---|
| 32.3 - 32.2 MPa | 314.0 - 314.3 K | 316 - 321 K |
| 4675 - 4650 psig | 105.6 - 106.2 F | 110 - 118 F |

CyA Std = 1.188 mg/mL -> HPLC area
23659759, 23911773
23514857, 22549989
23731012, 24105242
23573158, 22872947
24046855, 24471593
23852869, 23210120
22707340, 22739909
22716132, 23672243

ave = 23457862.38 ~ 587927.55 (2.51 % variation)

Biomass loaded, 1.0992 g extracted, 5 mL, 1.81e7 -> 4.5833 mg -> 4.1697 mg/g

8.9928 g -> 37.497 mg CyA loaded (estimate)

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed |
|---|---|---|---|---|---|---|
| 39-1 | 45 | 32.7 | 20 | 9.79e6 | 9.916 | 0.2768 |
| 39-2 | 90 | 67 | 20 | 7.51e6 | 7.607 | 0.4892 |
| 39-3 | 135 | 95.7 | 20 | 1.27e6 | 1.286 | 0.5251 |
| 39-4 | 185 | 152 | 20 | 230195 | 0.233 | 0.5316 |
| 39-5 | 225 | 199 | 20 | 325807 | 0.330 | 0.5408 |
| flow off for 15 min | | | | | | |
| 39-6 | 255 | 269 | 20 | 597305 | 0.605 | 0.5577 |
| 39-7 | 300 | 332 | 20 | 42979 | 0.044 | 0.5589 |
| 39-L1 | 330 | 373.1 | 20 | 43406 | 0.044 | 0.5602 |
| repressurized to 31.8 MPa | | | | | | |
| 39-8 | 450 | 438.5 | 20 | 335028 | 0.339 | 0.5696 |
| 39-L2 | 480 | 487 | 20 | 31750 | 0.032 | 0.5705 |
| 39-J | --- | --- | 25 | 68155 | 0.086 | 0.5729 |

20.522 mg removed

Appendix 2.5 (cont.)

39-E1 (1.3516g/6.9404g), 20 mL, 1.48e6 -> 1.4991 mg -> 1.1091 mg/g left

7.698 mg CyA left
20.522 mg CyA removed
---
28.220 mg initially

39-E2 (0.8249g/6.9404g), 5 mL, 7.18 S -> 1.8181 mg -> 2.2040 mg/g left

15.297 mg CyA left
20.522 mg CyA removed
---
35.819 mg initially

(95.5 % of 37.497 mg load estimated )

loss of mass 8.9928 - 6.9404 = 2.0524 g

Appendix 2.5 (cont.)

Experiment #40 done May 9, 1989 (pre-micrometering valve)

mycelial extraction, harvested March frozen till Apr. 19, biomass recovered by sieving, air dried 24 hr then oven dried 72 hr, ground and sieved to be 355 um or less

Extraction conditions letdown valve

32.0 MPa 314.8 K 300 - 323 K
4625 psig 107.0 F 81 - 121 F

Biomass loaded CyA Std = 1.188 mg/mL -> area = 22385952, 22413379
ave = 22399665

8.4600 g x 4.1697 mg/g = 35.276 mg loaded

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed |
|---|---|---|---|---|---|---|
| 40-1 | 15 | 24 | 20 | 1.13e7 | 11.986 | 0.3522 |
| 40-2 | 30 | 42 | 20 | 5.11e6 | 5.420 | 0.5114 |
| 40-3 | 45 | 64 | 20 | 939448 | 0.997 | 0.5407 |
| 40-4 | 60 | 83 | 20 | 235317 | 0.250 | 0.5481 |
| 40-5 | 105 | 125 | 20 | 31227 | 0.033 | 0.5490 |
| 40-6 | 150 | 222 | 20 | 1.08e6 | 1.146 | 0.5827 |
| 40-7 | 213 | 342 | 20 | 124458 | 0.132 | 0.5866 |
| 40-8 | 270 | 371.2 | 20 | 128884 | 0.137 | 0.5906 |
| 40-L | 300 | 411.5 | 20 | 760783 | 0.807 | 0.6143 |
| 40-J | --- | ----- | 20 | 38756 | 0.041 | 0.6155 |

20.949 mg removed

40-E1 (1.6361g/6.3930g), 5 mL, 9.06e6 -> 2.4026 mg -> 1.4685 mg/g (core)

40-E2 (1.0131g/6.3930g), 5 mL, 8.58e6 -> 2.2753 mg -> 2.2458 mg/g (outer annulus)

4.7569 g in annulus x 2.2458 mg/g + 2.4026 mg from core

= 13.086 mg CyA left
20.949 mg CyA removed
---
34.035 mg CyA initially

(96.5 % of 35.276 mg load estimate)

loss of mass 8.4600 - 6.3930 = 2.0670 g

---

remains 3.7438 g x 2.2458 mg/g = 8.4078 mg to expt #41-44

Appendix 2.5 (cont.)

Experiments #41,#42,#43,#44 done May 11, 12, 15, 16, 1989

(pre-micrometering valve)

mycelial extraction, reextraction of material from #39 and #40, material 850 um to 355 um and 355 um or less sized particles combined

CyA Std = 1.188 mg/mL -> area = 23318176, 23062480, 22973452, 23295077
ave = 23162296

Extraction conditions letdown valve

#41

32.3 - 31.8 MPa 313.7 - 314.3 K 308 - 317 K
4675 - 4600 psig 104.9 - 106.1 F 95 - 111 F

Biomass Loaded (combined mass of #39 + #40 = 7.2364 g total )

4.8168 g (66.56 % of total )

41-X (1.3548g/4.8168g), 5 mL, 1.09e7 -> 2.7953 mg -> 2.0633 mg/g ->
~9.5385 mg to start (estimate)

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed |
|---|---|---|---|---|---|---|
| 41-1 | 25 | 50 | 22 | 157905 | 0.178 | 0.0181 |
| 41-2 | 51 | 117 | 20 | 21884 | 0.022 | 0.0203 |
| 41-3 | 121 | 206 | 20 | 31738 | 0.033 | 0.0237 |
| 41-L | 138 | 270.6 | 20 | 54529 | 0.056 | 0.0293 |

0.289 mg removed

41-E1 (lost sample 90 - 125 um, 0.4495 g) x 1.3778 = 0.6193 mg (estimate)

41-E2 (63 - 90 um) (0.4107 g), 5 mL, 2129391 -> 0.5461 mg -> 1.3296 mg/g

41-E3 (0 - 63 um) (0.3890 g), 5 mL, 2.09e6 -> 0.5360 mg -> 1.3778 mg/g

41-E4 (355 - 855 um)(0.3740 g), 5 mL, 3.31e6 -> 0.8489 mg -> 2.2697 mg/g

(also 3.1028 g of 125 to 355 um to #42 ) x 2.26 = 7.0123 mg (estimate)

9.563 mg left
0.289 mg removed
9.852 mg initially
( 99.1 % of initial 9.9385 mg)

loss of mass 4.8168 - 4.7260 = 0.0908 g

Appendix 2.5 (cont.)

---

Extraction conditions letdown valve

#42

32.3 - 31.6 MPa 313.7 K 311 - 318 K
4675 - 4575 psig 105.0 F 100 - 112 F

Biomass Loaded

3.557 g (3.1028 g material from #41 -> 7.0123 mg CyA)
<-- (2.4196 g of other material from #39 & #40 -> 4.9924 mg CyA)
(-------- ----------)
7.732 mg (5.5224 g -> 2.1738 mg/g <- 12.005 mg CyA)
(estimated)
(material was ground to 125 um or less particles)

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed |
|---|---|---|---|---|---|---|
| 42-1 | 60 | 113 | 20 | 352829 | 0.362 | 0.0744 |
| 42-2 | 120 | 204.5 | 20 | 16564 | 0.017 | 0.0779 |
| 42-L | 140 | 240 | 20 | 19918 | 0.020 | 0.0820 |
| 42-J | --- | --- | 20 | 14103 | 0.015 | 0.0851 |

0.414 mg removed
#43 -> 4.450 mg left in #42
---
4.864 mg initially in #42
(experimental)

Appendix 2.5 (cont.)

---

Extraction conditions letdown valve

#43

32.2 - 31.8 MPa 313.4 - 314.2 K 317 - 319 K
4650 - 4600 psig 104.3 - 106.0 F 111 - 115 F

Biomass Loaded

~3.557 g (same material as #42 teflon sleeves removed from extractor)

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed | #42 + #43 combined runs total fraction removed |
|---|---|---|---|---|---|---|---|
| 43-1 | 60 | 63 | 20 | 255494 | 0.262 | 0.0589 | 0.1390 |
| 43-2 | 120 | 189.5 | 5.8 | 30292 | 0.009 | 0.0609 | 0.1408 |
| 43-L | 145 | 233 | 20 | 13730 | 0.014 | 0.0640 | 0.1437 |

0.285 mg removed
#44 -> 4.165 mg left in #43
---
4.450 mg initially in #43

(experimental)

Appendix 2.5 (cont.)

Extraction conditions letdown valve

#44

32.3 - 31.6 MPa 312.9 - 314.1 K 322 - 310 K
4675 - 4570 psig 103.6 - 105.7 F 120 - 99 F

Biomass Loaded

~3.557 g (same material as #42 & #43 except 0.5 mL H20 added)

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed | #42 + #43 + #44 combined runs Total fraction removed |
|---|---|---|---|---|---|---|---|
| 44-1 | 95 | 77 | 20 | 7786 | 0.008 | 0.0019 | 0.1453 |
| 44-2 | 180 | 198 | 6.8 | 7236 | 0.003 | 0.0026 | 0.1459 |
| 44-L | 205 | 238.7 | 16 | 6143 | 0.005 | 0.0038 | 0.1469 |
| 44-J | --- | ---- | 22.2 | 4632 | 0.005 | 0.0050 | 0.1479 |
| 44-D | --- | ---- | 5 | 70061 | 0.018 | 0.0094 | 0.1499 |

0.039 mg removed

44-E1 (0-63um) (0.2538 g), 5 mL, 1.28e6 -> 0.3283 mg -> 1.2934 mg/g

44-E2 (63-90um) (0.7870 g), 5 mL, 4.86e6 -> 1.2464 mg -> 1.5837 mg/g

44-E3 (+90um) (1.9774 g), 5 mL, 9.95e6 -> 2.5517 mg -> 1.2904 mg/g

3.0182 g

4.126 mg left
0.039 mg removed
4.165 mg initially
(experimental)

(+125 um only 0.0754g )
( or 2.5 % )

---

Appendix 2.5 (cont.)

overall yield calculations

#39 remains ~4.7637 g with 10.499 mg CyA remaining
#40 remains ~3.7438 g with 5.408 mg CyA remaining
8.5075 15.907 mg

1.3548 g used for CyA determination -> 2.0633mg/g

#39 + #40 7.2364 g with 14.9309 mg CyA in material
(~1.2 % variation)

#41 4.8168 g with 9.852 mg CyA (0.6656 x 7.2364 g)
yield 0.0293

#42 + #43 + #44 3.5570 g with 4.864 mg CyA ((0.6656 x 0.6442 x 7.2364
yield 0.150 + 0.3344 x 7.2364) x 0.6441)
= 3.5570 g (0.4916 x 7.2364)

14.9309 x 0.0293 = 0.438 mg removed

(14.9309 - 0.4380) x 0.150 = 2.174 mg removed
2.612 mg removed / 14.9309 mg =
0.175 yield

#39 35.819 mg CyA load 20.949 mg CyA removed
#40 34.035 mg CyA load 20.522 mg CyA removed
69.854 mg 41.471 mg combined yield -> 0.5937

fraction CyA remaining 1.0000 - 0.5937 = 0.4063

overall yield of #39,#40, #41 - #44 =
0.5937 + 0.4063 x 0.175 = 0.6648

Appendix 2.5 (cont.)

Experiment #45 done May 20, 1989 (pre-micrometering valve)

mycelial extraction, May 14 harvest, sieved to recover mycelia, partially dried material before loading

sample for moisture content 0.8522 g damp -> 0.6012 g dry , 29.5 %

| Extraction conditions | | letdown valve |
|---|---|---|
| 32.3 - 32.0 MPa | 313.4 - 314.6 K | ~318 K |
| 4675 - 4650 psig | 104.5 - 106.6 F | ~112 F |

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 22393473

11.0330 g damp -> 7.7834 g dry

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed |
|---|---|---|---|---|---|---|
| 45-1 | 60 | 45 | 20 | 176422 | 0.187 | 0.0166 |
| 45-2 | 102 | 104 | 20 | 1.73e6 | 1.836 | 0.1798 |
| 45-3 | 142 | 134 | 20 | 489326 | 0.519 | 0.2259 |
| 45-4 | 182 | 210 | 20 | 1.85e6 | 1.963 | 0.4004 |
| 45-5 | 222 | 272 | 20 | 503255 | 0.534 | 0.4478 |
| flow stopped overnight depressurized to 16.0 MPa, repressurized and restarted | | | | | | |
| 45-6 | 225 | 275 | 20 | 1.06e6 | 1.125 | 0.5478 |
| 45-7 | 321 | 360 | 20 | 566156 | 0.601 | 0.6012 |
| 45-8 | 402 | 424.5 | 20 | 125712 | 0.133 | 0.6130 |
| 45-9 | 476 | 481.5 | 20 | 49540 | 0.053 | 0.6178 |
| 45-L | 541 | 512.3 | 20 | 200815 | 0.213 | 0.6367 |
| 45-D | --- | ----- | 20 | 222266 | 0.236 | 0.6577 |
| 45-J | --- | ----- | 16.5 | 25369 | 0.022 | 0.6596 |
| | | | | | 7.422 mg removed | |

Appendix 2.5 (cont.)

45-E1 (top) (2.0334g/3.9278g), 5 mL, 3.06e6 -> 0.8117 mg ->

0.3992 mg/g wet or 1.5679 mg left

(moisture test 1.8944 g -> 1.3460 g, 29 %) 0.5618 mg/g dry

45-E2 (middle) (1.5189g/2.9629g), 5 mL, 1.99e6 -> 0.5279 mg ->

0.3475 mg/g wet or 1.0297 mg left

(moisture test 1.4440 g -> 1.1852 g, 18 %) 0.4234 mg/g dry

45-E3 (bottom) (1.1005g/2.3672g), 5 mL, 2.16e6 -> 0.5730 mg ->

0.5206 mg/g wet or 1.2324 mg left

(moisture test 1.2667 g -> 1.1232 g, 11 %) 0.5872 mg/g dry

total left = 3.830 mg

3.830 mg CyA left
7.422 mg CyA removed
---
11.252 mg CyA initially

loss of mass 11.0330 g - 9.2614 g = 1.7716 g wet basis

11.0330 x 0.7055 - 3.9278 x 0.7105 - 2.9629 x 0.8208 - 2.3672 x 0.8807

= 0.4763 g

moisture loss = 1.7716 - 0.4763 = 1.2953 g

Appendix 2.5 (cont.)

Experiment #46 done May 25, 1989 (pre-micrometering valve)

mycelial extraction, re-extraction of #45 some extractor internals removed

| Extraction conditions | | letdown valve |
|---|---|---|
| 32.3 - 31.8 MPa | 332.5 - 333.4 K | 319 K |
| 4675 - 4600 psig | 138.9 - 140.5 F | 114 F |

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 22393473

3.048 g

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed |
|---|---|---|---|---|---|---|
| 46-1 | 5 | 10 | 4/20 | 472272 | 0.501 | 0.2333 |
| (46-1 estimated, partially lost) | | | | | | |
| 46-2 | 44 | 66.3 | 20 | 8443 | 0.009 | 0.2375 |
| reset temperature to 311 K | | | | | | |
| 46-3 | 140 | 137 | 10 | 120011 | 0.064 | 0.2673 |
| reset temperature to 309 K | | | | | | |
| 46-4 | 207 | 184 | 10 | 100877 | 0.054 | 0.2925 |
| 46-5 | 267 | 221.7 | 10 | 28305 | 0.015 | 0.2995 |
| 46-L | 327 | 262 | 12.7 | 542502 | 0.366 | 0.4700 |
| 46-J | --- | --- | 54 | 4529 | 0.013 | 0.4760 |
| | | | | | 1.022 mg | |

46-E ( 2.9637 g total extract ),5 mL, 4.24e6 -> 1.125 mg left

1.125 mg CyA left
1.022 mg CyA removed
---
2.147 mg initially

loss of mass 3.0480 - 2.9637 = 0.0843 g

Estimate of CyA present as left from expt. #45

| | | |
|---|---|---|
| 0.5618 mg/g x | 1.3460 g | = 0.7562 mg |
| 0.4234 mg/g x | 1.1852 g | = 0.5018 mg |
| 0.5872 mg/g x | 1.1232 g | = 0.6595 mg |
| | 3.6544 g | 1.9175 mg |

Appendix 2.5 (cont.)

actual 3.0480 g loaded of 3.6544 g remaining (0.8341)

Cya #46 = 2.147 mg found versus 1.918 mg calculated as lef' in #45

which is 11.2 % higher

initial load of #45 11.0330 g x 0.7055 = 7.7838 g dry

3.0480/7.7838 = 0.3916 fraction extracted in #46

1/0.3916 = 2.5537 factor required to scale data of #46 to same range as #45

2.5537 x 1.125 mg left in #46 = 2.873 mg

11.252 mg initially in #45 - 2.873 mg left (calc) in #46

= 8.379 mg removed or a yield of 8.379/11.252 = 0.7447

but this does not account for the discrepency in CyA concentration in expt #46 with respect to expt #45 of 11.2 % due to experimental error

Calculation on the basis of yield in #46 gives

3.830 mg left from 45 extracted with a yield of 0.4760

gives 1.823 mg removed or 2.007 mg left

or a yield of (11.252 - 2.007)/11.252 = 0.8216

Appendix 2.5 (cont.)

Experiment #47 done May 30-31, 1989 (pre-micrometering valve)

mycelial extraction, May 14 harvest (156 Hr Ferm.), mycelia recovered by seiving, 'wet' material sample for moisture determination 2.2013 g wet -> 0.4410g dry -> 80.1%

| Extraction conditions | | letdown valve |
|---|---|---|
| 32.3 - 31.8 MPa | 332.0 - 332.9 K | 307 - 333 K |
| 4675 - 4600 psig | 138.1 - 139.5 F | 94 - 140 F |

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 22393473

19.7021 g wet -> 3.9470 g dry

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed |
|---|---|---|---|---|---|---|
| 47-1 | 10 | 4.75 | 20 | 41753 | 0.044 | 0.0119 |
| 47-2 | 23 | 7.75 | 20 | 27528 | 0.029 | 0.0198 |
| 47-3 | 29 | 8.75 | 20 | 17724 | 0.019 | 0.0250 |
| 47-4 | 50 | 11 | 20 | 16162 | 0.017 | 0.0296 |
| short hold | | | | | | |
| 47-5 | 112 | 14 | 20 | 14552 | 0.015 | 0.0337 |
| 47-6 | 172 | 19.7 | 20 | 8489 | 0.009 | 0.0361 |
| 47-7 | 287 | 29.2 | 40 | 178919 | 0.380 | 0.1393 |
| 47-8 | 335 | 80 | 16 | 91705 | 0.078 | 0.1604 |
| 47-9 | 360 | 123 | 14 | 35068 | 0.026 | 0.1675 |
| 47-10 | 398 | 161 | 12.5 | 14692 | 0.010 | 0.1702 |
| 47-11 | 428 | 221 | 5 | 78413 | 0.021 | 0.1759 |
| hold overnight pressure dropped to 16.0 MPa and temperature to 314.4 K | | | | | | |
| 47-12 | 513 | 372 | 5.4 | 143620 | 0.041 | 0.1870 |
| 47-13 | 584 | 426 | 13 | 7840 | 0.005 | 0.1884 |
| 47-L | 624 | 503 | 23 | 639476 | 0.780 | 0.4001 |

1.474 mg removed

47-E (total extract), 5mL, 8.33e6 -> 2.210 mg left

2.210 mg CyA left
1.474 mg CyA removed
---
3.684 mg initially

Appendix 2.5 (cont.)

Experiment #48 done June 2, 1989 (pre-micrometering valve)

mycelial extraction, May 14 harvest (156 Hr Ferm.), mycelia recovered by seiving, 'dry' material

| Extraction conditions | | letdown valve |
|---|---|---|
| 32.3 - 31.8 MPa | 332.0 - 332.8 K | 307 - 325 K |
| 4675 - 4600 psig | 138.1 - 139.3 F | 94 - 126 F |

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 21947945

4.6514 g

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed |
|---|---|---|---|---|---|---|
| 48-1 | 30 | 42 | 20 | 699625 | 0.757 | 0.1760 |
| 48-2 | 60 | 97 | 20 | 318517 | 0.345 | 0.2562 |
| 48-3 | 90 | 157 | 20 | 43936 | 0.048 | 0.2673 |
| 48-4 | 155 | 240 | 11.3 | 71493 | 0.044 | 0.2775 |
| 48-5 | 224 | 264 | 12.5 | 26135 | 0.018 | 0.2817 |
| temperature set point adjusted to 316 K ramped down during next interval | | | | | | |
| 48-6 | 391 | 284 | 17.8 | 18012 | 0.017 | 0.2857 |
| temperature set point adjusted to 312 K ramped down during next interval | | | | | | |
| 48-7 | 455 | 360 | 10.6 | 47637 | 0.027 | 0.2920 |
| 48-8 | 505 | 414 | 12.3 | 18468 | 0.012 | 0.2947 |
| 48-L | 530 | 456 | 48.5 | 468234 | 1.229 | 0.5804 |

2.497 mg removed

48-E (4.0711g total extract ), 5mL, 6.67e6 -> 1.805 mg left

1.805 mg CyA left
2.497 mg CyA removed
---
4.302 mg CyA initially

loss of mass 4.6514 - 4.0711 = 0.5803 g

Appendix 2.5 (cont.)

Experiment #49 done June 5-6, 1989 (pre-micrometering valve)

mycelial extraction, split load of material with variable histories
some remainders not used in previous experiments

| Extraction conditions | | letdown valve |
|---|---|---|
| 34.6 - 34.9 MPa | 310.9 K | 306 - 321 K |
| 5000 - 5050 psig | 100.0 F | 91 - 118 F |

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 22393473, 21947945
22908515, 23261543

b 8.2906 g of 0.3 - 0.6 mg/g material ~ 3.73 mg ave = 22627869
t 1.1981 g of 2.4 - 3.5 mg/g material ~ 3.53 mg
---
9.4887 g total with an estimated 7.26 mg of CyA present (b-> bottom, t-> top)

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed |
|---|---|---|---|---|---|---|
| 49-1 | 30 | 30.5 | 20 | 455792 | 0.479 | 0.0569 |
| 49-2 | 60 | 51 | 22 | 148915 | 0.172 | 0.0773 |
| 49-3 | 90 | 98 | 20 | 189852 | 0.199 | 0.1010 |
| 49-4 | 120 | 149 | 20 | 49366 | 0.052 | 0.1071 |
| 49-5 | 150 | 211.5 | 20 | 44637 | 0.047 | 0.1127 |
| flow stopped overnight pressure dropped to 10.8 MPa repressurized to 34.9 MPa | | | | | | |
| 49-6 | --- | ---- | 20 | 488030 | 0.512 | 0.1735 |
| 49-7 | 180 | 231.5 | 20 | 13440 | 0.014 | 0.1752 |
| ramped temperature to 332.6 K over next interval | | | | | | |
| 49-8 | 240 | 280 | 20 | 12980 | 0.014 | 0.1769 |
| 49-9 | 360 | 430 | 4.6 | 469070 | 0.113 | 0.1903 |
| 49-L | 400 | ~474 | 20 | 198781 | 0.209 | 0.2151 |
| 49-W | --- | ---- | 33.5 | 931769 | 1.639 | 0.4098 |

3.489 mg removed

bottom of extractor
49-E1a (7.0134 g total extracted), 5mL, 4.64e6 -\ 2.150 mg -\
b , 5mL, 3.55e6 -/ \
> 4.930
mg left
49-E2a (1.0596 g total extracted), 5mL, 9.57e6 -\ 2.780 mg /
b , 5mL, 1.02e6 -/ -/
top of extractor

4.930 mg CyA left
3.489 mg CyA removed
---
8.419 mg CyA initially
(more than initial estimate of 7.26 mg)

Appendix 2.5 (cont.)

Experiment #50 done June 7, 1989 (pre-micrometering valve)

mycelial extraction, harvested May 13, filtered out mycelia from broth, placed on aluminium foil and dried in oven at 80 C overnight, removed material from foil and ground with Wiley mill, sieved material to size range 212 um to 355 um using standard sieves.

| Extraction conditions | | letdown valve |
|---|---|---|
| 34.6 - 33.9 MPa | 310.9 - 311.0 K | 301 - 320 K |
| 5000 - 4900 psig | 100.0 - 101.2 F | 82 - 117 F |

Biomass Loaded

5.5114 g

CyA Std = 0.132 mg/mL -> area = 2581986, 2573104
2505213
ave = 2553434

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | cumulative fraction removed |
|---|---|---|---|---|---|---|
| 50-1 | 60 | 45.5 | 20 | 607774 | 0.628 | 0.1694 |
| 50-2 | 100 | 112.6 | 20 | 115949 | 0.120 | 0.2018 |
| 50-3 | 140 | 187 | 20 | 48293 | 0.050 | 0.2152 |
| 50-4 | 170 | 246.8 | 20 | 46794 | 0.048 | 0.2282 |
| held overnight pressure dropped to 14.6 MPa repressurized to 34.6 MPa | | | | | | |
| 50-5 | --- | 246.9 | 20 | 146780<br>152436 | 0.155 | 0.2700 |
| 50-6 | 230 | 338 | 10.4 | 34907<br>33370 | 0.018 | 0.2748 |
| 50-7 | 290 | 416 | 10.9 | 35041<br>35818 | 0.020 | 0.2802 |
| Temperature set point adjusted to 320 K then to 327.6 K | | | | | | |
| 50-8 | 355 | 478 | 12 | 28250 | 0.018 | 0.2851 |
| 50-L | 389 | 510.8 | 62.7 | 93912 | 0.304 | 0.3671 |

1.361 mg removed

50-Ea (1.8176g/4.9764g) , 5mL, 2.84e6 -\ 0.8570 mg -> 0.4715 mg/g
b 5mL, 475762 -/

2.347 mg CyA left
1.361 mg Cya removed
---
3.708 mg initially

loss of mass 5.5114 - 4.9764 = 0.5350 g
initial CyA determination

dried biomass 0.3877 g -> 5mL, 803261 -\ 0.2248 mg -> 0.5798 mg/g
5mL, 66468 -/ of initial load
5.5114g x 0.5798 mg/g = 3.196 mg loaded (low estimate)

Appendix 2.5 (cont.)

Experiment #51 done June 12, 1989 (pre-micrometering valve)

mycelial extraction, harvested May 13, material was stored frozen and thawed shortly before use , centrifuged 20 min. to recover mycelia, pellet loaded damp into extractor, very dense bed no channels

sample dried for moisture determination 1.1248 g wet -> 0.2595 g dry 23.1 % dry matter

| Extraction conditions | | letdown valve |
|---|---|---|
| 32.9 MPa | 313.3 - 314.3 K | 311 K |
| 4750 psig | 104.4 - 106.3 F | 100 F |

Biomass Loaded

14.1181 g wet

CyA Std = 0.132 mg/mL -> area = 2616565, 2671071, 2614826, 2731283
ave = 2658436

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed |
|---|---|---|---|---|---|---|
| 51-1 | 32 | 36 | 20 | 0 | | |
| 51-2 | 60 | 66 | 20 | 0 | Cyclosporine peaks for this experiment | |
| 51-3 | 120 | 108.3 | 20 | 0 | could not be detected though other | |
| 51-4 | 240 | 186.5 | 10 | 0 | peaks were present | |
| 51-5 | 353 | 348 | 4.8 | 0 | | |
| 51-6 | 496 | 567 | 5 | 0 | | |
| 51-L | 556 | 665 | 44 | trace | | |

51-E (total extracted) , 5ml, 9715576 -> 2.415 mg left

initial CyA approximately same as final = 2.415 mg CyA

initial CyA from pre-load sampling -> 1.5338 g wet biomass
-> 2 x 5mL, 1.22e6 + 149763 -> 0.3401 mg -> 0.2217 mg/g wet

-> 14.1181g x 0.2217 mg/g = 3.1302 mg CyA loaded
(estimates 23% higher)
(than remainder )

Appendix 2.5 (cont.)

Experiment #52 done June 20, 1989 (pre-micrometering valve)

mycelial extraction, white surface growth mycelia from an unagitated flask, oven dried on aluminium foil, flakes loaded for extraction

| Extraction Conditions | | letdown valve |
|---|---|---|
| 33.0 -> 31.8 MPa | 302.0 -> 313.6 K | 299.8 -> 283.2 K |
| 4775 -> 4600 psig | 83.9 -> 104.8 F | 80 -> 50 F |

Biomass Loaded CyA Std = 0.132 mg/mL -> area = 2753704,2752374
ave = 2753039

Not Determined (this experiment was done as a test)

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed |
|---|---|---|---|---|---|---|
| 52-1 | 30 | 42 | 20 | 22799 | 0.022 | 0.0132 |
| 52-2 | 90 | 176 | 20 | 10004 | 0.010 | 0.0193 |
| 52-3 | 150 | 381 | 7.8 | 12213 | 0.005 | 0.0223 |
| 52-4 | 223 | 644 | 5.5 | 18304 | 0.005 | 0.0253 |
| 52-5 | 306 | 942 | 3.5 | 0 | 0.0 | 0.0253 |
| 52-L | 390 | 1229 | 24 | 523256 | 0.602 | 0.3878 |

0.644 mg removed

52-E (total extract), 5mL, 4240214 -> 1.017 mg left

1.017 mg CyA left
0.644 mg CyA removed
---
1.661 mg CyA initially

Appendix 2.5 (cont )

Experiment #53 done June 22, 1989 (pre-micrometering valve)

mycelial extraction, harvested on May 13, 132 hr old fermentation, filtered out, dried on aluminium foil in drying oven at 80 C, and ground in Wiley mill, sieved for 212 to 355 um fraction

| Extraction Conditions | | letdown valve |
|---|---|---|
| 32.7 - 32.9 MPa | 312.4 - 314.0 K | ~309 K |
| 4725 - 4750 psig | 102.6 - 105.6 F | ~96 F |

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 23879586,23621322
ave = 23750454

13.6062 g

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | cumulative |
|---|---|---|---|---|---|---|---|
| 53-1 | 60 | 89 | 20 | 9943 | 0.995 | | 0.1074 |
| 53-2 | 120 | 192 | 20 | 22176 | 0.222 | | 0.1314 |
| 53-3 | 180 | 301 | 20 | 133656 | 0.134 | | 0.1459 |
| 53-4 | 286 | 507 | 20 | 176092 | 0.176 | | 0.1649 |
| 53-5 | 377 | 679 | 20 | 108490 | 0.109 | | 0.1767 |
| 53-6 | 465 | 861 | 20 | 80441 | 0.081 | | 0.1854 |
| 53-7 | 574 | 1034 | 20 | 62280 | 0.062 | | 0.1921 |
| 53-8 | 713 | 1204 | 20 | 37673 | 0.038 | | 0.1963 |
| 53-L | 743 | 1233 | 36.2 | 208036 | 0.377 | | 0.2370 |

2.194 mg removed

53-E (0.5003g/12.6048), 5mL, 1121438 -> 0.2805 mg -> 0.5606 mg/g left

7.066 mg CyA left
2.194 mg CyA removed
---
9.2604 mg CyA initially

loss of mass 13.6062 - 12.6048 = 1.0014 g

Appendix 2.5 (cont.)

Experiment #54 done June 28, 1989 (pre-micrometering valve)

mycelial extraction, methanol treated material from experiment #53
30 min. exposure to methanol, dried and ground

| Extraction Conditions | | letdown valve |
|---|---|---|
| 32.9 - 33.0 MPa | 313.4 - 314.0 K | 299.3 - 310.9 K |
| 4750 - 4775 psig | 104.5 - 105.6 F | 79 - 100 F |

Biomass Loaded CyA Std = 0.132 mg/mL -> area = 2872339

11.9580 g

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed cumulative | Cumulative of expt. #53+#54 |
|---|---|---|---|---|---|---|---|
| 54-1 | 80 | 75 | 11 | 206621 | 0.104 | 0.0147 | 0.2482 |
| 54-2 | 176 | 280 | 4.1 | 612407 | 0.115 | 0.0310 | 0.2607 |
| 54-3 | 244 | 411 | 9 | 417602 | 0.173 | 0.0555 | 0.2793 |
| 54-4 | 326 | 525 | 8 | 189424 | 0.070 | 0.0654 | 0.2869 |
| 54-5 | 404 | 609 | 10.2 | 120386 | 0.056 | 0.0734 | 0.2929 |
| 54-L | 421 | 647 | 64.6 | 425432 | 1.263 | 0.2522 | 0.4293 |

1.781 mg removed

54-E (3.1456 g/11.5621g), 5mL, 6253018 -> 1.437 mg -> 0.4568 mg/g left

5.281 mg CyA left

1.781 mg CyA removed
---
7.062 mg CyA initially

loss of mass 11.9580 - 11.5621 = 0.3959 g

( #53 -> 12.6048 g - 11.9580 g =
0.6468 g loss between runs -> 0.3626 mg CyA loss
7.066 mg CyA left - 0.3626 mg = 6.7034 mg to extract in Expt. #54
actually found 7.062 mg or 0.3586 mg (5.4%) more than expected.
This is due to the cumulative effect of experimental errors.
because of this no adjustment was made to the cumulative fraction
calculation for the combined experiments )

Appendix 2.5 (cont.)

Experiment #55 done June 29, 1989 (pre-micrometering valve)

mycelial extraction, (June 20 fermentor harvest), centrifuged 20 min., exposed pellet to methanol for 1 hr, dried 11 hr at 80 C

sample dried to determine moisture 0.0830 g wet -> 0.0733 g dry => 11.7 % moisture

| Extraction Conditions | | letdown valve |
|---|---|---|
| 32.9 - 33.0 MPa | 313.7 - 314.6 K | 317.5 - 322.5 K |
| 4750 - 4775 psig | 105.0 - 106.6 F | 112 - 122 F |

Biomass Load: 7.7400 g

CyA Std = 1.188 mg/mL -> area = 2.425e7, 2.433e7
ave = 2.429e7

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | fraction cumulative |
|---|---|---|---|---|---|---|---|
| 55-1 | 75 | 43.6 | 20 | 3370299 | 3.297 | | 0.3892 |
| 55-2 | 150 | 82.4 | 20 | 468414 | 0.458 | | 0.4433 |
| 55-3 | 225 | 112.8 | 20 | 255214 | 0.250 | | 0.4728 |
| 55-4 | 304 | 164.8 | 20 | 68259 | 0.067 | | 0.4807 |
| hold over night pressure dropped to 17.3 MPa repressurized before continuing | | | | | | | |
| 55-5 | --- | 168.9 | 20 | 266140 | 0.260 | | 0.5114 |
| 55-6 | 374 | 217.5 | 20 | 62674 | 0.061 | | 0.5186 |
| 55-L | 398 | 246 | 72.2 | 161635 | 0.571 | | 0.5861 |
| 55-W | --- | --- | 39.5 | 39995 | 0.077 | | 0.5951 |

5.0410 mg removed

55-E (3.5697g/5.8342g), 5mL, 7.16e6 -> 1.7509 _\ 2.0982 mg -> 0.5878 mg/g
, 5mL, 1.42e6 -> 0.3473 /

3.4292 mg CyA left
5.0410 mg CyA removed
---
8.4702 mg initially

loss of mass 7.7400 x 0.8831 - 5.8342 = 1.0012 g

initial biomass CyA conc. 0.8503 g -> 5mL,
3.93e6 -> 0.9611 mg -> 1.1303 mg/g

8.7482 mg CyA estimated in Load 3.3 % higher than actual experimental

Appendix 2.5 (cont.)

Experiment #56 done July 4, 1989 (pre-micrometering valve)

mycelial extraction, (June 20 fermenter harvested), centrifuged 20 min., dried pellets ~11 hr. still moist, ground to ~1 mm particles

sample dried to determine moisture 0.4144 g wet -> 0.3845 g dry => 7.2 % moisture

| Extraction Conditions | | letdown valve |
|---|---|---|
| 1st Run | | |
| 33.0 - 32.9 MPa | 313.6 - 314.5 K | 316.5 K |
| 4775 - 4750 psig | 104.8 - 106.4 F | 110 F |
| 2nd Run | | |
| 33.0 - 33.2 MPa | 327.5 K | 323 K |
| 4775 - 4800 psig | 129.8 F | 123 F |

Biomass Loaded 8.2978 g

CyA Std = 1.188 mg/mL -> area = 24189257,24562730 ave = 24375994

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | fraction cumulative |
|---|---|---|---|---|---|---|---|
| 56-1 | 75 | 62 | 20 | 4.27e6 | 4.162 | 0.4752 | |
| 56-2 | 150 | 138 | 20 | 394847 | 0.385 | 0.5191 | |
| 56-3 | 225 | 223 | 20 | 138713 | 0.135 | 0.5345 | |
| 56-4 | 300 | 306 | 20 | 96892 | 0.094 | 0.5453 | |
| 56-5 | 375 | 383 | 20 | 60636 | 0.059 | 0.5520 | |
| 56-L1 | 405 | 423.5 | 68.7 | 205691 | 0.689 | 0.6307 | |
| 56-W | --- | --- | 17.5 | 18830 | 0.016 | 0.6325 | |
| depressurization and repressurization to 33 MPa at 327.5 K | | | | | | | |
| 56b-1 | 1965 | 820 | 14.7 | 233706 | 0.167 | 0.6516 | |
| 56b-L2 | 1985 | 841.8 | 44.3 | 149755 | 0.323 | 0.6884 | |

6.030mg removed

56-E (1.3628g/6.2800g), 5mL, 2.43e6 -> 0.5921 mg -> 0.4345 mg/g

2.729 mg CyA left

6.030 mg CyA removed

---

8.759 mg CyA initially

loss of mass 8.2978 x 0.9280 - 6.2800 = 1.4204 g
(dry basis)

Appendix 2.5 (cont.)

Experiment #57 done July 7, 1989 (pre-micrometering valve)

mycelial extraction, 6.5 day old shake flask culture, centrifuged 20 min., ground pellet with morter and pestle and acid washed sand, washed out cells from sand with distilled water, centrifuged cells out again, pellet oven dried 4 hr. at 80 C ground in Wiley mill to pass 2 mm pore screening

sample dried to determine moisture 0.1247 g -> 0.0183 g => 14.7 %

| Extraction Conditions | | letdown valve |
|---|---|---|
| 1st Run | | |
| 32.9 MPa | 313.8 - 315.2 K | 313 - 320 K |
| 4760 psig | 105.2 - 107.6 F | 104 - 117 F |
| 2nd Run | | |
| 33.0 MPa | 327.3 K | 319 - 324 K |
| 4775 psig | 129.5 F | 116 - 124 F |

Biomass Loaded

5.3475 g

CyA Std = 1.188 mg/mL -> area = 24559125,24562730, 25355741
ave = 24825865

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | fraction cumulative |
|---|---|---|---|---|---|---|---|
| 57-1 | 75 | 104.5 | 21 | 2.64e6 | 2.653 | 0.4671 | |
| 57-2 | 150 | 188.7 | 20 | 168023 | 0.161 | 0.4954 | |
| 57-3 | 229 | 273.7 | 9.7 | 493179 | 0.229 | 0.5357 | |
| 57-4 | 300 | 362 | 9.5 | 194685 | 0.089 | 0.5514 | |
| 57-5 | 378 | 450 | 9.2 | 173913 | 0.077 | 0.5650 | |
| 57-L1 | 408 | 480 | 59 | 206042 | 0.582 | 0.6674 | |
| depressurized and repressurized to 33.0 MPa at 327.3 K | | | | | | | |
| 57b-1 | 676 | 671.5 | 10.8 | 33323 | 0.017 | 0.6704 | |
| 57b-J | 697 | 713 6 | 40.3 | 2590 | 0.005 | 0.6713 | |
| 57b-L2 | --- | ----- | 35.3 | 72631 | 0.123 | 0.6930 | |

3.936 mg removed

57-E (2.1603g/3.8074 g), 5mL, (2999497, 5.27e6 => ave. = 4.135e6 ) ->

0.9893 mg -> 0.458 mg/g left

1.744 mg CyA left
3.936 mg CyA removed
---
5.680 mg initially

loss of mass 5.3475 - 3.8074 = 1.5401 g

Appendix 2.5 (cont.)

Experiment #58 done July 17, 1989 (pre-micrometering valve)

mycelial extraction, 7.5 day old shake flask culture,
filtered onto glass wool, air dried 2 days

| Extraction Conditions | | letdown valve |
|---|---|---|
| 32.9 MPa | 313.5 - 314.0 K | 306.5 - 321 K |
| 4760 psig | 104.6 - 105.5 F | 92 - 118 F |

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 25225064

4.6252 g including glass wool

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed cumulative |
|---|---|---|---|---|---|---|
| 58-1 | 30 | 53 | 20 | 27251 | 0.026 | 0.0166 |
| 58-2 | 61 | 86 | 20 | 0 | 0.0 | 0.0166 |
| 58-3 | 127 | 143.5 | 20 | 0 | 0.0 | 0.0166 |
| 58-L | 254 | 299 | 24 | 458477 | 0.518 | 0.3470 |
| 58-J | --- | --- | 33.5 | 6892 | 0.011 | 0.3541 |

0.555 mg CyA removed

58-E total extract 5 mL, 4299905 -> 1.103 mg left

1.103 mg CyA left

0.555 mg CyA removed

---

1.568 mg initially

Appendix 2.5 (cont.)

Experiment #59 done July 19, 1989 (pre-micrometering valve)

mycelial extraction, 6.5 day old shake flask culture,
centrifuged 20 min., oven dried 3 hr. at 80 C
ground in Wiley mill to pass 0.5 mm pore screening

Extraction Conditions letdown valve

32.9 MPa 312.6 - 313.9 K 318 K
4750 psig 103.0 - 105.4 F 112 F

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 23559004, 23516550,
25534024
2.6086 g ave = 24203193

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | cumulative |
|---|---|---|---|---|---|---|---|
| 59-1 | 29 | 27 | 20 | 56921 | 0.056 | 0.0035 | |
| 59-2 | 59 | 65 | 20 | 10498 | 0.010 | 0.0042 | |
| 59-3 | 89 | 99.4 | 20 | 6157 | 0.006 | 0.0045 | |
| 59-4 | 119 | 135 | 20 | 10422 | 0.010 | 0.0052 | |
| 59-5 | 209 | 233 | 10 | 28788 | 0.014 | 0.0061 | |
| 59-6 | 300 | 330 | 9.6 | 16514 | 0.008 | 0.0066 | |
| 59-L1 | 340 | 405 | 31.3 | 1.71e6 | 2.627 | 0.1725 | |
| depressurized then repressurized after adding 2 ml methanol to extractor inlet | | | | | | | |
| 59-7 | 370 | 443 | 20 | 54943 | 0.054 | 0.1759 | |
| 59-8 | 430 | 520 | 20 | 6967 | 0.007 | 0.1763 | |
| 59-L2 | 450 | 560 | 30 | 515352 | 0.759 | 0.2243 | |
| 59-J | --- | --- | 28 | 20211 | 0.028 | 0.2260 | |

3.579 mg removed

59-E (1.4568g/2.3579g) , 5mL, 30849314 -> 7.571 mg -> 5.197 mg/g left

12.254 mg CyA left

3.579 mg CyA removed

---

15.833 mg CyA initially

loss of mass 2.6086 - 2.3579 = 0.2507 g

Appendix 2.5 (cont.)

Initial CyA concentration estimate
of #59

sample 1 (upper fraction in centrifuge tube)
wet biomass for dry weight 1.7369 g - 1.5364 g tray tare = 0.2005 g wet
after drying at 80 C 16 hr. 1.5482 g - 1.5364 g " " = 0.0118 g dry

fraction dry weight = 0.0118/0.2005 = 0.0589

wet biomass for CyA determination by ethyl acetate extraction

1.8106 g - 1.5549 g tray tare = 0.2557 g wet x 0.0589 =
0.0151 g dry biomass

-> 5mL, HPLC area = 164014 -> 0.0403 mg /0.0151 g = 2.676 mg/g

sample 2 (lower fraction in centrafuge tube)
wet biomass for dry weight 3.9989 g - 1.5341 g tray tare = 2.4648 g wet
after drying at 80 C 16 hr. 1.6959 g - 1.5341 g " " = 0.1618 g dry

fraction dry weight = 0.1618/2.4648 = 0.0656

wet biomass for CyA determination by ethyl acetate extraction

4.1877 g - 1.5526 g tray tare = 2.6351 g wet x 0.0656 =
0.1729 g dry biomass

-> 5mL, HPLC area = 7.43e6 -> 1.8235 mg / 0.1729 g = 10.549 mg / g

actual load was 2.6086 g dry biomass

sample 1 estimated CyA = 2.6086 x 2.676 = 5.285 mg
sample 2 estimated CyA = 2.6086 x 10.549 = 27.52 mg

average = 16.40 mg CyA loaded

actual found during experiment = 15.83 mg

Appendix 2.5 (cont.)

Experiment #60 done Aug. 2, 1989

mycelial extraction, blended in Virtis mixer for 30 min., centrifuged 20 min., oven dried 18 hr. @ 80 C, Wiley milled to pass 0.5 mm pore screening.

| Extraction Conditions | | letdown valve |
|---|---|---|
| 32.9 MPa | 313.8 - 314.6 K | 324.8 K |
| 4750 psig | 105.2 - 106.7 F | 125 F |

Biomass Loaded

2.8502 g

CyA Std = 1.188 mg/mL -> area = 25117344,25526371
ave = 25321858

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | fraction cumulative |
|---|---|---|---|---|---|---|---|
| 60-1 | 30 | 41.7 | 20 | 360248 | 0.338 | 0.0225 | |
| 60-2 | 60 | 75.1 | 20 | 503444 | 0.472 | 0.0539 | |
| 60-3 | 94 | 110.5 | 20 | 480096 | 0.451 | 0.0840 | |
| 60-4 | 134 | 146.5 | 20 | 278327 | 0.261 | 0.1014 | |
| 60-5 | 196 | 198 | 20 | 199655 | 0.187 | 0.1138 | |
| 60-6 | 259 | 251 | 11.1 | 245469 | 0.128 | 0.1224 | |
| 60-7 | 383 | 356.5 | 4 | 302399 | 0.057 | 0.1261 | |
| 60-L | 450 | 437 | 5 | 896132 | 0.210 | 0.1401 | |
| 60-W | --- | --- | 20 | 2.46e6 | 2.308 | 0.2939 | |
| 60-J | --- | --- | 23.3 | 47136 | 0.052 | 0.2974 | |
| | | | | | 4.464 mg totals | | |

60-E (0.3444g/2.7065g), 5 ml, 572289? -> 1.3425 mg -> 3.898 mg/g left

10.550 mg CyA left

4.464 mg CyA removed

15.014 mg initally

loss of mass 2.8502 - 2.7065 = 0.1437 g

Appendix 2.5 (cont.)

Experiment #61 done Aug. 3 - 7, 1989

mycelial extraction, blended in Virtis mixer for 30 min. with 0.4 % w/v Sodium Tripoly Phosphate centrifuged 20 min., oven dried 18 hr. @ 80 C, extracted without grinding first then Wiley milled to pass 0.5 mm pore screening and extracted again

Extraction Conditions

| 1st Run | | 2nd Run | |
|---|---|---|---|
| 33.1 MPa | 314.5 - 315.3 K | 33.0 MPa | 314.3 - 315.1 K |
| 4780 psi | 106.4 - 107.9 F | 4770 psi | 106.0 - 107.5 F |

| Biomass loaded | 1st Run | 2nd Run |
|---|---|---|
| | 2.9440 g | 2.7507 g |

CyA Std = 1.188 mg/mL -> area = 25526371, 26084695
ave = 25805533

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | | fraction removed |
|---|---|---|---|---|---|---|---|
| 61-1 | 45 | 35.5 | 20 | 31081 | 0.029 | | 0.0020 |
| 61-2 | 112 | 70 | 12.3 | 21587 | 0.012 | | 0.0028 |
| 61-3 | 246 | 172.5 | 4 | 120798 | 0.022 | | 0.0043 |
| 61-L | 300 | 243 | 7.8 | 17121 | 0.006 | | 0.0047 |
| 61-W | --- | --- | 20 | 754202 | 0.694 | | 0.0521 |
| 61-J | --- | --- | 33.8 | 5648 | 0.007 | | 0.0526 |
| grinding of material | | | | | | correction for material lost during grinding | |
| 61-4 | 418 | 368 5 | 6 | 1.27e6 | 0.351 | 0.376 | 0.0782 |
| 61-5 | 538 | 514.5 | 6.3 | 1.71e6 | 0.496 | 0.531 | 0.1145 |
| 61-6 | 663 | 642 | 4.2 | 1.03e6 | 0.199 | 0.213 | 0.1290 |
| 61-7 | 900 | 868.6 | 7.8 | 466489 | 0.168 | 0.180 | 0.1413 |
| 61-L2 | 930 | 910.3 | 15.2 | 235552 | 0.165 | 0.177 | 0.1534 |
| 61-W2 | --- | ---- | 10.8 | 3857616 | 1.918 | 2.053 | 0.2936 |
| 61-J2 | --- | ---- | 15.4 | 66496 | 0.047 | 0.050 | 0.2970 |
| | | | | | | 4.350 mg removed | |

61-E (0.3608g/2.6215g), 5 ml, 6157475 -> 1.417 mg -> 3.9283 mg/g left

10.298 mg CyA left
4.350 mg CyA removed
---
14.648 mg initially

loss of mass 2.9440 - 2.9440 g = 0.0 extraction #1
2.9440 - 2.7502 g = 0.1938 grinding
2.7502 - 2.6215 g = 0.1287 extraction #2

Appendix 2.5 (cont.)

Experiment #60-61x done Aug. 10, 1989

remains of experiments 60 and 61 were used together for re-extraction material was steam treated in autoclave for 12 min. with a 3 min. drying period, a glass wool filter separated material from experiment 60 from 61

Extraction Conditions

33.1 -> 32.4 MPa  314.9 -> 314.5 K
4780 -> 4680 psi  107.1 -> 106.4 K

Biomass loaded

2.3621g from # 60 dry -> 2.4674g (moisture gained between experiments)
2.2607g from # 61 dry -> 2.3571g "
---
4.6228g 4.8245g (actual load weight)

Initial CyA -> 9.2075 mg from #60, 8.8807 mg from #61 = 18.088 mg available

CyA std. 1.188 mg/mL -> area = 26925128

| Sample | t min | Vco2 L | Vmech mL | area | mg CyA | fraction removed | (60,61combined) Total Fraction removed (corrected for losses) |
|---|---|---|---|---|---|---|---|
| 6061x-1 | 120 | 161 | 4 | 381335 | 0.067 | 0.0037 | 0.2997 |
| 6061x-L | 215 | 286.7 | 24.4 | 268137 | 0.289 | 0.0197 | 0.3109 |
| 6061x-J | --- | ---- | 45.7 | 6988 | 0.014 | 0.0205 | 0.3115 |
| | | | | | 0.370 mg removed | | |

Biomass left

#60 2.3248 g -> 2.3621 - 2.3248 = 0.0373 g ( minor
#61 2.2221 g -> 2.2607 - 2.2221 = 0.0386 g losses )
---
4.5469 g

Appendix 2.5 (cont.)

Experiment #66 done Aug. 31, 1989

mycelial extraction, NaOH treated biomass from Experiments 60 and 61
agitated for 30 min. @ 70 C,
centrifuged 20 min., oven dried 18 hr. @ 80 C,
Wiley milled to pass 0.5 mm pore screening.

Extraction Conditions

33.2 MPa 314.5 - 315.0 K
4800 psi 106.3 - 107.4 F

Biomass Loaded

2.7007g (after treatment) CyA left from Expts. 60, 61 = 17.31 mg (calc)

Initial CyA for both Expt 60,61 = 28.82 mg

CyA Std = 1.188 mg/mL -> area = 23775710, 24411312
ave = 24093511

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | (60,61,66) Total Fraction removed (corrected for losses) |
|---|---|---|---|---|---|---|---|
| 66-1 | 66 | 117 | 8.6 | 305466 | 0.130 | 0.008 | 0.3164 |
| 66-2 | 126 | 222 | 10 | 470016 | 0.330 | 0.029 | 0.3292 |
| 66-3 | 180 | 308.5 | 11.1 | 455000 | 0.249 | 0.045 | 0.3390 |
| 66-4 | 253 | 417.5 | 8.4 | 280396 | 0.116 | 0.052 | 0.3433 |
| 66-5 | 322 | 529 | 10 | 800000 | 0.395 | 0.078 | 0.3592 |
| 66-6 | 402 | 638 | 8.2 | 266043 | 0.108 | 0.084 | 0.3629 |
| 66-7 | 462 | 732 | 10.4 | 106971 | 0.055 | 0.088 | 0.3653 |
| 66-8 | 697 | 1144 | 10.3 | 2059437 | 1.046 | 0.154 | 0.4057 |
| 66-9 | 882 | 1406 | 10.4 | 171526 | 0.088 | 0.160 | 0.4093 |
| 66-L | 1113 | 1666 | 30 | 1756004 | 2.598 | 0.325 | 0.5102 |

5.115 mg totals

66-E (0.4595g/2.4224g), 50 mL, 817256 -> 2.015 mg -> 4.385 mg/g left

10.62 mg CyA total left

5.115 mg CyA removed
---
15.735 mg total (91% of 17.31 mg inital calc.)
(some losses due to NaOH treatment)

loss of mass 2.7007 - 2.4224 = 0.2783 g

Appendix 2.5 (cont.)

Calculation of Cyclosporine Fraction Removed during multiple experiments

Experiments 60, 61, 6061x, 66:

total initial Cyclosporine in expt. #60 + #61 = 15.014 mg + 14.648 mg
= 29.662 mg

total Cyclosporine removed in expt. #60 + # 61 = 4.464 mg + 4.35 mg
= 8.814 mg

overall yield of extraction 8.814/29.662 = 0.2971

remaining fraction = 1.0000 - 0.2971 = 0.7029

total initial Cyclosporine in expt. #6061x = 18.088 mg
(lower due to sampling losses )

total Cyclosporine removed in expt. #6061x = 0.370 mg

yield of extraction in expt. #6061x = 0.370/18.088 = 0.0205

remaining fraction in expt. #6061x = 1.0000 - 0.0205 = 0.9795

overall yield of extractions #60, #61, #6061x = 0.2971 + 0.0205(0.7029)
= 0.3115

initial Cyclosporine before NaOH treatment = 17.718 mg

Cyclosporine left after NaOH treatment = 15.735 mg

fraction remaining after treatment = 15.735/17.718 = 0.8881
and before expt. #66

total Cyclosporine removed in expt. #66 = 5.115 mg

overall yield of expt. #66 = 5.115/15.735 = 0.3251

overall yield of extractions #60, #61, #6061x, #66 =

0.3115 + 0.3251 x 0.7029 x 0.9795 x 0.8881 = 0.5102

Appendix 2.5 (cont.)

Experiment #62 done Aug. 8, 1989

mycelial extraction, centrafuged 20 min., air dried mycelia +72 hours
Wiley milled to pass 0.5 mm pore screening.

| Extraction Conditions | | letdown valve |
|---|---|---|
| 33.1 MPa | 315.4 -> 315.8 K | 324.8 K |
| 4780 psig | 108.1 -> 108.7 F | 125 F |

Biomass loaded 3.4747 g

CyA Std = 1.188 mg/mL -> area = 26357111,26810784
ave = 26583948

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | cumulative |
|---|---|---|---|---|---|---|---|
| 62-1 | 114 | 198 | 6 | 2.57e6 | 0.689 | 0.0315 | |
| 62-2 | 228 | 311.5 | 6.6 | 166192 | 0.049 | 0.0338 | |
| 62-3 | 313 | 390.5 | 9.6 | 21405 | 0.009 | 0.0342 | |
| 62-L | --- | 430 | 8.6 | 2.83e6 | 1.088 | 0.0840 | |
| 62-W | --- | --- | 19.2 | 1.03e7 | 8.838 | 0.4886 | |
| 62-J | --- | --- | 29.4 | 58498 | 0.077 | 0.4921 | |

10.750 mg removed

62-E1 ((.3982g/3.1083g), 5 ml, 6.40e6 -> 1.430 mg -> 3.591 mg/g left
(ethyl acetate) (11.163 mg)

62-E2 (0.2935g/3.1083g), 60 ml, 394280 ->1.057 mg -> 3.602 mg/g left
(methanol) (11.195 mg)

62-EA (0.2969g/3.1083g), 5ml, 4.67e6 -> 1.043 mg -> 3.515 mg/g left
(autoclaved 12 min. -> ethyl acetate) (10.924 mg)

(ave) 11.094 mg CyA left
10.750 mg removed
---
21.844 mg initially

loss of mass 3.4747 - 3.1083 = 0.3664 g

Appendix 2.5 (cont.)

Experiment #63 done Aug. 14, 1989

mycelial extraction, centrafuged 20 min. ,
autoclaved for 12 min. 3 min. dry time,
air dried 5 days, ground to pass 0.5 mm pore screening.

| Extraction Conditions | | letdown valve |
|---|---|---|
| 33.0 MPa | 314.4 -> 314.5 K | 327.6 K |
| 4775 psig | 106.2 -> 106.7 F | 130.0 F |

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 2.4606e7

10.9303 g

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | cumulative |
|---|---|---|---|---|---|---|---|
| 63-1 | 60 | 38.6 | 20 | 2.90e6 | 2.800 | 0.297 | |
| 63-2 | 120 | 70.3 | 20 | 166754 | 0.161 | 0.314 | |
| 63-3 | 180 | 101.3 | 16 | 112693 | 0.087 | 0.324 | |
| 63-L | 240 | 151 | 32.6 | 380195 | 0.598 | 0.387 | |
| 63-W | --- | --- | 20 | 0 | 0 | | |

3.646 mg

63-E (0.4897g/10.0940g), 5mL, 1.16e6 -> 0.2800 mg -> 0.5718 mg/g left

5.772 mg CyA left

3.646 mg removed

---

9.418 mg initially

loss of mass 10 9303 - 10.094 = 0.8363 g

Appendix 2.5 (cont.)

Experiment #64 done Aug. 15,1989

mycelial extraction, freeze - thaw treated twice, dried 10 hr at 80 C, room temperature 11 hr, 2 hr drying 80 C, ground to pass 0.5 mm screening

| Extraction Conditions | | letdown valve |
|---|---|---|
| 32.9 MPa | 313.8 - 314.7 K | 322 K |
| 4750 psig | 105.2 - 106.7 F | 120 F |

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 24941628, 24593960
ave = 24767794

3.0927 g

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | fraction cumulative |
|---|---|---|---|---|---|---|---|
| 64-1 | 120 | 119.3 | 6.6 | 1366605 | 0.433 | 0.0264 | |
| 64-2 | 240 | 325.5 | 5.4 | 2526506 | 0.654 | 0.0664 | |
| 64-3 | 320 | 464.2 | 5.7 | 690904 | 0.189 | 0.0779 | |
| 64-4 | 410 | 603 | 5.8 | 426338 | 0.119 | 0.0852 | |
| 64-L | 432 | 658.6 | 35.5 | 1.08e6 | 1.839 | 0.1974 | |
| 64-J | --- | ---- | 7 | 86116 | 0.029 | 0.1992 | |

3.263 mg removed

64-E (0.3830g/2.7930g), 5 ml, 7.50e6 -> 1.791 mg -> 4.696 mg/g left

13.117 mg left

3.263 mg removed

---

16.380 mg initially

loss of mass 3.0927 - 2.7930 = 0.2997 g

Appendix 2.5 (cont.)

Experiment #65 done Aug. 28, 1989

mycelial extraction, French Pressed , lots of spores over 80% of biomass centrifuged for 20 min., oven dried for 16 hr. @ 80 C, milled to pass through 0.5 mm screening.

Extraction Conditions

30.9 MPa 314.3 - 314.8 K
4480 psi 106.0 - 107.0 F

Biomass Loaded
2.49g CyA Std = 1.188 mg/mL -> area = 22568795,22972437
ave= 22770616

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | |
|---|---|---|---|---|---|---|---|
| 65-1 | 97 | 138 | 5 | 1.45e6 | 0.378 | 0.0164 | |
| 65-2 | 174 | 251 | 7.8 | 316036 | 0.129 | 0.0220 | |
| 65-3 | 270 | 392.7 | 5 | 325282 | 0.085 | 0.0257 | |
| 65-4 | 366 | 531.3 | 5.2 | 113512 | 0.031 | 0.0271 | |
| 65-L | 424 | 628.3 | 58.5 | 474668 | 1.449 | 0.0900 | |
| 65-X | --- | ---- | 7.0 | 111896 | 0.041 | 0.0918 | (very low yield) |
| | | | | | 2.113 mg totals | | |

65-E (0.3186g/2.0244g) , 5mL, 12613609 -> 3.29 mg -> 10.33 mg/g

or

20.91 mg
total CyA left

loss of mass 2.49 - 2.0244 = 0.4656 g

Appendix 2.5 (cont.)

Experiment #67 done Sept. 28, 1989

mycelial extraction, (eight day old shake flask culture 280 - 300 mg/L), treated with 0.5 N NaOH, centrifuged 20 min., oven dried 4 - 6 hr at 80 C, ground to pass 0.5 mm pore screen.

| Extraction Conditions | | letdown valve |
|---|---|---|
| 33.2-33.5 MPa | 313.6 - 314.6 K | 322.6 K |
| 4800-4840 psig | 104.8 - 106.6 F | 121 F |

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 22962002, 23103284
ave = 23032643

2.502 g

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | cumulative |
|---|---|---|---|---|---|---|---|
| 67-1 | 61 | 52 | 13 | 67074 | 0.045 | | 0.0020 |
| 67-2 | 123 | 119 | 13.5 | 46723 | 0.033 | | 0.0035 |
| 67-3 | 180 | 181.7 | 13 | 36966 | 0.025 | | 0.0046 |
| 67-L | 230 | 254.2 | 31.5 | 3859165 | 6.270 | | 0.2838 |
| 67-W | --- | ---- | 33.5 | 101408 | 0.175 | | 0.2916 |

6.548 mg removed

67-E (0.5004g/2.1736g), 5 mL, 1.42e7 -> 3.6621 mg -> 7.3184 mg/g

15.907 mg left

6.548 mg removed

---

22.455 mg initially

loss of mass 2.5020 - 2.1736 = 0.3284g

Appendix 2.5 (cont.)

Experiment #68 done Oct. 31, 1989

mycelial extraction, centrifuged for 20 min., oven dried 4 hr at 80 C on filter paper ( thin layer), ground to pass 0.5 mm screening.

| Extraction Conditions | | letdown valve |
|---|---|---|
| 33.5 - 31.1 MPa | 313.9 - 314.5 K | ~318 K |
| 4840 -> 4500 psig | 105.4 - 106.5 F | ~114 F |

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 23352161

3.0780 g

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | cumulative |
|---|---|---|---|---|---|---|---|
| 68-1 | 67 | 50 | 12.8 | 944254 | 0.615 | | 0.0439 |
| 68-2 | 118 | 120 | 12.2 | 376390 | 0.234 | | 0.0605 |
| 68-3 | 179 | 198 | 12.2 | 210942 | 0.131 | | 0.0699 |
| 68-4 | 236 | 270 | 12.2 | 83041 | 0.052 | | 0.0736 |
| pressure dropped here to 31.7 MPa | | | | | | | |
| 68-5 | 266 | 345 | 13.8 | 47489 | 0.033 | | 0.0759 |
| pressure dropped here to 31.1 MPa | | | | | | | |
| 68-6 | 301 | 430 | 12.7 | 63223 | 0.041 | | 0.0789 |
| 68-L | 355 | 486.5 | 33.3 | 588616 | 0.997 | | 0.1500 |

2.103 mg removed

68-E (0.2422g/2.9602g), 5 mL, 3834606 -> 0.9754 mg -> 4.027 mg/g left

11.921 mg left

2.103 mg removed

---

14.024 mg initially

loss of mass 3.0780 - 2.9602 = 0.1178 g

Appendix 2.5 (cont.)

Experiment #69 done Nov.1, 1989

mycelial extraction, centrifuged for 20 min., air dried for 2 days on filter paper ( thin layer), ground to pass 0.5 mm screening.

| Extraction Conditions | | letdown valve |
|---|---|---|
| 33.7 -> 29.3 MPa | 314.0 - 314.6 K | 317 K |
| 4880 -> 4240 psig | 105.5 - 106.6 F | 111 F |

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 23879741,23777304
ave = 23828523

3.4685 g

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | cumulative |
|---|---|---|---|---|---|---|---|
| 69-1 | 45 | 69 | 12.2 | 864468 | 0.526 | | 0.0399 |
| 69-2 | 91 | 165 | 11.7 | 1.21e6 | 0.706 | | 0.0934 |
| 69-3 | 133 | 271 | 11.9 | 503094 | 0.299 | | 0.1161 |
| 69-4 | 183 | 399 | 10.5 | 542998 | 0.284 | | 0.1376 |
| 69-5 | 226 | 508 | 11.3 | 377990 | 0.213 | | 0.1537 |
| pressure dropped here to 30.6 MPa | | | | | | | |
| 69-6 | 271 | 632 | 12.7 | 123443 | 0.078 | | 0.1596 |
| pressure dropped here to 29.3 MPa | | | | | | | |
| 69-7 | 303 | 720 | 17.6 | 62033 | 0.054 | | 0.1637 |
| 69-L | 322 | 764 | 35.3 | 1248208 | 2.197 | | 0.3303 |
| 69-L2 | --- | --- | 9.0 | 293866 | 0.132 | | 0.3403 |

4.489 mg removed

69-E (0.1261g/3.1005g), 5mL, 1.42e6 -> 0.354 mg -> 2.807 mg/g

8.704 mg left

4.489 mg removed

13.193 mg initially

loss of mass 3.4685 - 3.1005 = 0.3680g

Appendix 2.5 (cont.)

Experiment #70 done Nov. 2, 1989

mycelial extraction, centrifuged for 20 min., methanol added
left over night, oven dried 4 hr at 80 C
on filter paper ( thin layer), ground to pass 0.5 mm screening.

| Extraction Conditions | | letdown valve |
|---|---|---|
| 33.6 MPa | 313.7 - 314.4 K | 319 K |
| 4860 psig | 105.0 - 106.3 F | 115 F |

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 23591176

C .225 g

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | cumulative |
|---|---|---|---|---|---|---|---|
| 70-1 | 58 | 148 | 11.8 | 695406 | 0.413 | | 0.0411 |
| 70-2 | 102 | 225 | 13.6 | 293866 | 0.201 | | 0.0611 |
| 70-3 | 148 | 303 | 13.5 | 102250 | 0.070 | | 0.0680 |
| 70-4 | 201 | 391 | 11.5 | 76009 | 0.044 | | 0.0724 |
| 70-L | 229 | 439 | 28.2 | 667415 | 0.948 | | 0.1667 |
| 70-W | --- | --- | 12.4 | 81228 | 0.051 | | 0.1717 |

1.727 mg removed

70-E (0.3820g/2.9662g), 5 mL, 4.26e6 -> 1.0726 mg -> 2.808 mg/g left

8.329 mg CyA left

1.727 mg CyA removed

10.056 mg initially

loss of mass 3.2225 - 2.9662 = 0.2563 g

Appendix 2.5 (cont.)

Experiment #71 done Nov. 3, 1989

mycelial extraction, centrifuged for 20 min., freeze dried on filter paper ( thin layer), course ground ~2 mm particles

Extraction Conditions letdown valve

33.3 -> 31.1 MPa 314.1 - 314.4 K ~317 K
4820 -> 4500 psig 105.7 - 106.4 F ~112 F

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 23777304

2.5525 g

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | fraction cumulative |
|---|---|---|---|---|---|---|---|
| 71-1 | 50 | 105 | 11.1 | 253032 | 0.140 | | 0.0115 |
| 71-2 | 90 | 205.5 | 12.6 | 611507 | 0.385 | | 0.0431 |
| 71-3 | 130 | 304 | 14.0 | 234879 | 0.171 | | 0.0571 |
| 71-4 | 170 | 400.5 | 12.9 | 228305 | 0.147 | | 0.0691 |
| 71-5 | 215 | 510 | 12.2 | 68940 | 0.042 | | 0.0726 |
| pressure dropped here to 31.1 MPa | | | | | | | |
| 71-6 | 281 | 666 | 9.6 | 65739 | 0.032 | | 0.0752 |
| 71-L | 308 | 727 | 29.7 | 1221141 | 1.812 | | 0.2238 |
| 71-L2 | --- | --- | 12.0 | 877446 | 0.534 | | 0.2676 |

3.263 mg removed

71-E (0.3302g/2.3283g), 5 mL, 5.07e6 -> 1.2666 mg -> 3.8358 mg/g left

8.931 mg CyA left

3.263 mg CyA removed

12.194 mg Cya initially

loss of mass 2.5525 - 2.3283 = 0.2242 g

Appendix 2.5 (cont.)

Experiment #72 done Nov. 7, 1989

mycelial extraction, centrifuged for 20 min., freeze dried
on filter paper ( thin layer), course flakes -10 mm particles

| Extraction Conditions | | letdown valve |
|---|---|---|
| 31.4 MPa | 313.8 - 314.3 -> 327.6 K | ~321 - ~326.5 K |
| 4540 psig | 105.1 - 106.1 -> 130 F | ~118 - ~128 F |

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 23432095,24029554
ave = 23730824

1.3140 g

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA |
|---|---|---|---|---|---|
| 72-1 | 60 | 84 | 10.8 | 101786 | 0.055 |
| 72-2 | 120 | 170 | 11.2 | 96805 | 0.054 |
| 72-3 | 180 | 257.5 | 11.9 | 103061 | 0.061 |
| Temperature set to 327.6 K | | | | | |
| 72-4 | 240 | 333 | 12.0 | 40731 | 0.025 |
| flow stopped overnight dropped to 11.1 MPa repressurized to 31.4 MPa | | | | | |
| 72-5 | --- | 333 | 19.6 | 93190 | 0.091 |
| 72-6 | 491 | 525 | 24.2 | 45154 | 0.055 |
| 72-L | lost sample | | | | |
| 72-W | --- | --- | 14.0 | 1.46e6 | 1.023 |
| | | | | | 1.364 mg removed |

1.2182 g material left

loss of mass = 1.3140 - 1.2182 = 0.0958 g

Appendix 2.5 (cont.)

Experiment #73 done Nov. 13, 1989

mycelial extraction, centrifuged for 20 min., air dried 13 days on filter paper ( thin layer), fine ground 90 um particles

Extraction Conditions letdown valve

31.5 MPa 313.1 - 314.7 K ~320 K
4550 psig 103.9 - 106.7 F ~117 F

Biomass Loaded CyA Std = 1.188 mg/mL -> area = 24547092,24380829
ave = 24463960

2.6547 g

| Sample | t min | Vco2 L | Vmeth mL | area | mg CyA | fraction removed | cumulative |
|---|---|---|---|---|---|---|---|
| 73-1 | 40 | 28 | 14.4 | 17348 | 0.012 | | 0.0033 |
| 73-2 | 80 | 73 | 14.2 | 395292 | 0.273 | | 0.0791 |
| 73-3 | 120 | 135 | 13.3 | 307943 | 0.199 | | 0.1343 |
| 73-4 | 160 | 193.3 | 13.8 | 71207 | 0.048 | | 0.1476 |
| 73-5 | 200 | 246.7 | 13.7 | 43628 | 0.029 | | 0.1557 |
| 73-L | 258 | 318 | 31.7 | 759926 | 1.179 | | 0.4803 |

1.731 mg removed

73-E (0.3846g/2.4523g), 5 mL, 1.21e6 -> 0.2938 mg -> 0.764 mg/g

1.873 mg CyA left

1.731 mg CyA removed
---
3.604 mg CyA initially

loss of mass 2.6547 - 2.4523 = 0.2024 g

Appendix 2.6 Cyclosporine Crystal Size Analysis Data

Appendix 2.6 Cyclosporine crystal size analysis data

| Particle size (um) | Number of particles: Set 1 | Set 2 | Set 3 | Set 4 | Totals | Fraction Cumulative |
|---|---|---|---|---|---|---|
| 2.0 | 3050 | 3100 | 5000 | 3250 | 14400 | 0.9571 |
| 6.25 | 50 | 37 | 82 | 100 | 269 | 0.9709 |
| 12.5 | 29 | 36 | 56 | 82 | 203 | 0.9843 |
| 50.0 | 24 | 12 | 44 | 22 | 102 | 0.9911 |
| 100 | 27 | 13 | 21 | 20 | 81 | 0.9964 |
| 150 | 8 | 9 | 26 | 11 | 54 | 1.0000 |

Analysis of microscope particle size data

| X | F | Psi | Xm | dx |
|---|---|---|---|---|
| 2.0000e+00 | 9.5310e-01 | 4.1882e-03 | 4.1250e+00 | 4.2500e+00 |
| 6.2500e+00 | 9.7090e-01 | 2.1440e-03 | 9.3750e+00 | 6.2500e+00 |
| 1.2500e+01 | 9.8430e-01 | 1.8133e-04 | 3.1250e+01 | 3.7500e+01 |
| 5.0000e+01 | 9.9110e-01 | 1.0600e-04 | 7.5000e+01 | 5.0000e+01 |
| 1.0000e+02 | 9.9640e-01 | 7.2000e-05 | 1.2500e+02 | 5.0000e+01 |
| 1.5000e+02 | 1.0000e+00 | 0.0000e+00 | 0.0000e+00 | 0.0000e+00 |

mean diam. = 1.259050 microns,
area mean diameter = 9.704831 microns
volume mean diam. = 21.169451 microns,
sauter mean diameter = 100.728617 microns
geometric mean diam. = 1.126239 microns, geometric std. dev. = 1.40290147

# APPENDIX 3

## COMPUTER PROGRAMS AND SUBROUTINES

# Appendix 3.1 Solubility Data Analysis Program Modules

Appendix 3.1.1 Datiupac.c, main data analysis module for solubility studies

Appendix 3.1.1 Datiupac main driver routines source code

```
/* DATIUPAC.C data analysis program with averageing
**
**
**      accepts filename for input on command line
**      if filename is not present program prompts user for a filename
**      if the file does not exist in either case the program
**     queries the user for the data required and stores it into the file
**
**      the naming convention for the files are:
**        (only the rootname is required the suffixes are appended)
**
**              rootname.DAT     for the data file
**              rootname.OUT     for the results of calculations
**
**
**      command line :-->  datiupac [rootname] [opt. output name]
**
**      program does the following after opening files:
**
**              it reads:  sample code, date and time, pressure(psi),
**                         temperature(F), peak area, solvent vol (ml),
**                         loop vol (ml), inj. vol.(uL)
**
**              the sample code 'STD' is used to flag standards
**          which must appear on the first line of the data file
**
**              it outputs: sample code, date and time,pressure(MPa),
**                          temperature(K), concentration (mg solute/ml
CO2),
**                          molar vol. (CO2 kmol/cubic meter),
**                    molar density (cubic meter/kmol CO2),
**                    reduced density (dimensionless),
**                          mole fraction of solute in CO2,
**                    plus the original data from the data file.
*/

#include <stdio.h>
#include <math.h>
#include <math1.h>
#include <float.h>
#include <string.h>
#include <cursors.h>

#define FALSE   0
#define TRUE    1
#define prompt(A) fprintf(stderr,A)

#define EOS_3   AG_HGK_EOS
```

Appendix 3.1.1 Datiupac main driver routines source code

```
/* set up data format strings

/* samp#,date,time,pres,temp,peak,solvol */
char *formatin = "%6s %8s %8s %lf %lf %lf %lf";
char *formatout = "%8s %8s %8s  %5.0lf %7.2lf %10.0lf  %9.2lf \n";

char *stdinfo = " HPLC analysis standard and other common information \n
\
 date     cyclosporine std.   injection    SCE loop vol.  \n \
            (mg/AREA) vol. (ul)        (ml)   \n\n  \
 %8s   %.4le      %6.1lf      %6.1lf \n\n";

char *titleout1  = "\n \n Raw experimental data:\n \n \
  sample   date     time   pres    temp    HPLC        solvent \n \
   code                    (psi)    (F)    peak area   vol. (ml) \n\n";

char *titleout2  = "\n  Calculated results: \n\n  \
   pressure  temperature  CO2vol.   density    loop conc  molefrac  \n \
     (MPa)       (K)     (m^3/kmol) (kmol/m^3) (mg/mlCO2)  \n\n";

            /*123456789012345678901234567890*/
char *mess1 = "standard deviations are shown as the second line of
results\n";

/*                  pres,  temp,  volCO2, density,loopconc,molefrac */
char *frmout1 = "    %7.3lf %10.2lf   %10.6lf %8.3lf %12.6lf %4.4le\n";
char *frmout2  = "       %7.3lf  %10.2lf     (red.den.=%8.5lf)   %12.6lf
%4.4le\n\n";

extern double AG_HGK_EOS();       /* listed as library file in cpac.lnk*/
                                  /* in iupac2.bin */
main(argc,argv,envptr)
int argc;
char **argv,**envptr;       /* envptr is ignored for now */
{
   FILE  *infp,*outfp,*fix_to_printer(),*infile_creat();
   char  infname[80],outfname[80];
   int   ret,processfiles();


   /* SET UP FILENAMES */
   prompt(CLEAR);

   if(argc  <  2)
   {
      retry1:
      prompt(" Enter input filename (without extention) \n");
      if(!scanf(" %s",infname))
      {
         prompt(" FILENAME ENTRY ERROR !!! ... RETRY\n");
         goto retry1;
      }
```

Appendix 3.1.1 Datiupac main driver routines source code

```
      strmfe(infname,infname,"DAT");
      strmfe(outfname,infname,"OUT");
   }
   else
   {
      strmfe(infname,argv[1],"DAT");
      if(argc >= 3)
         strmfe(outfname,argv[2],"OUT");
      else
      {
         strmfe(outfname,argv[1],"OUT");
      }
   }

   if((infp = fopen(infname,"r")) == NULL)
   {
      infp = infile_creat(infname);   /* create a data file if possible
*/
   }                                  /* exits if not possible */

   if((outfp = fopen(outfname,"w")) == NULL)
   {
      outfp = fix_to_printer(); /* sends results to the printer or screen
*/
   }

   ret =  processfiles(infp,outfp); /* do all the work here */

   fclose(infp);
   fclose(outfp);

   return(ret);
}

#include <error.h>

FILE *infile_creat(fname)
char *fname;
{
   FILE *tmp;
   char  samcd[7],date[9],time[9]; /* allow for nulls */
   double pres,temp,peak,solvol,lpvol,injvol;
   int   i;

   if(errno != ENOENT)exit(1);   /* exit condition */
   if((tmp = fopen(fname,"w"))==NULL)exit(1);

   prompt(" File does not exist: ");
   prompt(fname);
   prompt("\n CREATED NEW FILE FOR DATA ENTRY \n");

      prompt(" Enter STANDARD's  Date  ");
```

Appendix 3.1.1 Datiupac main driver routines source code

```
      scanf(" %8s ",date);
      prompt(" Enter standard  calibration   mg/AREA  ");
      scanf(" %lf ",&peak);

      prompt(" Enter Loop volume (ml) ");
      scanf(" %lf",&lpvol);

      prompt(" Enter injection volume (ml) ");
      scanf(" %lf",&injvol);

   fprintf(tmp," STD     %8s %le %le %le\n",date,peak,lpvol,injvol);


   do {
      prompt("\n\n Enter Sample Code:   ");
      i = scanf(" %6s",samcd);

      prompt(" Enter date  MM/DD/YY ");
      i *= scanf(" %8s",date);

      prompt(" Enter time  HH:MM ");
      i *= scanf(" %8s",time);

      prompt(" Enter pressure in (psi)  ");
      i *= scanf(" %lf",&pres);

      prompt(" Enter temperature in degrees F  ");
      i *= scanf(" %lf",&temp);

      prompt(" Enter HPLC peak area   ");
      i *= scanf(" %lf",&peak);

      prompt(" Enter Sample Solvent Volume (ml)   ");
      i *= scanf(" %lf",&solvol);
      fprintf(tmp,formatin,samcd,date,time,pres,temp,
                                      peak,solvol);

      fprintf(tmp,"\n");
        /* added new line for the human reader/editor  of the data file
*/

      prompt(" Are you Done ?   y/n  ");
      scanf(" %s",samcd);
      if(samcd[0] == 'y') i = FALSE; /* exit loop here */

   } while(i);

   fclose(tmp);
   if((tmp = fopen(fname,"r"))==NULL)exit(1);

   return(tmp);
}
```

Appendix 3.1.1 Datiupac main driver routines source code

```
FILE *fix_to_printer()
{
   FILE *tmp;
   if((tmp = fopen("PRN:","w"))==NULL) exit(1); /* file problems */
   return(tmp);
}
```

```
double ave_std( array,ar_siz,stddev )
double *array,*stddev;
int ar_siz;
{
  int i;
  double sum,sumsq;

   sum = 0.0;
   sumsq = 0.0;

  for ( i = 0; i < ar_siz; i++)
  {
      sum += array[i];
      sumsq += array[i] * array[i];
  }

  if( ar_siz == 1) *stddev = 0.0;
  else
   {
   sumsq = ((sumsq - sum * sum /(double)ar_siz)/((double)ar_siz - 1.));

   *stddev = sqrt(fabs(sumsq));
   }
   return sum/(double)ar_siz;
}
```

```
/* process files subroutine next */
```

Appendix 3.1.1 Datiupac main driver routines source code

```
int processfiles(infp,outfp)
FILE *infp,*outfp;
{
   /* data definitions */

                                    /* allow for nulls */
   char  stdcd[7],stddate[9],samcd[10][7],date[10][9],time[10][9];
   doublepres,temp,peak,solvol,lpvol,injvol,stdpeak,vol,concCO2,molfrac;
   double presar[10],tempar[10],peakar[10],solvar[10],tmp;
   double presdev,solvdev,tempdev,peakdev,concdev,moldev,density;
   int   i,j;

   /* get standard calibration data from beginning of file */
   /* linear standard curve */

      f s c a n f ( i n f p , "   % 6 s   % 8 s   % l f   % l f
%lf",stdcd,stddate,&stdpeak,&lpvol,&injvol);
   fprintf(outfp,stdinfo ,stddate,stdpeak,injvol*1000.0,lpvol);
   fprintf(outfp, messl);

   j = 0;
      /* preread one data set to start */
                                                                  i      =
fscanf(infp,formatin,samcd[j],date[j],time[j],&presar[j],&tempar[j],
                                    &peakar[j],&solvar[j]);

   prompt(" read 1st data\n");
   do {

      fprintf(outfp,titleoutl); /* new set of data */

        do {    /* averaging values routine  */
            /* write out previously read values */

fprintf(outfp,formatout,samcd[j],date[j],time[j],presar[j],tempar[j],
                                    peakar[j],solvar[j]);
         j++ ;
                                                                  i      =
fscanf(infp,formatin,samcd[j],date[j],time[j],&presar[j],&tempar[j],
                                    &peakar[j],&solvar[j]);


        prompt("in read loop\n");
        } while ( i != EOF && j < 10 &&
                      fabs(1.0 - presar[j-1]/presar[j]) < 0.05
                    && fabs(1.0 - tempar[j-1]/tempar[j]) < 0.05 );
         prompt(" calculate averages \n");

         pres = ave_std(presar,j,&presdev);
         peak = ave_std(peakar,j,&peakdev);
         temp = ave_std(tempar,j,&tempdev);
         solvol = ave_std(solvar,j,&solvdev);
```

Appendix 3.1.1 Datiupac main driver routines source code

```
        prompt(" done averages \n");

      /* perform units conversions */
      pres *= 101325./14.696;
      pres += 101325.;     /* correct for guage to absolute pressure */
      presdev *= 101325.0/14.696; /* convert to Pascals */

      temp -=32;
      temp *= 5.0/9.0;        /* now in degrees C */
      temp += 273.15;         /* now in degrees K */

      tempdev *= 5.0/9.0;     /* convert to degrees C or K */

      vol = EOS_3(pres,0.0,temp,1);      /* m3/kmol */

      concCO2 = peak*stdpeak*solvol/(injvol*lpvol); /* mg/ml CO2 */

      concdev = peakdev * stdpeak *solvol/(injvol*lpvol);

      molfrac = concCO2*vol/1202.6; /* kmol/kmol */
      moldev  = concdev*vol/1202.6;

      tmp = moldev/molfrac;    /* assume constant % variation */
                               /* adjust for true molefraction */
      molfrac = 1.0/(1.0+1.0/molfrac);

      moldev  = molfrac*tmp;
    /* send it o t */

      density = 1.0/vol;

      pres *= 1.0e-6;          /* convert to MegaPascals */
      presdev *= 1.0e-6;

     fprintf(outfp,titleout2);
     fprintf(outfp,frmout1,pres,temp,vol,density,concCO2,molfrac);
     fprintf(outfp,frmout2,presdev,tempdev,density/10.59,concdev,moldev);


     prompt("output calculation results \n");
      if ( i != EOF )
      {
       strncpy(samcd[0], samcd[j],7);
       strncpy(date[0], date[j],9);
       strncpy(time[0], time[j],9);

         presar[0] = presar[j];
         tempar[0] = tempar[j];
         solvar[0] = solvar[j];
         peakar[0] = peakar[j];
```

Appendix 3.1.1 Datiupac main driver routines source code

```
            j = 0;
        }

    } while (i != EOF);

    return(0); /* okay from process */
}
```

Appendix 3.1.2 Iupac.c, routines Equation of State evaluation

Appendix 3.1.2 iupac eos subroutines source code

```
/*      IUPAC.C
**
**      this equation of state is based on the paper of K.S. Pitzer
**      and D.R. Schreiber , Fluid Phase Equilibria 41,1-17,1988.
**
**      it is an improvement on the original iupac equations.
**      instead of a switching function it uses the Haar, Gallagher,
**      and Kell (1980,1984) function to correct the behaviour of the
**      Altunin and Gadetskii (1971) function (0.1% accuracy outside
**      the critical density region)
*/

#include <math.h>
#include <math1.h>
#include <float.h>

#ifdef TEST3

#define D(x)  x

#else

#define D(x)

#endif
#define TOL     (1.0e-08)

static double   Tc   =  304.210;    /* Kelvin  critical temperature */

static double   Pc   =  7382500.;  /* Pascals critical pressure    */

static double   Zc   =  0.2744223;  /* ?    critical compressibility */

static double   rhoc =  10.59;      /* kgmol/cubic meter critical density
*/

static double   Vc   =  1.0/10.59;  /* m3/kgmol critical volume     */

static double   mw   =   44.009;    /* g/mol or kg/kmol */
```

Appendix 3.1.2 iupac eos subroutines source code

```
#define NEW2
#ifdef NEW2
static double  bcoef[10][7] =
{

-.7255896770,-1.669856633,0.4191613578,1.154058547,1.145027582,1.1488455
13,0.7069388840,
0.4481451002,1.269083933,6.057811911,15.85978978,20.21837027,9.190077144
,0.0,
-.1743673384,-1.954404447,-5.615197965,-6.976816915,-.5761694929,3.00728
4937,0.0,
-4.243816093e-4,-1.788455844,-11.34629367,-29.10403562,-30.02663937,-8.3
61282386,0.0,
0.2668130548,2.718574223,9.462288816,10.60317379,0.1567993789,-2.7232168
50,0.0,
0.07340283381,1.154789219,7.450988805,16.00143047,10.97104869,0.0,0.0,
-.1756082074,-2.114184586,-6.144768702,-4.667566118,0.0,0.0,0.0,
8.844271016e-3,0.01488945560,-1.445010207,-1.997943186,0.0,0.0,0.0,
0.06107749242,0.6239980516,1.194066295,0.0,0.0,0.0,0.0,
-0.01994277669,-0.1666138543,5.923888289e-3,0.0,0.0,0.0,0.0
};
#else


static double bcoef[10][7] =
{

-0.72585443,-1.68332974,0.259587221,0.376945574,-0.670755370,-0.8714561
26,-0.149156928,
0.447869183,1.26050691,5.96957049,15.4645885,19.4449475,8.64880497,0.0,
-0.172011999,-1.83458178,-4.61487677,-3.82121926,3.60171349,4.92265552,0
.0,
0.446304911e-2,-1.76300541,-11.1436705,-27.8215446,-27.1685720,-8.421778
72,0.0,
0.255491571,2.37414246,7.50925141,6.61133318,-2.42663210,-2.57944032,0.0,
0.0594667298,1.16974683,7.43706410,15.0646731,9.57496845, 0.0, 0.0,
-0.147960010,-1.69233071,-4.68219937,-3.13517448, 0.0, 0.0, 0.0,
0.0136710441,-0.100492330,-1.63653806,-1.87082988, 0.0, 0.0, 0.0,
0.0392284575,0.441503812,0.886741970, 0.0, 0.0, 0.0, 0.0,
-0.0119872097,-0.0846051949,0.0464564370, 0.0, 0.0, 0.0, 0.0
};

#endif
static int Ji[10] = {6,5,5,5,5,4,3,3,2,2}; /* limits of bcoef[][] */

double R = 8314.34;  /* gas constant (Pa m3)/(K kgmol) */
#define NEW1
#ifdef NEW1
static double const1  = (44.009/468.0);
#else
static double const1  = (1.0/10.63);
#endif
```

Appendix 3.1.2 iupac eos subroutines source code

```
static double   Z_AG(V,T)
double   V,T;
{
   double   dtau,ww,dww,sum,sumi;
   int   i,j;

   ww = const1/V;

   dww = (ww-1.0);

   dtau = ((304.2/T) - 1.0);

   sum = 0.0;

   for(i=9;i>=0;i--)
   {
      sum *= dww;    /* 1st time through the loop equals zero */
      sumi = 0.0;    /* reset for next intermediate sumation */

      for(j=Ji[i] ; j>=0 ; j--)
      {
         sumi *= dtau;  /* 1st time through equals zero */
         sumi += bcoef[i][j]; /* do it do it to it etc. */
      }

      sum += sumi;
   }

   sum *= ww;

   sum += 1.0;   /* compressibility calculated so far */

        D(printf("pressure = %10.8g \n",R*T*sum/V);)

   return(sum);   /* returning compresssibility */

}
/*****************************************************************************/

static double  alf[3] = { 34.0,40.0,30.0 };
static double  li[3]  = { 0.0,2.0,0.0 };
static double  beta[3] = { 20000.0,20000.0,40000.0 };
static double  gi[3]  = { -7.53e-4,-5.73e-3,1.84e-4 };
```

Appendix 3.1.2 iupac eos subroutines source code

```
static double Z_HGK(V,T)
double V,T;
{
   double del,taul[3],suml,rrho,tmp1,tmp2,tmp3;
   int i;

   rrho = Vc/V;

   del = rrho - 1.0;

   taul[0] = taul[1] = (Tc/T) - 1.011;

   taul[2] = (Tc/T) -1.009;

   suml = 0.0;

   for(i=0;i<3;i++)
   {
      tmp1 = alf[i]*del*del;
      tmp2 = -tmp1 - beta[i]*taul[i]*taul[i];
      tmp3 = (isodd(i))? del : (1.0/del) ;        /* ternary operator */
      suml += gi[i]*tmp3*(li[i]-2.0*tmp1)*exp(tmp2);
   }

   suml *= rrho;
        D(printf("cor. pres. = %10.8g \n", R*T*suml/V);)
   return(suml);   /* return compressibility correction */
}

static double pres(V,T)
double V,T;
{
   double tmp;

   tmp = Z_AG(V,T) + Z_HGK(V,T);

   return(R*T*tmp/V);   /* return corrected pressure */
}

static double S1,S2;    /* temporary global values */

static double vv(vt)    /* function for zbrentrt() on volume  call */
double vt;              /* S1 = P, S2 = T */
{
   return(S1*vt/R/S2 - Z_AG(vt,S2) - Z_HGK(vt,S2) );
}

static double tt(ts)    /* function for zbrentrt() on temperature  call*/
double ts;              /* S1 = P, S2 = V */
{
   return(S1*S2/R/ts - Z_AG(S2,ts) - Z_HGK(S2,ts) );
}
```

Appendix 3.1.2 iupac eos subroutines source code

```
static double volume(P,T)
double P,T;
{
   double vol,CO2PR_vol(),lowbnd,upbnd;  /* note: function declared here
*/
   int i,cnt,cnt2;
   S1 = P;
   S2 = T;

   /* use Peng Robinson Equation for initial guess */

   vol = CO2PR_vol( P,T,1 ); /* 1 = Vapor volume or supercritical vol */


   cnt2 = cnt = 0;
   lowbnd = upbnd = vol;

loopb:
   lowbnd = 0.9*lowbnd;
   upbnd = 1.1*upbnd;
loop:
   i = zbrentrt(vv,&vol,TOL,lowbnd,upbnd,200);
   if( i == NOTBRAC && cnt <= 100)  /* allow 100x spread in range */
   {
      cnt++;
      goto loopb; /* increase range till we bracket a value */
   }
   if(i == MAXITER && cnt2 != 1) /* we fail on this once only */
   {
      cnt2++;
      goto loop;
   }
   else if(i == MAXITER) vol = -vol;   /* unsoluble problem */

   return(vol);   /* return a value */
}

static double temp(P,V)
double P,V;
{
   double tmp = P*V/R;
   S1 = P;
   S2 = V;
   if(zbrentrt(tt,&tmp,TOL,tmp*0.5,1000.0,1000))
   {
      tmp = -tmp;
   }
   return(tmp);   /* return a temperature value */
}
```

Appendix 3.1.2 iupac eos subroutines source code

```
static double comp_Z(P,V,T)
double P,V,T;
{
   double ret;

   if(V > 0.0 && T > 0.0) ret = Z_AG(V,T) + Z_HGK(V,T);
   else if (P > 0.0 && T > 0.0) ret = P*volume(P,T)/R/T;
   else if (P > 0.0 && V > 0.0) ret = P*V/R/temp(P,V);
   else ret = -1.0;  /* error condition -->  fail */

   return(ret);
}

/* the only thing the outside world sees */

double AG_HGK_EOS(P,V,T,mode)
double P,V,T;
int mode;
{
   double ret;

   switch(mode)   /* select method here */
   {
      case 0:  /* pressure */

         ret = pres(V,T);
         break;

      case 1:  /* volume gas | supercritical */

         ret = volume(P,T);
         break;

      case 2:  /* temperature */

         ret = temp(P,V);
         break;

      case 3:  /* compressibility */

         ret = comp_Z(P,V,T);
         break;

      default:

         ret = -1.0;
         break;

   }

   return(ret);
}
```

Appendix 3.1.2 iupac eos subroutines source code

```
#ifdef TEST

/* minimal testing function */

#include <stdio.h>

void main()
{
   double vvv,ppp,ttt,ret;
   int mode,i;

   printf("\033E");  /* clear screen */

   do {
      printf(" enter test values of pressure (Pa), volume (M3/kgmol), \n
\
               temperature (kelvin), mode (0-3) (pvtz) \n");
      i = scanf(" %lf %lf %lf %d", &ppp,&vvv,&ttt,&mode);

      ret = AG_HGK_EOS(ppp,vvv,ttt,mode);

      printf("\npressure = %10.8g , volume = %10.8g, temperature = %10.8g
\n \
            return = %10.8g, mode = %d \n",ppp,vvv,ttt,ret,mode);
   } while(i);
}

#endif
```

Appendix 3.1.3 CO2pr_v1.c, Peng-Robinson Equation of State Volume function for initial guesses in Iupac.c

Appendix 3.1.3  CO2PR_VOL( )  Subroutine source code.

```
/* CO2PR_vol (); in CO2PR_VL.C
**
**      calculates the Peng Robinson equation volume exclusively for
**      Carbon Dioxide. Primary purpose is to precalculate a close
**      volume for other more complex equations of state
**
**
**      Stryjek and Vera 1986a J.Chem.Eng. 64,325 form of kappa used
**
*/

#include <math.h>
#include <float.h>
#include <math1.h>

extern double R;     /* defined externally as 8314.34 */

/* specific to CO2 */

/* define static constants   can not use external reference to R here */
static double AAA = (0.457235*8314.34*8314.34*304.21*304.21/7382430.0);
static double BBB = (0.077796*8314.34*304.21/7382430.0);

#define  WW     (0.225)
s   t   a   t   i   c   d   o   u   b   l   e   K   0   =
(0.378893+WW*(1.4897153+WW*(-0.17131848+WW*0.0196554)));
#define  K1     (0.04285)

#define  square(D)      ((D)*(D))

double kappa(Tr)
double Tr;
{

        if(Tr < 1.0)
        {
           return (K0 +  K1*(1.0-sqrt(Tr))*(0.7-Tr));

        }
        return(K0);
}
```

Appendix 3.1.3  CO2PR_VOL( )  Subroutine source code.

```
double CO2PR_vol(P,T,ret_flg)
double P,T;
int ret_flg;    /* 1 = supercritical or vapor volume, 2 = liquid vol */
{
   double carry[4],roots[3],aa,bb,aaa,Tr = T/304.21,RT;
   int cflg;   /* complex roots flg */

   RT = R*T;

   aaa = kappa(Tr);   /* NOTE: reuse of variables */
   aaa *= (1.0-sqrt(Tr));
   aaa = AAA*square(1.0+aaa);

   aa = aaa*P/square(RT);
   bb = BBB*P/RT;


   carry[3] = 1.0;
   carry[2] = bb-1.0;
   carry[1] = aa-bb*(2.0+3.0*bb);
   carry[0] = bb*(bb*(1.0+bb)-aa);


   if((cflg = cubicrt(carry,roots))<=0 && cflg != RLCMPLXRT )
                        return(RT/P);

   /* handle liquid volume return first */
   if((cflg >= TWORT)&&(ret_flg == 2))
   {  /* conditions for returning roots from  */
      /* A.S.Lawal, 1987, Ind. Eng. Chem. Res.26,859-860    */
      /* A Consistent Rule for Selecting Roots in Cubic Equations of
            State*/
      if(roots[2] >= bb) return ( roots[2]*RT/P );
      if(roots[1] >= bb) return ( roots[1]*RT/P );
                              /* if roots[2] is -ve */
   }                          /* or less than bb    */
/* do the default largest root is in roots[0]... feature of cubicrt  */
   return((roots[0] < bb)? RT/P : roots[0]*RT/P);
                              /* only one possibility left */
}
```

Appendix 3.1.4 Cubicrt.c, Used in CO2pr_vl.c

Appendix 3.1.4 cubicrt() subroutine source code

```
/*    cubicrt(a,x)
*
*     returns:     RLCMPLXRT   1 real root,& 1 complex conjugate pair
*                    (-3)      x[0] = real root,
*                              x[1] = real part, x[2] = imag. part;
*
*                  THREERT     3 real roots in sorted order
*                    (3)       x[0]  = largest root (most +ve)
*                              x[1]  = next real root
*                              x[2]  = smallest root
*
*                  TWORT (2)   2 real roots x[0] = largest
*
*                  SNGLRT (1)  1 real root only, x[0] = root
*
*                  CMPLXRT     complex conjugate pair
*                     (-2)     x[0] = real part, x[1] = imag. part
*
*                 RTERR  (-1) one constant value, a[0]= constant, rest=0
*
*                  NOROOT (0)  no roots possible all zero coefficients
*
*     equation is of form   a[3]*x*x*x+a[2]*x*x+a[1]*x+a[0]
*
*/

#include <math.h>
#include <float.h>
#include <math1.h>


int cubicrt(a,x)
double a[],x[];
{
   double q,r,d,s,t,a2;
   int i,j,ret = THREERT; /* normal exit */
   if(a[3]==0.0)return(quadrt(a,x)); /* roots of quadratic apply */

   a2 = a[2]/a[3]/3.0;

   q = a[1]/a[3]/3.0 -a2*a2;

   r = (a2*a[1] - a[0])/a[3]/2.0 - a2*a2*a2;

   d = q*q*q+r*r;

#ifdef DEBUG
 printf("a2 = %lg  q = %lg  r = %lg\n d = %lg \n",a2,q,r,d);
#endif
```

Appendix 3.1.4 cubicrt() subroutine source code

```
   if(d>0.0)   /* 1 real  root ,complex conjugate pair */
   {
      d = sqrt(d);
      s = cubrt(r+d);
      t = cubrt(r-d);
#ifdef DEBUG
 printf("d = %lg s = %lg t = %lg \n  a2 = %lg\n",d,s,t,a2);
#endif

      x[0] = s+t-a2;
      x[1] = -((s+t)/2.0+a2); /*real part*/
      x[2] = RT3D2*(s-t);     /*imag part*/
      ret = RLCMPLXRT;        /*one real one complex flag for return code
*/
   }
   else if(d == 0.0) /* all real roots at least two are equal*/
   {
      r = cubrt(r);
#ifdef DEBUG
      printf("a2 = %lg r = %lg \n",a2,r);
#endif

      x[0] = 2.0*r-a2;
      x[2] = x[1] = -(r+a2);
      if(x[0]<x[1])
      {
        x[2] = x[0];
        x[0] = x[1]; /* reorder so biggest is first*/
      }
   }
   else              /* d<0.0 condition : all real and unequal */
   {
#ifdef DEBUG
      d = -q*q*q;

      printf("-q*q*q = %.15lg \n",d);

      s = sqrt(d);

      printf(" r = %.15lg sqrt(-q*q*q)= %.15lg \n",r,s);


      if((d=fabs(s = r/s))>0.999999999999&&d<1.00000000001)
              s=((r>0.0)?1.0:-1.0) ;


      printf("s= %.15lg \n",s);


      s = acos(s);
```

Appendix 3.1.4 cubicrt() subroutine source code

```
        printf("acos(s) = %.15lg \n",s);

        s /= 3.0;
#else
                    /*   round off to unity if it's close */
        if((d=fabs(s = r/sqrt(-q*q*q)))>0.99999999999&&d<1.00000000001)
                s = ((r>0.0)?1.0:-1.0);

        s = acos(s)/3.0;

#endif

        q = 2.0*sqrt(-q);

#ifdef DEBUG
     printf("a2 = %lg s = %lg q = %lg \n ",a2,s,q);
#endif
        for(i=0;i<3;i++)
                /* calc values and sort in order largest to smallest */
        {
           x[i] = q*cos(s+((double)i)*TWOPID3)-a2;

           for(j=0;j<i;j++) if(x[j]<x[i]){ r = x[j]; x[j]=x[i]; x[i] = r;
}
        }
    }
    return(ret);

}
```

Appendix 3.1.5 Cubrt.c, Used in Cubicrt.c

Appendix 3.1.5 cubrt() subroutine source code

```
/* CUBRT.C  cubrt(x) find cubic root of a number */

/* algorythm from cheney and kincaid */
/* uses newtons method     */

#include <math.h>
#include <math1.h>
#include <float.h>

/* first version here is probably the best and fastest */

#define VER1
#ifdef  VER1

static int __cuiter  = 4;

double cubrt(x)
double x;
{
   double x0,xsgn;
   int i,m;

   xsgn = fsign(x);
   x = frexp(fabs(x),&m); /* x = r*2^3m , 0.125 <= r < 1.0 */

   if(i=m%3)
   {
      if(i == 1){ m+=2; x*=0.25;}        /* x/=4.0 */
      else { m++; x*=0.5; }              /* x/=2.0 */
   }

   m/=3;

   /* special approximation to root */

   x0 = x+0.3877552;   /* use as tmp variable */

   x0 = 2.502926 - (8.045125*x0)/((x+4.612244)*x0-0.3598496);

   for(i=0;i<__cuiter;i++) x0 = (2.0/3.0)*(x0+0.5*x/(x0*x0));

   return(xsgn*ldexp(x0,m));
}

#else

double cubrt(x)   /* this was the first attempt */
double x;         /* gives wrong sign for answer */
{
   double x1,x2,xdiv3,twothirds=2.0/3.0;

   xdiv3 = x2 = x/3.0; /* initial guess */
```

Appendix 3.1.5 cubrt() subroutine source code

```
   do {
      x1 = x2;
      x2 = twothirds*x1-xdiv3/(x1*x1);  /* compressed newtons formula */
   } while((fabs(x2-x1))>(fabs(x2*EPS*2.0)));
                  /*allow 15 digits of accuracy */
   return(x2);  /* exit with specified value */
}
#endif

#ifdef TEST
#include <stdio.h>

main()
{
   double x;
   int i;

   do {

        printf("enter x \n");

        i = scanf("%lf",&x);

      printf("x = %.16le\n cubrt(x)  = %.16le\n pow(x,1/3) = %.16le\n ",
            x,cubrt(x),( fsign(x)*pow(fabs(x),1.0/3.0) ) );

        if(getchar()=='y')
        {
            printf("enter new interation count \n");

            scanf("%d",&__cuiter);
        }

    } while(i);
}
#endif
```

Appendix 3.2 Cyclosporine Crystal Size Determination Program
Sizan.c, Used for particle size analysis

Appendix 3.2 Partical size analysis program source code

```
/* SIZAN.C translation by Derk W. te Bokkel
** of original fortran program   by J. Beeckmans
**
**      Original description follows: (slightly modified for 'C')
**
**      Program to compute population parameters from the discrete
**      cumulative distribution function
**
**      F(i) equals fraction of sample, by number, smaller than x(i)
**      x(0) equals the hypothetical smallest size (microns), at F(0)=0.0
**      x(n-1) equals the hypothetical largest size (microns),
**                                              at F(n-1) = 1.0
**
**      n equals the number of data points (size catagories)
**
**      1st line read =  the number of size catagories  (integer)
**      2nd line read =  x(i), i=0,n-1
**      3rd line read =  F(i), i=0,n-1
**
*/

#include <stdio.h>
#include <stdlib.h>
#include <math.h>
#include <mathl.h>
#include <float.h>
#include <cursors.h>

#ifdef TEST
#define D(x) x
#define Dl(x) x
#else
#define D(a)  dummy(x);
#define Dl(a)
#endif


/* we will employ stdio redirected to feed in the data file */

void dummy(dptr)
double *dptr;
{
    int j=0,k=0;
                        /* nochanges really */
        dptr +=j;
        dptr +=k;
}
```

Appendix 3.2 Partical size analysis program source code

```
void main()
{
   double *f,*x,*dx,*xm,*psi,*x1,d2,d3,d1,xg,tmp,sigg,dva;
   int i,n1,n;

   scanf(" %d ",&n);    /* get catagory count */

   D(fprintf(stderr," n = %d\n",n);)
   /* allocate memory */

   if((x = (double *)calloc(5*n+1,sizeof(double)))==NULL)
   {
      fprintf(stderr,"not enough memory\n"); /* allow for redirection */
      exit(1);
   }

   f =x+n;     /* x+n  */          /* initialize array pointers */
   dx = f+n;   /* x+2n */
   xm = dx+n;  /* x+3n */
   psi = xm+n; /* x+4n */

   D(fprintf(stderr,"OK for memory\n");)

   /* read in both x and f array in same loop,since they ajoin */

   for(i=0; i<2*n ;i++){ scanf(" %lf ",x+i);
                         D(fprintf(stderr," x[%d] = %lf \n",i,x[i]);)
                       }
   D(fprintf(stderr,"got data\n");)

   n1 = n-1;

   d1 = 0.0;
   d2 = 0.0;
   d3 = 0.0;
   xg = 0.0;
   sigg = 0.0; /* zero values */

   D(fprintf(stderr,"\nzero'd values... going to first calc. loop\n");)

       x1 = x;
       x1++;
   for(i=0; i < n1 ;i++)
   {
      D(fprintf(stderr,"top of loop i = %d \n",i);)
      (*(xm+i)) = ((*(x1+i)) + (*(x+i)))/2.0;

      /* here we insert  dummy function calls to fake out optimizer*/
      /* optimizer in this compiler has a bad bug so we trick it */

        dummy(x);
```

Appendix 3.2 Partical size analysis program source code

```
      Dl(fprintf(stderr,"after xm assign\n");)
      (*(dx+i)) = (*(xl+i)) - (*(x+i));
      D(fprintf(stderr,"mid of top \n");)
      (*(psi+i)) = ((*(f+i+l))- (*(f+i)))/(*(dx+i));
      tmp = (*(xm+i))*(*(psi+i))*(*(dx+i));
      D(fprintf(stderr,"middle of loop tmp = %lf\n",tmp);)
      dl += tmp;
      tmp *= (*(xm+i));
      d2 += tmp;
      d3 += tmp*(*(xm+i));
      D(fprintf(stderr,"bottom of loop i = %d\n",i);)
   }

   D(fprintf(stderr," out of first calculation loop ... func calls \n");)

   dva = d3/d2;

   d3 = cubrt(d3);

   d2 = sqrt(d2);

   D(fprintf(stderr," last 2 loops next ...\n");)

   for(i=0; i<nl; i++)xg += log(xm[i])*psi[i]*dx[i];

   for(i=0; i<nl; i++)sigg += pow((log(xm[i])- xg),2.0)*psi[i]*dx[i];

   xg = exp(xg);
   sigg = exp(sigg);

   fprintf(stderr,CLEAR);

   printf("\n\n\n Analysis of microscope particle size analisis data \n");

   printf("    X           F           Psi           Xm          dx \n");

   for(i=0; i<n; i++)
        printf("    %.4le    %.4le    %.4le    %.4le    %.4le
\n",x[i],f[i],psi[i],xm[i],dx[i]);

   printf("\n\n mean diam. = %.6lf microns,  area mean diameter = %.6lf\
 microns\n",dl,d2);
   printf(" volume mean diam. = %.6lf microns,  sauter mean diameter =
%.6lf\
 microns\n",d3,dva);
   printf(" geometric mean diam. = %.6lf microns, geometric std. dev. =
\
%.8lf\n",xg,sigg);

   fprintf(stderr,"ALL DONE !!!!\n");

}
```

# Appendix 3.3 Other Programs Used

Appendix 3.3.1 Regresum.c, Used for linear regression of solubility data

Appendix 3.3.1 Linear regression program source code

```
/*  REGRESUM.C
**
** reads temp????.sum files for analysis by linear regression
** between reduced density of 1.4 to 2.0  with log (mole fraction) as
** the dependent variable
**
*/


#include <stdio.h>
#include <math.h>

main(argc,argv)
int argc;
char **argv;
{
    char dummy[20],test[20],single[2],tild1,tild2,tild3,echar;
                                    f       l       o       a       t
temp,pres,presdev,redden[30],conc,concdev,molfrac[30],molfrdev[30];
    float power,inter,slope,adev,bdev,chi2,q,cutoff;
    int count,i;
    FILE *fp;

        if(argc > 1) fp = fopen(argv[1],"r");
        else exit(1);

        fprintf(stderr," enter reduced density cutoff ");

      cutoff = 1.3;

        scanf(" %f ",&cutoff);

    do {        /* handle aribitrary file header */
        fscanf(fp," %s ",test);
    }while(strcmp("frac.",test));  /* catch end of title to key reads */

    i = 1;

    printf(
    " reduced    mole        standard \n\
 density   fraction     deviation \n\n");

  while( fscanf(fp," %1s %lf %lf%c",single, &temp,&pres,&tild1) != EOF )
   {
    if(tild1 == '~')    /* handle standard deviations */
        fscanf(fp,"%f %f %f%c",&presdev,&redden[i],&conc,&tild2);
    else
        {
            presdev = 0.0;
            fscanf(fp,"%f %f%c",&redden[i],&conc,&tild2);
        }
```

Appendix 3.3.1 Linear regression program source code

```
                        /* ditto */
    if(tild2 == '~')
        fscanf(fp,"%f %f%c",&concdev,&molfrac[i],&tild3);
    else
        {
        concdev = 0.0;
        fscanf(fp,"%f%c",&molfrac[i],&tild3);
        }
                        /* once more */
    if(tild3 == '~')
    {
        fscanf(fp,"%3f%c%f\n",&molfrdev[i],&echar,&power );

        if(echar == 'e') /* set up proper values when exponent is there*/
        {
            molfrdev[i] *= pow(10.0,power);
            molfrac[i]  *= pow(10.0,power);
        }
        else printf("ERROR in READING mole fraction info\n");
    }
    else
    {
        fscanf(fp,"\n");
        molfrdev[i] = 1e-8;  /* very small error assumed */
    }


    printf("  %5.2f      %.3e       %.3e \n"
           ,redden[i],molfrac[i],molfrdev[i]);

    molfrac[i] = log10(molfrac[i]);
   molfrdev[i] = log10(molfrdev[i]);



    if (redden[i] >= cutoff)   i++;
                    /* clobber values that are out of range*/
  } /* end of reading while */



    printf("\n\n Reduced density low end cutoff = %5.2f\n",cutoff);



    count = i-1;  /* total number of values read */
    printf("\n\n count = %d \n\n",count);

    for(i = 1; i <= count ; i++)
        printf(" red den. = %f log10(molefrac) = %f log10(std. dev.) =
%f\n",
```

Appendix 3.3.1 Linear regression program source code

```
        redden[i],molfrac[i],molfrdev[i]);
    /* done getting a complete data set */
    /* now process it accordingly */

fit(redden,molfrac,count,molfrdev,0,&inter,&slope,&adev,&bdev,&chi2,&q);

    printf(
   "\n\n equation of line is %f + %f x   = y  \n   adev =%f  bdev= %f\n",
                    inter,slope,adev,bdev);
    printf("chi -squared = %f  q= %f \n \n",chi2,q);

    printf("line trials table using fixed densities 1.4 1.6 1.8 2.0\n");

    for(i=0; i<4;i++)
    {
        cutoff = inter + slope*(i*0.2 + 1.4);

        printf("   %lf     ",pow(10.0,cutoff));
    }
    printf(" \n\n ");

    printf(" fit using log ( std.dev.) \n\n");


fit(redden,molfrac,count,molfrdev,1,&inter,&slope,&adev,&bdev,&chi2,&q);

    printf(" equation of line is %f + %f x   = y \n     adev =%f  bdev=
%f\n",
                    inter,slope,adev,bdev);
    printf("chi -squared = %f  q= %f \n \n",chi2,q);

    printf("line trials table using fixed densities 1.4 1.6 1.8 2.0\n");

    for(i=0; i<4;i++)
    {
        cutoff = inter + slope*(i*0.2 + 1.4);

        printf("   %lf   ",pow(10.0,cutoff));
    }
    printf(" \n\n ");

    fclose(fp);

}
```

Appendix 3.3.2 Sumstrip.c, Used to prepare data for BSTAT program (Shareware)

Appendix 3.3.2 program to strip unwanted characters from files to be submitted to BSTAT program (Shareware)

```
/* sumstrip.c
**
** reads temp????.sum files  and strips out unnecessary text to prepare
** a daughter file for further analysis
*/


#include <stdio.h>
#include <math.h>

main(argc,argv)
int argc;
char **argv;
{
    char test[20],single[2],tild1,tild2,tild3,echar;
    float temp,pres,presdev,redden,conc,concdev,molfrac,molfrdev;
    float power;
    int count,i;
    FILE *fp;

        if(argc > 1) fp = fopen(argv[1],"r");
        else exit(1);

    do {        /* handle aribitrary file header */
        fscanf(fp," %s ",test);
    )while(strcmp("frac.",test));  /* catch end of title to key reads */



  while( fscanf(fp," %ls %f %f%c",single, &temp,&pres,&tild1) != EOF )
  { /* 1 */

   if(tild1 == '~')    /* handle standard deviations */
       fscanf(fp,"%f %f %f%c",&presdev,&redden,&conc,&tild2);
   else
       {
           presdev = 0.0;
           fscanf(fp,"%f %f%c",&redden,&conc,&tild2);
       }
                       /* ditto */
   if(tild2 == '~')
       fscanf(fp,"%f %f%c",&concdev,&molfrac,&tild3);
   else
       {
       concdev = 0.0;
       fscanf(fp,"%f%c",&molfrac,&tild3);
       }
```

Appendix 3.3.2 program to strip unwanted characters from files to be submitted to BSTAT program (Shareware)

```
                        /* once more */
    if(tild3 == '-')
    {
        fscanf(fp,"%3f%c%f\n",&molfrdev,&echar,&power );

        if(echar == 'e') /* set up proper values when exponent is there*/
        {
            molfrdev *= pow(10.0,power);
            molfrac  *= pow(10.0,power);
        }
        else printf("ERROR in READING mole fraction info\n");
    }
    else
    {
        fscanf(fp,"\n");
        molfrdev = 1e-8;  /* very small error assumed */
    }
    printf("%7.2f %7.2f %7.2f", temp, pres, presdev);
    printf(" %5.4f %7.2f %7.5f %7.5f %.3e %.3e \n",redden,redden*10.59,
                                    conc,concdev,molfrac,molfrdev);
  }
}
```

## REFERENCES


Adams,W.R., J.A.Zollweg, W.B Streett, and S.S.H.Rizvi, 1988, New Apparatus for Measurement of Supercritical Fluid-Liquid Phase Equilibria, A.I.Ch.E.J. 34(8), 1387-1391.

Anonymous, 1981, $CO_2$ replaces hexane in soy oil extraction, Chem. Eng. News May 25.

Anonymous, 1986, A newly patented supercritical process gives fine results for powders, Chem. Eng. 93(18), 18.

Angus,S., B.Armstrong, and K.M. Reuck, 1976, "Thermodynamic Tables Project ( IUPAC ), International Thermodynamic Tables of the Fluid State. Vol. 3 Carbon Dioxide", Don Mills, Permagon of Canada Ltd.

Armistead,J.D., 1988, "Temperature and Mineral effects on Cyclosporin A Biosynthesis by Tolypocladium inflatum", M.E.Sc. Thesis, Univ. Western Ont., London, Canada

Balenovic,Z., M.N.Myers, and J.C.Giddings, 1970, Binary Diffusion in Dense Gases to 1360 atm by the Chromatographic Peak-Broadening Method, J. Chem. Phys. 52, 915-922.

Bamberger,T., J.C.Erickson, C.Cooney, and S.K.Kumar, 1988, Measurement and Model Prediction of Solubilites of Pure Fatty Acids, Pure Triglycerides, and Mixtures of Triglycerides in Supercritical Carbon Dioxide, J. Chem. Eng. Data 33, 327-333.

Barker,I.K., K.D.Bartle, and A.A.Clifford, 1988, Measurement of Solubilites in Fluids at Supercritical Temperatures and Lower Pressures Using Chromatographic Retention, Chem. Eng. Commun. 68, 177-184.

Bartle,K.D., D.W.Jones, and H.Pakdel, 1982, Mild Supercritical-Gas Extraction from Low-Rank Coals: Separation, Spectroscopy, and Composition of Alkane Products, Sep. Sci. Technol. 17(1), 167-181.

Bartmann,D., and G.M.Schneider, 1973, Experimental results and Physico-chemical aspects of Supercritical Fluid Chromatography with Carbon Dioxide as the Mobile Phase, J. Chromato. 83, 135-145.

Basta,N., and S.McQueen, 1985, Supercritical Fluids: still seeking acceptance Chem. Eng. 92(2) ,14-17.

Bertucco,A., M.Fermeglia, and I.Kikic, 1986, Modified Carnahan-Starling-van der Waals Equation for Supercritical Fluid Extraction, Chem. Eng. J. 32, 21-30.

Bevia,F.R., R.R.Zapata, A.F.M.Gomis and D.P.Rico, 1986, Model for Phase Equilibria Correlation and Prediction. Characteristics and Application to Binary Liquid-Vapor and Binary, Ternary and Quaternary Liquid-Liquid Equilibria, Can.J.Chem.Eng.,64,311-322.

Borel,J.F., 1982, "The History of Cyclosporine A and Its Significance" in, White,D.G.J., ed., Cyclosporin A, Elsevier Biomedical Press, Amsterdam.

Bott,T.R., 1982, Fundamentals of carbon dioxide in solvent extraction, Chem. Ind. 19, 394-396.

Bowman,Jr.,L.M., M.N.Myers, J.C.Giddings, 1982, Supercritical Fluid (Dense Gas) Chromatography/Extraction with Linear Density Programming, Sep. Sci. Technol. 17(1), 271-287.

Brady,B.O., C-P.C.Kao, K.M.Dooley, F.C.Knopf, and R.P.Gambrell, 1987, Supercritical Extraction of Toxic Organics from Soils, Ind. Eng. Chem. Res. 26(2), 261-268.

Braun,G., and H.Schmidt, High Pressure Extraction of Crude Montan Wax, Ber. Bunsenges. Phys. Chem. 88, 891-894.

Brignole,E.A., 1986, Supercritical Fluid Extraction, Fluid Phase Equilibria 29, 133-144.

Brignole,E.A., P.M.Andersen, and A.Fredenslund, 1987, Supercritical Fluid Extraction of Alcohols from Water, Ind. Eng. Chem. Res. 26, 254-261.

Brignole,E.A., S.Skjold-Jorgensen, and Aa.Fredenslund, 1984, Application of a Local Composition Equation of State to Supercritical Fluid Phase Equilibrium Problems, Ber. Bunsenges. Phys. Chem. 88, 801-806.

Briones,J.A., J.C.Mullins, M.C.Thies, and B-U.Kim, 1987, Ternary Phase Equilibria for Acetic Acid-Water Mixtures with Supercritical Carbon Dioxide, Fluid Phase Equilibria, 36, 235-246.

Brogle,H., 1982 $CO_2$ as a Solvent: Its properties and applications, Chem. Ind. 19, 385-390.

Brown,B.O., A.J.Kishbaugh, and M.E.Paulaitis, 1987, Experimental Determination of Enhancement Factors from Supercritical Chromatography, Fluid Phase Equil. 36, 247-261.

Brunner,G., and S.Peter, 1981, Zum Stand Der Extraktion mit komprimierten Gasen, Chem.Ing.Tech.,53,529- .

Brunner,G., and S.Peter, 1982, On the Solubility of Glycerides and Fatty Acids in Compressed Gases in the Presence of an Entrainer, Sep. Sci. Technol. 17(1), 199-214.

Brunner,G., 1983, Selectivity of Supercritical Compounds and Entrainers with respect to Model Substances, Fluid Phase Equil. 10, 289-298.

Brunner,G., 1984, Mass Transfer from Solid Material in Gas Extraction, Ber. Bunsenges. Phys. Chem. 88, 887-891.

Brunner,G., 1985, Mass Transfer in Gas Extraction, in Supercritical Fluid Technology, Ed. J.M.L. Penninger, M. Radosz, M.A. McHugh and V.J. Krukonis, Elsevier Sci. Publ. B.V., Amsterdam.

Brunner,G., and K.Kreim, 1986, Separation of Ethanol from Aqueous Solutions by Gas Extraction, Ger. Chem. Eng. 9, 246-250.
Calimli,A., and A.Olcay, 1982, Supercritical Dioxane Extraction of Spruce Wood and of Dioxane-Lignin and Comparison on the Extracts with the Pyrolysis Products, Sep. Sci. Technol. 17(1), 183-197.

Canjar,L.N. and F.S.Manning, 1967, "Thermodynamic Properties and Reduced Correlations for Gases", Gulf Publ. Co.,Houston.

Capriel,P., A.Haisch, and S.U.Khan, 1986, Supercritical Methanol: An Efficacious Technique for the Extraction of Bound Pesticide Residues from Soil and Plant Samples, J. Agric. Food. Chem. 34, 70-73.

Caragay,A.B., 1981, Supercritical fluids for extraction of flavors and fragrances from natural products, Perfumer & Flavorist 6, 43-55.

Chang,H., and D.G.Morrell, 1985, Solubilities of Methoxy-1-tetralone and Methyl Nitrobenzoate Isomers and Their Mixtures in Supercritical Carbon Dioxide, J. Chem. Eng. Data 30, 74-78.

Charoensombut-Amon,T., R.J.Martin, and R.Kobayashi, 1986, Application of a Generalized Multiproperty Apparatus to Measure Phase Equilibrium and Vapor Phase Densities of Supercritical Carbon Dioxide in n-Hexadecane systems up to 26 MPa, Fluid Phase Equil. 31, 89-104.

Chen, S.H., 1983, A Rough-Hard-Sphere Theory for Diffusion in Supercritical Carbon Dioxide, Chem. Eng. Sci. 38, 655-660.

Cheng,H., M.E.P.de Fernandez, J.A.Zollweg, and W.B.Streett, 1989, Vapor-Liquid Equilibrium in the System Carbon Dioxide + n-Pentane from 252 to 458 K at Pressures to 10 MPa, J. Chem. Eng. Data 34, 319-323.

Cheong,P.L., D.Zhang, K.Ohgaki, and B.C-Y.Lu, 1986, High Pressure Phase Equilibria for Binary Systems Involving a Solid Phase, Fluid Phase Equilibria 29, 555-562.

Chimowitz,E.H., and K.J. Pennisi, 1986, Process Synthesis Concepts for Supercritical Gas Extraction in the Crossover Region, A.I.Ch.E.J. 32, 1665-1676.

Chimowitz,E.H., F.D.Kelly, and F.M.Munoz, 1988, Analysis of Retrograde Behavior and the Cross-over Effect in Supercritical Fluids, Fluid Phase Equilibria 44, 23-52.

Choi,K.J., Z.Nakhost, V.J.Krukonis, and M.Karel, 1987, Supercritical Fluid Extraction and Characterization of Lipids from Algae Scenedesmus obliquus, Food Biotech. 1(2), 263-281.

Chrastil,J., 1982, Solubility of Solids and Liquids in Supercritical Gases, J. Phys. Chem. 86, 3016-3021.

Cisternas,L.A., 1988, A Simple and Accurate Technique to Obtain Pure Component Parameters for Three-Parameter Eqution of State, Fluid Phase Equilibria,39,75-87.

Cochran,H.D., L.L.Lee and D.M.Pfund, 1987, Application of the Kirkwood-Buff Theory of Solutions to Dilute Supercritical Mixtures, Fluid Phase Equilibria,34,219-234.

Cotterman,R.L., D.Dimitrelis, and J.M.Prausnitz, 1984, Supercritical-Fluid Extraction Calculations for High-Boiling Petroleum Fractions Using Propane. Applications of Continuous Thermodynamics, Ber. Bunsenges. Phys. Chem. 88, 796-801.

Cullen,E.J. and K.A.Kobe, 1955, Benedict Equation of State: Application to Vapor-Liquid Equilibria, A.I.Ch.E.J.,1(4),452-455.

Cygnarowicz,M.L., and W.D.Seider, 1989, Effect of Retrograde Solubility on the Design Optimization of Supercritical Extraction Processes, Ind. Eng. Chem. Res. 28, 1497-1503.

Daubert,T.E., and R.Bartakovits, 1989, Prediction of Critical Temperature and Pressure of Organic Compounds by Group Contribution. Ind. Eng. Chem. Res. 28(5) 638-641.

Debenedetti,P.G., and R.C. Reid, 1985, Binary Diffusion in Supercritical Fluids, in Supercritical Fluid Technology, Ed. J.M.L. Penninger, M. Radosz, M.A. McHugh and V.J. Krukonis, Elsevier Sci. Publ. B.V., Amsterdam.

Debenedetti,P.G., and R.C. Reid, 1986, Diffusion and Mass Transfer in Supercritical Fluids, AIChE J. 32,2034-2046.

Debenedetti,P.G., 1987, Clustering in Dilute, Binary Supercritical Mixtures: A Fluctuation Analysis, Chem. Eng. Sci. 42(9), 2203-2212.

Debenedetti,P.G., and S.K.Kumar, 1988, The Molecular Basis of Temperature Effects in Supercritical Extraction, A.I.Ch.E.J. 34(4), 645-657.

de Fernandez,M.E.P., J.A.Zollweg, and W.B.Streett, 1989, Vapor-Liquid Equilibrium in the Binary System Carbon Dioxide + n-Butane, J. Chem. Eng. Data 34, 324-328.

de Filippi,R.P., 1982, $CO_2$ as a solvent: application to fats, oils and other materials, Chem. Ind. 19, 390-394.

de Filippi,R.P., and J.M.Moses, 1982, Extraction of Organics from Aqueous Solutions Using Critical-Fluid Carbon Dioxide, Proc. 4th Symp. Biotechnol., ed. C.D.Scott, p,206-219, New York, J.Wiley & Sons.

Deiters,U.K., 1983, Calculation and Prediction of Fluid Phase Equilibria from an Equation of State, Fluid Phase Equil. 10, 173-182.

Deiters,U.K., 1987, Density-Dependent Mixing Rules for the Calculation of Fluid Phase Equilibria at High Pressures, Fluid Phase Equilibria, 34, 309-310.

Deiters,U.K., and I.Swaid, 1984, Calculation of Fluid-Fluid and Solid-Fluid Phase Equilibria in Binary Mixtures at High Pressures, Ber. Bunsenges. Phys. Chem. 88, 791-796.

Deiters,U.K., and G.M.Schneider, 1986, High Pressure Phase Equilibria: Experimental Methods, Fluid Phase Equilibria 29, 145-160.

de Loos,Th.W., J.H.van Dorp, and R.N.Lichtenthaler, 1983, Phase Equilibria and Critical Phenomena in Fluid (n-Alkane + Water) Systems at High Pressures and Temperatures, Fluid Phase Equil. 10, 279-287.

de Loos,Th.W., W.Poot, and R.N.Lichtenthaler, 1984, Fluid Phase Equilibria in Binary Ethylene + n-Alkane Systems, Ber. Bunsenges. Phys. Chem. 88, 855-859.

del Valle,J.M., and J.M. Aguilera, 1988, An Improved Equation for Predicting the Solubility of Vegetable Oils in Supercritical $CO_2$, Ind. Eng. Chem. Res. 27, 1551-1553.

Dhalewadikar,S.V., A.J.Seckner, M.A.McHugh, and T.L.Guckes, 1987, Separation of Dodecane-Biphenyl Mixtures using Supercritical Ethane, Carbon Dioxide, and Ammonia, Ind. Eng. Chem. Res. 26, 976-982.

Di Andreth,J.R. and M.E.Paulaitis, 1987, An Experimental Study of Three- and Four-Phase Equilibria for Isopropanol-Water-Carbon Dioxide Mixtures at Elevated Pressures, Fluid Phase Equilibria 32, 261-271.

Dimitrelis,D., and J.M.Prausnitz, 1989, Solubilities of n-Octadecane, Phenanthrene, and n-Octadecane/Phenathrene Mixtures in Supercritical Propane at 390 and 420 K and Pressures to 60 bar, J. Chem. Eng. Data 34, 286-291.

Dobbs,J.M., J.M.Wong, and K.P.Johnson, 1986, Nonpolar Co-Solvents for Solubility Enhancement in Supercritical Fluid Carbon Dioxide, J. Chem. Eng. Data 31, 303-308.

Dobbs,J.M., and K.P.Johnson, 1987, Selectivities in Pure and Mixed Supercritical Fluid Solvents, Ind. Eng. Chem. Res. 26, 1476-1482.

Dobbs,J.M., J.M.Wong, R.J.Lahiere, and K.P.Johnson, 1987, Modification of Supercritical Fluid Phase Behavior Using Polar Cosolvents, Ind. Eng. Chem. Res. 26, 56-65.

Dooley,K.M., and F.C.Knopf, Oxidation Catalysis in a Supercritical Fluid Medium, Ind. Eng. Chem. Res. 26, 1910-1916.

D'Souza,R., and A.S.Teja, 1988, High-Pressure Phase Equilibria in the System Glucose + Fructose + Water + Ethanol + Carbon Dioxide, Fluid Phase Equilibria 39, 211-224.

D'Souza,R., J.R.Patrick, and A.S.Teja, 1988, High Pressure Phase Equilibria in the Carbon Dioxide - n-Hexadecane and Carbon Dioxide - Water Systems, Can. J. Chem. Eng. 66, 319-323.

Dziezak,J.D., 1986, Innovative Separation Process Finding Its Way into the Food Industry, Food Technol. 40(6), 66-69.

Ebeling,H., and E.U.Franck, 1984, Spectroscopic Determination of Caffeine Solubility in Supercritical Carbon Dioxide, Ber. Bunsenges. Phys. Chem. 88, 862-865.

Eckert,C.J., and S.I.Sandler, 1986, Vapor-Liquid Equilibria for the Carbon Dioxide-Cyclopentane System at 37.7, 45.0, and 60.0 °C, J. Chem. Eng. Data 31, 26-28.

Eggers,R., 1980, Large-Scale Industrial Plant for Extraction with Supercritical Gases, in "Extraction with Supercritical Gases", Ed. G.M.Schneider, E.Stahl, and G.Wilke, Weinham, Deer Beach (Florida), Basel, Verlag Chemie.

Eggers,R., and R.Tschiersch, 1980, Development and Design of Plant for High- Pressure Extraction of Natural Products, in "Extraction with Supercritical Gases", Ed. G.M.Schneider, E.Stahl, and G.Wilke, Weinham, Deer Beach (Florida), Basel, Verlag Chemie.

Eisenbach,W., P.J.Gottsch, K.Niemann, and K.Zosel, 1983, Extraction with Supercritical Gases: The First Twenty Years, Fluid Phase Equil. 10, 315-318.

Eisenbach,W.O., K.Niemann, and P.J.Gottsch, 1983, Supercritical Fluid Extraction of Oil Sands and Residues from Oil and Coal Hydrogenation, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Eisenbach,W., 1984, Supercritical Fluid Extraction: A Film Demonstration, Ber. Bunsenges. Phys. Chem. 88, 882-887.

Eldridge,A.C., J.P.Friedrich, K.Warner, and W.F.Kwolek, 1986, Preparation and Evaluation of Supercritical Cqarbon Dioxide Defatted Soybean Flakes, J. Food Sci. 51(3), 584-587.

Ellis,S.R.M., 1971, Vapor Phase Extraction Process, Br.Chem. Eng. 16, 358-.

Ely,J.F. and J.K. Baker, 1983, A Review of Supercritical Fluid Extraction, NBS Tech. Note. 1070.

Ender,U., and S.Peter, 1989, 'Pressure Pulsation Enhances Mass Transfer in Supercritical Extraction': Verbesserung des Stoffaustausches bei der uberkritischen Fluid-Extraktion durch Druckpulsation, Chem. Ing. Tech. 61, 324-326.

Fall, J.L., and K.D.Luks, 1986, Effect of Additive Gases on the Liquid-Liquid- Vapor Immiscibility of the Carbon Dioxide + n-Nonadecane Mixture, J. Chem. Eng. Data 31, 332-336.

Farah,N. and R.W.Missen, 1986, The Computer-Derivation of Thermodynamic Equations Part I. First and Second Derivatives for Simple Systems, Can.J.Chem.Eng.,64,154-157.

Feist,R., and G.M. Schneider, 1982, Determination of Binary Diffusion Coefficients of Benzene, Phenol, Naphthalene, and Caffeine in Supercritical $CO_2$ between 308 and 333 K in the Pressure Range 80 to 160 Bar with Supercritical Fluid Chromatography (SFC), Sep. Sci. Tech. 17,261-270.

Fleming,P.D. and R.J.Brugman, 1987, Toward a Molecular Equation of State for Real Materials, A.I.Ch.E.J.,33(5),729-740.

Fong,W.S., P.C.F.Chan, P.Pichaichanarong, W.h.Corcoran, and D.D.Lawson, 1983, Experimental Observations on a Systematic Approach to Supercritical Extraction of Coal, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Frank,E.U., 1983, Thermophysical Properties of Supercritical Fluids with Special Consideration of Aqueous Systems, Fluid Phase Equil. 10, 211-222.

Franck,E.U., 1984, Physicochemical Properties of Supercritical Solvents, Ber. Bunsenges. Phys. Chem. 88, 820-825.

Fujimoto,C., H.Yoshida, and K.Jinno, 1989, The Use of Polar Modifiers in Microbore Supercritical Fluid Chromartography Combined with Inductively Coupled Plasma Spectrometry, J. Microcolumn Sep. 1(1), 19-22.

Gahrs, H.J., 1984, Applications of Atmospheric Gases in High Pressure Extraction, Ber. Bunsenges. Phys. Chem. 88, 894-897.

Gahrs,H.J., 1985, High Pressure Extraction - Increase of Range of Application by use of Multicomponent Gaseous Solvents, Ger. Chem. Eng. 8, 1-7.

Gere, D.R., 1983, Supercritical Fluid Chromatography, Science 222, 253-259.

Gilbert,M.L., and M.E.Paulaitis, 1986, Gas-Liquid Equilibrium for Ethanol-Water- Carbon Dioxide Mixtures at Elevated Pressures, J. Chem. Eng. Data 31, 296-298.

Gouw,T.H., and R.E.Jentoft, 1972, Supercritical Fluid Chromatography, J. Chromatogr. 68, 303-323.

Hall,C.K., and B.A.Hacker, 1983, Corresponding States Theories for Chain Molecules, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Hamann, H., C. Hoheisel and H. Richtering, 1972, Nuclear Magnetic Resonance Studies and Self-Diffusion at Critical Points in Fluid Systems, Ber. Bunsenges. Phys. Chem. 76, 249-253.

Hannay,J.B. and J.Hogarth, 1979, On the solubilty of solids in Gases, Proc. R. Soc. London 29, 324- .

Hannay,J.B. and J.Hogarth, 1980, On the solubilty of solids in Gases, Proc. R. Soc. London 30, 178- .

Harri,E., and A.Reugger, 1978, Organic Compounds, U.S. Patent 4,117,118.

Harri,E., and A.Reugger, 1980, Antibiotic Production, U.S. Patent 4,215,199.

Hederer,H., and H.Heidemeyer, 1985, Process Design for High-pressure Extraction with Supercritical Gases, Ger. Chem. Eng. 8,112-118.

Herrick,D.E., G.D.Holder, Y.T.Shah, and J.Bruggeman, 1988, Acceleration of Chlorination of Alumina Using Supercritical CCL4, A.I.Ch.E.J. 34(4), 669-671.

Hirata,Y., and Y.Okamoto, 1989, Supercritical Fluid Extraction Combined with Microcolumn Liquid Chromatography for the Analysis of Polymer Additives, J. Microcolumn Sep. 1(1),46-50.

Hirschfelder,J.O., R.J.Buehler, H.A.McGee,Jr., and J.R.Sutton, 1958a, Generalized Equation of State for Gases and Liquids, Ind. Eng. Chem., 50(3), 375-385.

Hirschfelder,J.O., R.J.Buehler, H.A.McGee,Jr., and J.R.Sutton, 1958b, Generalized Thermodynamic Excess Functions for Gases and Liquids, Ind. Eng. Chem., 50(3), 386-390.

Hoffman,S., and T.Greibrokk, 1989, Packed Capillary Supercritical Fluid Chromatography with Mixed Mobile Phases and Light-Scattering Detection, J. Microcolumn Sep. 1(1), 35-40.

Holder,G.D., 1986, Phase Behavior in Fluid-Solid Systems, Fluid Phase Equilibria,29,447-455.

Holscher,I.F., M.Spee, and G.M.Schneider, 1989, Fluid-Phase Equilibria of Binary and Ternary Mixtures of $CO_2$ with Hexadecane, 1-Dodecanol, 1-Hexadecanol, and 2-Ethoxy-Ethanol at 333.2 and 393.2 K and at Pressures up to 33 MPa, Fluid Phase Equilibria 49, 103-113.

Holste,J.C., K.R.Hall, P.T.Eubank, and K.N.Marsh, 1986, High Pressure PVT Measurements, Fluid Phase Equilibria,29,161-175.

Hong,G.T., and M.Modell, 1983, Binary Phase Diagrams from a Cubic Equation of State, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Hong,J.H., and R.Kobayashi, 1988, Vapor-Liquid Equilibrium Studies for the Carbon Dioxide-Methanol System, Fluid Phase Equilibria 41, 269-276.

Huang,F-H., 1984, "An Accurate Equation of State for Carbon Dioxide at Supercritical Conditions", M.Sc. Thesis, Univ. Oklahoma, Norman,Oklahoma.

Huang,F-H., M-H.Li, L.L.Lee, K.E.Starling, and F.T.H.Chung, 1985, An Accurate Equation of State for Carbon Dioxide, J. Chem. Eng. Japan, 18(5), 490-496.

Hubert,P., and O.G.Vitzthum, 1980, Fluid Extraction of Hops, Spices, and Tobacco with Supercritical Gases, in "Extraction with Supercritical Gases", Ed. G.M.Schneider, E.Stahl, and G.Wilke, Weinham, Deer Beach (Florida), Basel, Verlag Chemie.

Ikushima,Y., K.Hatakeda, S.Ito, N.Saito, T.Asano, and T.Goto, 1988, A Supercritical Carbon Dioxide Extraction from Mixtures of Triglycerides and Higher Fatty Acid Methyl Esters Using a Gas-Effusion-Type System, Ind. Eng. Chem. Res. 27, 818-823.

Ikushima,Y., N.Saito, and T.Goto, 1989, Selective Extraction of Oleic, Linoleic and Linolenic Acid Methyl Esters from Their Mixture with Supercritical Carbon Dioxide-Entrainer Systems and a Correlation of the Extraction Efficiency with a Solubility Parameter, Ind. Eng. Chem. Res. 28, 1364-1369.

Inomata,H., K.Arai, and S.Saito, 1986, Measurement of Vapor-Liquid Equilibria at Elevated Temperatures and Pressures using a Flow Type Apparatus, Fluid Phase Equilibria 29, 225-232.

Inomata,H., T.Kondo, S.Hirohama, K.Arai, Y.Suzuki, and M.Konno, 1989, Vapor-Liquid Equilibria for Binary Mixtures of Carbon Dioxide and Fatty Acid Methyl Esters, Fluid Phase Equilibria 46, 41-52.

Irani,C.A., and E.W.Funk, 1977, Separations using Supercritical Gases. in CRC Handbook: Recent Developments in Separation Science vol. 3, Part A,171 - Boca Raton, FL, CRC Press.

Jennings,D.W., and A.S.Teja, 1989, Vapor-Liquid Equilibria in the Carbon Dioxide - 1-Hexane and Carbon Dioxide - 1-Hexyne Systems, J.Chem. Eng. Data 34, 305-309.

Johnson,K.P., S.E.Barry, N.K.Read, and T.R.Holcomb, 1987, Separation of Isomers Using Retrograde Crystallization from Supercritical Fluids, Ind. Eng. Chem. Res. 26(11), 2372-2377.

Johnson,K.P. and C.A.Eckert, 1981, An Analytical Carnahan-Starling-van der Waals Model for Solubility of Hydrocarbon Solids in Supercritical Fluids, A.I.Ch.E.J.,27(5),773-779.

Johnson,K.P., D.H.Ziger, and C.A.Eckert, 1982, Solubilities of Hydrocarbon Solids in Supercritical Fluids. The Augmented van der Waals Treatment, Ind. Eng. Chem. Res. 21, 191-197.

Johnson,K.P., 1984, Supercritical Fluids. in Encyclopedia of Chemical Technology, 3rd Ed., Suppl. vol., p. 872- ,New York, John Wiley & Sons.

Johnson,K.P., D.G.Peck, and S.Kim, 1989, Modeling Supercritical Mixtures: How Predictive Is It?, Ind. Eng. Chem. Res. 28, 1115-1125.

Jonah,D.A., K.S.Shing, V.Venkatasubramanian, and K.E.Gubbins, 1983, Molecular Dynamics of Dilute Solutes in Supercritical Solvents, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Jonah,D., 1989, A Linear Correlation for Solid Solubilities in Supercritical Gases, Chem. Eng. Comm. 79, 1-12.

Jones,M.C., and P.J.Giarratano, 1988, Latent Heats of Supercritical Fluid Mixtures, A.I.Ch.E.J.,34(12),2059-2062.

Josten,H., and H.Hartmann, 1987, Liquified Gases as a Medium for the Extractive Separation of Liquid Mixtures, Chem. Eng. Process. 21, 217-227.

Kalra,H., S.Y-K. Chung and C-J.Chen, 1987, Phase Equilibrium Data for Supercritical Extraction of Lemon Flavors and Palm Oils with Carbon Dioxide, Fluid Phase Equilibria 36, 263-278.

Kander,R.G., and M.E.Paulaitis, 1983, The Absorption of Phenol from Dense Carbon Dioxide onto Activated Carbon, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Kaminishi,G-I., C.Yokoyama, and S.Takahashi, 1987, Vapor Pressures of Binary Mixtures of Carbon Dioxide with Benzene, n-Hexane, and Cyclohexane up to 7 MPa, Fluid Phase Equilibria 34, 83-99.

Kates, M., 1986, Techniques of Lipidology, Vol 3 part 2, Isolation, Analysis, and Identification of Lipids, 2nd revised Ed., in the series 'Laboratory Techniques in Biochemistry and Molecular Biology', Ed. R.H. Burden, and P.H.Knippenberg, Elsevier Pulb., Amsterdam, New York, Oxford.

Kennedy,J.T. and G.Thodos, 1960, Reduced Density Correlation for Carbon Dioxide, Gaseous and Liquid States, J.Chem.Eng.Data 5(3),293-297.

Kershaw,J.R., and J.Jezko, 1982, Supercritical Gas Extraction of South African Coals, Sep. Sci. Technol. 17(1), 151-166.

Kiatkowski,J., Z.Lisicki, and W.Majewski, 1984, An Experimental Method for Measuring Solubilities of Solids in Supercritical Fluids, Ber. Bunsenges. Phys. Chem. 88, 865-869.

Killesreiter,H., 1984, Continuous Viscosity Measurements in Order to Observe the Solubility and Miscibility of Carbon Dioxide in a Crude Oil, Ber. Bunsenges. Phys. Chem. 88, 838-841.

Kim,S., and K.P.Johnson, 1987, Molecular Interactions in Dilute Supercritical Fluid Solutions, Ind. Eng. Chem. Fund. 26, 1206-1213.

Kim-E,M.E., and R.C.Reid, 1983, The Rapid Depressurization of Hot, High Pressure Liquids or Supercritical Fluids, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

King,M.B., and T.R.Bott, 1982, Problems Associated with the Development of Gas Extraction and Similar Processes, Sep. Sci. Technol. 17(1), 119-150.

King, M.B., K. Kassim and T.R. Bott, 1983, Mass Transfer into Near-Critical Extractants, Fluid Phase Equil. 10, 249-260.

King,M.B., D.A.Alderson, F.H.Fallah, D.M.Kassim, K.M.Kassim, J.R.Sheldon, and R.S.Mahmud, 1983, Some Vapor/Liquid and Vapor/Solid Equilibrium Measurements of Relevance for Supercritical Extraction Operations, and Their Correlation, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

King,M.B., K.Kassim, T.R.Bott, J.R.Sheldon, and R.S.Mahmud, 1984, Prediction of Mutual Solubilites of Heavy Components with Super-Critical and Slightly Sub-Critical Solvents: The Role of Equations of State and Some Applications of a Simple Expanded Lattice Model at Subcritical Temperatures, Ber. Bunsenges. Phys. Chem. 88, 812-820.

King,M.B., T.R.Bott, and J.H.Chami, 1987, Extraction of bio-materials with compressed carbon dioxide and other solvents under near critical conditions, in Separations for Biotechnology, Eds. M.S. Verrall and M.J. Hudson, Chichester, Ellis Horwood Ltd.

Klein,T., and S.Schulz, 1989, Phase Equilibria in Mixtures of Glycerides and Carbon Dioxide and Application of Continous Thermodynamics to Mixtures of Rapeseed Oil and Carbon Dioxide, Fluid Phase Equil. 50, 79-100.

Kleintjens,L.A., and R.Koningsveld, 1982, Mean-Field Lattice-Gas Description of the System $CO_2/H_2O$, Sep. Sci. Technol. 17(1), 215-233.

Kleintjens,L.A., and R.Koningsveld, 1983, Mean-Field Lattice-Gas Description of Fluid Phase Equilibria, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Kleintjens,L.A., 1983, Mean-Field Lattice Gas Description of Vapor-Liquid and Supercritical Equilibria, Fluid Phase Equil. 10, 183-190.

Klesper,E., 1980, Chromatography with Supercritical Fluids, in "Extraction with Supercritical Gases", Ed. G.M.Schneider, E.Stahl, and G.Wilke, Weinham, Deer Beach (Florida), Basel, Verlag Chemie.

Klesper,E., and F.P.Schmidt, 1988, Recent Developments in Supercritical Fluid Chromatography (SFC), Chimicaoggi, (11), 9-15.

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Krynicki, K., A.-L. Meragi and J.G. Powles, 1981, Self Diffusion in Carbon Dioxide Near the Critical Point, Bunsenges. Phys. Chem. 85, 1153-1154.

Kuk, M.S., and J.C.Montagna, 1983, Solubility of Oxygenated Hydrocarbons in Supercritical Carbon Dioxide, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Kumar,S.K., U.W.Suter, and R.C.Reid, 1986, Fractionation of Polymers with Supercritical Fluids, Fluid Phase Equilibria, 29, 373-382.

Kurnik,R.T., S.J.Holla, and R.C.Reid, 1981, Solubility of Solids in Supercritical Carbon Dioxide and Ethylene, J. Chem. Eng. Data 26, 47-51.

Kurnik,R.T., and R.C.Reid, 1982, Solubility of Solid Mixtures in Supercritical Fluids, Fluid Phase Equilibria 8, 93-105.

Kwak,T.Y. and G.A.Mansoori, 1986, Van der Waals Mixing Rules for Cubic Equations of State. Applications for Supercritical Fluid Extraction Modelling, Chem.Eng.Sci.,41(5),1303-1309.

Lahiere,R.J., and J.R.Fair, 1989, Novel Techniques to Measure Equilibria of Supercritical Solvents and Liquid Mixtures, J. Chem. Eng. Data 34, 275-278.

Larson,K.A., and M.L.King, 1986, Evaluation of Supercritical Fluid Extraction in the Pharmaceutical Industry, Biotechnol. Prog. 2(2), 73-82.

Lauer, H.H., D. McManigill and R.D. Board, 1983, Mobile-Phase Transport Properties of Liquified Gases in Near-Critical and Supercritical Fluid Chromatography, Anal. Chem. 55, 1370-1375.

Lawal,A.S., 1987, A Consistent Rule for Selecting Roots in Cubic Equations of State, Ind.Eng.Chem.Res.,26,857-859.

Lee,M.L., B.Xu, E.C.Haung, N.M.Djordjevic, H-C.K Chang, and K.E.Markides. 1989, Liquid Sample Introduction Methods in Capillary Column Supercritical Fluid Chromatography, J. Microcolumn Sep. 1(1), 7-13.

Lee,R.L., and K.C.Chao, 1988, Extraction of 1-Methylnaphthalene and m-Cresol with Supercritical Carbon Dioxide and Ethane, Fluid Phase Equilibria 43, 329-340.

Lemert,R.M., and K.P.Johnson, 1989, Solid-Liquid-Gas Equilibria in Multicomponent Supercritical Fluid Systems, Fluid Phase Equilibria 45, 265-286.

Le Neindre,B., Y.Garrabos, and R.Tufeu, 1984, Thermal Conductivity in Supercritical Fluids, Ber. Bunsenges. Phys. Chem. 88, 916-920.

Lentz,H., and E.U.Franck, 1980, Phase Equilibria and Critical Curves of Binary Ammonia-Hydrocarbon Mixtures, in "Extraction with Supercritical Gases", Ed. G.M.Schneider, E.Stahl, and G.Wilke, Weinham, Deer Beach (Florida), Basel, Verlag Chemie.

Leu,A-D., and D.B.Robinson, 1988, Equilibrium-Phase Properties of the Neopentane-Carbon Dioxide Binary System, J. Chem. Eng. Data 33, 313-316.

Levy,G.B., 1986, Process Supercritical Chromatography: an Introduction, American. Lab. (12),62-71.

Levy,E.J., S.Lurcott, S.O'Neill, S.Yocklovich, R.Cohen, K.Pfeiffer, T.P.Wampler, and S.A.Liebman, 1987, Advances in supercritical Fluid Systems, American Lab. (8), 66-72.

Li,L., and E.Kiran, 1988, Interaction of Supercritical Fluids with Lignocellulosic Materials, Ind. Eng. Chem. Res. 27, 1301-1312.

Li,S.F.Y., 1989, Experimental Studies on Supercritical Fluid Separation Processes, J. Chem. Tech. Biotechnol. 46, 1-10.

Mak,P.C.N. and J.Lielmezs, 1989, Correlation for the Second Virial Coefficient for Nonpolar Compounds Using Cubic Equation of State, Ind.Chem.Eng.Res.,28,127-130.

Mansoori,G.A., K.Schulz, and E.E.Martinelli, 1988, Bioseparation using Supercritical Fluid Extraction/Retrograde Condensation, Bio/Technol 6, 393-396.

Margaritis, A., and P.S.Chahal, 1989, Development of a fructose-based medium for biosynthesis of Cyclosporin-A by Beauvaria nivea. Biotechnol. Lett. 11(11), 765-768.

Marshall,J.W., 1986, "Factors for the Process Development of Cyclosporin Fermentations", M.E.Sc. Thesis, Univ. Western Ont., London, Canada.

Martin,J.J., 1984, Correlation of Second Viral Coefficients Using a Modified Cubic Equation of State, Ind.Eng.Chem.Fundam.23,454-459.

Mathias,P.M. and M.S.Benson, 1986, Computational Aspects of Equations of State: Fact and Fiction, A.I.Ch.E.J. 32(12), 2087-2090.

Matson,D.W., J.L.Fulton, R.C.Peterson, and R.D.Smith, 1987, Rapid Expansion of Supercritical Fluid Solutions: Solute Formation of Powders, Thin Films, and Fibers, Ind. Eng. Chem. Res. 26(11), 2298-2306.

McDonald,E., and J.Howard, 1982, Chemicals from Forest Products by Supercritical Gas Extraction, Proc. of Fourth Bioenergy R&D Seminar March 29-31, Winnipeg, Manitoba.

McDonald,E.C., J.Howard, and B.Bennett, 1983, Chemicals from Forest Products by Supercritical Extraction, Fluid Phase Equil. 10, 337-344.

McHugh,M.A., and M.E.Paulaitis, 1980, Solid Solubilities of Naphthalene and Biphenyl in Supercritical Carbon Dioxide J. Chem. Eng. Data 25, 326-329.

McHugh,M.A., M.W.Mallet, and J.P.Kohn, 1983, High Pressure Fluid Phase Equilibria of Alcohol-Water-Supercritical Solvent Mixtures, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

McHugh,M.A., and T.J.Yogan, 1984, Three-Phase Solid-Liquid-Gas Equilibria for Three Carbon Dioxide-Hydrocarbon Solid Systems, Two Ethane - Hydrocarbon Solid Systems, and Two Ethylene-Hydrocarbon Solid Systems, J. Chem. Eng. Data 29, 112-115.

McHugh,M.A., A.J.Seckner, and T.J.Yogan, 1984, High-Pressure Phase Behavior of Binary Mixtures of Octacosane and Carbon Dioxide, Ind. Eng. Chem. Res. 23, 493-499.

McHugh,M.A., 1986, Extraction with Supercritical Fluids. in Recent Developments in Separation Science, vol. 9, ed. N.N.Li and J.M.Calo., 75 - 105, Boca Raton, FL, CRC Press.

McHugh,M.A. and V.J.Krukonis, 1986, "Supercritical Fluid Extraction. Principles and Practice", Butterworths, Boston.

Melham,G.A., R.Saini, and B.M.Goodwin, 1989, A Modified Peng-Robinson Equation of State, Fluid Phase Equil. 47, 189-237.

Metzger,J.O., J.Hartmanns. D.Malwitz, and P.Koll, 1983, Thermal Organic Reactions in Supercritical Fluids, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Miller,J.F., E.L.Almond, N.N.Shah, J.M.Ludlow, J.A.Zollweg, W.B.Streett, S.H.Zinder, and D.S.Clark, 1988, High-Pressure-Temperature Bioreactor for Studying Pressure-Temperature Relationships in Bacterial Growth and Productivity, Biotechnol. Bioeng. 31, 407-413.

Miller,M.M., A.Jangkamolkulchai, and K.D.Luks, 1989, Reexamination of the Multiphase Equilibria of the System Carbon Dioxide + n-Butylbenzene + n-Eicosane, Fluid Phase Equil. 50, 189-199.

Mitra,S., J.W.Chen, and D.S.Viswanath, 1988, Solubility and Partial Molar Volumes of Heavy Aromatic Hydrocarbons in Supercritical $CO_2$, J. Chem. Eng. Data 33, 35-37.

Mohamed,R.S., and G.D.Holder, 1987, High Pressure Phase Behavior in Systems Containing $CO_2$ and Heavier Compounds with Similar Vapor Pressures, Fluid Phase Equilibria 32, 295-317.

Mohamed,R.S., P.G.Debenedetti, and R.K.Prud'homme, 1989, Effects of Process Conditions on Crystals Obtained from Supercritical Mixtures, A.I.Ch.E.J. 35(2), 325-328.

Monge,A., and J.M.Prausnitz, 1983, An Experimental Method for Measuring Solubilities of Heavy Fossil-Fuel Fractions In Compressed Gases to 100 Bar and 300 C, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds.,

Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Moradinia,I., and A.S.Teja, 1988, Solubilities of Solid n-Nonacosane and n-Tritriacontane in Supercritical Ethane, J. Chem. Eng. Data 33, 240-242.

Naik,S.N., and H.Lentz, 1989, Extraction of Perfumes and Flavours from Plant Materials with Liquid Carbon Dioxide under Liquid-Vapor Equilibrium Conditions, Fluid Phase Equilibria 49, 115-126.

Nakanishi,K., Y.Adachi and I.Fujihara, 1986, PVT relation and Density Dependent Local Composition for Super-critical and Sub-critical Fluid Mixtures of Lennard-Jones Molecules, Fluid Phase Equilibria,29,347-355.

Nakamura,K., Y.M.Chi, Y.Yamada, and T.Yano, 1986, Lipase Activity and Stability in Supercritical Carbon Dioxide, Chem. Eng. Commun. 45, 207-212.

Nezbeda,I., and K.Aim, 1987, Perturbed Hard-Sphere Equations of State of Real Fluids-III. Residual Parameter 'ap' of Non-Polar Liquids, Fluid Phase Equilibria, 34,171-188.

Nighswander,J.A., N.Kalogerakis, and A.K.Mehrotra, 1989, Solubilities of Carbon Dioxide in Water and 1 wt % NaCl Solution at Pressures up to 10 MPa and Temperatures from 80 to 200 °C, J. Chem Eng. Data 34, 355-360.

Nisson,W.B., E.J.Gauglitz Jr., J.K.Hudson, V.F.Stout, and J.Spinelli, 1988, Fractionation of Menhaden Oil Ethyl Esters Using Supercritical Fluid $CO_2$, JAOCS 65(1), 109-117.

Novotny,M., S.R.Springston, P.A.Peaden, J.C.Fjeldsted, and M.L.Lee, Capillary Supercritical Fluid Chromatography, Anal.Chem. 53(3), 407a-415a.

Oellrich,L.R., H.Knapp and J.M.Prausnitz, 1978, A Simple Perturbed-Hard-Sphere Equation of State Applicable to Subcritical and Supercritical peratures, Fluid Phase Equilibria,2,163-171.

Ohgaki,K., I.Tsukahara, K.Semba, and T.Katayama, 1989, A fundamental study of extraction with a supercritical fluid. Solubilities of α-tocopherol, palmitic acid, and tripalmitin in compressed carbon dioxide at 25 °C and 40 °C, Intern. Chem. Eng. 29(2), 302-308.

Olcay,A., T.Tugrul, and A.Calimli, 1983, The Supercritical Extraction of Lignites and Wood, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Orbey,H. and J.H.Vera, 1983, Correlation for the Third Virial Coefficient Using $T_c$,$P_c$ and $\omega$ as Parameters, A.I.Ch.E.J.,29(1),107-113.

Orbey,H. and J.H.Vera, 1986, Rational Construction of an Augmented Hard Core Equation of State for Pure Compounds and Study of its Application to Mixtures, Chem. Eng. Commun. 44, 95-106.

Orye,R.V., 1969, Prediction and Correlation of Phase Equilibria and Thermal Properties with the BWR Equation of State, Ind. Eng. Chem. Proc. Des. Develop, 8(4), 579-588.

O'Toole,C., P.Richmond, and J.Reynolds, 1986, Extracting foodstuffs using supercritical $CO_2$, Chem. Engr. 426, 74-79.

Panzner,F., S.R.M.Ellis, and T.R.Bott, 1979, The Extraction and Separation at Near Critical Conditions of Components in Some Natural Products, Proc. Int. Solv. Ext. Conf. Vol. 2, CIM Special vol. 21, 685-692, Ste-Anne-de-Bellevue, Harpell's Press.

Patel,M.R., J.C.Holste, K.R.Hall, and P.T.Eubank, 1987, Thermophysical Properties of Gaseous Carbon Dioxide-Water Mixtures, Fluid Phase Equil. 36, 279-299.

Patel,M.R., and P.T.Eubank, 1988, Experimental Densities and Derived Thermodynamic Properties for Carbon Dioxide-Water Mixtures, J. Chem. Eng. Data 33, 185-193.

Paul,P.M.F., and W.S.Wise, 1971, The Principles of Gas Extraction, London, Mills and Boon Ltd.

Paulitis,M.E., R.G.Kander, and J.R.DiAndreth, 1984, Phase Equilibria Related to Supercritical-Fluid Solvent Extractions, Ber. Bunsenges. Phys. Chem. 88, 869-875.

Paulitis,M.E., V.J.Krukonis, R.T.Kurnik, and R.C.Reid, 1983a, Supercritical Fluid Extraction, Rev.Chem.Eng. 1, 179-250.

Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds. 1983b, Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Paulitis,M.E., M.A.McHugh, and C.P.Chai, 1983, Solid Solubilities In Supercritical Fluids at Elevated Pressures, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Peng,D-Y., 1986, An Empirical Method for Calculating Vapor-Liquid Critical Points of Multicomponent Mixtures, Can.J.Chem.Eng.,64,827-830.

Pennisi,K.J., and E.H.Chimowitz, 1986, Solubilities of Solid 1,10-Decanediol and a Solid Mixture of 1,10-Decanediol and Benzoic Acid in Supercritical Carbon Dioxide, J. Chem. Eng. Data 31, 285-288.

Peschel,W. and H.Wenzel, 1984, Equation-of-State Predictions of Phase Equilibria at Elevated Pressures in Mixtures Containing Methanol, Ber. Bunsenges. Phys. Chem. 88, 807-812.

Peter,S., and G.Brunner, 1980, The Separation of Nonvolatile Substances by Means of Compressed Gases in Countercurrent Processes, in "Extraction with Supercritical Gases", Ed. G.M.Schneider, E.Stahl, and G.Wilke, Weinham, Deer Beach (Florida), Basel, Verlag Chemie.

Peter,S., 1984, Chemical Engineering Applications of Supercritical Solvents, Ber. Bunsenges. Phys. Chem. 88, 875-822.

Peter,S., M.Schneider, E.Weidner, and R.Ziegelitz, 1987, The Separation of Lecithin and Soy Oil in a Countercurrent Column by Near Critical Fluid Extraction, Chem. Eng. Technol. 10, 37-42.

Pfund,D.M., L.L.Lee and H.D.Cochran, 1988, Application of the Kirkwood-Buff Theory of Solutions to Dilute Supercritical Mixtures. II. The Excluded Volume and Local Composition Models, Fluid Phase Equilibria,39,169-192.

Pitzer,K.S. and D.R.Schreiber, 1988, Improving Equation of State Accuracy in the Critical Region: Equations for Carbon Dioxide and Neopentane as Examples, Fluid Phase Equilibria,41,1-17.

Poirier,M.G., A.Ahmed, J-L.Grandmaison, and S.C.F.Kaliaguine, 1987, Supercritical Gas Extraction of Wood with Methanol in a Tubular Reactor, Ind. Eng. Chem. Res. 26, 1738-1743.

Prange,M.M., and W.H.Riepe, 1987, Studies on Phase Equilibria of a Multicomponent Model Mixture in Supercritical Carbon Dioxide and Trifluoromethane, Chem. Eng. Process. 22(4), 183-191.

Prausnitz,J.M., R.N.Lichtenthaler, and E.G.de Azevedo, 1986, Molecular Thermodynamics of Fluid-Phase Equilibria, 2nd Ed., Englewood Cliffs, N.J., Prentice-Hall Inc.

Quesniaux,V., R.Tees, M.H.Schreier, R.M.Wenger, P.Donatsch, and M.H.V. Van Regenmortel, 1986, Monoclonal Antibodies to Ciclosporin, Prog. Allergy 38, 108-122.

Radosz,M., 1984, Variable-Volume Circulation Apparatus for Measuring High-Pressure Fluid-Phase Equilibria, Ber. Bunsenges. Phys. Chem. 88, 859-862.

Radzyminski,I.F, and W.B.Whiting, 1987, Fluid Phase Stability and Equations of State, Fluid Phase Equilibria,34,101-110.

Rainwater,J.C., and M.R.Moldover, 1983, Thermodynamic Models of Dilute Solutions in Supercritical Solvents, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Rance,R.W., and E.L.Cussler, 1974, Fast Fluxes With Supercritical Solvents, A.I.Ch.E.J. 20(2), 353-356.

Randall,L.G., 1982, The Present Status of Dense (Supercritical) Gas Extraction and Dense Gas Chromatography: Impetus for DGC/MS Development, Sep. Sci. Technol. 17(1), 1-118.

Randall,L.G., 1983, Analysis of Dense (Supercritical) Gas Systems. Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Randolph,T.W., H.W.Blanch, J.M.Prausnitz, and C.R.Wilke, 1985, Enzymatic Catalysis in a Supercritical Fluid, Biotechnol. Lett. 7(5), 325-328.

Randolph,T.W., H.W.Blanch, and J.M.Prausnitz, 1988, Enzyme-Catalyzed Oxidation of Cholesterol in Supercritical Carbon Dioxide, A.I.Ch.E.J. 34(8), 1354-1360.

Recasens,F., B.J.McCoy, and J.M.Smith, 1989, Desorption Processes: Supercritical Fluid Regeneration of Activated Carbon, A.I.Ch.E.J. 35(6), 951-958.

Reid,R.C., J.M.Prausnitz, and B.E.Poling, 1987, "The Properties of Liquids and Gases", 4th Ed. McGraw - Hill Book Co., New York

Rizvi,S.S.H., A.L.Benado, J.A.Zollweg, and J.A.Daniels, 1986, Supercritical Fluid Extraction: Fundamental Principles and Modeling Methods, Food Tech. 40(6), 55-65.

Roach,M.S., 1988, "Solvent Extraction of Cyclosporin A from Tolypocladium inflatum", M.E.Sc. Thesis, Univ. Western Ont., London, Canada.

Roop,R.K., and A.Akgerman, 1989, Entrainer Effect for Supercritical Extraction of Phenol from Water, Ind. Eng. Chem. Res. 28, 1542-1546.

Rowlinson,J.S., 1983, Critical and Supercritical Fluids, Fluid Phase Equil. 10, 135-139.

Saad,H., and E.Gulari, 1984, Diffusion of Liquid Hydrocarbons in Supercritical $CO_2$ by Photon Correlation Spectroscopy, Ber. Bunsenges. Phys. Chem. 88, 834-837.

Scarrah,W.P., 1983, Liquefaction of Lignite Using Low Cost Supercritical Solvents, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Schaeffer,S.T., L.H.Zalkow, and A.S.Teja, 1988, Solubility of Monocrototaline in Supercritical Carbon Dioxide and Carbon Dioxide-Ethanol Mixtures, Fluid Phase Equilibria 43, 45-56.

Schmitt,W.J., R.A.Greiger-Block, and T.W.Chapman, 1983, The Preparation of Acid-Catalyzed Silica Aerogel, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Schmitt,W.J., and R.C.Reid, 1986a, Solubility of Monofunctional Organic Solids in Chemically Diverse Supercritical Fluids, J. Chem. Eng. Data 31, 204-212.

Schmitt,W.J., and R.C.Reid, 1986b, The use of Entrainers in Modifying the Solubility of Phenanthrene and Benzoic Acid in Supercritical Carbon Dioxide and Ethane, Fluid Phase Equilibria, 32, 77-99.

Schmitz,F.P., D.Leyendecker, and E.Klesper, 1984, Chromatography with Mobile Phases in the Liquid and Supercritical State, Ber. Bunsenges. Phys. Chem. 88, 912-915.

Schneider,G.M., E.Stahl, and G.Wilke, Eds. 1980, Extraction with Supercritical Gases, Weinheim, W. Germany, Verlag Chemie.

Schneider,G.M., 1980, Physicochemical Principles of Extraction with Supercritical Gases, in "Extraction with Supercritical Gases", Ed. G.M.Schneider, E.Stahl, and G.Wilke, Weinham, Deer Beach (Florida), Basel, Verlag Chemie.

Schneider,G.M., 1983, Physicochemical Aspects of Fluid Extraction, Fluid Phase Equil. 10, 141-157.

Schneider,G.M., 1984, Phase Equilibria in Fluid Systems, Ber. Bunsenges. Phys. Chem. 88, 841-848.

Schneider, G.M., 1978,1980, Physicochemical Principles of Extraction with Supercritical Gases, in Extraction with Supercritical Gases, Ed. Scheinder, G.M., E. Stahl and G. Wilke Weinheim, Deerfield Beach Fl., Basel, Verlag Chemie, 1980. (Angew. Chem. Int. Ed. Engl. 17,701-754, 1978)

Shimshick,E.J., 1983, Extraction with supercritical CO2, Chemtech 13(6), 374-375.

Simon.R., 1983, Measuring the Properties of Petroleum Reservoir Fluids up to 20,000 psia (138 MPa) and 400 F (200 C), Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Simon,R. and R.L.Schmidt, 1983, A System for Determining Fluid Properties up to 136 MPa and 473 K, Fluid Phase Equil. 10, 233-248.

Sims,M., 1982, Process uses liquid $CO_2$ for botanical extractions, Chem. Eng. 89(2), 50-51.

Skinner,R.N., 1983, Compressed Gas Extraction, The Ultimate Answer in the Food Industry, Chem. Australia 50(3), 89-92.

Somayajulu,G.R., 1989, Estimation Proceedures for Critical Constants, J.Chem. Eng.Data,34,106-120.

Squires,T.G., C.G.Venier, and T.Aida, 1983, Supercritical Fluid Solvents in Organic Chemistry, Fluid Phase Equil. 10, 261-268.

Stahl,E., and E.Schutz, 1980, Extraktion labiler Naturstoffe mit uberkritischen Gasen, Planta Medica 40, 12-21.

Stahl,E., E.Schutz, and H.K.Mangold, 1980a, Extraction of Seed Oils with Liquid and Supercritical Carbon Dioxide, J. Agric. Food Chem. 28, 1153-1157.

Stahl,E., W.Schilz, E.Schutz, and E.Willing, 1980b, A Quick Method for the Microanalytical Evaluation of the Disolving Power of Supercritical Gases, in "Extraction with Supercritical Gases", Ed. G.M.Schneider, E.Stahl, and G.Wilke, Weinham, Deer Beach (Florida), Basel, Verlag Chemie.

Stahl,E., and K.W.Quirin, 1983, Dense Gas Extraction on a Laboratory Scale: A Survey of Some Recent Results, Fluid Phase Equil. 10, 269-278.

Stahl,E., K.W.Quirin, A.Glatz, D.Gerard, and G.Rau, 1984, New Developments in the Field of High-Pressure Extraction of Natural Products with Dense Gases, Ber. Bunsenges. Phys. Chem. 88, 900-907.

Stephan,K., and K.H.Schaber, 1982, High Pressure Phase Equilibria for Vapor Phase Extraction Processes, Sep. Sci. Technol. 17(1), 235-260.

Streett,W.B., 1983, Phase Equilibria In Fluid and Solid Mixtures at High Pressure, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Stryjek,R. and J.H.Vera, 1986a, PRSV: An Improved Peng-Robinson Equation of State for Pure Compound and Mixtures, Can.J.Chem.Eng.,64,323-333.

Stryjek,R. and J.H.Vera, 1986b, PRSV: An Improved Peng-Robinson Equation of State with New Mixing Rules for Strongly Nonideal Mixtures, Can.J.Chem.Eng.,64,334-340.

Stryjek,R. and J.H.Vera, 1986c, PRSV: A Cubic Equation of State for Accurate Vapor-Liquid Equilibria Calculations, Can. J. Chem. Eng., 64, 820-826.

Stutzer,D., G.Brunner, and S.Peter, 1983, Separation of Finely Dispersed Solids from Low-Volatile Viscous Media by Gas Extraction, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Subramaniam,B., and M.A.McHugh, 1986, Reactions in Supercritical Fluids - A Review, Ind. Eng. Chem. Process Des. Dev. 25, 1-12.

Sun, C.K.J. and S.H. Chen, 1985a, Tracer Diffusion of Aromatic Hydrocarbons in n-Hexane up to Supercritical Region Chem. Eng. Sci. 40, 2217-2224.

Sun, C.K.J. and S.H. Chen, 1985b, Tracer Diffusion of Aromatic Hydrocarbons in Liquid Cyclohexane up to its Critical Temperature, A.I.Ch.E.J. 31, 1510-1515.

Sun, C.K.J. and S.H. Chen, 1985c, Diffusion of Benzene, Toluene, Naphthalene, and Phenanthrene in Supercritical Dense 2,3- Dimethylbutane, A.I.Ch.E.J. 31, 1904-1910.

Sun, C.K.J. and S.H. Chen, 1986, Tracer Diffusion in Dense Ethanol: A Generalized Correlation for Nonpolar and Hydrogen-Bonded Solvents, A.I.Ch.E.J. 32, 1367-1371.

Sun, C.K.J. and S.H. Chen, 1987, Tracer Diffusion in Dense Methanol and 2-Propanol up to Supercritical Region: Understanding of Solvent Molecular Association and Development of an Empirical Correlation, Ind. Eng. Chem. Res. 26, 815-819.

Suppes,G.J., and M.A.McHugh, 1989, Phase Behavior of the Carbon Dioxide-Styrene System, J. Chem. Eng. Data, 34, 310-312.

Suppes,G.J., R.N.Occhiogrosso, and M.A.McHugh, Oxidation of Cumene in Supercritical Reaction Media, Ind. Eng. Chem. Res. 28,1152-1156.

Swaid,I., and G.M.Schneider, 1979, Determination of Binary Diffusion Coefficients of Benzene and some Alkylbenzenes in Supercritical $CO_2$ between 308 and 328 K in the Pressure Range 80 to 160 bar with Supercritical Fluid Chromatography (SFC), Ber. Bunsenges. Phys. Chem. 83,969-974.

Swientek,R.J., 1987, Supercritical fluid extraction separates components in foods, Food Proc. (7), 32-36.

Tan,C-S., and J-Y.Weng, 1987, Solubility Measurements of Naphthol Isomers in Supercritical $CO_2$ by a Recycle Technique, Fluid Phase Equilibria 34, 37-47.

Tan,C-S., and D-C.Liou, 1988, Desorption of Ethyl Acetate from Activated Carbon by Supercritical Carbon Dioxide, Ind. Eng. Chem. Res. 27, 988-991.

Tan,C-S., and D-C.Liou, 1989, Modeling of Desorption at Supercritical Conditions, A.I.Ch.E.J. 35(6), 1029-1031.

Tan,C-J., and Y-C.Wu, 1988, Supercritical Fluid distribution in a packed column, Chem. Eng. Commun. 68, 119-131.

Tan,C-S., S-K.Liang, and D-C.Liou, 1988, Fluid-Solid Mass Transfer in a Supercritical Fluid Extractor, Chem. Eng. J. 38, 17-22.

Tavana,A., J.Chang, A.D.Randolph, N.Rodriguez, 1989, Scanning of Cosolvents for Supercritical Fluids Solubilization of Organics, A.I.Ch.E.J. 35(4), 645-648.

Teja,A.S., and R.L.Smith, 1983, The Correlation and Prediction of Critical States of Mixtures Using a Corresponding States Principle, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Teja,A.S., and R.L.Smith,Jr., 1987, Critical Properties of Thermally Unstable Substances from Mixture Data, A.I.Ch.E.J.,33(9),1560-1562

Tiltscher,H., H.Wolf, and J.Schelchshorn, 1984, Utilization of Supercritical Fluid Solvent-Effects in Heterogeneous Catalysis, Ber. Bungenges. Phys. Chem. 88, 897-900.

Traber,R.P., M.Kuhn, H.Hofmann, and E.Harri, 1978, Ger. Offen. 28 19 094.

Traber,R.P., H.Hofmann, E.Harri, and M.Kuhn, 1980, Ger. Offen. 29 41 080.

Traber,R.P., H.Hofmann, and E.Harri, 1982, Patentschrift (Switz.) 633 826.

Traber,R.P., H.Hofmann, and E.Harri, 1983, Patentschrift (Switz.) 637 123.

Trebble,M.A. and P.R.Bishnoi, 1986, Accuracy and Consistenc Comparisons of Ten Cubic Equations of State for Polar and Non-Polar Compounds, Fluid Phase Equilibria,29,465-474.

Trebble,M.A. and P.R.Bishnoi, 1987, Development of a New Four-Parameter Cubic Equation of State, Fluid Phase Equilibria,35,1-18.

Trebble,M.A. and P.R.Bishnoi, 1988, Thermodynamic Property Predictions with the Trebble-Bishnoi Equation of State, Fluid Phase Equilibria, 39, 11-128.

Trebble,M.A., 1989, Calculation of Constants in the Trebble-Bishnoi Equation of State with an Extended Corresponding States Approach, Fluid Phase Equilibria, 45, 165-172.

Triday,J., and J.M.Smith, 1988, Dynamic Behavior of Supercritical Extraction of Kerogen from Shale, A.I.Ch.E.J. 34(4), 658-668.

Tsonopoulos,C., 1974, An Empirical Correlation of Second Virial Coefficients, A.I.Ch.E.J.,20(2),263-272.

Tsonopoulos,C., and J.L.Heidman, 1986, High-Pressure Vapor-Liquid Equilibria with Cubic Equations of State, Fluid Phase Equilibria, 29, 391-414.

Tucker,J.C., 1986, "Batch Production of Cyclosporin A by Tolypocladium inflatum", M.E.Sc. Thesis, Univ. Western Ont., London, Canada.

Valteris,R.L., 1966, The Solubility of Materials in Compressed Hydrocarbon Gases, Birmingham Univ. Chem. Eng. 17, 38 - .

van der Haegen, R., R.Koningsveld, L.A.Kleintjens, and L.van Opstal, 1988, Solubility of Solids in Supercritical Solvents. IV. Mean-Field Lattice Gas Description for the p-T-x Space Diagram of the System Ethylene - Naphthalene, Fluid Phase Equilibria 43, 1-19.

Van Leer,R.A., and M.E.Paulaitis, 1980, Solubilities of Phenol and Chlorinated Phenols in Supercritical Carbon Dioxide, J. Chem. Eng. Data 25, 257-259.

van Wasen,U., and G.M.Schneider, 1975, Pressure and Density Dependence of Capacity Ratios in Supercritical Fluid Chromatography (SFC) with Carbon Dioxide as Mobile Phase, Chromatogr. 8(6), 274-276.

van Wasen, U., I. Swaid, and G.M. Schneider, 1980, Physicochemical Principles and Application of Supercritical Fluid Chromatography (SFC), Angew. Chem. Int. Ed. Engl. 19, 575-587.

Vetere,A., 1987, Estimation of the Critical Temperatures and Pressures of Organic Compounds by Using the Rackett Equation, Chem.Eng.J.,35,211-214.

Vidal,J., 1984, Phase Equilibria and Density Calculations for Mixtures in the Critical Range with Simple Equations of State, Ber. Bunsenges. Phys. Chem. 88, 784-791.

Wagner,Z., and I.Wichterle, 1987, High-Pressure Vapor-Liquid Equilibrium in Systems Containing Carbon Dioxide, 1-Hexene, and n-Hexane, Fluid Phase Equil. 33, 109-123.

Walsh,J.M., G.D.Ikonomou, and M.D.Donohue, 1987, Supercritical Phase Behavior: The Entrainer Effect, Fluid Phase Equilibria 33, 295-314.

Weder,J.K.P., 1984, Studies on Proteins and Amino Acids Exposed to Supercritical Carbon Dioxide Extraction Conditions, Food Chem. 15, 175-190.

Weder,J.K.P., 1984, Influence of Supercritical Carbon Dioxide on Proteins, J. Am. Oil Chem. Soc. 61, 673.

Widom,B., 1983, Three-Phase Equilibrium and the Tricritical point, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Williams,D.F., 1981, Extraction with Supercritical Gases, Chem.Eng.Sci. 36, 1769 - 1788.

Wilsak,R.A. and G.Thodos, 1985, An Equation of State: Its Development from Argon Data and Its Application to Other Substances, A.I.Ch.E.J., 31(5), 729-740.

Wilsch, A., R. Feist and G.M. Schneider, 1983, Capacity Ratios and Diffusion Coefficients of Low-Volatile Organic Compounds in Supercritical Carbon Dioxide From Supercritical Fluid Chromatography (SFC), Fluid Phase Equil. 10, 299-306.

Won,K.W., 1983a, Thermodynamic Calculation of Supercritical-Fluid Equilibria: New Mixing Rules for Equations of State, Fluid Phase Equil. 10, 191-210.

Won,K.W., 1983b, Phase Equilibria of High-Boiling Organic Solutes in Compressed Supercritical Fluids - Equations of State with New Mixing Rule, Paulitis,M.E., J.M.L.Penninger, R.D.Gray, and P.Davidson, Eds., Chemical Engineering at Supercritical Conditions, Ann Arbor, MI, Ann Arbor Science.

Wong,J.M., and K.P.Johnson, 1986, Solubilization of Biomolecules in Carbon Dioxide based Supercritical Fluids, Biotechnol. Prog. 2(1), 29-39.

Wong,J.O. and J.M.Prausnitz, 1985, Comments Concerning a Simple Equation of State of the van der Waals Form, Chem.Eng.Commun.,37,41-53.

Wormald,C.J., C.N.Colling, and G.Smith, 1983, Thermodynamics of Supercritical Steam + Carbon Dioxide Mixtures, Fluid Phase Equil. 10, 223-231.

Wormald,C.J., 1984, Heats of Mixing of Water + Hydrocarbons at High Temperatures and Pressures, Ber. Bunsenges. Phys. Chem. 88, 826-834.

Wu,A.H-Y., 1988, Phase Equilibria for Fluid Mixtures Containing Small and Large Molecules, Ph.D Dissertation, Univ. Calif. Berkley Calif., 238p.

Xu,Z. and S.I.Sandler, 1987, Application to Mixtures of the Peng-Robinson Equation of State with Fluid-Specific Parameters, Ind.Eng.Chem.Res.,26,1234-1238.

Xu,Z. and S.I.Sandler, 1987, Temperature-Dependent Parameters and the Peng- Robinson Equation of State, Ind.Eng.Chem.Res.,26,601-606.

Yamaguchi,K., M.Murakami, H.Nakano, S.Konosu, T.Kokura, H.Yamamoto, M.Kosaka, and K.Hata, 1986, Supercritical Carbon Dioxide Extraction of Oils from Antartic Krill, J. Agric. Food Chem. 34, 904-907.

Yokoyama,C., K.Arai, S.Saito, and H.Mori, 1988, Bubble-Point Pressures of the $H_2$-CO-$CO_2$ System, Fluid Phase Equilibria 39, 101-110.

Yonker,C.R., B.W.Wright, H.R.Udseth, and R.D.Smith, 1984, New Methods for Characterization of Supercritical Fluid Solutions, Ber. Bunsenges. Phys. Chem. 88, 908-911.

Yu,J-M., and B.C-Y.Lu, 1987, A Three-Parameter Cubic Equation of State For Asymmetric Mixture Density Calculations, Fluid Phase Equilibria,34,1-19.

Zerda,T.W., B.Wiegand, and J.Jonas, 1986, FTIR Measurements of Solubilities of Anthracene in Supercritical $CO_2$, J. Chem. Eng. Data 31, 274-277.

Zosel,K., 1980, Separation with Supercritical Gases: Practical Applications, in "Extraction with Supercritical Gases", Ed. G.M.Schneider, E.Stahl, and G.Wilke, Weinham, Deer Beach (Florida), Basel, Verlag Chemie.