PN-ABB-107

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON D.C. 20523

DATE: 9/15/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 7.1.3

PR #2

Attachment

CENTRO DE QUÍMICA ESTRUTURAL
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PROJECT Nº: 936-5542

PROJECT TITLE: "SUPERCRITICAL - FLUID EXTRACTION OF NATURAL AND BIOLOGICAL PRODUCTS"

GRANT Nº: DPE-5542-G-SS-7048-00

PROJECT OFFICE: S&T / SCI

OBLIGATION Nº: 736 1213

PRINCIPAL INVESTIGATOR: MANUEL NUNES DA PONTE

GRANTEE: CENTRO DE QUÍMICA ESTRUTURAL

Instituto Superior Técnico

Av. Rovisco Pais

1096 Lisboa, Portugal

Rec d [illegible] SEP 14 1988

PROGRESS REPORT No. 2

Period from Feb.20 to Aug.19 1988

During the second semester, work has been carried out following the results already obtained in the first semester and as stated in our Progress Report No. 1. A summary of the principal activities is given below.

1) Training Program and Consultation

The cooperation with the University of California at Berkeley has continued.

Dr.Susana Barreiros has just come back from Berkeley, where she spent a year on a post-doc assignement. The graduate student Margarida Goncalves has spent a two-month period also with the group of Professor Prausnitz. Both collaborators of this project have been involved in the study of enzymatically catalyzed reactions in supercritical fluids, a field of large interest to our project. Ms. Goncalves' visit to Berkeley was partially funded by this project (under the item "Training and Travel").

The collaboration with Cornell University has not involved mutual visits this year, but a quite intensive program has been prepared for the second half of the coming year. This will tentatively involve a post-doc stay in Cornell, a Summer-term stay by a graduate student, also in Cornell, and a visit to Lisbon of Prof. Zollweg one of the leaders of the Cornell group.

Prof. Daniel Jonah, of the University of Sierra Leone, will spend the coming month of October in Lisbon. This visit was programmed for the first year, but was slightly delayed.

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## 2) Separation of eucalyptus oil components

### i) Solubility Measurements

Using the apparatus descr bed in our previous report, we have been measuring vapor-liquid equilibrium (VLE) data on systems containing limonene, cineole and supercritical fluids.

As briefly mentioned in Progress Report No. 1, we started to obtain VLE data on the ternary system limonene+cineole+carbon dioxide. These measurements were made at 40 C for different total pressures,and for a mixture of overall mass ratio of (approximately) 0.80 cineole to 0.20 limonene, a composition chosen to simulate eucalyptus oil, as obtained after the first step of purification. The attached pre-print, entitled Phase Equilibria of Systems containing Limonene, Cineole and Supercritical Carbon Dioxide, summarises the results obtained for the above mentioned ternary, as well as those previously reported in our progress report no. 1 for the binaries. It will be presented in October, in Nice, France, to the International Symposium on Supercritical Fluids, and published in its proceedings.

The results obtained so far with supercritical carbon dioxide indicate that this solvent shows no selectivity in extraction of either limonene or cineole. We have therefore selected other solvents to carry out this extraction. In particular, we looked for solvents that could enrich the vapor phase in limonene, which is the impurity to be removed. As limonene is a hydrocarbon, while cineole is an ether, ethane and

ethylene (ethene) seemed to be a good choice. Moreover, each of these gases forms azeotropic mixtures with carbon dioxide, which we thought could be tried as solvents, conserving the advantages of the use of carbon dioxide as a supercritical solvent.

Figure 1 shows VLE data obtained at 30 C for limonene and for cineole in an azeotropic mixture of ethane with carbon dioxide (which has a composition of about 21 mol % in ethane). As it can be seen, the behavior of both components in the supercritical mixture is similar. We anticipated that this behavior meant, following our experience with carbon dioxide, that the selectivity was not improved with the addition of ethane.

A decision was taken to use one of the pure hydrocarbons as solvent. The results at 25 C for limonene+ethylene are shown in Figure 2. This figure suggests that the observed behavior is quite different from our previous results. The pressure-composition diagram of the limonene+ethylene system shown in Figure 2 seems to indicate that at (about) 68 bar there are three phases in equilibrium: a vapor phase, a limonene-rich liquid phase, and an ethylene-rich liquid phase. Confirmation of this kind of liquid-liquid equilibrium would probably lead to a much improved selectivity in the separation of cineole and limonene. Further studies are now in progress in order to understand the phase diagram of this system.

ii) Countercurrent Extraction Apparatus

We have started the assembling of this apparatus. Tests have

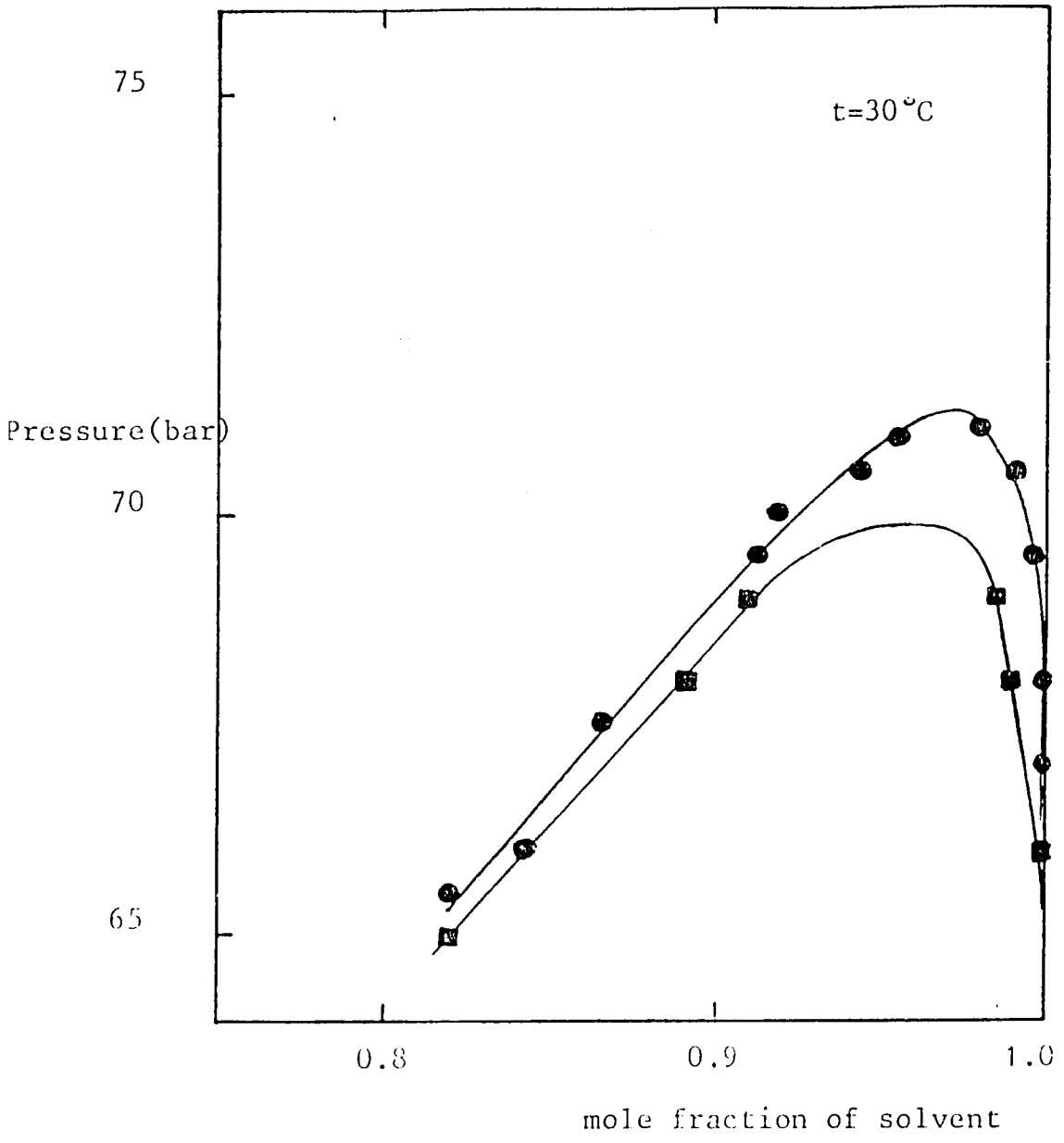


Figure 1. Vapor-liquid equilibria of limonene ( ● ) and of cineol in an azeotropic mixture ethane+carbon dioxide at 30°C

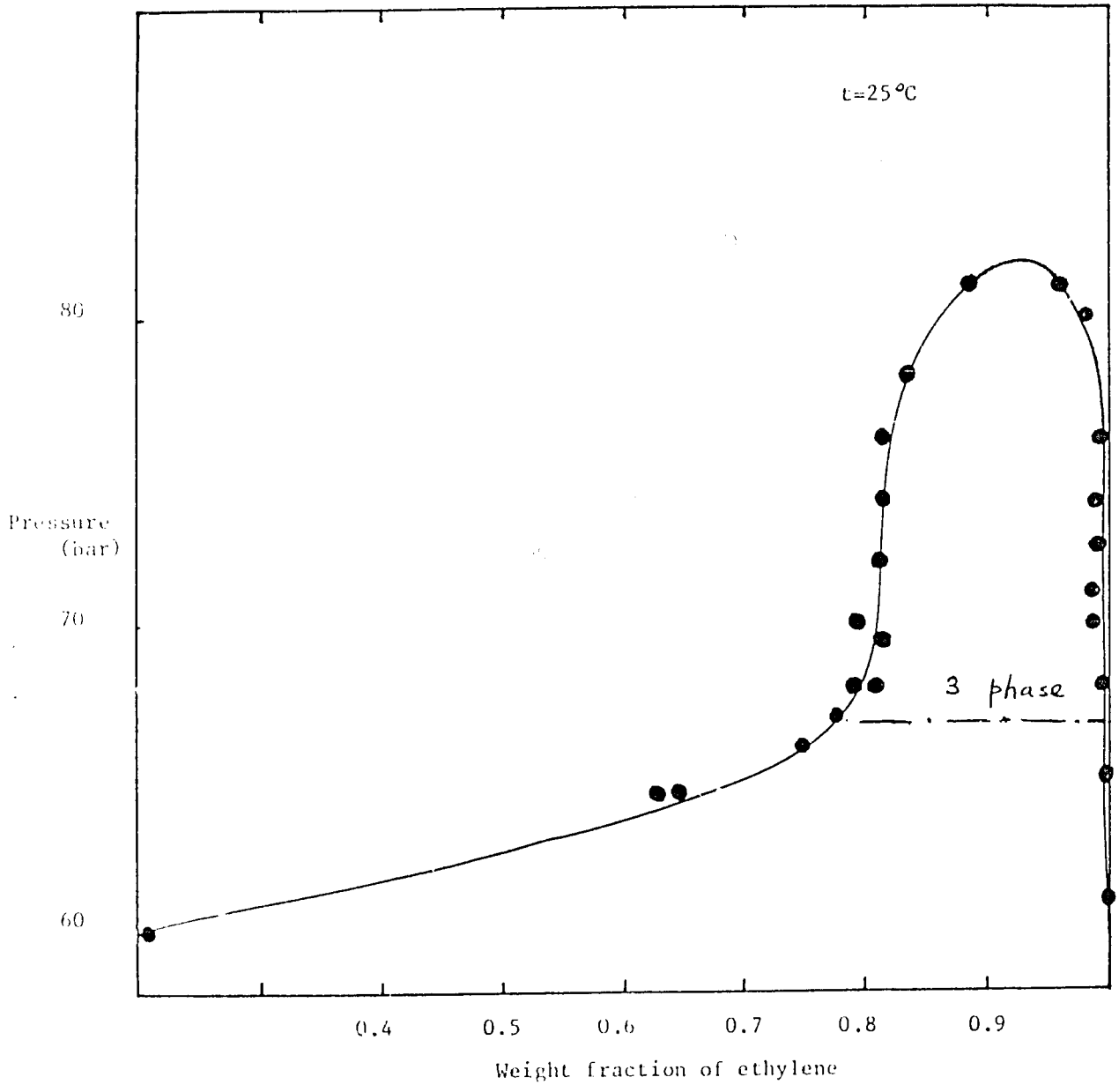


Figure 2. Vapor-liquid equilibria of limonene in supercritical ethylene at 25 °C.

been carried out to verify the specifications of the compressor and of the high-pressure liquid pump, two of the main components of this apparatus.

There have been difficulties related with the acquisition of some items of equipment, and in particular pressure gages. After a pre-selection of American makers of this kind of equipment, attempts to obtain technical information and proforma invoices have failed. Existing equipment belonging to Centro de Quimica Estrutural will be used until these difficulties can be overcome.

iii) Thermodynamic modelling of vapor-liquid equilibrium

Computer programs have been written to use the well-known Peng-Robinson equation in the correlation of the vapor-liquid equilibrium data described under i). In the above mentioned pre-print, a full description of the results so far obtained is given.

3) Conclusion

A comparison between the time schedule included in our proposal and the actual development of the research work performed during the first year of this project shows that:

a) in Step 1, the development of a solubility apparatus and the measurement of solubilities are slightly ahead of the submitted timetable;

b) in Step 2, the implementation of calculation programs was started as planned;

c) in Step 3, the design and construction of extraction

columns has been completed and the assembly of apparatus is ahead of the proposed schedule, while some preliminary tests have already been performed.

As setbacks to the planned sequence of the research work, we had:

a) a recent publication of research work performed at MIT, that led us to change from propionic acid to lactic acid as model substance for extractions from fermentation broths;

b) our findings about lack of selectivity of pure carbon dioxide as an extraction solvent in the purification of eucalyptus oil;

c) difficulties of communication with American scientific equipment makers, that generate delays in acquisitions.

The steps taken to overcome these problems are either described above or in our previous report.

FINANCIAL REPORT No. 2/88

Period from 02/20/88 to 08/19/88

The total expenditure during the second semester of this project (corresponding to our Vouchers No.2 and No.3) is summarized below.

| | |
|---|---|
| i) Item: 'Equipment' | |
| High-pressure fittings | US $ 94 |
| Metering pump (freight and handling charges) | $ 395 |
| Microcomputer | $ 4,344 |
| High-pressure tubing, valves, crosses, etc. | $ 2,403.5 |
| Mass flowmeter | $ 3,011 |
| Shelter for compressor | $ 924 |
| ii) Item: 'Materials and Reagents' | $ 27 |
| iii) Item: 'Training and Travel' | $ 2,009 |
| iv) Item: 'Overheads' | $ 2,641.5 |
| TOTAL | $15,849.0 |

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The following table compares the actual expenses occured during the first year of this project (August 20, 1987 to August 19, 1988) with the budget as stated in our proposal.

Table 1: Budgeted and actual expenses for the first year of this project.

| Item | Budget (1st. year) (US $) | Actual Expenses (1st. year) (US $) |
|---|---|---|
| Equipment | 31,000 | 29,999.5 |
| Materials and Reagents | 3,000 | 2,708 |
| Training and Travel | 4,000 | 2,009 |
| Consultation | 2,000 | -- |
| Overheads | 8,000 | 6,943.5 |
| TOTAL | $ 48,000 | $ 41,660.0 |

As this table shows, the actual expenses have followed closely the expenses predicted in our initial budget. The larger differences are found in the items 'Travel' and 'Consultation'. In this last item, a visit to Lisbon is planned for the coming month of October, that will spend the budgeted amount, while the Training and Travel differences will be spent during the second semester of the second year of the project, in the development of the already existing close links with the

University of Cornell.

# PHASE EQUILIBRIA OF SYSTEMS CONTAINING LIMONENE, CINEOLE, AND SUPERCRITICAL CARBON DIOXIDE

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## SUMMARY

Using a constant-volume, static cell we measured the vapour-liquid equilibrium at 313 K for the mixtures carbon dioxide + limonene and carbon dioxide + cineole in the vicinity of the mixture critical point (pressures up to 8.2 MPa). Data were also obtained for the ternary $CO_2$+ limonene + cineole at the same temperature and for a fixed overall mass ratio of approximately 0.80 cineole to 0.20 limonene.

The solubility behaviour of limonene and cineole in supercritical carbon dioxide was found to be quite similar. The results obtained with the ternary mixture show no selectivity in extraction of either component from a liquid sample with carbon dioxide.

Our data were well correlated with the Peng-Robinson equation of state using one interaction parameter obtained from regression of the binary vapour-liquid equilibrium data.


## 1. INTRODUCTION

The well-known characteristics of supercritical fluids, namely their suitability to the extraction of heat-labile biochemical and natural products, no residues being left in either extract or raffinate, make them adequate to the extraction of essential oil components.

Essential oils are largely used in food, pharmaceutical, and cosmetic industries. Numerous processes for supercritical extraction of oils have been presented. In particular, the high solubility of terpenes in supercritical carbon dioxide has been reported by a number of authors.

Stahl and collaborators [1,2] measured solubilities as a function of pressure in a flow apparatus for a diversity of essential oil components. Coppelia and Barton [3] and Kalra et al. [4] measured the vapour-liquid equilibrium of lemon oil, a multicomponent mixture that includes several terpenes. However no vapour-liquid equilibrium data are available for mixtures of carbon dioxide with pure

terpenes.

The main purpose of this work is to examine the possibility of purifying eucaliptus oil by supercritical fluid extraction. The main component of this oil is 1-8 cineole ($C_{10}H_{18}O$). In the final stages of purification it must be separated from d-limonene ($C_{10}H_{16}$), a process usually carried out by fractional crystallization. Relevant thermodynamic data are needed to evaluate the feasibility of replacing this extraction process by supercritical fluid extraction.

We report here measurements of vapour-liquid equilibrium at 313 K for the binaries $CO_2$ + limonene and $CO_2$ + cineole in the vicinity of the mixture critical point. Data are also presented at the same temperature for the ternary $CO_2$ + limonene + cineole with a fixed overall mass ratio of approximately 0.80 cineole to 0.20 limonene.

## 1 EXPERIMENTAL

Measurements were performed in the apparatus schematically shown in Figure 1.

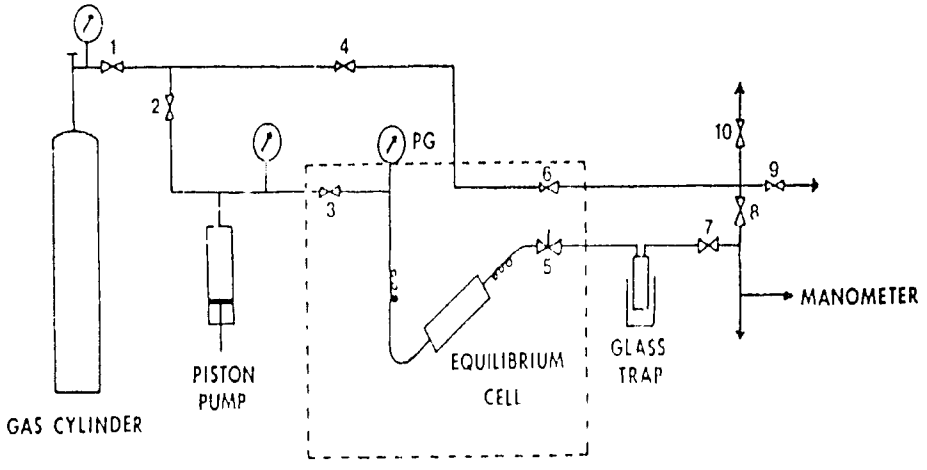


Figure 1. Schematic diagram of the experimental apparatus.

The main piece of equipment is a static vapour-liquid equilibrium cell of approximately 30 $cm^3$. It is connected to the rest of the apparatus through flexible tubing that allows considerable freedom of movement of the cell for a convenient mixing of its contents.

Samples can be withdrawn from the phases on top and bottom of the cell through a capillary line. These samples are collected by depressurization and expansion through the micrometering valve 5 into a small glass trap connected to

large steel bottles of calibrated volume. When valve 5 is open, the liquid solute condenses in the cold glass trap while the carbon dioxide expands into the steel bottles. Pressure in these bottles is measured with a mercury manometer and is used to calculate the amount of solvent gas, while the amount of the liquid solute is obtained by weighing of the glass trap. In the case of measurements with ternary mixtures, the ratio cineole/limonene is subsequently obtained by gas chromatography.

After filling at atmospheric pressure with a small amount (about 6 $cm^3$) of liquid (limonene, cineole, or limonene + cineole), the cell is closed and connected to the apparatus in a thermostated water bath.

Carbon dioxide is pressurized by a piston hand pump into the cell through valves 2 and 3, and equilibrium between liquid and vapour is attained after about 40 minutes of vigorous stirring of the whole cell. After about the same time of rest (no stirring), the pressure is read on a Bourdon tube manometer of 50 kPa precision, and the temperature is measured with a calibrated mercury thermometer of 0.1 K precision.

A sample from the top of the cell (gas phase) is then withdrawn and the amount of substances obtained using the procedure described above. Since these amounts were always very small, they were corrected for the vapor pressures of the solutes. The cell is inverted stirring of the system is resumed to restore equilibrium, and then allowed to settle again for 40 minutes and a new sample is taken from the bottom of the cell (liquid phase).

The carbon dioxide used in this work was 99.998 mole % pure supplied by Air Liquide, d-limonene was 99 mole % pure supplied by Sarsyntex, and 1-8 cineole was also 99 % pure supplied by EGA-Chemie.

## III. RESULTS AND DISCUSSION

Experimental vapour-liquid equilibrium results at 313 K are shown in Figure 2 for $CO_2$ + limonene and in Figure 3 for $CO_2$ + cineole.

As shown in Figures 2 and 3, the vapour pressures read for the liquid samples are slightly lower than for the gas samples. This pressure drop is the result of the adopted sequence of experiments, as described in the preceding section.

Above the highest measured pressure indicated for each system, samples taken from top and bottom of the cell had the same composition (within experimental error), an indication of the existence of one (supercritical) phase only inside the cell.

The results show that the solubility behaviour of limonene and cineole in dense carbon dioxide is quite similar. This is confirmed by the results obtained for the ternary system which indicate that dense $CO_2$ dissolves equally well both components. Actually, the ratio cineole/limonene in the liquid and vapour phases in equilibrium is the same within experimental error, indicating no selectivity for either of them in an extraction process.

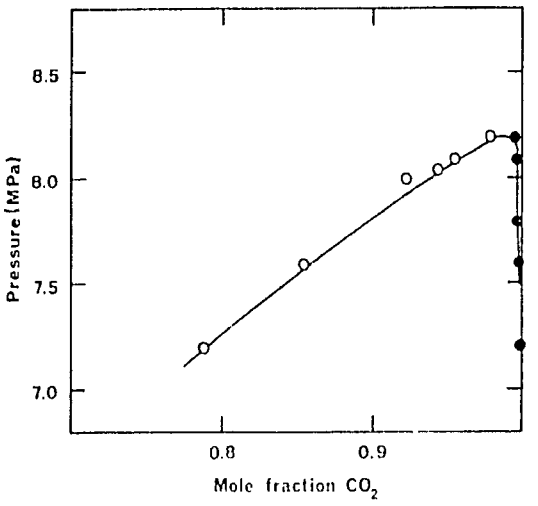


Figure 2. Experimental and calculated vapour-liquid equilibria for $CO_2$+limonene at 313 K:

o liquid ; ● vapour

—— calculated from Peng-Robinson equation with $k_{12}$=0.080.

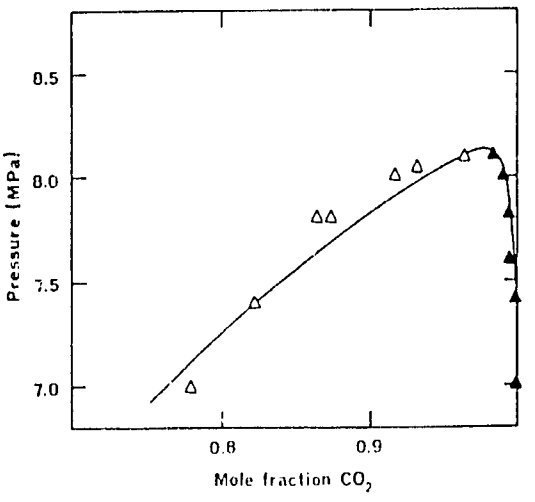


Figure 3. Experimental and calculated vapour-liquid equilibria for $CO_2$+cineole at 313 K.

Δ liquid ; ▲ vapour.

—— calculated from Peng-Robinson equation with $k_{12}$=0.075.

Figure 4 compares solubility data (expressed in mg of limonene per NL of $CO_2$, 1 NL corresponding to 1 $dm^3$ of $CO_2$ at 100 kPa and 293.15 K ) for limonene in supercritical carbon dioxide obtained by several authors. As shown, our results for the liquid and the vapour phases are in good agreement with the equilibrium data obtained by Coppella and Barton for lemon oil, a mixture of terpenes with approximately 70 mole % of limonene. Agreement is also found between the data of Stahl and Gerard and our results for the vapour phase. However, since the latter authors have determined the solubilities by means of the dynamic flow principle, they could not detect the mixture critical point (about 8.3 MPa for $CO_2$ + limonene at 313 K ). It makes no sense to define solubilities above it as at these conditions $CO_2$ and limonene have one (supercritical) phase only. Their curve above 8.3 MPa

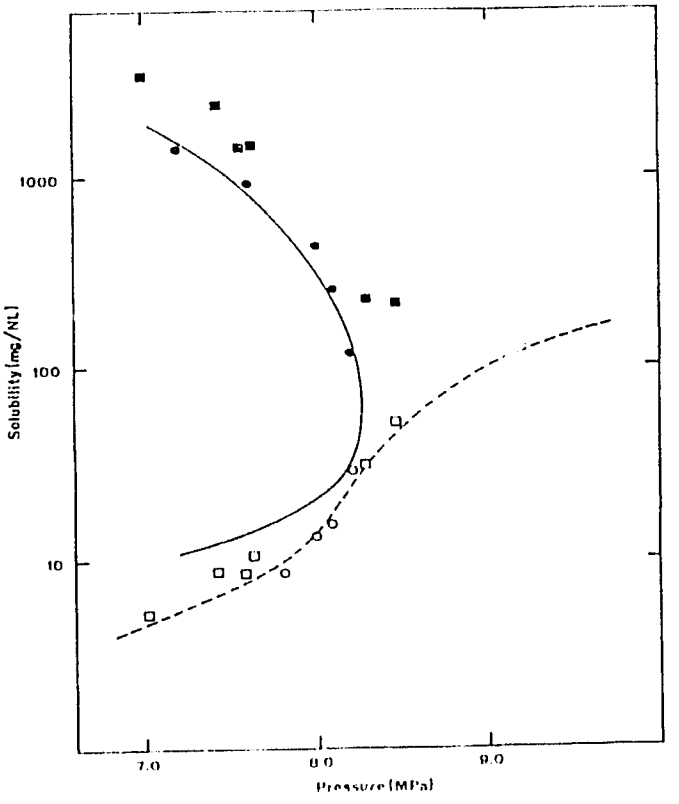


Figure 4. Solubility of limonene in dense carbon dioxide at 313 K.
Experimental:
Coppella and Barton: ■ liquid; □ vapour.
This work: ● liquid; ○ vapour.
Stahl and Gerard: - - -
Calculated: —— Peng-Robinson equation with $k_{12}$=0.080.

is therefore meaningless, as can be seen by comparison with our results and Coppella and Barton's for the liquid phase.

For a quantitative description of the phase equilibrium of the systems studied we used the Peng-Robinson equation of state.

For the pure components, equation of state parameter **b** was obtained from critical data. Parameter **a** was calculated at a given temperature from critical data and acentric factor.

For limonene and cineole $T_c$ and $P_c$ were estimated by the methods of Nokay and Lydersen [5,6], respectively. The acentric factor was estimated by the method of Pitzer et al. [6]. Table 1 shows the pure component constants used in this work.

| | Molecular Weight | Critical Pressure (MPa) | Critical Temperature (K) | Acentric Factor |
|---|---|---|---|---|
| $CO_2$ | 44.01 | 7.38 | 304.2 | 0.225 |
| Limonene | 136.24 | 2.75 | 654.4 | 0.327 |
| Cineole | 154.25 | 2.77 | 665 | 0.312 |

Table 1. Pure component constants.

For mixtures, **a** and **b** are composition dependent and obtained from the usual quadratic mixing rules. The binary constant $a_{12}$ is given by $a_{12}=(1-k_{12})(a_{11}a_{22})^{1/2}$, being $k_{12}$ an adjustable parameter which characterize the interactions of unlike molecules. This parameter was obtained from regression of the binary phase equilibrium data.

As shown in Figures 2, 3, and 4 the Peng-Robinson equation of state can reproduce quantitatively the observed phase equilibrium behaviour with a binary interaction parameter determined from the binary data.

It was also found that the same thermodynamic model can reproduce semi-quantitatively the ternary data, using the interaction parameters obtained from the binary data (and assuming $k_{23}=0$ for the binary limonene+cineole). As an example, Table 2 shows a comparison between experimental ternary data at 313 K and the predictions of the Peng-Robinson equation of state using $k_{12}=0.080$, $k_{13}=0.075$ and $k_{23}=0$.

| P (MPa) | x($CO_2$) exp. | x($CO_2$) calc. | x(limonene) exp. | x(limonene) calc. | x(cineole) exp. | x(cineole) calc. |
|---|---|---|---|---|---|---|
| 7.4 | 0.888 | 0.819 | 0.025 | 0.081 | 0.087 | 0.100 |
| 7.6 | 0.859 | 0.856 | 0.030 | 0.024 | 0.111 | 0.120 |
| 7.75 | 0.867 | 0.888 | 0.031 | 0.012 | 0.102 | 0.100 |
| 8.0 | 0.914 | 0.942 | 0.023 | 0.008 | 0.063 | 0.050 |
| 8.2 | 0.950 | 0.972 | 0.012 | 0.008 | 0.038 | 0.020 |

Table 2. Experimental and calculated liquid compositions for the ternary $CO_2$ + limonene + cineole at 313 K.

## IV. ACKNOWLEDGEMENTS

The authors are grateful to Professor M. Paulaitis and Dr.C.-P. Kao (University of Delaware, Newark, U.S.A.) for helpful discussions and to JNICT, Franco-Farmaceutica, Favorita (Lisboa, Portugal) and A.I.D. (Washington D.C., U.S.A.) for financial support.

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