Solubilities of Anti-Cancer and Anti-AIDS Drugs in Supercritical Carbon Dioxide

By

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Abstract

Solubility data for pharmacological drugs in supercritical fluids (SCFs) is of great importance, because there is an environmental and cost effective need for alternative specialty separation methods.

This research was focused on the study and determination of solubilities of some anti-cancer (*e.g.,* Taxol, 5-Fluorouracil) and anti-aids drugs (*e.g.,* Azodicarbonamide, Thymidine and 2-Phenyl-4H-3,1-benzoxazin-4-one) in supercritical carbon dioxide. These measurements were made using a Supercritical Fluid Chromatograph (SFC) coupled to a high pressure UV detector online. The solubility of these drugs were studied as a function of temperature (35.1°C – 55.1°C) and pressure (100 – 300 bar).

This technique was initially validated using phenanthrene and compared with the data of several other investigators. The technique proved to be fast, reliable and reproducible. The order of magnitude of the obtained solubilities was 10^{-6} to 10^{-4} mole fraction. The drug with the highest solubility was 2-Phenyl-4H-3,1-benzoxazin-4-one and the less soluble was taxol. These results correlated well with the volatility of the drugs (indicated by their melting point).

This research also studied the effect of pressure (100 – 300 bar) and temperature (35.1°C – 55.1°C) on the solubility of the drugs. The effect of pressure on the solubility of the drugs followed the expected trend of increasing solubility with an isothermal increase in the pressure for all temperatures studied. This is explained since as pressure is increased, carbon dioxide density increases, and the intermolecular mean distance of carbon dioxide molecules decreases; thereby, increasing the specific interaction between the solute and solvent molecules. The temperature effect always showed a proportional effect in solubility. This indicated that the temperature effect in solute volatility (proportional effect) was more significant than the temperature effect in solvent density (inversely proportional effect).

This study showed that it is possible to determine relatively fast a large number of solubility measurements for the studied systems by retention in SFC.

Resumen

Los datos de solubilidad para drogas farmacológicas en fluidos supercríticos son de suma importancia debido a que es necesario buscar alternativas en métodos de separación que sean ambientalmente y económicamente efectivos.

Esta investigación fue dirigida al estudio y determinación de solubilidades para algunas drogas anti-cáncer (*e.j.,* taxol, 5-fluorouracil) y anti-sida (*e.j.,* azodicarbonamide, thymidine and 2-phenyl-4H-3,1-benzoxazin-4-one) en bióxido de carbono supercrítico. Las medidas fueron realizadas en un cromatógrafo de fluidos supercríticos el cual tenía acoplado en línea un detector UV de alta presión. Las solubilidades de las drogas fueron estudiadas como función de la temperatura (35.1°C - 55.1°C) y presión (100 – 300 bar).

La técnica fue validada usando medidas de solubilidad de fenantreno y comparadas con datos de solubilidad obtenidos de otros investigadores. La técnica fue rápida, confiable y reproducible. Los datos de solubilidad obtenidos para las drogas investigadas fueron del orden de magnitud de 10^{-6} a 10^{-4} en fracciones molares. De las drogas estudiadas, la que presentó mayor solubilidad fue 2-Phenyl-4H-3,1-benzoxazin-4-one y la de menor fue taxol. Esta tendencia se correlaciona bien con la volatilidad de las drogas (indicada por su punto de fusión).

El efecto de la presión sobre la solubilidad siguió la tendencia esperada de un incremento de la solubilidad con el incremento isotermal de la presión para las temperaturas estudiadas. Esta tendencia se explica debido a que cuando la presión aumenta, la densidad del bióxido de carbono aumenta y por tanto la distancia intermolecular promedio de las moléculas de bióxido de carbono disminuye, y por consiguiente las interacciones entre las moléculas de soluto y solvente aumentan.

El efecto de la temperatura siempre mostró una tendencia proporcional a la solubilidad. Esto refleja que el efecto de la temperatura es más significativo en la volatilidad del soluto (efecto proporcional) que en la densidad del solvente (efecto inversamente proporcional).

Acknowledgments

I wish to express my most sincere acknowledgment to Dr. David Suleiman, first for being my friend and second my advisor; for his friendship, his excellent advices and support in the course of my graduate studies.

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List of Symbols and Abbreviations

$C_i(T)$	Constant for a particular solute, column and temperature
dv	Volume of CO_2 used in the extraction
dw	Difference of weight in the cell
H_i^o	Henry's constant at the reference pressure P^o
k_i	Capacity factor for solute i
n_i^m	Moles of solute i in the mobile phase.
n_i^s	Moles of solute i in the stationary phase.
P^{sat}_i	Saturation pressure of the solute i
P_o	Reference pressure
P_p	Pump's pressure
R	Constant of the ideal gases
SFC	Supercritical Fluid Chromatography
SCF	Supercritical Fluid
SCFs	Supercritical Fluids
T	Temperature in Kelvin degrees
T_{a1}	Room initial temperature
T_{a2}	Room final temperature
$T_{cham.}$	Temperature of the chamber in the ISCO extractor
T_{p1}	Initial temperature of the pump
T_{p2}	Final temperature of the pump
T_{av}	Average temperature of the pump
t_i	Retention time of a solute i
t_o	Retention time of the solvent
v_1	Initial volume in the pump
v_2	Final volume in the pump
V^m	Total volume of mobile phase
V^s	Total volume of stationary phase
w_1	Initial weight of the cell
w_2	Final weight of the cell

x_i^s	Mole fraction of the solute i in the stationary phase
y_i^m	Mole fraction of the solute i in the mobile phase (solubility)
y_i	Solubility of the solute i

Greek Letters

ϕ_i	Fugacity coefficient of solute i
ν_i	Molar volume of the solute i
ν_i^*	Partial molar volume of solute i in the stationary phase
ν^m	Molar volume of the mobile phase in the column
ν^s	Molar volume of the stationary phase in the column
ρ	Carbon dioxide density (mol/L)

Subscript

i	Wherever solute
o	Standard conditions

CHAPTER I : INTRODUCTION

Many industrial processes involve separation steps; some of them are very difficult due to current stringent purity requirements. Pharmaceutical companies are searching for new alternatives to their existing processes for the synthesis and separation of their drugs and procedures to be effective, environmentally safe and with lower cost. Our focus in this investigation is to study the possibility of separation of anti-Cancer and anti-AIDS drugs using supercritical carbon dioxide. The conventional production of these drugs comprises a series of multiple separation and purification processes, with a series of organic solvent extraction and precipitation processes. The solute-solvent separation stage is very important due to the large volume of organic solvent to be eliminated, and difficulties in the solvent separation and recovery stage. These stages are not only energy intensive but often technically challenging and with numerous environmental and health related issues. The interest in using a supercritical fluid (SCF) is due to the possibility of developing a process for the extraction, separation and purification that simplifies the number of stages of the actual production processes, which minimize the cost of industrial production and reduces the risk of environmental impact by eliminating the use of the organic solvents, and assuring the quality of the extracted drug.

The present research has been entitled “Solubilities of anti-Cancer and anti-AIDS Drugs in Supercritical carbon dioxide”. This research is focused to the study of the solubility of some anti-Cancer and anti-AIDS drugs in supercritical carbon dioxide. Supercritical Fluid Chromatography (SFC) is used to measure the capacity factors for several drugs and from these to obtain the solubilities. These measurements were made coupling the SFC with a high pressure ultraviolet (UV) detector online. The solubility, and hence, the specific intermolecular interactions were studied as a function of temperature and pressure. The retention times of the solute of interest and of the solvent were recorded. These times were used to obtain the capacity factors,

which in turn were used to estimate the solubilities. Experimental measurements of the drugs' solubilities were taken between 100 and 300 bars and from 35.1°C to 55.1°C. The study evaluated the effect of pressure and temperature in the phase equilibria of these drugs in supercritical carbon dioxide. The determination of solubilities will be a critical step to evaluate the feasibility of the supercritical extraction as an alternative process to the current separation methods (*e.g.,* extraction, drying, crystallization, etc.)

A second technique for measuring the solubility was the conventional flow method that measured solubilities gravimetrically. This technique was utilized as an independent method for the measurements of the solubilities of the studied drugs. This second method was used to determine the value of the calibration parameter needed in the SFC method.

This investigation provided a database of the solubility of Anti-cancer and Anti-AIDS drugs in supercritical carbon dioxide. The knowledge of the phase equilibria of these drugs could not only be vital for the design of alternative separation methods for these drugs using supercritical carbon dioxide, but could provide further insight about intermolecular interactions in supercritical fluids.

I.1. Objectives

The objectives of this research are:

- To validate the reliability of the Supercritical Fluid Chromatography (SFC) with a well studied substance, Phenanthrene.
- To measure the solubilities of various anti-Cancer (*e.g.,* Taxol) and anti-AIDS (*e.g.,* Sustiva) drugs in supercritical carbon dioxide.
- To study how various operating variables (*i.e.,* temperature and pressure) influence the solvating capacity of the supercritical carbon dioxide with these drugs.
- To evaluate and elucidate with statistical techniques the effect of these variables and their interactions on the solubility.

CHAPTER II : LITERATURE REVIEW

II.1. Previous Work

Conventional methods for measuring solid solubilities in supercritical fluids have various experimental limitations. The transpiration technique (Eckert and Jhonston, 1981) is the most common classical method. In this technique a stream of the supercritical fluid is pumped over a bed of pure solid, then depressurized and the solute collected and weighed. Since the solute is heavier, the time required is large, and the amounts weighed are very small. Therefore the conventional experiments for measuring solubilities are very time consuming and require large amounts of pure solute, which are often unavailable, or can be very expensive.

One alternative to the conventional method is the chromatographic technique used in this research, which has been used extensively by several investigators to determine solubilities (Smith *et al.,* 1987; Yonker *et al.,* 1987; Barker *et al.,* 1988; Bartle *et al.,* 1990) with measurements of capacity factors. This method has many advantages over the more conventional methods: rapidity, small sample size, low purity requirements (the impurities are separated in the chromatographic column). However this method is limited by the need of one actual solubility datum with a conventional method at each temperature (calibration parameter).

In the last years, a large amount of studies have been conducted to evaluate solubilities of some drugs such as: antibiotic, anti-cancer (Vandana and Teja, 1997), anti-hypertensive, (Knez *et al.,* 1995) anti-inflammatory (García *et al.,* 1998) and anti-depressive (Jara *et al.,* 1999) in supercritical carbon dioxide and supercritical water. Some recent papers have shown the utilization of the SCFs in the preparation of micro-particles and nano-particles of different materials for controlled drug release or

drug delivery application (*e.g.,* polymers and proteins) (Charoenchaitrakool *et al*., 2000).

Bartle *et al*., (1990), and Suleiman *et al*., (1992, 1994, 1995a, 1995b), suggested the technique of SFC over conditional methods, because of its ability to adjust the process variables and produce fast and accurate solubility measurements. This method determines the capacity factors and with these, some phase equilibrium properties, such as solubilities, partial molar volumes and enthalpies can be calculated. Suleiman *et al.,* (1994, 1995a, 1995b) determined solubilities of heavy paraffins, n-alkanes, cyclic and tricyclic alkanes in methane, ethane and carbon dioxide. Regular solution theory was used to characterize the stationary phase to permit the conversion of the capacity factors to estimate the solubility. The solubility results obtained in this research were in good agreement with those available from more conventional techniques. However, this method extended the database of phase equilibria in various SCFs. Marks *et al.,* (1996), extended the technique in carbon dioxide for aliphatic substances allowing for a better description of entropic contributions in SCFs.

Ting S. *et al.,* (1993) determined the solubility of naproxen (a non-steroidal anti-inflammatory drug) in pure supercritical CO_2 and the influence of six polar cosolvents (acetone, methanol, ethanol, ethyl acetate, 1-propanol and 2-propanol) at various concentrations (from 1.75 to 5.25 mol%), on the solubility of this drug. The solubility of naproxen increased with the use of these cosolvents at 60°C. They used the Peng-Robinson and Soave-Redlich-Kwong equations of state to correlate these ternary systems. They regressed negative binary interaction parameters, which according to reported by them, indicated strong interactions between naproxen and the cosolvents.

Knez Z. *et al.,* (1995) studied the solubility of nifedipine and nitrendipine (anti-hypertensive drugs) in supercritical carbon dioxide, using a static-analytical method. The study was carried out in the pressure range from 100 to 300 bar and temperatures

of 60, 80 and 100°C. The solubility, in mole fraction, at 300 bars and 100°C, of nifedipine was 7.1 x 10^{-5} and for nitrendipine was 10.6 x 10^{-5}. The difference in the solubilities of both compounds was small due to the similar chemical structure and physical properties.

Macnaughton S. *et al.,* (1996) investigated the solubilities of three inhibitors of inflammatory activity: ketoprofen, piroxicam and nimesulide, in supercritical carbon dioxide. This research was performed using a dynamic saturation technique over the pressure range 100 to 220 bar and temperatures of 40°C and 60°C. These anti-inflammatory drugs showed solubilities ranging from $4x10^{-6}$ to $1.5x10^{-3}$ mole fraction. These solubilites exhibited a clear dependence with the solvent density. The reliability and efficiency of this technique was previously established by measuring the solubility of salicylic acid in supercritical CO_2 and comparing it with the literature data: there was an excellent agreement.

Mojica *et al.,* (1997) used retention measurements in SFC to obtain the solubility data for two different systems: naphtalene and phenanthrene in supercritical CO_2. In this investigation the reliability and validity of the technique was confirmed at various temperatures (35.1°C – 55.1°C), and pressures (120 - 300 bar). This research used this system to measure the solubility of the ibuprofen in supercritical carbon dioxide, but the technique failed, due to extremely high retention times for chromatographic column/restriction system used. The flow restriction system was not fully studied at this time.

Vandana V. *et al.,* (1997) also worked with Taxol, but using both carbon dioxide and nitrous oxide as supercritical solvents. Taxol was found to be more soluble in nitrous oxide than in carbon dioxide and the solubilities in both cases were very low and at a range of 1.1 x 10^{-6} – 7.4 x 10^{-6} mole fraction.

García J. *et al.,* (1998) used the technique of SFC to measure the solubility of some anti-inflammatory drugs such as: acetaminophen, naproxen and ibuprofen at temperatures between 40°C and 50°C and pressures between 120 and 300 bars. This research used a different column (C_8 instead of C_{18}) to reduce the long retention times obtained by Mojica *et al*. The dimensions of the column were also varied. A shorter column was used (50 mm instead of 100 mm in length and 2 mm instead of 4 mm in diameter). With this new column, the anti-inflammatory drugs were studied with reasonable retention times (less than 15 minutes) and with excellent reproducibility. The study with the new column was first validated with phenanthrene at 50°C and the results were compared to those measured by Bartle (1990), Suleiman (1992) and Mojica *et al*. (1997).

Nalesnik C. *et al.,* (1998) researched the solubility of the Taxol (Paclitaxel, an anti-cancer drug) in supercritical carbon dioxide using a high-pressure ultraviolet-visible-light-transmission static cell at pressures from 200 to 480 bar and temperatures between 35°C and 45°C. The solubility of this drug increased from 0.7×10^{-7} to 5.0×10^{-7} mole fraction at the pressure range mentioned. The researchers modeled the experimental data using the Peng-Robinson equation of state and the Chrastil's method. The method of the equation of state did not provide an adequate fit for the data, but the empirical method (Chrastil's) provided a good fit with low average absolute relative deviations.

Jara-Morante, E. (1999) studied the solubility of imipramine HCl, one of the first Tricyclic anti-Depressants synthesized and utilized in the treatment of depression, in supercritical carbon dioxide. She utilized the conventional solubility measurement method along with a recovery technique in a solvent (Methanol). The extract was quantified by a spectrophotometer. She worked at a range of pressures of 300 to 500 bar and using two temperatures, 40 and 50°C; obtaining a solubility isotherm for each temperature. She observed that under these conditions the drug did not decompose and that it was only soluble in carbon dioxide in the ppm range.

Gordillo M. D. *et al.,* (1999) measured the solubility of the antibiotic penicillin G in supercritical carbon dioxide at pressures from 100 to 350 bars and temperatures from 40 to 60°C using a dynamic flow apparatus. The solubility for this drug was 1.1×10^{-6} mole fraction and from the obtained results, it was concluded that Penicillin G solubility increased with pressure. The experimental data were correlated using the Redlich-Kwong and Soave-Redlich-Kwong equations of state, with Lorent-Berthelot mixing rules. These equations provided a good prediction for the solid-fluid equilibrium of the penicillin and supercritical carbon dioxide system.

Stassi A. *et al.,* (2000) carried out an assessment of solubility of ketoprofen and vanillic acid in supercritical CO_2 using a plant operated under dynamic conditions. The reliability of the equipment was preliminarily checked by comparing solubility data obtained for salicylic acid and naphthalene with literature ones. Solubility measurements for ketoprofen and vanillic acid were carried out at 40°C and 55°C in the 90-250 bar pressure range. A crossover region (150-160 bar) was found for ketoprofen and vanillic acid, respectively. The fitting of the experimental data for this research was found to be largely sensitive to the value of the sublimation pressure of the solute.

Teutenberg T. *et al.,* (2001) developed a method for the separation of the anti-cancer drugs: 5-fluorouracil, methotrexate, 7-hydroxymethotrexate, using superheated water as the mobile phase. The retention factors were satisfactory for all of the compounds that were investigated. This investigation was evaluated within the temperature range from ambient temperature to 160°C. At these conditions no degradation of the stationary phase of the PS-DVB (polystyrene-divinylbenzene) column was observed. The separation of these substances was optimized by adjusting the pH from 11.5 to 3.5.

As it can be seen from the previous pages, numerous investigators have studied the solubility of various pharmacological drugs in supercritical fluids. Only one anti-

cancer drug (Taxol) and no anti-aids drugs have been studied in supercritical carbon dioxide. This investigation will not only provide data for a few anti-cancer and anti-aids drugs but will also complement a very interesting database to understand intermolecular interactions in supercritical carbon dioxide.

II.2. Drugs Used in this Research

1. **Taxol:** Medicament used for treatment of ovary cancer and lung cancer.

- Generic name: Paclitaxel
- Molecular formula: $C_{47}H_{51}NO_{14}$
- Molecular weight : 853.9 g/gmol
- Freezing point: 216-217°C
- Physical state: solid
- Chemical structure

$R = CH_3$

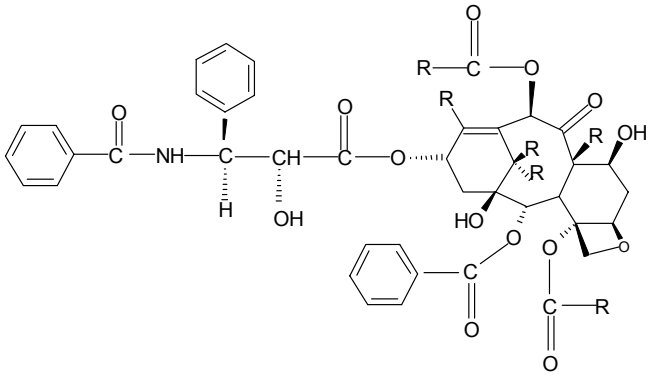


Figure 1. Chemical structure of taxol.

2. **5-Fluorouracil:** Medicament used for the treatment of rectal and colon cancer, breast cancer, stomach cancer, and head and neck cancer

- Generic name: Fluoracilo (5-FU, 2,4-Dihydroxy-5-fluoropyrimidine)

- Molecular formula: $C_4H_3FN_2O_2$
- Molecular weight : 130.1 g/gmol
- Freezing point: 282-286°C
- Physical state: solid
- Chemical structure

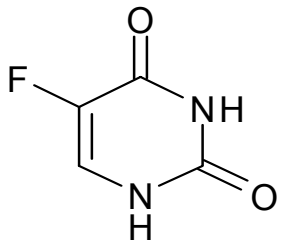


Figure 2. Chemical structure of 5-fluorouracil.

3. **Thymidine:** This compound has a chemical structure very similar to the anti-cancer drug 4'-cyanothymidine. Since 4-cyanothymidine was not available, thymidine was selected for this investigation.

- Molecular formula: $C_{10}H_{14}N_2O_5$
- Molecular weight : 242.23 g/gmol
- Melting point: 187-189°C
- Physical state: solid
- Chemical structure

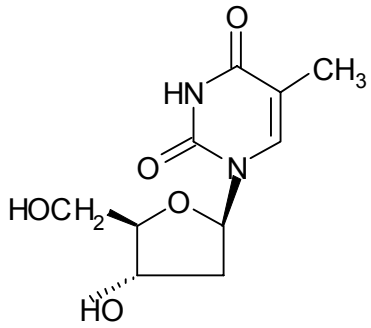


Figure 3. Chemical structure of thymidine.

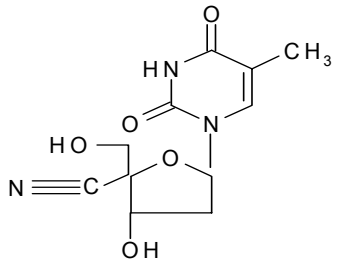


Figure 4. Chemical structure of 4'- cyanothymidine.

4. Azodicarbonamide: This medicament inhibits a wide variety of HIV-1 strains, HIV-2 strains and SIV (1,2). In addition to its antiviral effects, ADA also has virucidal activity.

- Generic name: ADA
- Molecular formula: $C_2H_4N_4O_2$
- Molecular weight : 116.08 g/gmol
- Melting point: 225°C
- Physical state: solid
- Chemical structure

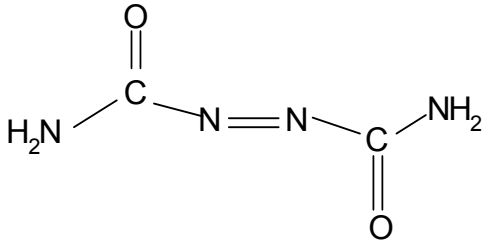


Figure 5. Chemical structure of azodicarbonamide.

5. **2-Phenyl-4H-3,1-benzoxazin-4-one:** This compounds was studied since it not was possible to obtain Efavirenz (Sustiva), an anti-AIDS. This compound has in its structure the benzoxazin group, therefore it was used as a substitute compound.

- Molecular formula: $C_{14}H_9NO_2$
- Molecular weight : 223.23 g/gmol
- Melting point: 123-125°C
- Physical state: solid
- Chemical structure

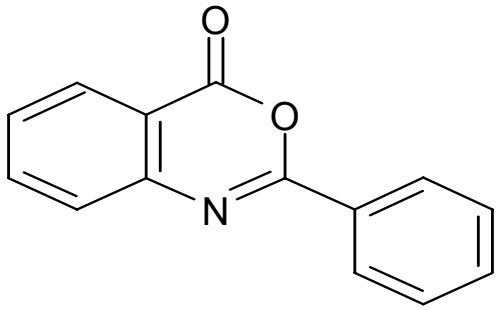


Figure 6. Chemical structure of 2-phenyl-4H-3, 1-benzoxazin-4-one.

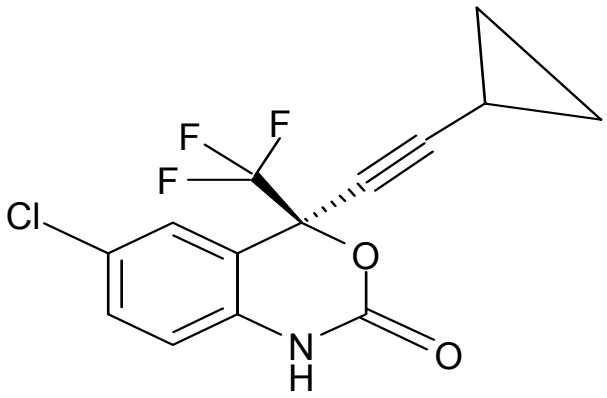


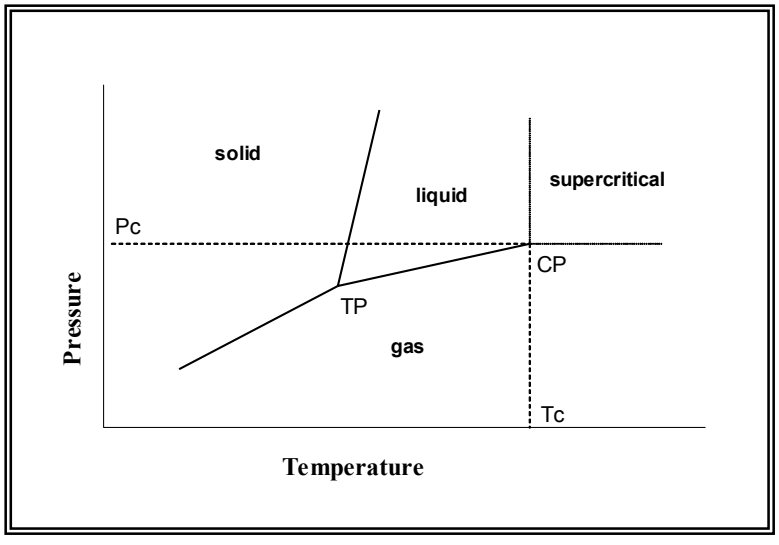
Figure 7. Chemical structure of sustiva.

CHAPTER III : THEORY

III.1. Supercritical Fluids

Supercritical fluids (SCFs) were discovered in 1879, but they were first utilized in applications of extraction in the 1950's[1]. The 1980's saw an increase in their use as mobile phase for most commonly used SCF, but the non-polar nature of the CO_2 limited the growth of the technique.

For any pure component, there is a point at a particular temperature and pressure called the critical point. At this point, the gas and the liquid regions coincide into one phase. Above this point, but close to it, the fluid is neither a gas nor a liquid, but a substance with physical characteristics of both. A supercritical fluid (SCF) is a substance heated and pressurized above this point, which shows unique properties that are different from those of either gases or liquids under standard conditions. (Figure 1).



TP: Triple point
CP: Critical point
Tc: Critical temperature
Pc: Critical pressure

Figure 8. Phase Diagram for a Pure Substance.

[1] Smith, R. M. Supercritical Fluid Chromatography. Royal Society of Chemistry. 1988.

A SCF has both the gas-like property of being able to penetrate almost anything, due to higher diffusivity and viscosity than normal liquids, and the liquid-like property of being able to dissolve materials. A SCF is capable of extracting like a liquid, but without the difficulties of removing liquids; the solvent can be simply removed by depressurization. Also, SCFs offer the advantage of being able to significantly change density, which affects the solubility, with small variations in the pressure and temperature of the system. The following table compares physical properties of gases, liquids and SCFs.

Table 1. Physical properties of gases, liquids, and supercritical fluids.[2]

Property	Symbol	Units	Gas	Supercritical Fluid	Liquid
Density	ρ	g/cm^3	10^{-3}	0.3	1
Diffusivity	$\mathcal{D}$	cm^2/s	10^{-1}	10^{-3}	$5x10^{-6}$
Viscosity	μ	g/cm•s	10^{-4}	10^{-3}	10^{-2}

III.2. Common Supercritical Fluids

Many substances have critical points which are very high and require large demands of energy. Other substances are contaminants and very expensive. On this account, the use of carbon dioxide in the form of a supercritical fluid offers a substitute to organic solvents due to its low cost, non toxic nature (user-friendly to both human beings and the environment), can be reused, which means a lower operating cost, can be manipulated at room temperature, (which makes handling heat-vulnerable substances easy and safe). As this solvent is not organic, it can be safely used in food processing and pharmaceutical processes. Table 2 shows the substances more commonly used as SCFs.

[2] McHugh, M.; Krukonis, V. Supercritical Fluid Extraction Principles and Practice; Butterworths: London, 1993.

Table 2. Common supercritical fluids.[3]

Compound	Tc [°K]	Pc [bar]
CO_2	304.3	73.9
C_2H_4	282.9	51.2
N_2O	309.5	73.5
NH_3	405.5	114.0
n-C_5	469.6	33.8
n-C_4	425.0	38.0
CCl_2F_2	384.8	41.25
CHF_3	298.9	47.52
H_2O	647.1	221.2

III.3. Applications of the Supercritical Fluids

The dissolving power of a SCF offers a safe solvent for the extraction processes in comparison with the conventional extraction. This is due to its high diffusivity and density. Knowledge of the solubility of the solute in a SCF is of interest in the extraction process to identify the conditions of optimum solubility and to verify that the substance (drug or other compound) does not degrade or decompose under supercritical conditions. Products dissolved in a SCF may be separated out by pressure reduction and/or by changing the temperature. SCF processes are being commercialized in the polymers, food and pharmaceutical industries among others. But there are two main applications for the supercritical fluids: extraction and chromatography.

[3] McHugh, M.; Krukonis, V. Supercritical Fluid Extraction Principles and Practice; Butterworths: London, 1993.

The supercritical fluid extraction (SFE) has great advantages such as: removal of extracting solvent is very easy and can be performed by a simple depressurizing. It is possible to control the extraction manipulating variables like pressure, temperature and composition, the yield of the process can be increased by addition of co-solvents (modifying) to the supercritical fluid to enhance its solubility, and use non-toxic and inexpensive solvents (like carbon dioxide). Another advantage of SFE (mainly when carbon dioxide is used as a supercritical fluid) is that it permits one process materials at ambient temperature and under relatively high pressures and the products obtained are of better quality than those obtained by organic solvent extraction.

Supercritical fluid chromatography (SFC) is a technique of analysis, suggested first by Lovelock in 1958, which fits between HPLC chromatography and GC chromatography, using as mobile phase a supercritical fluid like the carbon dioxide. This chromatographic technique has some advantages over conventional chromatographic techniques, such as: higher selectivity, faster separations, it can be operated at room temperature (no temperature ramping needed), can use packed and capillary columns, and the properties of the mobile phase can be 'tuned' manipulating variables such as pressure and temperature for an optimum separation. Also, it can use the detectors utilized in both types of chromatography (HPLC and GC).

III.4. Supercritical Fluid Chromatography Theory

The theory of separation in SFC is based on the density of SCF, which relates to the solvating power of the SCF. If the pressure of the system is increased, the density of the SCF increases and therefore its solvating power increases too. Therefore if the density of the mobile phase of a SCF is increased, the components retained in the column can elute faster.

The solubility indicates the affinity of the fluid for the solute and it is an important factor in the supercritical extraction process. The solubility of a substance in a supercritical fluid is affected by three main factors: First, the volatility (vapor pressure) of the solute; second, the solvating effect of the supercritical fluid and last, the solute-solvent interactions.

In a chromatographic medium, the equilibrium is reached between the solute dissolved in the mobile phase (supercritical CO_2) and the retained in the stationary phase. The degree of retention of a solute i in a supercritical fluid chromatography (SFC) is characterized by the capacity factor, k_i, defined as:

$$k_i = \frac{n_i^s}{n_i^m} = \frac{t_i - t_o}{t_o} \qquad (1)$$

The capacity factor is inversely related to the solvating power of the mobile phase for that solute i; the more soluble it is in the mobile phase, the less it will be retained. The capacity factor is also related to the equilibrium distribution of the solute between both phases (mobile and stationary) of the following form (Suleiman *et al*., 1993):

$$k_i = \frac{n_i^s}{n_i^m} = \frac{x_i^s V^s \nu^m}{y_i^m \nu^s V^m} \qquad (2)$$

Phase equilibrium considerations between the pure solid, the SCF (mobile phase) and the stationary phase in the chromatographic column shows (Prausnitz, 1986):

$$y_i \phi_i P = x_i H_i \qquad (3)$$

where ϕ_i is the fugacity coefficient of solute i in the mobile phase (supercritical carbon dioxide), P is the system pressure, H_i is the Henry's constant between solute i and the stationary phase (packing of the column). H_i is affected by pressure and this effect can be described in the following form:

$$H_i = H_i^o \exp\left[\frac{\nu_i^* (P - P^o)}{RT}\right] \qquad (4)$$

where H_i^o is the Henry's constant between solute i and the stationary phase (packing of the column) at the reference pressure P^o, and ν_i^* is the partial molar volume of solute i in the stationary phase. If we consider that ν_i^* is equal to the liquid molar volume ν_i and combining equations 2, 3 and 4, we can obtain for ϕ_i:

$$\phi_i = \frac{k_i H_i^o}{P \nu^m} \exp\left(\frac{\nu_i (P - P^o)}{RT}\right)\left[\frac{V^m \nu^s}{V^s}\right] \qquad (5)$$

Note that the ratio inside brackets, $[V^m \nu^s / V^s]$, depends exclusively on the chromatographic column, and does not vary significantly with pressure. The conventional solubility expression for SCFs (Prausnitz *et al.,* 1986) is:

$$y_i = \frac{P_i^{sat}}{P \phi_i} \exp\left[\frac{\nu_i \left(P - P_i^{sat}\right)}{RT}\right] \qquad (6)$$

Equation 6 clearly shows the factors that influence on the solubility: volatility, P_i^{sat}, solvating effect of the SCF (pressure and temperature) and solute-solvent interactions (ϕ_i). Where P_i^{sat} is the vapor or sublimation pressure depending on the physical state of the solute at the studied conditions. To calculate the solubility of a solute in a SCF, the fugacity coefficient (ϕ_i) of solute must to be determined or calculated by equation 5.

Combining the equations 5 and 6 we obtain an easier expression for the solubility of a solute i, from the chromatographically determined capacity factors, k_i:

$$y_i = [C_i(T)]\frac{v^m}{k_i} \qquad (7)$$

where

$$C_i(T) = \frac{P_i^{sat} V^s}{H_i^o V^m v^s} \exp\left[\frac{v_i (P^o - P_i^{sat})}{RT}\right] \qquad (8)$$

$C_i(T)$ is a constant that is only a function of temperature, chromatographic column and the nature of solute i. Once it is characterized $C_i(T)$ for a given solute, temperature and chromatographic column, it can obtain the solubilities from chromatographic capacity factors, k_i. Recall that capacity factors are fast and with small amounts of solute required. The determination of the value of this constant, $C_i(T)$, was performed making measurements of solubilities for a determined temperature by an independent method. Once $C_i(T)$ known, the entire solubility isotherm can be determined rapidly from the chromatographic capacity factors.

The equation 7 can also be expressed in the following way:

$$S = y_i \rho = \frac{[C_i(T)]}{k_i} \qquad (9)$$

where S is defined as the solubility of the solute i, which in turn is the mole fraction times the mobile supercritical phase density. If the values 1/S are plotted against the values of the capacity factors we can to obtain a straight line, where the slope of this line is $1/C_i(T)$. This approach minimizes the error obtaining $C_i(T)$.

$$\frac{1}{S} = \left(\frac{1}{C_i(T)} \right) k_i \qquad (10)$$

Once we evaluate the parameter $C_i(T)$, then we can easily determine the values of solubilities using equation 7 or 9, with the estimated values of the capacity factors. The chromatographic technique is very easy and faster than conventional methods. It is possible to obtain numerous determinations of solubilities in a few minutes (rather than several hours for the conventional method) and using only a few milligrams of solute (compared to the several grams required with high purity in the conventional approach).

CHAPTER IV : MATERIALS AND METHODS

IV.1. Equipment and Materials used

The experiments described were performed in a supercritical chromatograph, which is an adaptation of conventional HPLC chromatograph, utilizing as mobile phase of carbon dioxide in supercritical conditions. This equipment consists of the following parts (Figure 2):

- A syringe pump model 260D ISCO with its pump controller series D ISCO. This pump has a maximum capacity of 500 atm. It was used to pressurize the carbon dioxide over its critical pressure. It has various programmable operating modes (constant flow rate, constant pressure, and ramping either pressure or flow rate) that offer excellent flexibility.
- A water temperature open bath with its thermostat (temperature controller) and a temperature indicator for 10 thermocouples (throughout the system). This apparatus is a Model 2067 Forma Scientific, Inc. It has a Heise pressure transducer, model 901B, with a pressure digital display (Model 901) to monitor the system pressure in the inlet of the chromatographic column.
- The sample injection system consists of a Valco Model A90 injection valve, which is operated with a pneumatic actuator (operated with an air line of about 110 psig of pressure), and it is capable of injecting 0.05 or 0.10 μL depending on the selected rotor. This pneumatic actuator that operates the injection valve is controlled by a DVI (Digital Valve Interface) with inject/load switch (mark VICI). The carbon dioxide in supercritical conditions enters to the injection valve, where a 0.1 μL injection loop permits introduction of small amounts of the solute dissolved in a particular solvent (*e.g.,* pentane).
- A carbon dioxide tank with a dip tube, (Scott Specialty Gases, Inc. with purity: 99.9995% CO_2) with the following specifications:

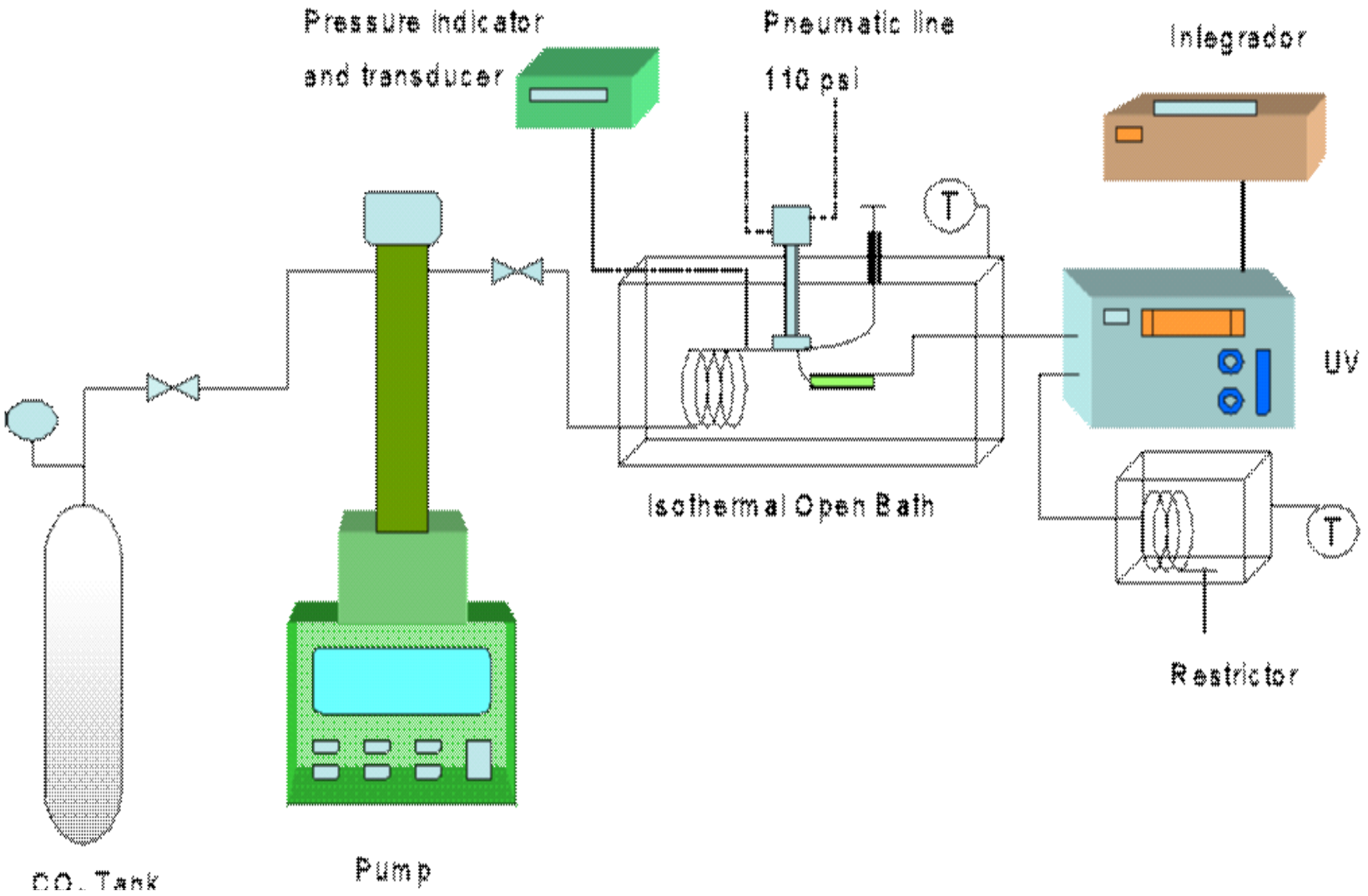


Figure 9. Diagram of the Supercritical Fluid Chromatograph

Table 3. Carbon Dioxide Specifications (SFC grade).

Component	Max. concentration [ppm]	Component	Max. concentration [ppm]
Hydrogen	5	Methane	2
Carbon monoxide	5	Water	3
Oxygen	2	Non-volatile organic	0.1
Nitrogen	50	Particulate	1
Argon	5	UV cutoff @ 200 nm (au)	0.1

- High pressure stainless steel tubing of 1/8" and 1/16" was used to connect the different apparatus of the equipment of SFC. The smaller diameter tubing was selected to minimize the volume in the system.
- A variable restrictor (Model VR100) was used to depressurize the effluent that comes out the UV system. The restrictor is immersed in a thermal bath at a constant temperature of 35°C.
- A VWR Model 1130A thermostatic bath was utilized to heat the restrictor to overcome the cooling upon expansion associated with depressurization of the carbon dioxide (Joule-Thompson effect).
- A high-pressure in line ultraviolet detector, which measures the signal emitted by the sample injected to the system, was used for this investigation. It has an outlet to an integrator to record the signal of the detector. The detector is a V^4 Absorbance Detector ISCO Inc. with a variable wavelength system (two lamps, deuterium and tungsten). The integrator is a Hewlett Packard HP 3395 model.
- The column employed in this equipment was a C_{18} BDS (Hypersil bonded phase to a silica support) packed column with a maximum capacity of pressure of 5000 psig. The column is submerged in the Form Scientific Thermostatic Bath (Model 2067) to control the temperature.
- The temperatures at various points of the equipment were monitored by a resistence temperature dependence (RTD) elements from Omega, connected to an Omegarometer DP 2000.

To assure the thermal equilibrium of the system, the supercritical fluid is conducted through 2 m of stainless steel tubing (1/16" ID) immersed in the constant temperature bath.

IV.1.1. Chemicals

Carbon dioxide SFC grade (99.9995% purity) was obtained from Scott Specialty Gases products. The drugs used were obtained from Aldrich Chemical Company with a stated of purity of 95% or better. They were used without further purification, since the chromatographic column separated out the impurities, in all experiments. Table 4 shows the materials used in the experiments, the purity, molecular weight, wavelength of absorption and their sources.

Table 4. Properties of Materials utilized in this research.

Material	Source	Purity	Molecular Weight (g/mol)	Wavelength (nm)
CO_2	Scott Specialty Gases	99.9995	44	200
Pentane HPLC Grade	Sigma Chemical	99+%	77	210
Acetone HPLC Grade	Sigma Chemical	99+%	32	330
Benzene	Sigma Chemical	99%	78	204
Naphthalene	Aldrich	98%	128.16	275
Phenanthrene	Aldrich	98%	178.23	251
Paclitaxel	Aldrich	95%	853.9	226
Methanol	Fisher ChemAlert	99.9%	32.04	205
Ethanol	Fisher ChemAlert	99.9%	46	205
5-Fluorouracil	Aldrich	99%	130.1	254
Thymidine	Aldrich	99%	242.23	260
Azodicarbonamide	Aldrich	97%	116.08	245
2-Phenyl-4H-3,1-benzoxazin-4-one	Aldrich	98%	223.23	245

The wavelengths shown in Table 4 were obtained from the literature and others were determined analytically in a PowerWaxe$_x$ Microplate Scanning Spectrophotometer (Bio-Tek Instruments, Inc.) doing a scanning to a sample of the solute from 190 nm up to 350 nm.

IV.2. The Chromatographic Column

In this research, a HPLC column with typical reversed-phase material containing long chain hydrocarbon groups was used. This column is packed with particles of Hypersil BDS C_{18}. The column is submerged in the thermostatic bath for maintaining its temperature constant during the experimental run. Also, the chromatographic column was maintained under pressure continuously to maintain constant its performance (activity).

The followings are the main characteristics of the column utilized, specifications provided by the manufacturer (Alltech):

Tabla 5. Specifications of the column.

Bonded Phase	**Dimensions (mm)**	**End Cap**	**Particle size (µm)**	**Surface area (m^2/g)**	**Mean pore size (Å)**	**Bonded phase coverage (mol/m^2)**	**Pore volume (cc/g)**
Hypersil BDS C_{18}	100 x 4.6	Fully	4.57	169	125	$2.84x10^{-6}$	0.57

IV.3. UV Detector

Due to the high pressure nature of our research, an ISCO V^4 UV-Vis variable wavelength detector in the line of flow was used. This equipment has a high-pressure cell that can support pressures up to 6000 psig. The specifications of high pressure

cell are: ISCO Series 0080-73, SFC Cell, dead volume of 1 μL, path length of 5 mm. The detector can measure as either absorbance, or percent of transmittance of light in a range of wavelengths between 190 and 750 nm. For the ultraviolet light source, a deuterium lamp was used, while for the visible light a tungsten lamp was selected. The switch for the selection of the lamp is easily performed in the front panel of the detector. The signal of the UV detector was sent to an integrator, a Hewlett Packard Model 3395, for accurate quantification of retention times and response.

The followings are the guidelines for the operation of the UV detector:

- Set the desired wavelength.
- Select the deuterium lamp as the light emission source (UV source).
- If the equipment has been connected for at least 2.5 hours, it will be ready for use within 30 minutes after switching ON. Otherwise, it should be left running for at least 20 to 30 minutes before a run is made.
- The flow rate of the system should not exceed 0.40 mL/min at STP (measured with manual meter Fisher).
- Set the front and rear panel controls as follows:

Rise time switch:	0.80
Recorder switch:	Photometer
Chart speed switch:	0.20
Sensivity switch:	0.02
Collect/Auto/Waste switch:	Waste (An off position)

IV.4. Experimental Procedure

Before the experimental runs with the drugs were performed, we needed to evaluate the experimental procedure utilized. We validated the technique making several runs with phenanthrene (a very well studied substance) to compare with results obtained by others investigators. To evaluate the capacity of the signal of the UV detector we used

benzene and acetone were used to observe the operation of this equipment by comparison with measurements performed with other spectrophotometers.

The drugs used in this investigation were: taxol, 5-fluorouracil, thymidine, azodicarbonamide and 2-phenyl-4H-3,1-benzoxazin-4-one. Solutions of each of these drugs were prepared using methanol, acetone, benzene and ethanol as solvent and injected in the supercritical chromatograph. The use of a solvent was simply to inject a liquid sample. The solvent selected had to be of small molecular weight and volatile to go unretained through the column without affecting the equilibrium between the drug and the column or the drug and the SCF.

Before the injection of the sample, it was required that the equipment be stabilized, the temperature in the bath be set to the desired value, and in the same way, the value of the pressure in the pump (equilibrium). The equipment was run at least one hour before any injection was made. This assured a stable flow and thermal equilibrium. The pressure in the line of the equipment was monitored by a pressure transducer and a digital pressure indicator (Heise) before the column. This way the pressure in various places of the system was accurately measured.

IV.4.1. Filling the Syringe Pump and Operation of the Equipment

Carbon dioxide was withdrawn out of the CO_2 tank (with a dip tube) in liquid form and the syringe pump was filled for the SFC runs. To ensure that CO_2 is filled in the pump in the liquid state, water at 6°C was passed through the cooling jacket of the pump (an ice-water set up was used and the temperature was measured with an Omega Type K surface thermocouple).

The following is the procedure for filling-up the syringe pump:

- Close out the valves of the pump.

- Circulate water at 6 °C through of the cooling jacket of the pump for sure that the carbon dioxide that into be liquid. The temperature of the CO_2 should be as low as possible to prevent vaporizing of this into of the pump.
- Open the valve of the carbon dioxide tank.
- Open the inlet valve of the pump.
- Operate pump refill to a rate of 5 or 6 mL/min (slow flow rates provide better refill).
- When the refill is completed, wait for 20-30 minutes approximately for achieve the equilibrium, before closing the inlet valve of the pump.
- With both valves closed, run the pump to the desired value of pressure. Wait a time for the stabilization of the pump pressure and then open the outlet valve of the pump.

Once the pump was full, we could start our regular runs. From the pump the carbon dioxide flowed at either constant pressure or constant flow through 2 m of stainless steel tubing, immersed in a constant temperature bath (this is to assure thermal equilibrium). The sample was then injected into the injection valve and this passes through the chromatographic column. From the column, the substance goes to the UV detector where the signal is sent to the integrator for it to be recorded and to provide retention times.

These retention times of the solutes studied and un-retained markers (*e.g.,* pentane, acetone, methanol, etc.) were recorded and utilized to calculate the capacity factors (equilibrium partition coefficients).

IV.5. Direct Measurements of Solubilities in the Supercritical Extractor

In order to calculate the solubilities, with the capacity factors obtained in the chromatograph, it was necessary to use an independent technique for the evaluation of

the solubility of each drugs to a known temperature and pressure. This is done with the goal of obtaining a calibration value Ci(T) for the systems studied.

An ISCO extraction apparatus was modified to carry out these measurements (Figure 3). The equipment consists of a syringe pump (ISCO 260D), a thermostatic chamber, an equilibrium cell, a variable-flow-rate restrictor, and an ice trap. Experiments were conducted by allowing the supercritical CO_2 to slowly flow through the cell to ensure the equilibrium, where an amount of the drug was previously loaded.

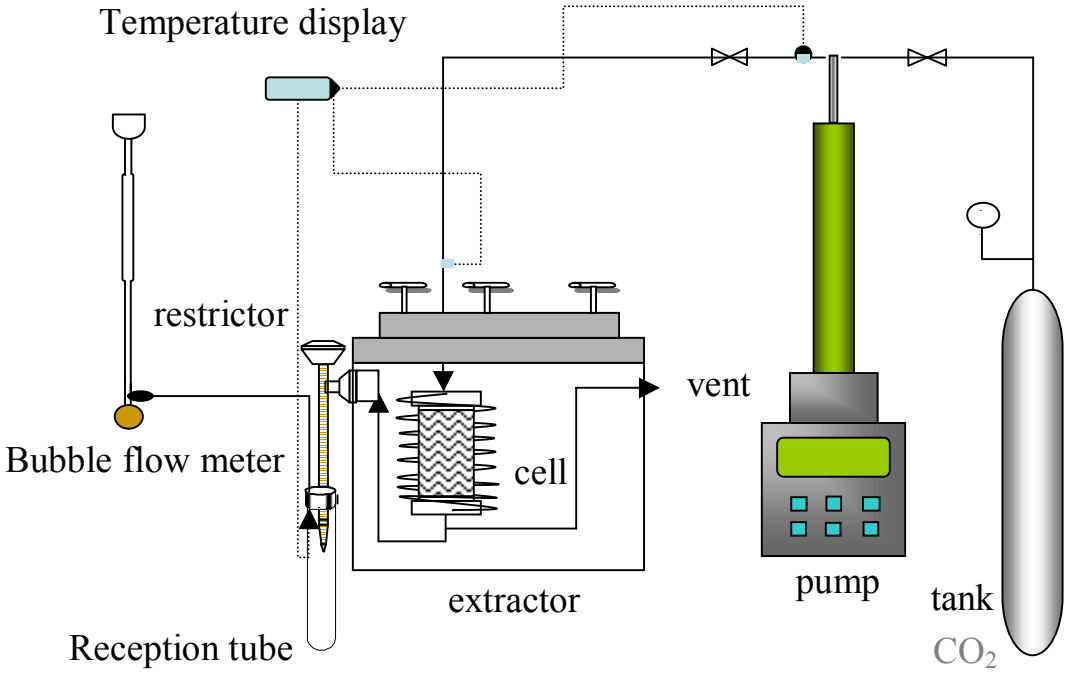


Figure 10. Diagram of the Supercritical Extractor ISCO[4]

The chamber pressure was kept constant and controlled by the pump, and the flow rate was controlled by the variable restrictor. The temperature of the solvent leaving the pump was measured by a thermocouple (Omega DP24-T).

[4] Eliana Jara M. Solubilidad de Imipramine HCl en Dióxido de Carbono Supercrítico. Tesis Maestría en ciencias en Ingeniería Química. Universidad de Puerto Rico, Mayagüez. 1999. Páginas 43-44.

For the measurement of the solubility, the cell was weighed before and after the experiment. This allowed the calculation of the amount dissolved by the difference of weights (gravimetric technique). The amount of solvent was measured by the difference in volume readings in the syringe pump (the density of the carbon dioxide is determined at the pump conditions).

To assure phase equilibrium in the cell, the solvent flow rate was very low, and therefore the runs were rather long (*e.g.,* 6-8 hours). The mass of the cell after the experiment was determined using a Sartorious balance model (with a ±10 μg accuracy).

CHAPTER V : RESULTS AND DISCUSSION

V.1. Validation of the Chromatographic Method

In order to verify the reliability of the chromatographic method it was first validated the technique using a well studied system. We selected phenanthrene due to the extensive literature available about this compound. The results are shown in Figure 11 and the data in Table 6. The deviation from Mojica, (1998) was only 2.25% and 9.8% and 5.5% with respect to the data of Suleiman et al., (1992) and Bartle et al., (1990) respectively.

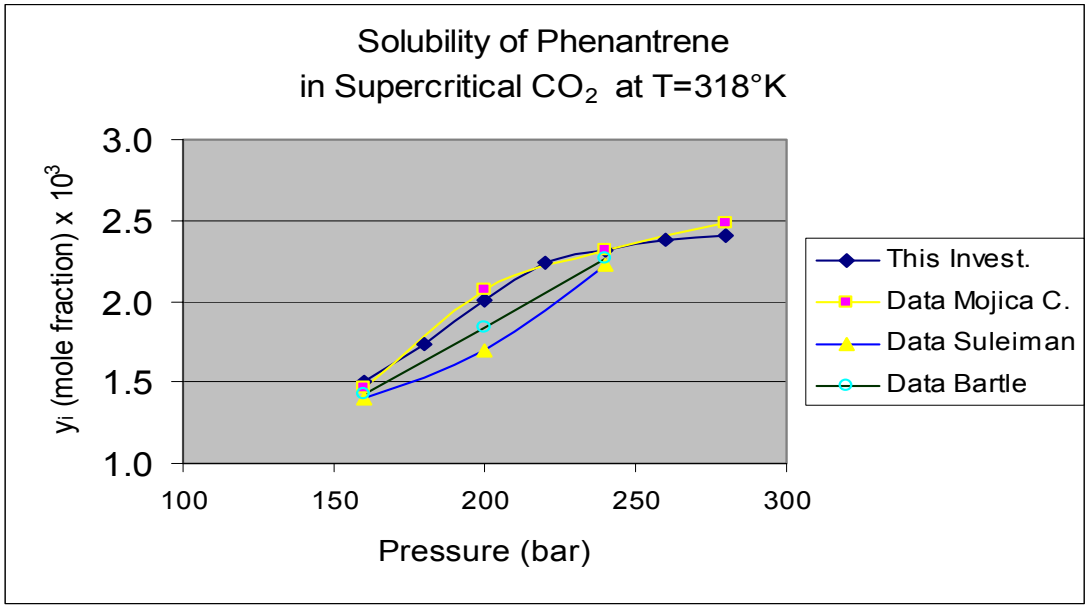


Figure 11. Solubility of Phenanthrene in Supercritical Carbon Dioxide

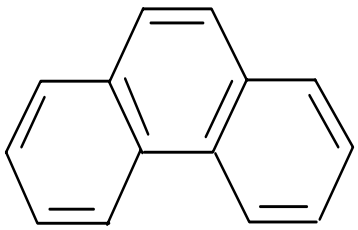


Figure 12. Chemical Structure of Phenanthrene

Solvent: CO_2 **Temperature:** 318°K

Solute: Phenanthrene **$C_i(T)$:** 0.0362 mol/L

Table 6. Results of the validation of the equipment with phenanthrene in Supercritical CO_2.

Pressure system (bar)	t_1 (minute)	t_0 (minute)	Capacity factor, k_i	CO_2 Density (gmol/L)	$y_i * 10^3$ (mole fraction)
280	2.5	1.5	0.695	19.98	2.61
260	2.7	1.6	0.749	19.67	2.46
240	3.0	1.6	0.819	19.32	2.29
220	3.3	1.7	0.894	18.94	2.14
200	3.7	1.8	1.010	18.50	1.94
180	4.3	2.0	1.163	17.96	1.73
160	5.2	2.2	1.400	17.30	1.50

V.2. Experimental Data of the Supercritical Fluid Chromatograph

The experimental results for this research (obtained in the supercritical chromatograph) are shown numerically in tables A1-A15 (Appendix A). The data presents: solubility (mole fraction), capacity factors, solvent density, pressure, flow, and retention times. The data covers the range of 100 – 300 bar and 35.1 – 55.1°C. The solubility values for each drug were estimated with equations 1 and 8 respectively. Before it was necessary evaluate the values of the calibration parameter $C_i(T)$ with the solubility data obtained of the experimental runs by the gravimetric technique in the supercritical fluid extractor.

Table 7 includes the solubility data obtained by the gravimetric technique using the ISCO Supercritical Fluid Extractor (SFE) for each drug and temperature studied. The values are an average of two measurements performed at the same temperature and

pressure. All solubility values shown in the following table were measured at a pressure of 300 bar and only at 35 and 55°C. In Appendix B are shown the data obtained of the run performed in the ISCO supercritical fluid extractor and with this information the sample's calculation of the solubility was achieved for each drug studied, shown in Appendix C. Table 7 summarizes the calculations of Appendix C.

Table 7. Solubility obtained by the gravimetric technique (ISCO SFE).

Drug	**Temperature (°C)**	**Solubility (mole fraction)**
2-phenyl-4H-3,1-benzoxazin-4-one	35	3.8×10^{-4}
	55	4.5×10^{-4}
5-fluorouracil	35	1.6×10^{-5}
	55	4.3×10^{-5}
Azodicarbonamide	35	2.55×10^{-5}
	55	1.95×10^{-5}
Thymidine	35	7.35×10^{-6}
	55	8.0×10^{-6}
Taxol	35	3.72×10^{-6}
	55	5.91×10^{-6}

V.3. Data obtained of the Supercritical Extractor and Estimation of $C_i(T)$

The values of the calibration parameter $C_i(T)$, for each drug and temperature studied, were estimated using a conventional method (gravimetric) as explained in chapter IV (section IV.5). Table 8 shows the values obtained for the calibration parameter $C_i(T)$, evaluated with the solubility values given in the Table 7. In Appendix D there is a sample's calculation of these values for $C_i(T)$ at each temperature for the drug studied. The direct estimations for $C_i(T)$ were performed only at 35.1°C and 55.1°C, the value for 45.1°C was obtained by mean of a regression of $\log[C_i(T)]$ versus $1/T$. This was

done, since $C_i(T)$ represents physical data for the column, and a Henry's constant between the solute and the stationary phase. Henry's constants tend to follow a Van't Hoff trend with temperature. Also, this method was used to interpolate between a very small range of temperatures.

Table 8. Calibration Parameter $C_i(T)$ for each drug studied.

Drug	**Temperature (°C)**	**$C_i(T)$ (mol/L)**
2-phenyl-4H-3,1-benzoxazin-4-one	35.1	2.81×10^{-4}
	45.1	2.78×10^{-4}
	55.1	2.75×10^{-4}
5-fluorouracil	35.1	1.3×10^{-5}
	45.1	1.7×10^{-5}
	55.1	2.0×10^{-5}
Azodicarbonamide	35.1	2.27×10^{-5}
	45.1	2.0×10^{-5}
	55.1	1.8×10^{-5}
Thymidine	35.1	3×10^{-6}
	45.1	4.9×10^{-6}
	55.1	7.8×10^{-6}
Taxol	35.1	5.4×10^{-6}
	45.1	1.1×10^{-5}
	55.1	2.2×10^{-5}

As it can observe for some drugs, the $C_i(T)$ presents an inverse response with the temperature, for example in: taxol, azodicarbonamide and 2-phenyl-4H-3,1-benzoxazin-4-one. While that for 5-fluorouracil and thymidine increases with an increasing in the temperature.

V.4. Plotting of the Solubility Isotherms

The retention times used in this investigation (for the calculation of the capacity factors) were an average of several measurements obtained at the maxima of their response. The densities used in the calculations corresponded to pure fluid densities because the systems studied were considered highly diluted. These densities were obtained using a computer program, the modified Bennedict-Webb-Rubin equation of state[5] (MBWR EOS)[6] being used to calculate the properties of gases and light hydrocarbons.

Isotherms shown in the Figures 13-17 were plotted with the data tabulated in the Appendix A, where for each drug are shown variability of the solubility with pressure for the same temperature.

The order of magnitude for the solubilities studied was of 10^{-6} to 10^{-4} mole fraction; the drug that showed the highest solubility in supercritical CO_2, of according the results obtained, was 2-phenyl-4H-3,1-benzoxazin-4-one (Figure 13). This is due to the non-polar character of this compound and its low melting point. In contrast to the 2-phenyl-4H-3,1-benzoxazin-4-one, the drug that presented the lowest solubility was taxol, this is explained by its polar character and low volatility (Table 9). However, taxol shows an increase of its solubility with temperature (Figure 14) and its solubility is also better with an increase of pressure. The results for taxol agree with other literature values in order of magnitude, but they were not compared because of the pressure range studied (Vandana and Teja, 1997).

5-Fluorouracil also presents a tendency of higher solubility to high pressures and temperatures (Figure 15). However, the solubility is considered very low because of this compound is of polar character and the CO_2 does not have a very high solvating

[5] Benedict, M., G. B. Webb, and L. C. Rubin: *J. Chem. Phys.,* **8**: 334 (1940).
[6] Benedict, M., G. B. Webb, and L. C. Rubin: *J. Chem. Phys.,* **10**: 747 (1942).

power with polar compounds. It should be noticed that above 250 bar, the solubility increases, especially at higher temperatures.

Thymidine showed better solubility to a temperature of 318°K and pressures higher than 200 bar (Figure 16). The increasing of its solubility was appreciable to pressure of 300 bar.

Azodicarbonamide showed that its solubility is influenced by low temperatures (308.1°K), at this temperature the increase is higher compared with 318.1°K and 328.1°K (Figure 17).

The estimated error in the solubility for taxol is approximately 10% (Vandana and Teja, 1997) and the error in the estimation of the solvent density is approximately 5%. The estimation of the error for the density was performed by comparison with data from the literature (*The International Thermodynamic Tables for the Fluid State Carbon Dioxide*, IUPAC, Volume 3, 1976).

Table 9 summarizes the range in solubility shown by the drugs in comparison with their molecular weight and melting point. The lowest melting point substance (2-phenyl-4H-3,1-benzoxazin-4-one) has the highest solubility. This is explained by the higher sublimation pressure.

Table 9. Comparison of solubility between the drugs studied.

Drug	**Chemical Formula**	**Molecular Weight (gmol)**	**Melting Point (°C)**	**Solubility Range (mole fraction)**
Azodicarbonamide	$C_2H_4N_4O_2$	116.08	225	$(7.6 – 25) \times 10^{-6}$
5-fluorouracil	$C_4H_3FN_2O_2$	130.10	282 - 286	$(3.5 – 43) \times 10^{-6}$
2-phenyl-4H-3,1-benzoxazin-4-one	$C_{14}H_9NO_2$	223.23	123 - 125	$(7.1 – 46) \times 10^{-5}$
Thymidine	$C_{10}H_{14}N_2O_5$	242.23	187 - 189	$(1.2 – 20) \times 10^{-6}$
Taxol	$C_{47}H_{51}NO_{14}$	853.90	216 - 217	$(1.2 – 11) \times 10^{-6}$

Taxol had the lowest solubility, because it had the highest molecular weight (significantly higher than the others). The other drugs had different functional groups and different molecular weights, but similar melting points and therefore similar solubilities.

Although no error bars are presented, the overall error is estimated to be around 10%. The largest contributor to the error comes from the pressure drop through the column (1-3 bars).

It should also be pointed out that at the lowest pressures (*e.g.,* 100-120 bars). The solubility often increased with decreasing pressure. This is explained by the fact that around those pressures ν_i^* is significantly different from ν_i (Molecular charisma, Ref.). This assumption in the chromatographic analysis is weaker around this area.

CHAPTER VI : CONCLUSIONS AND RECOMMENDATIONS

A supercritical chromatograph was used to measure the solubility of anti-cancer and anti-aids drugs over a temperature range of 35.1°C-55.1°C and pressures between 100 bar to 300 bar. The main advantage of this method was the utilization of the measurements of the retention times to calculate the capacity factors and quantitatively estimate the solubilities fast and acurrately.

The solubility of benzoxazin in supercritical carbon dioxide was appreciable, this is explained considering its high non-polarity, which facilitates salvation by carbon dioxide solvating. It was found that benzoxazin could be dissolved in supercritical carbon dioxide with concentrations in the order of magnitude of 10^{-4} mole fraction and its solubility increases with temperature. The observed solubility of benzoxazin was due to the low melting point and thus to its higher vapor pressure.

Taxol, presented opposite results due to its low solubility with the supercritical carbon dioxide. This is explained because this compound presents in its structure three hydroxyl groups, it is a relatively large biomolecule, has a high molecular weight and is also mildly polar. The solubility obtained for taxol was in the order of magnitude of 10^{-6} mole fraction at 55.1°C (Figure 14). The knowledge of the solubility of this drug in supercritical carbon dioxide is of great importance in the extractions to identify the conditions of optimum solubility and to verify that drug does not degrade or decompose under supercritical conditions. Due to low solubility of the taxol, we suggest a reverse-extraction approach to remove the solvent, while leaving dried taxol undissolved in supercritical carbon dioxide at 125 bar and 308.2°K. Further studies should focus on the study of crystallization kinetics while removing the solvent.

The effect of pressure on the solubility of the other drugs studied followed the expected trend of increasing solubility with an isothermal increase in the pressure for all three temperatures studied. This is explained due to that as pressure is increased, carbon dioxide density increases and the intermolecular mean distance of carbon dioxide molecules decreases, thereby increasing the specific interaction between the solute and solvent molecules. However, taxol showed an unusual behavior due to high solubility to low pressures, this can be explained because of effect of the vapor pressure of this compound.

There is another factor that affects the solubility of these drugs; the effect of the system temperature, which influences the solute vapor pressure (or better sublimation pressure), the solvent density and the intermolecular interactions (drug-CO_2) in the fluid phase. Some drugs showed an increase in solubility with temperature. This indicates that volatility of the drug (vapor pressure – proportional effect) was dominant temperature effect (over solvent density - inversely proportional).

However, we have to say, that the method is still limited by the requirement of a solubility datum estimated at the same temperature, but by an independent technique. Nevertheless this study showed that it is possible to determine relatively rapid a large number of solubility measurements for the studied systems by retention in SFC.

We must to recommend, that the column be kept continuously under pressure, so that it does not loose its activity. Also, use a variable restrictor (with flows less or equal 1 mL/min.). We also suggest a study relating the influence of the flow on the equilibrium of solubility. Other type of detectors could extend the method to other compound, which are not UV-active, for example, an FT-IR detector, and this way provide a wider applications of this technique.

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APPENDIXES

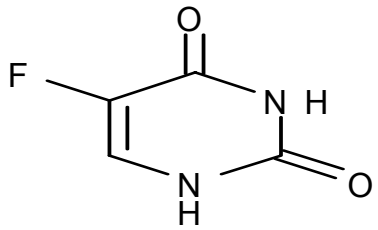
Appendix A

Data of the Capacity Factors and Solubilities of the Drugs Studied

Solvent: CO_2

Solute: Fluorouracil

Temperature: 308.1 °K



5-Fluorouracil

$C_i(T)$: 1.3345×10^{-5} mol/L

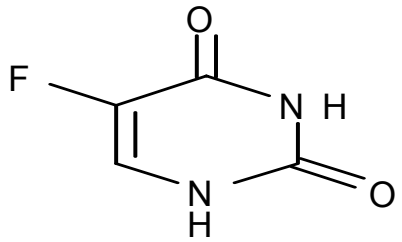
Table A1. Data for 5-fluorouracil to 308.1°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^6$ (mole fraction)
100.33	9.820	7.965	0.118	16.207	0.233	3.54
125.01	8.191	6.815	0.155	17.645	0.202	3.75
150.18	7.017	6.035	0.185	18.522	0.163	4.43
175.51	5.713	5.017	0.352	19.170	0.139	5.02
200.10	6.320	5.623	0.220	19.676	0.124	5.47
225.31	6.010	5.450	0.290	20.113	0.103	6.46
250.18	4.831	4.432	0.357	20.488	0.090	7.24
275.22	4.477	4.165	0.410	20.823	0.075	8.56
300.35	3.629	3.490	0.515	21.125	0.040	15.90

Solvent: CO_2

Solute: Fluorouracil

Temperature: 318.1 °K



5-Fluorouracil

$C_i(T)$: 1.6675×10^{-5} mol/L

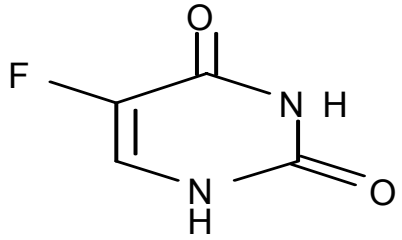
Table A2. Data for 5-fluorouracil to 318.1°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^6$ (mole fraction)
100.30	11.15	9.050	0.131	11.350	0.232	6.33
124.95	7.250	6.087	0.157	15.389	0.191	5.67
149.78	6.835	5.895	0.227	16.855	0.159	6.20
175.45	6.055	5.286	0.229	17.798	0.145	6.44
200.20	5.643	4.928	0.251	18.472	0.145	6.22
224.98	5.549	4.890	0.310	19.017	0.135	6.50
250.32	5.150	4.692	0.365	19.486	0.098	8.77
275.21	4.485	4.250	0.342	19.885	0.055	15.16
299.78	4.090	3.946	0.375	20.235	0.036	22.58

Solvent: CO_2

Solute: Fluorouracil

Temperature: 328.1 °K

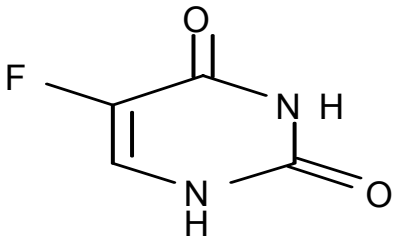


5-Fluorouracil

$C_i(T)$: 2.0553×10^{-5} mol/L

Table A3. Data for 5-fluorouracil to 328.1°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^6$ (mole fraction)
100.35	8.75	7.230	0.123	7.422	0.210	13.17
125.52	7.45	6.313	0.165	12.366	0.180	9.23
150.45	6.25	5.513	0.253	14.902	0.134	10.32
175.45	5.84	5.203	0.235	16.253	0.122	10.33
200.35	5.41	4.855	0.311	17.164	0.114	10.48
225.45	4.95	4.550	0.310	17.864	0.088	13.09
250.82	4.804	4.459	0.334	18.438	0.077	14.41
275.27	4.55	4.350	0.372	18.908	0.046	23.64
300.51	4.18	4.079	0.375	19.330	0.025	42.94



5-Fluorouracil

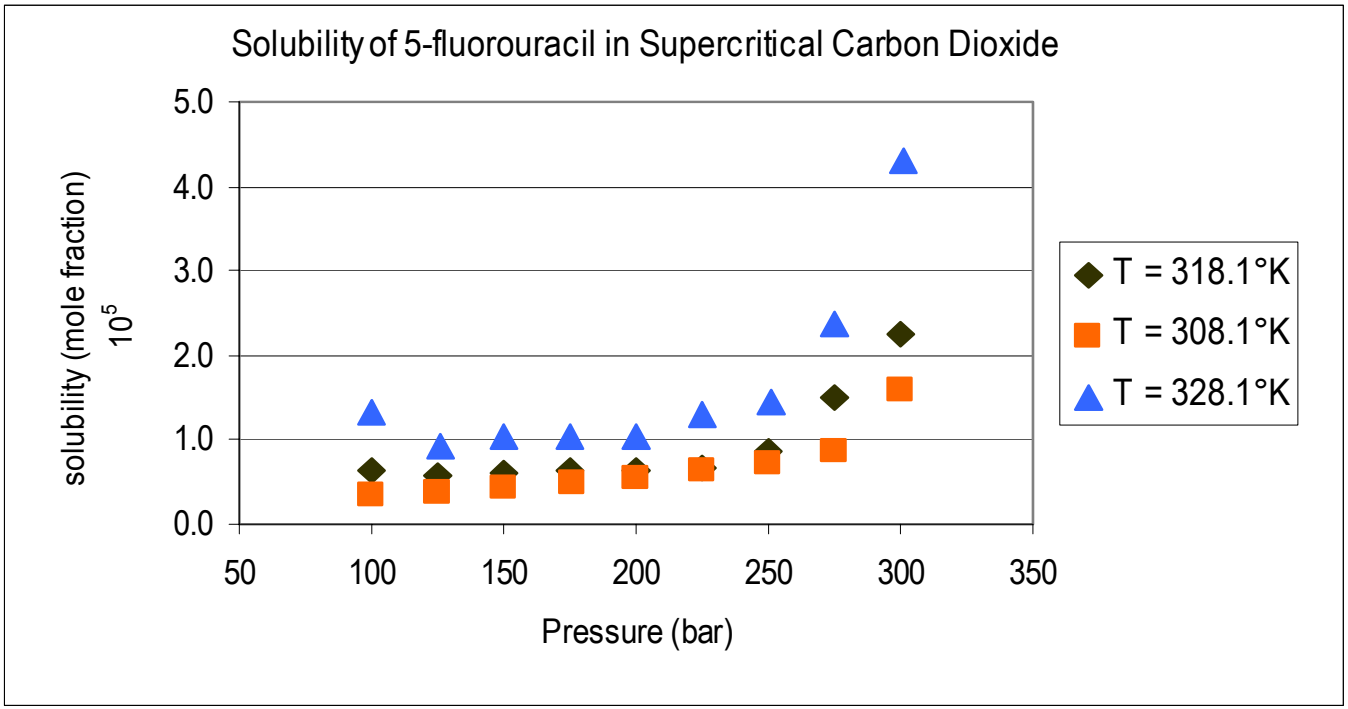
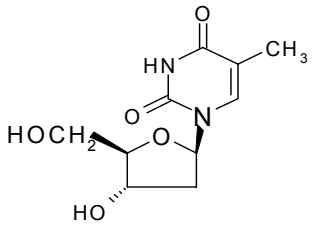


Figure 15. Solubility of Anti-cancer Drug 5-fluorouracil in Supercritical Carbon Dioxide.

Solvent: CO_2

Solute: Thymidine

Temperature: 308.1 °K



Thymidine

$C_i(T)$: 2.9788×10^{-6} mol/L

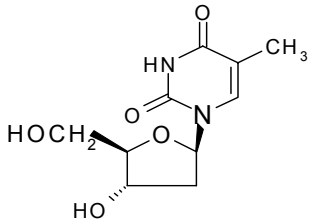
Table A4. Data for thymidine to 308.1°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^6$ (mole fraction)
100.81	9.360	8.151	0.173	16.247	0.148	1.24
125.01	8.504	7.480	0.202	17.645	0.137	1.23
150.33	7.330	6.590	0.237	18.526	0.112	1.43
175.35	6.620	5.997	0.281	19.166	0.104	1.50
199.98	5.770	5.320	0.285	19.674	0.084	1.79
225.28	5.297	4.910	0.285	20.113	0.079	1.88
249.55	4.865	4.543	0.345	20.479	0.071	2.05
275.28	4.456	4.223	0.368	20.823	0.055	2.59
300.01	4.035	3.959	0.395	21.121	0.019	7.34

Solvent: CO_2

Solute: Thymidine

Temperature: 318.1 °K



Thymidine

$C_i(T)$: 4.8992×10^{-6} mol/L

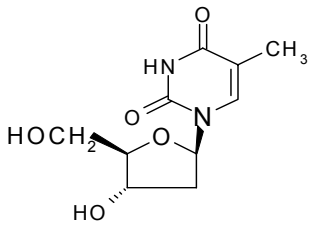
Table A5. Data for thymidine to 318.1°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^6$ (mole fraction)
100.71	8.242	7.012	0.186	11.483	0.175	2.43
124.89	7.380	6.515	0.245	15.384	0.133	2.40
150.09	6.785	6.050	0.285	16.869	0.121	2.39
175.51	6.105	5.540	0.295	17.80	0.102	2.70
200.25	5.417	5.083	0.365	18.473	0.066	4.04
224.37	4.968	4.683	0.336	19.004	0.061	4.24
250.05	4.852	4.670	0.338	19.48	0.039	6.45
274.72	4.461	4.355	0.361	19.878	0.024	10.13
299.45	4.175	4.124	0.405	20.23	0.012	19.60

Solvent: CO_2

Solute: Thymidine

Temperature: 328.1 °K

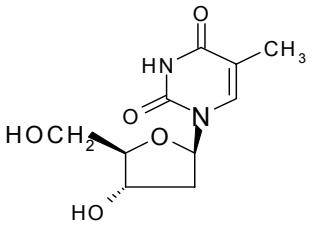


Thymidine

$C_i(T)$: 7.8151 x 10^{-6} mol/L

Table A6. Data for thymidine to 328.1°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^6$ (mole fraction)
99.010	8.780	6.450	0.135	7.178	0.361	3.01
125.09	7.350	5.961	0.175	12.301	0.233	2.73
150.40	6.245	5.29	0.253	14.898	0.180	2.90
175.35	6.027	5.155	0.375	16.249	0.169	2.84
200.15	5.434	4.743	0.327	17.158	0.146	3.13
225.05	5.205	4.650	0.335	17.854	0.120	3.67
249.26	4.635	4.160	0.345	18.406	0.114	3.72
275.05	4.155	3.790	0.375	18.904	0.096	4.29
299.96	4.018	3.819	0.378	19.321	0.052	7.76



Thymidine

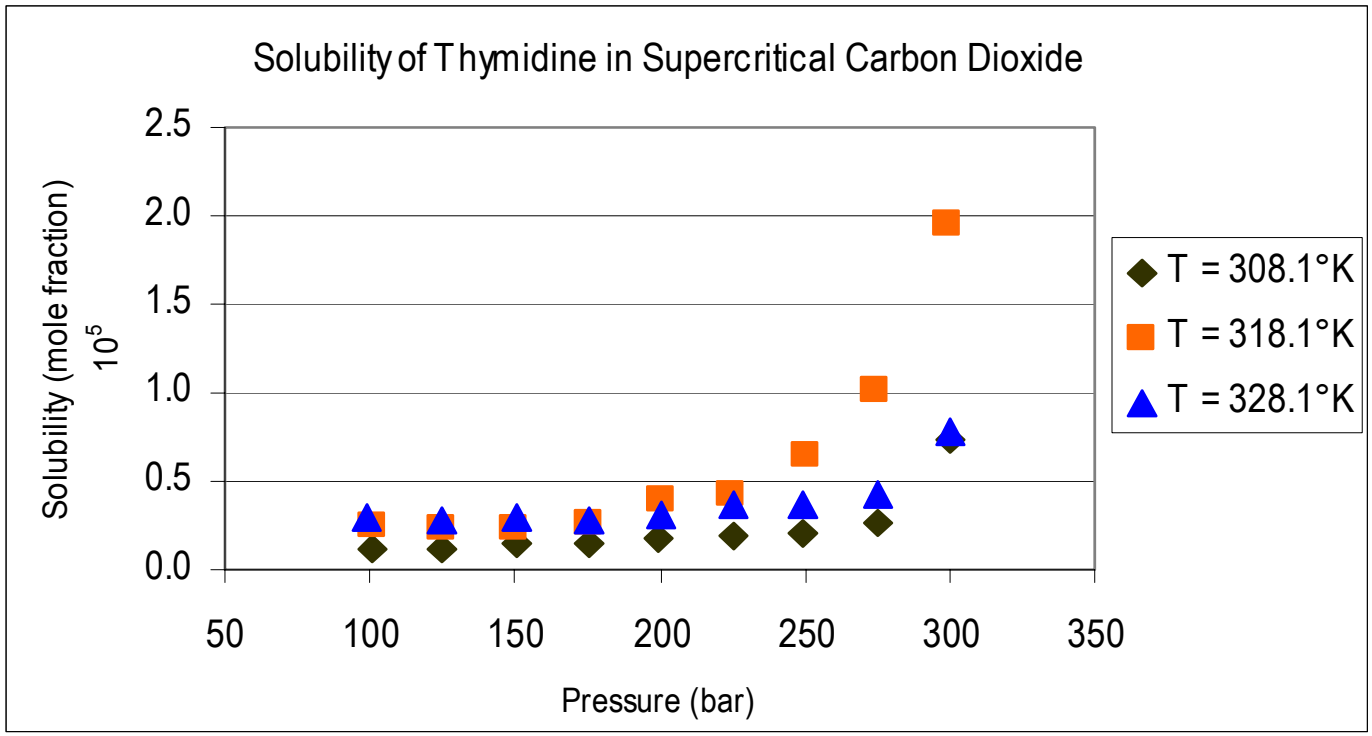
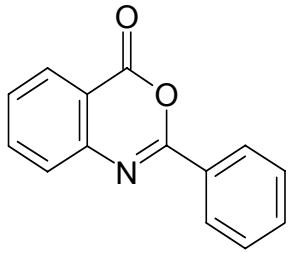


Figure 16. Solubility of Anti-cancer Drug Thymidine in Supercritical Carbon Dioxide.

Solvent: CO_2

Solute: Benzoxazin

Temperature: 308.2 °K



2-Phenyl-4H-3,1-benzoxazin-4-one

$C_i(T)$: 2.8129×10^{-4} mol/L

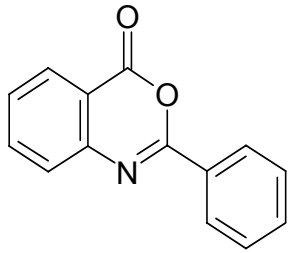
Table A7. Data for 2-phenyl-4H-3,1-benzoxazin-4-one to 308.2°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^5$ (mole fraction)
100.95	13.506	11.005	0.116	16.229	0.227	7.63
125.03	10.885	8.895	0.185	17.627	0.224	7.13
149.98	8.324	6.935	0.225	18.501	0.200	7.59
175.15	7.253	6.293	0.265	19.149	0.152	9.63
200.45	6.875	6.078	0.301	19.671	0.131	10.90
225.22	6.405	5.714	0.322	20.101	0.121	11.57
250.05	5.881	5.273	0.345	20.476	0.115	11.91
275.70	5.425	4.953	0.365	20.819	0.095	14.18
300.50	4.588	4.432	0.382	21.118	0.035	37.84

Solvent: CO_2

Solute: Benzoxazin

Temperature: 318.2 °K



2-Phenyl-4H-3,1-benzoxazin-4-one

$C_i(T)$: 2.7830×10^{-4} mol/L

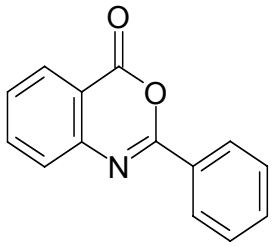
Table A8. Data for 2-phenyl-4H-3,1-benzoxazin-4-one to 318.2°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^5$ (mole fraction)
100.35	10.453	8.153	0.185	11.429	0.282	8.63
125.85	9.907	7.954	0.223	15.488	0.246	7.32
150.20	8.730	7.181	0.315	16.892	0.216	7.64
175.21	7.775	6.552	0.336	17.805	0.187	8.37
200.62	7.014	6.052	0.318	18.495	0.159	9.47
225.40	6.615	5.750	0.293	19.036	0.150	9.72
250.31	5.954	5.337	0.346	19.496	0.116	12.35
274.96	5.213	4.801	0.335	19.891	0.086	16.30
300.05	5.450	5.293	0.372	20.247	0.029662	46.3400

Solvent: CO_2

Solute: Benzoxazin

Temperature: 328.2 °K

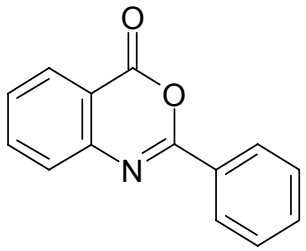


2-Phenyl-4H-3,1-benzoxazin-4-one

$C_i(T)$: 2.7542×10^{-4} mol/L

Table A9. Data for 2-phenyl-4H-3,1-benzoxazin-4-one to 328.2°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^5$ (mole fraction)
100.18	8.541	7.105	0.113	7.390	0.202	18.44
125.24	7.959	6.805	0.134	12.323	0.170	13.18
150.64	7.245	6.358	0.157	14.915	0.140	13.24
175.51	6.892	6.180	0.195	16.256	0.115	14.70
200.15	6.425	5.965	0.253	17.158	0.077	20.82
225.55	6.330	5.942	0.293	17.866	0.065	23.60
250.20	5.409	5.214	0.327	18.426	0.037	39.97
275.25	5.054	4.896	0.345	18.907	0.032	45.14
300.08	4.575	4.435	0.357	19.323	0.032	45.15



2-Phenyl-4H-3,1-benzoxazin-4-one

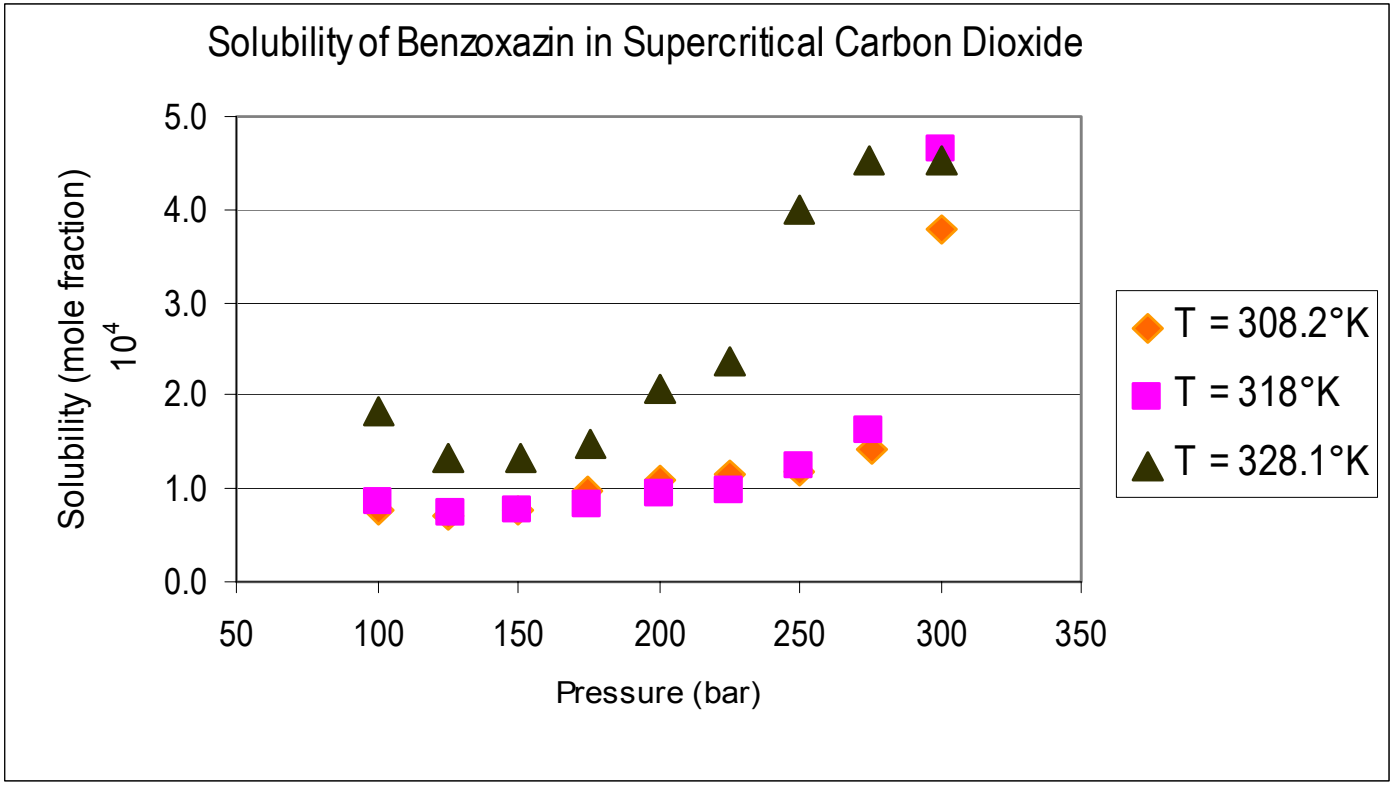
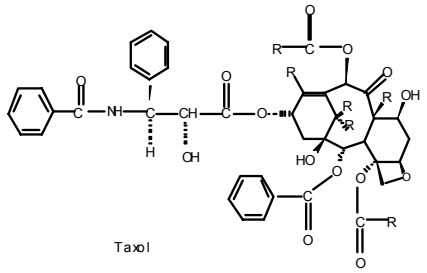


Figure 13. Solubility of Anti-AIDS Drug Benzoxazin in Supercritical Carbon Dioxide.

Solvent: CO_2

Solute: Taxol

Temperature: 308.2 °K



Taxol

$C_i(T)$: 5.4372 x 10^{-6} mol/L

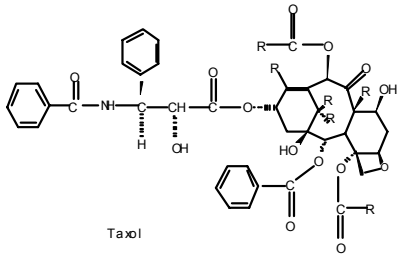
Table A10. Data for taxol to 308.2°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^6$ (mole fraction)
100.23	10.815	8.627	0.116	16.167	0.254	1.33
125.12	9.914	8.008	0.131	17.631	0.238	1.30
150.31	9.195	7.435	0.143	18.51	0.237	1.24
175.06	8.076	6.883	0.185	19.147	0.173	1.64
199.45	7.182	6.385	0.243	19.652	0.125	2.22
225.14	6.342	5.738	0.295	20.100	0.105	2.57
250.05	6.051	5.630	0.312	20.476	0.075	3.55
275.93	5.580	5.214	0.337	20.822	0.070	3.72
300.85	5.056	4.857	0.355	21.122	0.040	6.28

Solvent: CO_2

Solute: Taxol

Temperature: 318.1 °K



Taxol

$C_i(T)$: 1.1364×10^{-5} mol/L

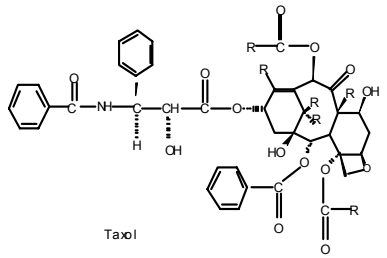
Table A11. Data for taxol to 318.1°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^6$ (mole fraction)
100.18	11.482	9.085	0.128	11.310	0.264	3.81
125.70	10.32	8.204	0.149	15.449	0.258	2.85
150.35	8.776	7.050	0.164	16.880	0.245	2.75
175.35	8.064	6.491	0.215	17.795	0.242	2.64
200.10	7.203	5.824	0.245	18.470	0.237	2.60
225.61	6.468	5.252	0.287	19.029	0.232	2.58
250.45	6.021	4.953	0.316	19.488	0.216	2.70
275.25	5.653	4.721	0.330	19.886	0.197	2.89
300.45	5.182	4.670	0.357	20.244	0.109	5.12

Solvent: CO_2

Solute: Taxol

Temperature: 328.1 °K

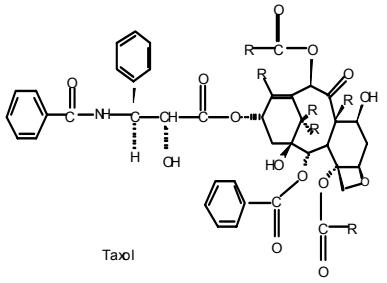


Taxol

$C_i(T)$: 2.2404×10^{-5} mol/L

Table A12. Data for taxol to 328.1°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^6$ (mole fraction)
100.11	9.843	7.730	0.146	7.378	0.273	11.10
125.20	8.730	6.958	0.181	12.317	0.255	7.14
149.95	8.208	6.595	0.241	14.867	0.244	6.16
175.62	7.391	5.959	0.253	16.261	0.240	5.73
200.80	6.717	5.477	0.321	17.178	0.226	5.76
226.40	6.042	4.951	0.279	17.887	0.220	5.68
250.10	5.657	4.685	0.293	18.423	0.207	5.86
275.45	5.174	4.310	0.324	18.911	0.200	5.91
300.08	4.653	4.120	0.358	19.323	0.129	8.96



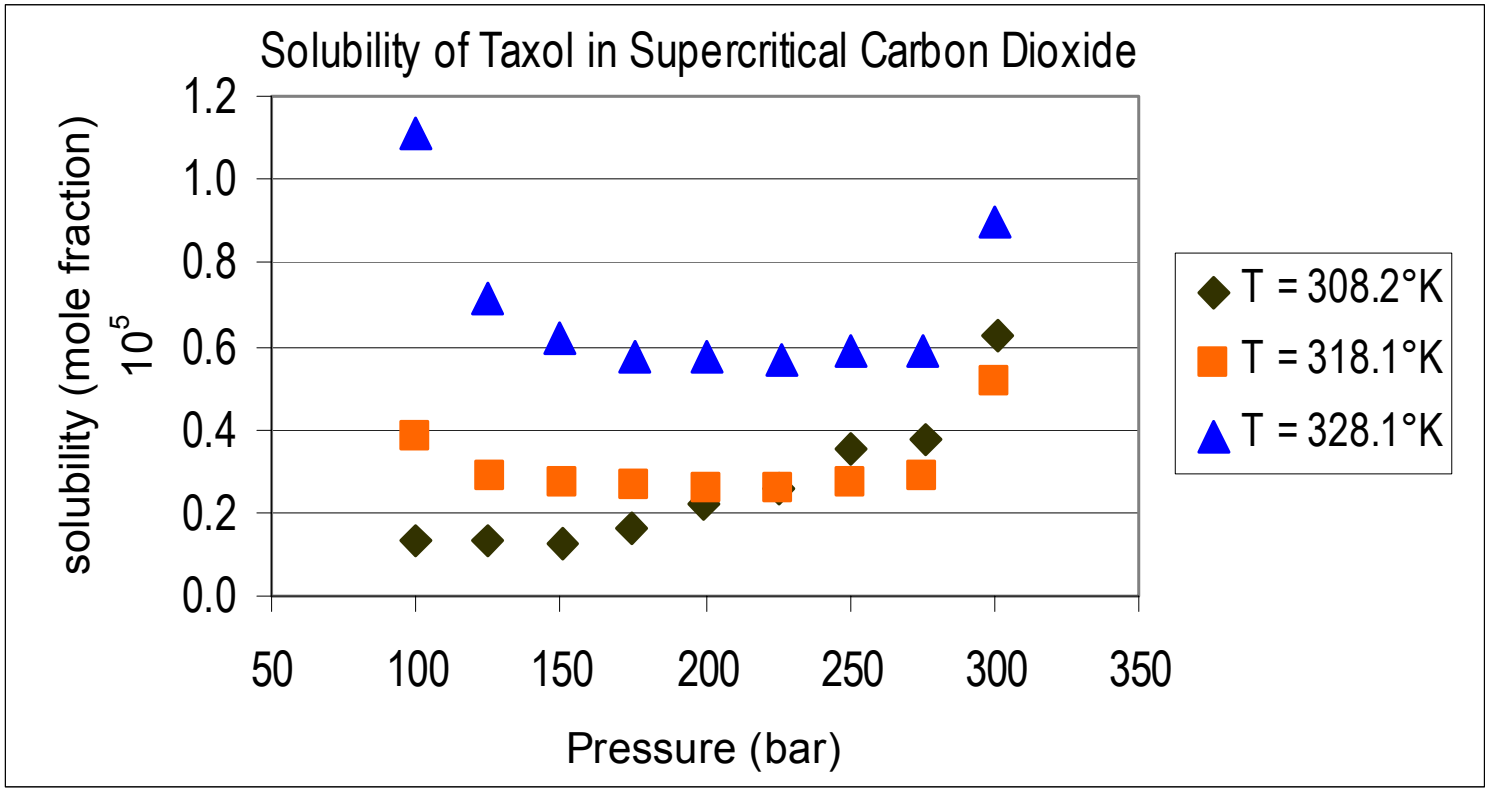
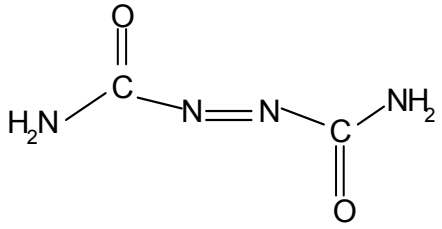


Figure 14. Solubility of Anti-cancer Drug Taxol in Supercritical Carbon Dioxide.

Solvent: CO_2

Solute: Azodicarbonamide

Temperature: 308.1 °K



Azodicarbonamide

$C_i(T)$: 2.2680×10^{-5} mol/L

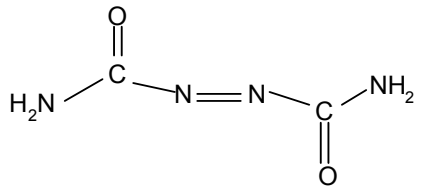
Table A13. Data for azodicarbonamide to 308.1°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^6$ (mole fraction)
100.92	12.065	10.43	0.105	16.256	0.157	8.90
125.70	9.244	8.156	0.136	17.674	0.133	9.62
150.20	8.567	7.646	0.185	18.522	0.120	10.16
174.86	7.490	6.863	0.212	19.155	0.091	12.96
200.51	6.950	6.379	0.246	19.684	0.090	12.87
225.31	6.420	5.908	0.301	20.113	0.087	13.01
250.51	5.867	5.571	0.315	20.493	0.053	20.83
275.68	5.542	5.282	0.325	20.828	0.049	22.12
298.35	5.156	4.948	0.348	21.102	0.042	25.57

Solvent: CO_2

Solute: Azodicarbonamide

Temperature: 318.1 °K



Azodicarbonamide

$C_i(T)$: 2.0419 x 10^{-5} mol/L

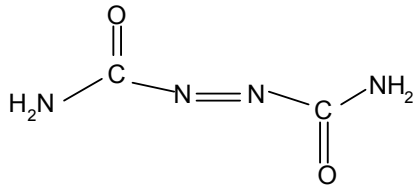
Table A14. Data for azodicarbonamide to 318.1°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^6$ (mole fraction)
100.51	9.585	7.766	0.108	11.418	0.23	7.64
125.42	8.605	7.581	0.187	15.427	0.14	9.80
150.28	8.097	7.193	0.141	16.877	0.126	9.63
174.33	7.110	6.320	0.205	17.763	0.125	9.20
200.05	6.776	6.097	0.252	18.468	0.111	9.93
225.85	6.007	5.440	0.276	19.034	0.104	10.29
250.28	5.664	5.153	0.303	19.485	0.010	10.57
275.64	5.280	4.924	0.335	19.891	0.072	14.20
300.55	5.016	4.744	0.345	20.245	0.057	17.59

Solvent: CO_2

Solute: Azodicarbonamide

Temperature: 328.1 °K

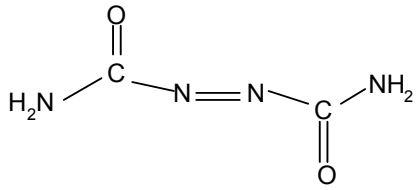


Azodicarbonamide

$C_i(T)$: 1.8501×10^{-5} mol/L

Table A15. Data for azodicarbonamide to 328.1°K

System pressure (bar)	t_1 (minutes)	t_0 (minutes)	Flow (mL/min.)	CO_2 Density (gmol/L)	Capacity factor k_i	$y_i * 10^6$ (mole fraction)
100.32	9.247	7.986	0.114	7.416	0.158	15.80
125.27	8.227	7.250	0.153	12.328	0.135	11.14
149.75	7.560	6.725	0.193	14.853	0.124	10.03
175.35	6.993	6.290	0.232	16.249	0.112	10.19
200.01	6.055	5.461	0.268	17.153	0.109	9.92
224.82	5.580	5.172	0.301	17.848	0.079	13.14
248.75	5.451	5.095	0.312	18.395	0.070	14.39
275.66	5.047	4.735	0.335	18.915	0.066	14.84
300.25	4.840	4.610	0.354	19.326	0.050	19.19



Azodicarbonamide

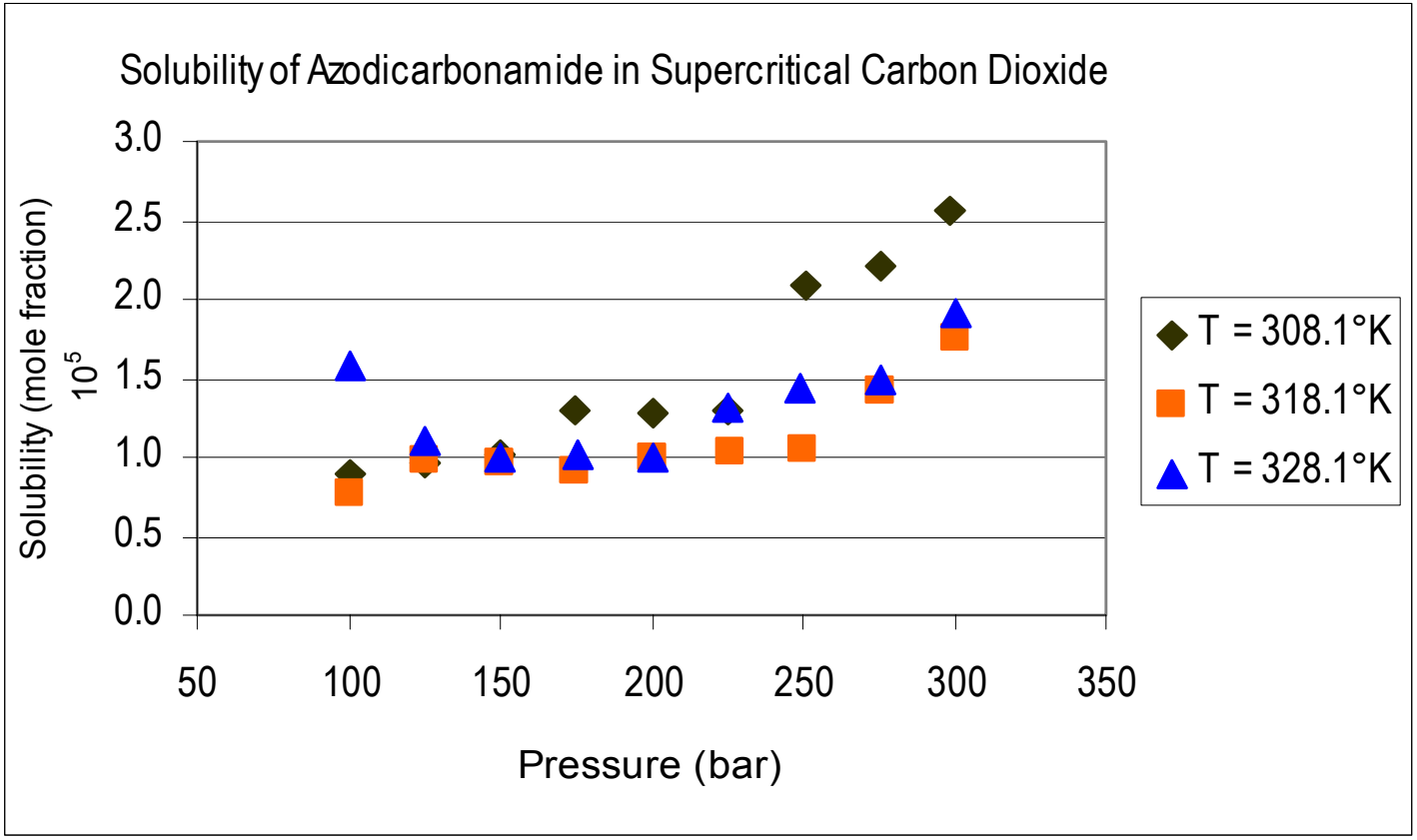


Figure 17. Solubility of Anti-AIDS Drug Azodicarbonamide in Supercritical Carbon Dioxide.

Appendix B

Data obtained from Extractions in the Supercritical Fluid Extractor

- Fluorouracil

$T_{cham.}$: 35°C P_p: 300 bar

T_{p1} (°C)	T_{p2} (°C)	$T_{av.}$ (°C)	T_{a1} (°C)	T_{a2} (°C)	w_1 (g)	w_2 (g)	dw (g)	v_1 (mL)	v_2 (mL)	dv (mL)
30.9	31.5	31.2	31.4	31.8	16.7482	16.7461	0.0021	145.38	94.70	50.68
28.6	30.6	29.6	30	31.5	16.7124	16.7100	0.0024	218.40	167.78	50.62

$T_{cham.}$: 55°C P_p: 300 bar

T_{p1} (°C)	T_{p2} (°C)	$T_{av.}$ (°C)	T_{a1} (°C)	T_{a2} (°C)	w_1 (g)	w_2 (g)	dw (g)	v_1 (mL)	v_2 (mL)	dv (mL)
32.5	31.7	32.1	30.5	32.4	16.7946	16.7892	0.0054	219.15	168.35	50.8
29	30.6	29.8	29.6	31.5	16.5933	16.5867	0.0066	214.58	165.25	49.33

- Benzoxazin

$T_{cham.}$: 35°C P_p: 300 bar

T_{p1} (°C)	T_{p2} (°C)	$T_{av.}$ (°C)	T_{a1} (°C)	T_{a2} (°C)	w_1 (g)	w_2 (g)	dw (g)	v_1 (mL)	v_2 (mL)	dv (mL)
30.4	31.2	30.8	30.8	31.7	17.0565	16.9773	0.0792	184.75	99.82	50.50
31.2	31.7	31.45	32.0	33.0	16.5027	16.4086	0.0941	78.75	33.0	45.75

$T_{cham.}$: 55°C P_p: 300 bar

T_{p1} (°C)	T_{p2} (°C)	$T_{av.}$ (°C)	T_{a1} (°C)	T_{a2} (°C)	w_1 (g)	w_2 (g)	dw (g)	v_1 (mL)	v_2 (mL)	dv (mL)
31	31.3	31.15	31.2	31.7	16.4160	16.2904	0.1256	228.01	176.94	51.07
31.5	31.0	31.25	31.7	32.0	16.4944	16.4010	0.0934	224.18	174.15	50.03

- Azodicarbonamide

$T_{cham.}$: 35°C P_p: 300 bar

T_{p1} (°C)	T_{p2} (°C)	$T_{av.}$ (°C)	T_{a1} (°C)	T_{a2} (°C)	w_1 (g)	w_2 (g)	dw (g)	v_1 (mL)	v_2 (mL)	dv (mL)
30.6	30.2	30.4	31.4	30.1	16.3609	16.3584	0.0025	147.0	96.84	50.16
27.9	29.8	28.85	28.5	30.5	16.6331	16.6288	0.0043	221.21	166.36	54.85

$T_{cham.}$: 55°C P_p: 300 bar

T_{p1} (°C)	T_{p2} (°C)	$T_{av.}$ (°C)	T_{a1} (°C)	T_{a2} (°C)	w_1 (g)	w_2 (g)	dw (g)	v_1 (mL)	v_2 (mL)	dv (mL)
29.8	31.0	30.4	30.8	31.5	16.4794	16.4765	0.0029	147.32	96.02	51.3
24.8	30.7	27.75	29.0	31.0	16.7060	16.7040	0.0020	208.20	157.90	50.3

- Thymidine

$T_{cham.}$: 35°C P_p: 300 bar

T_{p1} (°C)	T_{p2} (°C)	$T_{av.}$ (°C)	T_{a1} (°C)	T_{a2} (°C)	w_1 (g)	w_2 (g)	dw (g)	v_1 (mL)	v_2 (mL)	dv (mL)
32.3	32.0	32.15	32.5	33.0	16.3399	16.3375	0.0024	139.04	86.22	52.82
30.7	32.5	31.6	31.5	32.8	16.5697	16.5681	0.0016	203.62	151.52	52.10

$T_{cham.}$: 55°C P_p: 300 bar

T_{p1} (°C)	T_{p2} (°C)	$T_{av.}$ (°C)	T_{a1} (°C)	T_{a2} (°C)	w_1 (g)	w_2 (g)	dw (g)	v_1 (mL)	v_2 (mL)	dv (mL)
30.5	29.0	29.75	31.0	30.0	16.1261	16.1248	0.0013	138.01	87.07	50.94
30.0	31.4	30.7	30.7	32.8	16.2430	16.2400	0.0030	212.75	158.62	54.13

Appendix C

Determination of solubilities by gravimetric technique using the ISCO Extractor

- Benzoxazin (bzx)

M = 223.23 g/gmol

$T_{cham.}$ = 35°C P_p = 300 bar

dw = 0.0792 g dv = 50.5 mL

$T_{av.}$= 30.8°C $\rho(T_{av.}, P_p)$ = 21.49 mol/L

The density of the carbon dioxide was determined to the pump pressure (P_p) and to the average temperature (T_{av}).

Moles of CO_2 = $\rho(T_{av.}, P_p)$ * dv/1000

Moles of CO_2 = 1.08

Moles of bzx = dw/M

Moles of bzx = 3.5×10^{-4}

Solubility : y_i = Moles of bzx/Moles of CO_2

$y_i = 3.3 \times 10^{-4}$

$T_{cham.}$ = 35°C P_p = 300 bar

dw = 0.0941 g dv = 45.75 mL

$T_{av.}$= 31.45°C $\rho(T_{av.}, P_p)$ = 21.435 mol/L

Moles of CO_2 = 0.98

Moles of bzx = 4.2×10^{-4}

$y_i = 4.3 \times 10^{-4}$

$T_{cham.}$ = 55°C P_p = 300 bar

dw = 0.1256 g dv = 51.07 mL

$T_{av.}$= 31.15°C $\rho(T_{av.}, P_p)$ = 21.46 mol/L

Moles of CO_2 = 1.09

Moles of bzx = 5.6×10^{-4}

y_i = 5.1×10^{-4}

$T_{cham.}$ = 55°C P_p = 300 bar

dw = 0.0934 g dv = 50.03 mL

$T_{av.}$= 31.25°C $\rho_{co_2}(T_{av.}, P_p)$ = 21.452 mol/L

Moles of CO_2 = 1.07

Moles of bzx = 4.2×10^{-4}

y_i = 3.9×10^{-4}

The following are the results for other drugs studied:

- Fluorouracil (flr)

M = 130.1 g/gmol

$T_{cham.}$ = 35°C P_p = 300 bar

Moles of CO_2 = 1.09

Moles of flr = 1.6×10^{-5}

y_i = 1.5×10^{-5}

$T_{cham.}$ = 35°C P_p = 300 bar

Moles of CO_2 = 1.09

Moles of flr = 1.84×10^{-5}

$y_i = 1.7 \times 10^{-5}$

$T_{cham.}$ = 55°C P_p = 300 bar

Moles of CO_2 = 1.09

Moles of flr = 4.15×10^{-5}

$y_i = 3.8 \times 10^{-5}$

$T_{cham.}$ = 55°C P_p = 300 bar

Moles of CO_2 = 1.06

Moles of flr = 5.1×10^{-5}

$y_i = 4.8 \times 10^{-5}$

- Azodicarbonamide (azd)

M = 116.08 g/gmol

$T_{cham.}$ = 35°C P_p = 300 bar

Moles of CO_2 = 1.08

Moles of azd = 2.15×10^{-5}

$y_i = 2 \times 10^{-5}$

$T_{cham.}$ = 35°C P_p = 300 bar

Moles of CO_2 = 1.19

Moles of azd = 3.7×10^{-5}

y_i = 3.12×10^{-5}

$T_{cham.}$ = 55°C P_p = 300 bar

Moles of CO_2 = 1.10

Moles of azd = 2.5×10^{-5}

y_i = 2.3×10^{-5}

$T_{cham.}$ = 55°C P_p = 300 bar

Moles of CO_2 = 1.09

Moles of azd = 1.7×10^{-5}

y_i = 1.6×10^{-5}

- Thymidine (thy)

M = 242.23 g/gmol

$T_{cham.}$ = 35°C P_p = 300 bar

Moles of CO_2 = 1.13

Moles of thy = 9.9×10^{-6}

y_i = 8.8×10^{-6}

$T_{cham.}$ = 35°C P_p = 300 bar

Moles of CO_2 = 1.12

Moles of thy = 6.6×10^{-6}

y_i = 5.9×10^{-6}

$T_{cham.}$ = 55°C P_p = 300 bar

Moles of CO_2 = 1.1

Moles of thy = 5.4×10^{-6}

y_i = 4.9×10^{-6}

$T_{cham.}$ = 55°C P_p = 300 bar

Moles of CO_2 = 1.16

Moles of thy = 1.2×10^{-5}

y_i = 1.1×10^{-5}

Appendix D

Estimation of the parameter $C_i(T)$ for each temperature and studied drug

- Fluorouracil

T = 35.1°C k = 0.039829 ρ_{co_2} = 21.1250 gmol/L

We estimated the $C_i(T)$ values with the equation 8:

$y_1 = 1.5 \times 10^{-5}$ $C_1(T) = 1.2 \times 10^{-5}$ mol/L

$y_2 = 1.7 \times 10^{-5}$ $C_2(T) = 1.4 \times 10^{-5}$ mol/L

$C(T) = 1.3 \times 10^{-5}$ mol/L (media value)

T = 55.1°C k = 0.024761 ρ_{co_2} = 19.33 gmol/L

$y_1 = 3.8 \times 10^{-5}$ $C_1(T) = 1.8 \times 10^{-5}$ mol/L

$y_2 = 4.8 \times 10^{-5}$ $C_2(T) = 2.3 \times 10^{-5}$ mol/L

$C(T) = 2.0 \times 10^{-5}$ mol/L (media value)

The value for 45.1°C was calculated by means of a regression equation obtained as: $\log[C_i(T)]$ versus 1/T (T in Kelvin degree) with the values obtained experimentally.

The regression equation for the values of fluorouracil is:

$$\log [C_i(T)] = -947.98/T - 1.7978$$

The value for 318.1°K (45.1°C) of $C_i(T)$ was of 1.7×10^{-5} mol/L

- Benzoxazin

$T = 35.2°C$ $k = 0.035199$ $\rho_{co_2} = 21.12$ gmol/L

$y_1 = 3.3 \times 10^{-4}$ $C_1(T) = 2.4 \times 10^{-5}$ mol/L

$y_2 = 4.3 \times 10^{-4}$ $C_2(T) = 3.2 \times 10^{-4}$ mol/L

$C(T) = 2.8 \times 10^{-4}$ mol/L (media value)

$T = 55.1°C$ $k = 0.031567$ $\rho_{co_2} = 19.32$ gmol/L

$y_1 = 5.1 \times 10^{-4}$ $C_1(T) = 3.1 \times 10^{-4}$ mol/L

$y_2 = 3.9 \times 10^{-4}$ $C_2(T) = 2.4 \times 10^{-4}$ mol/L

$C(T) = 2.8 \times 10^{-4}$ mol/L (media value)

The regression equation for the values of benzoxazin is:

$$\log [C_i(T)] = 46.511/T - 3.7017$$

The value for 318.1°K (45.1°C) of $C_i(T)$ was of 2.8×10^{-4} mol/L

- Azodicarbonamide

$T = 35.1°C$ $k = 0.042037$ $\rho_{co_2} = 21.102$ gmol/L

$y_1 = 2 \times 10^{-5}$ $C_1(T) = 1.8 \times 10^{-5}$ mol/L

$y_2 = 3.1 \times 10^{-5}$ $C_2(T) = 2.8 \times 10^{-5}$ mol/L

$C(T) = 2.268016 \times 10^{-5}$ mol/L (media value)

T = 55.1°C k = 0.049892 ρ_{co_2} = 19.326 gmol/L

$y_1 = 2.3 \times 10^{-5}$ $C_1(T) = 2.2 \times 10^{-5}$ mol/L

$y_2 = 1.6 \times 10^{-5}$ $C_2(T) = 1.5 \times 10^{-5}$ mol/L

$C(T) = 1.8 \times 10^{-5}$ mol/L (media value)

The regression equation for the values of azodicarbonamide is:

$$\log [C_i(T)] = 447.04/T - 6.0953$$

The value for 318.1°K (45.1°C) of $C_i(T)$ was of 2.0×10^{-5} mol/L

- Thymidine

T = 35.1°C k = 0.019197 ρ_{co_2} = 21.121 gmol/L

$y_1 = 8.8 \times 10^{-6}$ $C_1(T) = 3.6 \times 10^{-6}$ mol/L

$y_2 = 5.9 \times 10^{-6}$ $C_2(T) = 2.4 \times 10^{-6}$ mol/L

$C(T) = 3 \times 10^{-6}$ mol/L (media value)

T = 55.1°C k = 0.052108 ρ_{co_2} = 19.321 gmol/L

$y_1 = 4.\ 9 \times 10^{-6}$ $C_1(T) = 4.9 \times 10^{-6}$ mol/L

$y_2 = 1.1 \times 10^{-5}$ $C_2(T) = 1.1 \times 10^{-5}$ mol/L

$C(T) = 7.8 \times 10^{-6}$ mol/L (media value)

The regression equation for the values of thymidine is:

$$\log [C_i(T)] = -2117.2/T + 1.3459$$

The value for 318.1°K (45.1°C) of $C_i(T)$ was of 4.9×10^{-6} mol/L

- Taxol

T = 35°C P = 275 bar k = 0.070196 ρ_{co_2} = 20.822 gmol/L

$y = 3.72 \times 10^{-6}$ $C(T) = 5.4 \times 10^{-6}$ mol/L

T = 55°C P = 275 bar k = 0.200464 ρ_{co_2} = 18.911 gmol/L

$y = 5.91 \times 10^{-6}$ $C(T) = 2.2 \times 10^{-5}$ mol/L

The regression equation for the values of taxol is:

$$\log [C_i(T)] = -3106.3/T + 4.8207$$

The value for 318.1°K (45.1°C) of $C_i(T)$ was of 1.1×10^{-5} mol/L

Appendix E

Experimental Design

In the present research we have worked with the following variables: pressure, temperature and flow, but due to considerations of time and economics, in the experiments carried out, we have varied only two variables, temperature and pressure of the system.

Therefore, for study how these variables (pressure and temperature) and their interaction affect the solvating power of the supercritical CO_2 with the studied drugs, we have chosen only 5 levels in the variable pressure (we had 9 levels, from 100 to 300 bar with increments of 25 bar) and 3 levels in the variable temperature (35, 45 and 55 °C). Every data was replicated twice. The analysis was realized using a factorial experiment and lineal regression model (Ordinary least square method).

These are the following variables without encode:

X_A: Pressure (5 levels: 100, 150, 200, 250, 300 bar)
X_B: Temperature (3 levels: 35.1, 45.1, 55.1 °C)
y: solubility (mole fraction)

The proposed model for the study is the following:

$$Y = \beta_0 + \beta_1 X_A + \beta_2 X_B + \beta_3 X_A X_B + \varepsilon \qquad (C1)$$

Where β's are the coefficients of the model and ε is the error.

For obtain the β's values, we must to resolve the following equation:

$$\beta's = (X'*X)^{-1}X'*y \qquad (C2)$$

X : Codified matrix of the levels of every variable.

X' : Transposed matrix

y : Response matrix (solubility values)

Data for the encoded matrix obtained of the levels for the variables pressure and temperature. These data was obtained in the following way:

$$Xi = (\epsilon i - \epsilon p)/(Ri/2) \qquad (C3)$$

Where:

ϵi: Variable in its natural state

ϵp: Average value of that variable

Ri: Range of the variable

For temperature the encoded levels are:

$\epsilon p = (35.1+45.1+55.1)/3$ $\epsilon p = 45.1$

$Ri = 55.1 - 35.1$ $Ri = 20$

Applying the equation (C3):

For T = 35.1°C $X_{35.1} = (35.1 - 45.1)/(20/2)$ $X_{35.1} = -1$

For T = 45.1°C $X_{45.1} = (45.1 - 45.1)/(20/2)$ $X_{45.1} = 0$

For T = 55.1°C $X_{55.1} = (55.1 - 45.1)/(20/2)$ $X_{55.1} = 1$

For pressure the encoded levels are:

$\epsilon_p = (100+150+200+250+300)/5 \quad \epsilon_p = 200$

$Ri = 300 - 100 \qquad Ri = 200$

Applying the equation (C3):

For P = 100 bar $\quad X_{100} = (100 - 200)/(200/2) \quad X_{100} = -1$

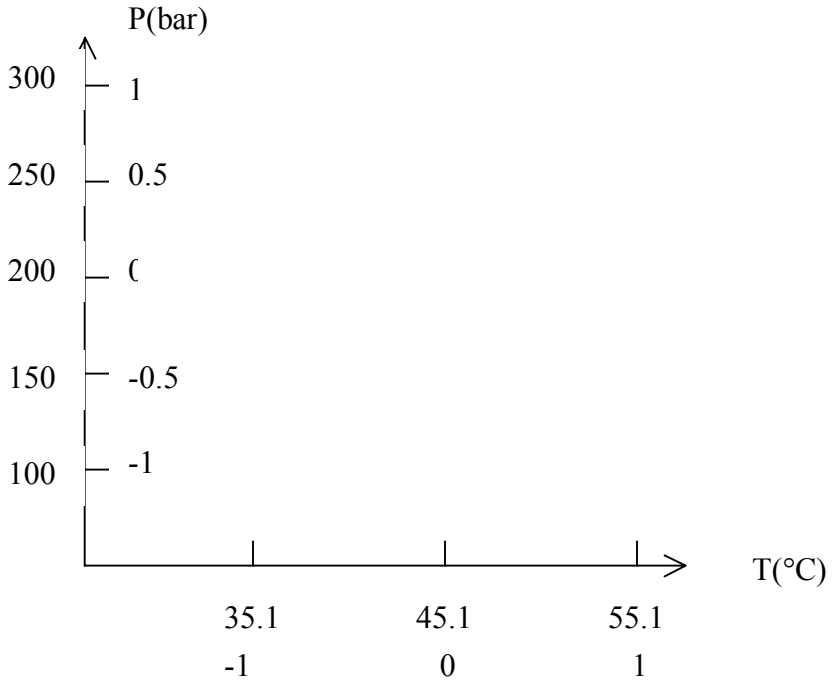
For P = 150 bar $\quad X_{150} = (150 - 200)/(200/2) \quad X_{150} = -0.5$

For P = 200 bar $\quad X_{200} = (200 - 200)/(200/2) \quad X_{200} = 0$

For P = 250 bar $\quad X_{250} = (250 - 200)/(200/2) \quad X_{250} = 0.5$

For P = 300 bar $\quad X_{300} = (300 - 200)/(200/2) \quad X_{300} = 1$

Levels without encoded and encoded for each variable:



For fluorouracil the encoded matrix X and y (values of solubility) are:

Matrix X

β_0	β_1	β_2	β_3
1	0	0	0
1	-0.5	1	-0.5
1	-0.5	-1	0.5
1	1	1	1
1	0.5	-1	-0.5
1	-1	1	-1
1	-0.5	0	0
1	-1	-1	1
1	0.5	1	0.5
1	0.5	0	0
1	0	1	0
1	-1	0	0
1	1	-1	-1
1	0	-1	0
1	1	0	0

Matrix $y*10^6$

6.2218
10.3170
4.4279
42.9417
7.2352
13.1720
6.2043
3.5356
14.4073
8.7667
10.4751
6.3314
15.8612
5.4717
22.5818

These calculations were performed in the Matlab program of the following way:

It was calculated the transpose (X') of the matrix X, the product X'*X.

The result of the product X'*X is the same for all experiments performed, without to consider the order of the runs (the runs were performed randomize):

X'*X =

15	0	0	0
0	7.5	0	0
0	0	10	0
0	0	0	5

The inverse of the product X'*X is:

$(X'*X)^{-1} =$

0.0667	0	0	0
0	0.1333	0	0
0	0	0.1000	0
0	0	0	0.2000

The product of the transpose matrix X' and the matrix y is:

$X'*_V =$

177.9507
63.0757
54.7815
18.0856

The coefficient values for β (obtained with the equation C2) are the following:

β_0	β_1	β_2	β_3
11.8634	8.4101	5.4781	3.6171

The model obtained is: $y*10^6 = 11.8634 + 8.4101X_A + 5.4781X_B + 3.6171X_AX_B$

For thymidine the encoded matrix X and y (values of solubility) are:

Matrix X

β_0	β_1	β_2	β_3
1	-1	-1	1
1	-0.5	1	-0.5
1	0.5	0	0
1	0	-1	0
1	-1	1	-1
1	-0.5	0	0
1	1	-1	-1
1	0.5	1	0.5
1	0	0	0
1	0.5	-1	-0.5
1	1	1	1
1	-0.5	-1	0.5
1	0	1	0
1	1	0	0
1	-1	0	0

Matrix $y*10^6$

1.2361
2.9058
6.4534
1.7900
3.0140
2.3906
7.3470
3.7186
4.0362
2.0523
7.7626
1.4319
3.1264
19.5832
2.4323

These calculations were performed in the Matlab program of the following way:

It was calculated the transpose (X') of the matrix X, the product X'*X.

The result of the product X'*X is the same for all experiments performed, without to consider the order of the runs (the runs were performed randomize):

X'*X =

15	0	0	0
0	7.5	0	0
0	0	10	0
0	0	0	5

The inverse of the product X'*X is:

$(X'*X)^{-1} =$

0.0667	0	0	0
0	0.1333	0	0
0	0	0.1000	0
0	0	0	0.2000

The product of the transpose matrix X' and the matrix y is:

$X'*y =$

69.2804
30.7584
6.6701
-1.2661

The coefficient values for β (obtained with the equation C2) are the following:

β_0	β_1	β_2	β_3
4.6187	4.1011	0.6670	-0.2532

The model obtained is: $y*10^6 = 4.6187 + 4.1011X_A + 0.6670X_B - 0.2532X_AX_B$

For benzoxazin the encoded matrix X and y (values of solubility) are:

Matrix X

β_0	β_1	β_2	β_3
1	0.5	1	0.5
1	0	-1	0
1	-1	0	0
1	1	1	1
1	0.5	0	0
1	-0.5	1	-0.5
1	0.5	-1	-0.5
1	1	0	0
1	-1	-1	1
1	0	1	0
1	-0.5	-1	0.5
1	-0.5	0	0
1	1	-1	-1
1	-1	1	-1
1	0	0	0

Matrix $y*10^5$

39.9682
10.9053
8.6317
45.1544
12.3476
13.2368
11.9144
46.3400
7.6269
20.8159
7.5912
7.6378
37.8429
18.4406
9.4664

$X'*X =$

15	0	0	0
0	7.5	0	0
0	0	10	0
0	0	0	5

$(X'*X)^{-1} =$

0.0667	0	0	0
0	0.1333	0	0
0	0	0.1000	0
0	0	0	0.2000

The product of the transpose matrix X' and the matrix y is:

$$X'*y = \begin{bmatrix} 297.9201 \\ 112.5203 \\ 61.7352 \\ 7.7019 \end{bmatrix}$$

The coefficient values for β are the following:

β_0	β_1	β_2	β_3
19.8613	15.0027	6.1735	1.5404

The model obtained is: $y*10^5 = 19.8613 + 15.0027X_A + 6.1735X_B + 1.5404X_AX_B$

For azodicarbonamide the encoded matrix X and y (values of solubility) are:

Matrix X

β_0	β_1	β_2	β_3
1	0.5	-1	-0.5
1	-0.5	1	-0.5
1	0	0	0
1	0.5	1	0.5
1	-1	0	0
1	-0.5	-1	0.5
1	1	0	0
1	0.5	0	0
1	1	-1	-1
1	-1	-1	1
1	0	1	0
1	1	1	1
1	0	-1	0
1	-0.5	0	0
1	-1	1	-1

Matrix $y*10^6$

20.8297
10.0321
9.9282
14.3944
7.6352
10.1656
17.5915
10.5678
25.5675
8.9002
9.9162
19.1881
12.8721
9.6270
15.7995

$X'*X =$

15	0	0	0
0	7.5	0	0
0	0	10	0
0	0	0	5

$(X'*X)^{-1} =$

0.0667	0	0	0
0	0.1333	0	0
0	0	0.1000	0
0	0	0	0.2000

The product of the transpose matrix X' and the matrix y is:

$$X'*y = \begin{bmatrix} 203.0151 \\ 37.9958 \\ -9.0048 \\ -16.4296 \end{bmatrix}$$

The coefficient values for β are the following:

β_0	β_1	β_2	β_3
13.5343	5.0661	-0.9005	-3.2859

The model obtained is: $y*10^5 = 13.5343 + 5.0661X_A - 0.9005X_B - 3.2859X_AX_B$

For taxol the encoded matrix X and y (values of solubility) are:

Matrix X

β_0	β_1	β_2	β_3
1	0.5	-1	-0.5
1	-0.5	1	-0.5
1	0	0	0
1	0.5	1	0.5
1	-1	0	0
1	-0.5	-1	0.5
1	1	0	0
1	0.5	0	0
1	1	-1	-1
1	-1	-1	1
1	0	1	0
1	1	1	1
1	0	-1	0
1	-0.5	0	0
1	-1	1	-1

Matrix $y*10^6$

3.5511
6.1616
2.5985
5.8617
3.8083
1.2409
5.1201
2.7043
6.2829
1.3261
5.7609
8.9626
2.2165
2.7498
11.1091

$X'*X =$

15	0	0	0
0	7.5	0	0
0	0	10	0
0	0	0	5

$(X'*X)^{-1} =$

0.0667	0	0	0
0	0.1333	0	0
0	0	0.1000	0
0	0	0	0.2000

The product of the transpose matrix X' and the matrix y is:

$$X'*y = \begin{bmatrix} 69.4544 \\ 5.1045 \\ 23.2384 \\ -8.4083 \end{bmatrix}$$

The coefficient values for β are the following:

β_0	β_1	β_2	β_3
4.6303	0.6806	2.3238	-1.6817

The model obtained is: $y*10^5 = 4.6303 + 0.6806X_A + 2.3238X_B - 1.6817X_AX_B$