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**SUPERCRITICAL FLUID EXTRACTION OF PLANT
AND ENVIRONMENTAL SAMPLES**

by

WAN AINI WAN IBRAHIM

B.Sc.(Hons), PGCE, M.Sc.

A Doctoral Thesis

**submitted in partial fulfilment of the
requirements for the award of the Doctor of Philosophy
of Loughborough University of Technology**

October 1994

Supervisor: Dr. R. M. Smith

Department of Chemistry

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**Dedicated to my husband, Zuhaimy,
my children, Hanis, Hanan, Attiyaa and Aliyaa,
my late mum, Wan Zubaidah Wan Salleh
and my father, Haji Wan Ibrahim Haji Wan Ismail.**

ACKNOWLEDGEMENTS

First and foremost my gratitude goes to Almighty Allah for the mercy and strength given to me throughout my entire study and for the completion of this work.

I would like to thank my supervisor, Dr. Roger M. Smith for his invaluable help and advice in the project and in the preparation of this thesis. I would also like to thank University Teknologi Malaysia for a study leave and the Public Service Department of Malaysia for financial support throughout my studies. I thank Dow Elanco for the gift of Dursban granules and chlorpyrifos and Wessex Water plc. for the sludge sample and for the analysis.

My thanks also goes to the technical staff, Elaine, Bev, John, Alan, Simon and Mr. Bower and also to the friends in the lab, Dave, Hasan, Yuan, Li, Tanya, Arjinder, Nil, Shereen, Archana, Paul, Andy, Alan and Ramin. Also to friends who have helped to look after my children.

Last but not least, many thanks to my family for their support and encouragement throughout my studies and most importantly I want to thank my husband, Zuhaimy for his endless support and help with the children throughout my entire studies and to my children, Hanis, Hanan, Attiyaa and Aliyaa for their patience in putting up with my studies. May Allah bless you all.

ABSTRACT

Since the inception of analytical supercritical fluid extraction (SFE) in the early 80's, this technique has garnered great attractions in the extractions of variety of analytes from variety of matrices. In this study, supercritical carbon dioxide (SC CO₂) has been examined as a sample preparation method for the extraction of eugenol from plant matrix prior to high performance liquid chromatography (HPLC) analysis and for the extraction of organochlorine pesticides (OCPs) from sewerage sludges and chlorpyrifos from formulation and soil samples prior to capillary gas chromatography (GC) analysis. This is an area of considerable interest as many current methods use environmentally hazardous chlorinated solvents and alternative methods are required.

Although numerous studies have examined the potential application of SFE to isolate pesticides and plant products, the work has been qualitative rather than quantitative. The present work describes studies which have examined the supercritical conditions needed for complete extraction of the pesticides and plant product, eugenol. Initially a complex matrix, sludge was chosen. Later a simple matrix, soil was chosen and a single pesticide, chlorpyrifos was used as the SFE of sludge was unsuccessful. In the extraction of chlorpyrifos, problems were encountered in the trapping of the extract on depressurisation of the SC CO₂. The effect of collection solvent, CO₂ flow rate, solvent depth, and restrictor heating on the trapping efficiency have been investigated. Two methods of trapping were evaluated. Once a quantitative trapping method was

established, the effect of different soil matrices on the recovery of chlorpyrifos at different chlorpyrifos spiking level was investigated. The SFE of soil was compared to Soxhlet extraction.

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CHAPTER 1

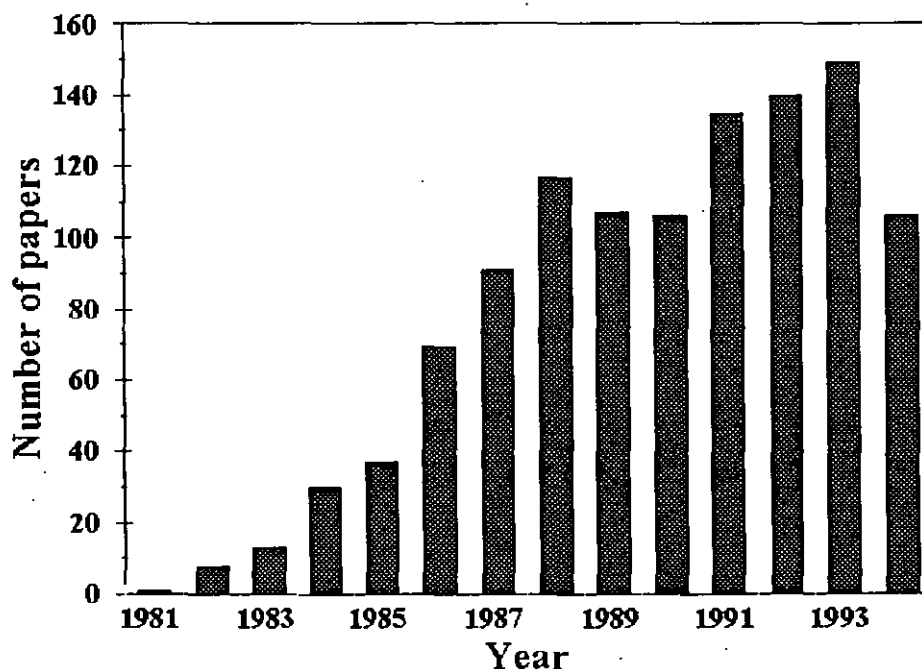
INTRODUCTION

1.0 INTRODUCTION

In the last ten years or so, there has been considerable interest in the use of supercritical fluids (SFs) as an extraction medium and they are becoming an important tool in analytical science (1). This is reflected in the numbers of papers published since the early 80's (Figure 1). The main driving force, behind the growth of the use of SFs as an extraction medium, is the need to replace existing traditional extraction methods (liquid-liquid and liquid-solid extractions) with alternative methods which are faster, more selective, non-destructive, cost effective, use non-hazardous solvents and offer the possibility for automation. The need for alternative extraction methods is emphasised by current efforts of the US Environmental Protection Agency (EPA) to reduce the use of methylene chloride, as an extraction fluid for environmental sample preparation, by 95% over the next few years (2). The adoption of the 1987 Montreal Protocol (3) which eliminates or bans the use of environmentally hazardous solvents such as chlorofluorocarbons (CFCs), which deplete the ozone layer, has also fuelled the search for alternative extraction media. The use of SF fulfils these requirements because most SFE uses carbon dioxide as solvent. Carbon dioxide is non-hazardous, non-toxic, inexpensive and it is easily

available and since it is a gas it is vented to the atmosphere (where it was taken from) on depressurisation of the SF.

Figure 1. Frequency of published articles on SFE based on a survey, August, 1994 (4).



1.1 HISTORY OF SUPERCRITICAL FLUIDS IN ANALYTICAL CHEMISTRY

Historically the first person to note the nature of a SF was Caignard de la Tour in 1822 (5). In 1879, Hannay and Hogarth (6) studied the solubility of cobalt and iron chlorides in supercritical ethanol and in 1958

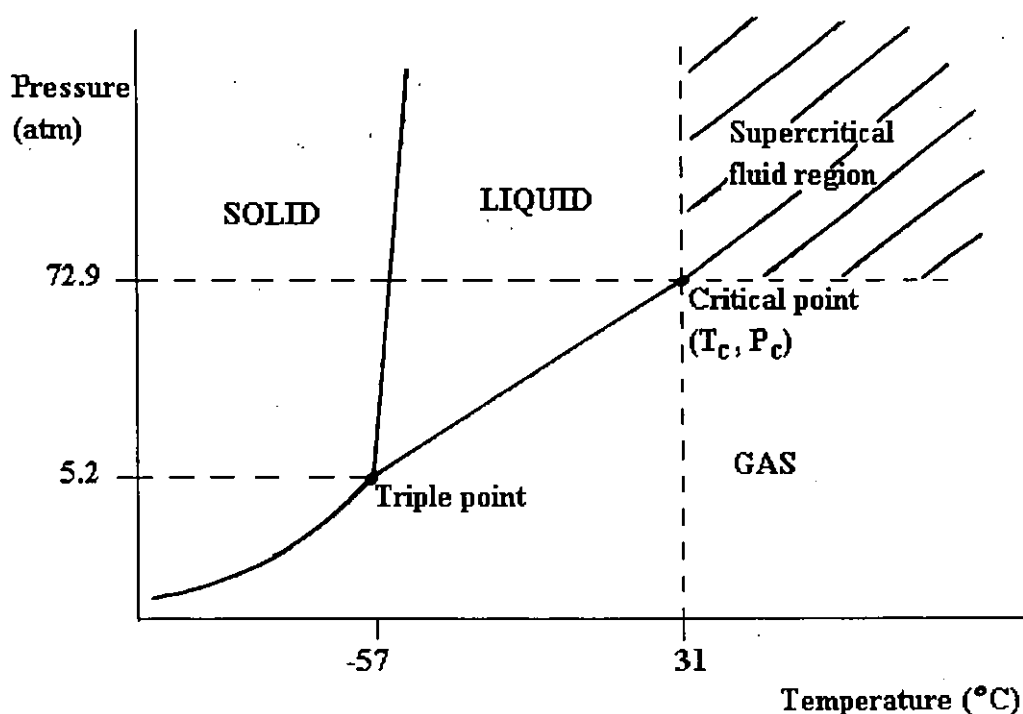
Lovelock (7) suggested that a SF might be used as a mobile phase in chromatography. In 1962, Klesper, Turner, Corwin and co-workers (8) used SF Freon as the mobile phase to transport metal porphyrins through a chromatographic column. Sie and Rijnders (9) and Giddings (10) developed the technique further. In 1972, Jentoft and Gouw (11) analysed petroleum derived mixtures by supercritical fluid chromatography (SFC) and in 1981, Novotny and Lee (12) demonstrated the first use of capillary SFC. In the same year, commercially packed column SFC were made available and four years later the first capillary column SFC instrument was available on the market. In the area of extraction, Zosel (13) was the first to use SF as an extraction medium in the early 60s. Since then this method has been developed as an industrial scale extraction techniques as reported by many research groups, such as Hubert and Vitzthum (14), Peter and Brunner (15) and Stahl *et al.* (16, 17).

1.2 SUPERCRITICAL FLUIDS

A supercritical fluid is an element or compound which has been raised above its critical temperature (T_c) and critical pressure (P_c). As long as these two parameters exceeded their critical values then a SF results. Once a SF is formed it cannot be characterised as either a gas or a liquid. The density of the liquid and gaseous phases becomes identical as these two phases coalesce into a fluid. The supercritical fluid region is denoted by the dashed lines in the phase diagram of carbon dioxide (Figure 2) since no phase changes occurs on crossing them. There is a distinctive change in

going from a solid to a liquid or from a solid to a gas (both with increasing pressure) hence the solid line dividing that boundary.

Figure 2. Phase diagram of carbon dioxide.



SF have several characteristic properties which make them suitable for extraction purposes. Their often lower viscosity and higher diffusion rate than liquids make the mass transfer during extraction rapid. The solvent power of a SF can be controlled by changing the pressure and to some extent the temperature. Many SF have a low critical temperature and this enables extractions to be carried out at a low temperature and may

avoid decomposition of thermally labile compounds. Concentration of the extract by distillation of solvent is also eliminated since many SF are gases at ambient temperature and are lost by vaporisation on depressurisation. SFE can also be coupled to chromatographic (HPLC, GC, SFC) and spectroscopic techniques (IR and MS) to provide specific identification or structural information, with the advantage that solvent is readily removed.

1.2.1 PROPERTIES OF SUPERCRITICAL FLUIDS

SF are sometimes considered to be "super solvents", but this is not true when their solvating power is compared with that of liquids. The solvent strengths of SF approach those of liquids only as their density is increased. The density at critical point of CO₂ is 0.47 g ml⁻¹, the density of CO₂ at 400 atm, 31.3°C is 0.96 g ml⁻¹ and the density of liquid CO₂ at 63.4 atm, 25°C is 0.93 g ml⁻¹ (18). Although SF do not have any advantage over liquid solvents in overall solvating power, there are several other characteristics of SFs which makes them an ideal solvent for analytical extraction. The physical properties of a SF are generally intermediate between those of a liquid and a gas (Table 1). The SF possess a liquid-like density over much of the range of industrial interest and generally has a viscosity which is an order of magnitude smaller and diffusivities an order of magnitude higher than a liquid. Their mass transfer characteristics are therefore much better than a liquid and this provides appreciable penetrating power into the solute matrix. Generally SFE takes about 10-60 minutes for complete extraction, whereas liquid extraction may take several hours or even days to perform.

Table 1. Typical physical properties associated with different fluid states of carbon dioxide (19).

Phase	Density (g cm^{-3})	Diffusivity ($\text{cm}^2 \text{s}^{-1}$)	Viscosity ($\text{g cm}^{-1}\text{s}$)
Gas P=1atm, T=15-30°C	$(0.6-2) \times 10^{-3}$	0.1 - 0.4	$(1 - 3) \times 10^{-4}$
SF P=73-300 atm T=31°C	0.2 - 0.9	$(0.2 - 0.7) \times 10^{-3}$	$(1 - 9) \times 10^{-4}$
Liquid P=1atm, T= 15-30°C	0.6 - 1.6	$(0.2 - 2) \times 10^{-6}$	$(0.2 - 3) \times 10^{-2}$

1.2.2 SELECTION OF SOLVENT FOR EXTRACTION

Currently, many SFs have been examined for use in chromatography and extraction. Table 2 lists some of them along with their critical properties. Most are gaseous at ambient temperature, which makes their disposal easy (i.e. no disposal fees to pay). The selection of a SF for extraction is dictated by several factors. Because of practical considerations, the majority of SFE investigations reported to date have used CO_2 . This is because it is relatively non-reactive, non-toxic, non-flammable, available at high purity at a low cost and has relatively low critical parameters. CO_2 is also capable of dissolving a wide range of non-polar and moderately polar molecules. Unfortunately, CO_2 is not a good solvent for polar analytes. Nitrous oxide has similar critical properties to CO_2 but its use should be discouraged since it may form an explosive

mixture when combined with easily oxidised organic material (20). Ammonia, being a polar molecule would be good as a SF solvent for polar molecules. However, its use is limited because of corrosion or toxicity hazards (21).

Table 2. Characteristics of representative SFs used for SFE. The values given for the Hildebrand solubility parameter (δ) are the maximum at high pressure (22).

Fluid	P_c (atm)	T_c ($^{\circ}\text{C}$)	δ (cal/cm^2) ^{1/2}	Dipole moment (db)
CO ₂	72.8	31.0	10.7	0.0
N ₂ O	71.5	36.4	10.6	0.2
NH ₃	112.0	132.2	13.2	1.5
SF ₆	37.1	45.6	7.6	0.0
Xe	57.6	16.5	9.5	0.0
CHF ₃	48.0	26.1	8.7	1.6
CHClF ₂	49.1	96.1	8.8	1.4
C ₂ H ₆	48.2	32.2	8.7	0.0
n-C ₄ H ₁₀	37.5	152.0	7.7	0.0

1.3 USE OF MODIFIERS

The solvating power of CO₂ is limited to non-polar analytes such as aliphatic hydrocarbons with up to 20 carbon atoms, most small aromatic

hydrocarbons, halogenated hydrocarbons, steroids, fats, organochlorine pesticides, aldehydes, ketones, esters and low alcohols, but can be increased by the addition of polar organic compounds known as modifiers (also known as entrainers, co-solvents, moderators). Most liquid co-solvents have solubility parameters which are larger than that of CO₂, so that they may be used to increase extraction strength. Table 3 gives a summary of the large increase in solubility that may be obtained with a simple co-solvent (23, 24). Co-solvents, unlike carbon dioxide, can form electron donor-acceptor complexes (e.g. H-bonds) with certain polar solutes to influence solubilities and selectivities beyond those that would be expected based on volatilities alone.

Table 3. Effects of co-solvents on solubilities in SFs at 35°C (23, 24).

Solute	Co-solvent	$y_{\text{binary}}/y_{\text{CO}_2}$
acridine	3.5% methanol	2.3
2-aminobenzoic acid	3.5% methanol	7.2
cholesterol	9% methanol	100
hydroquinone	2% tributyl phosphate	>300
hydroquinone	0.65% AOT, W _o = 10, 6% octanol	>>100

In packed column SFC small (<1%) amounts of modifier produce large retention changes, presumably because the modifier also competes

with the solute for active sites in the packed column (25, 26). These effect also affect capillary SFC. However, Yonker and Smith demonstrated that modifier concentrations need to be of the order of 5-20 mol% in carbon dioxide to effect large retention changes in capillary SFC (27). This could be due to the more effective solvation of the bonded phase by the solvent modifier for the packed column than for the capillary column or the elimination of active sites by the polar modifier.

The mechanisms of the interaction between analytes and modified fluids are not well understood, but there appears to be an unusually high concentration (clustering) of the co-solvent in the vicinity of some solutes. Solvatochromic dye studies indicate that polar analytes tend to concentrate within such clusters (23, 24, 28 - 30). A modifier may act by increasing the solubility of the analyte in the extraction fluid, or by competing with the target analyte for active sites on the matrix.

1.3.1 SELECTION OF MODIFIER

The use of different modifiers can yield quite different recoveries (Figure 3), and no clear criteria exist for the selection of the best modifier for a particular extraction (31). The type and quantity of modifier used with CO₂ in SFE is generally arrived at by trial and error as little solubility data for analytes in modified CO₂ exists. A logical first choice for a modifier would be a liquid that is itself a good solvent for the target analytes. Specific interactions expected between the modifier and the sample matrix may also be useful to consider. Some of the modifiers that

have been used in supercritical fluid technology with carbon dioxide as the primary SF are listed in Table 4 (31). To date, the most common modifier employed in SFE has been methanol, chosen because of its high solvent polarity parameter, and its ability to deactivate the active sites on column support in SFC (32 -34).

Figure 3. Recoveries of alkyl benzenesulphonates from municipal wastewater treatment sludge using a 15 minute SFE at 380 atm with pure CO₂ and N₂O, and different polarity modifiers in CO₂ (35).

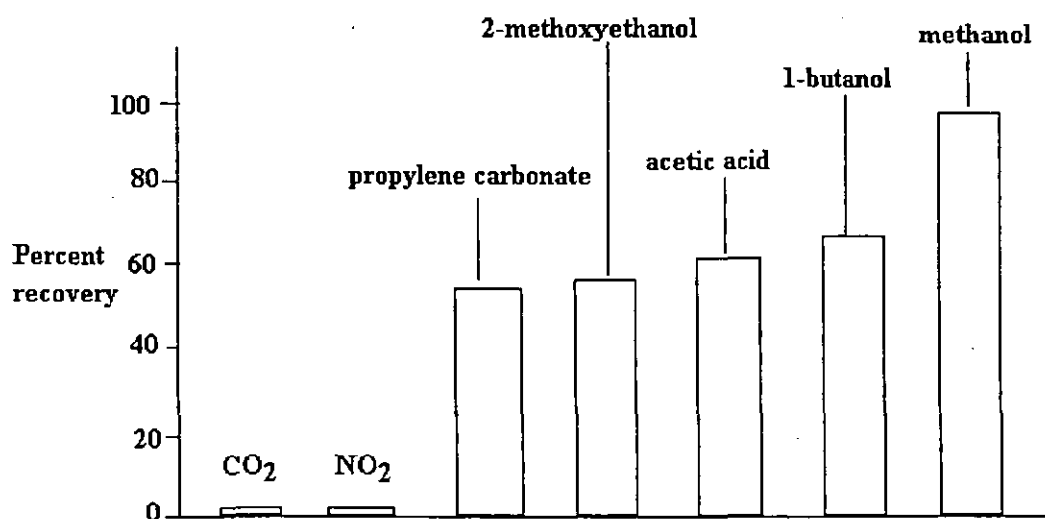


Table 4. Modifiers for use in SF technology with carbon dioxide as the primary mobile phase and their physical properties (31).

Modifier	T _c (°C)	P _c (atm)	Dielectric constant	Polarity index
Methanol	239.4	79.9	32.7	5.1
Ethanol	243.0	63.0	24.3	4.3
1-propanol	263.5	51.0	20.33	4.0
2-Propanol	235.1	47.0	18.3	3.9
1-Hexanol	336.8	40.0	13.3	3.5
2-Methoxyethanol	302	52.2	16.93	5.5
Tetrahydrofuran	67.0	51.2	7.58	4.0
1,4-Dioxane	314	51.4	2.25	4.8
Acetonitrile	275	47.7	37.5	5.8
Dichloromethane	237	60.0	8.93	3.1
Chloroform	263.2	54.2	4.81	4.1
Water	374.1	217.6	80.1	10.2
Formic acid	307	-	58.5	-
Dimethyl sulphoxide	465.0	-	46.68	7.2

1.3.2 MODIFIER ADDITION

In practice, modifiers can be introduced in a number of ways in SFC and SFE. The first method is by the use of pre-mixed cylinders which can be obtained commercially (Table 5) (36). The cylinder contains a discrete concentration level of a specific modifier in CO₂. This method eliminates the complexity and expense of operating a second high-pressure pump in the SFE or SFC system. However, this approach is clumsy and expensive

for selecting modifiers since a new cylinder must be purchased for each different modifier and modifier concentration. Also there appear to be serious limitations to the use of premixed cylinders as a result of the shifts in the vapour-liquid equilibria (VLE) as the liquid phase contents are depleted. It has been found that the concentration of methanol in a premixed cylinder increased as the contents of the cylinder were depleted (36, 37). As the liquid phase is withdrawn, CO₂ (the more volatile component) vaporises, disproportionately occupying the liberated volume. As a result, the concentration of the less volatile modifier in the liquid phase and thus in the head space gas increases. Users of such cylinders for SFC may see negative shifts in analyte retention time, loss of selectivity, and occasional reversals in elution order during the normal life of the cylinder. In SFE, it may result in the extraction of undesirable sample components or reduced extraction efficiency for the analytes of interest or both. Trapping and recovery may be affected adversely since trap temperatures are usually optimised for a specific modifier concentration (38).

The second method of modifier addition is by using a second pump. With the use of a second pump, the modifier concentration can be changed immediately. The CO₂ and modifier is mixed downstream of the pumps in a T-piece (static mixer) (39) or a dynamic mixer (40) before going to the extraction vessel.

The third method of modifier addition is to add the modifier to the sample matrix to be extracted before filling the extraction vessel (41) or

while the sample matrix is already in the extraction vessel but prior to the extraction (42). This method of addition is especially good for screening a number of different modifiers (It is quick as there is no need to flush the entire system between different experimental runs) but it is not suitable for dynamic extraction as the modifier is quickly flushed out of the matrix and system. However, it is particularly useful for static extraction.

Table 5. Modifiers in commercially available cylinder-stored CO₂ mixtures for analytical SFC (36).

Acetic acid	Ethanol	n-Propanol
Acetone	Formic acid	Sulphur dioxide
Acetonitrile	n-Hexane	Tetrahydrofuran
Carbon disulphide	Methanol	Toluene
Carbon tetrachloride	Methylene chloride	Trifluorochloromethane
Chloroform	iso-Propanol	Water

1.4 CHARACTERISATION OF THE SOLVENT STRENGTH OF SUPERCRITICAL FLUIDS

Several parameters have been used to characterise the solvent strength of a SF. Among these parameters are dipole moment, dielectric constant, refractive index, polarisability per volume, solubility parameter and solvatochromic scales. In this section, the solubility parameter and solvatochromic scales will be described.

1.4.1 SOLUBILITY PARAMETER

The solvent strength of a SF may be continuously adjusted from gas-like to liquid-like values, as described qualitatively by the solubility parameter. The solubility parameter, δ , of a SF is calculated according to the empirical formula developed by Giddings *et al.* (43, 44).

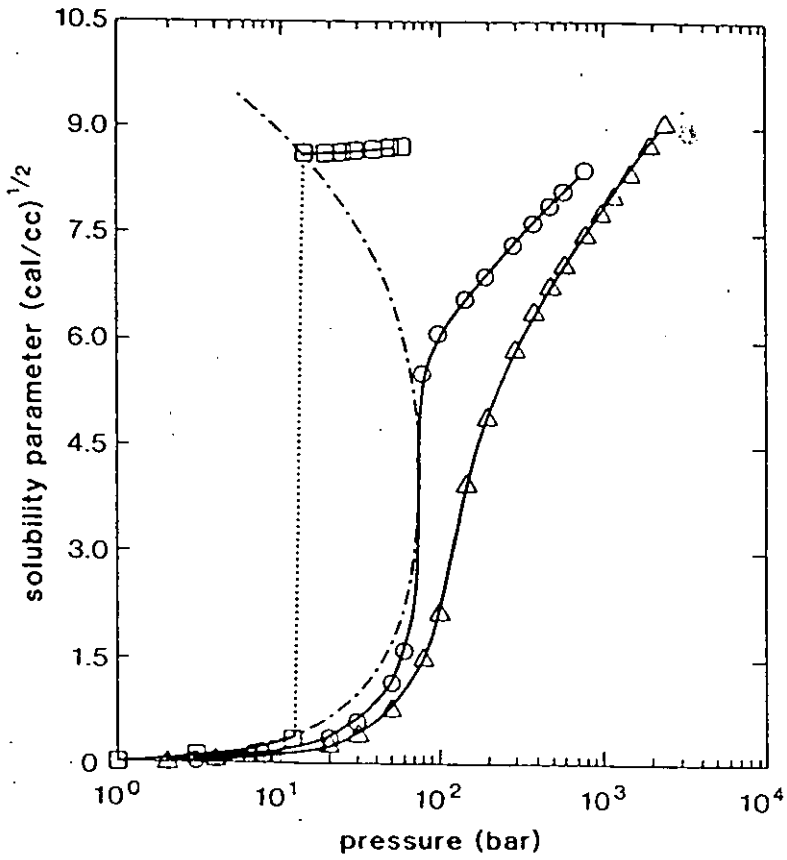
$$\delta = 1.25 P_c^{1/2} (\rho_{r, SF} / \rho_{r, liq.})$$

where P_c = fluid critical pressure, $\rho_{r, SF}$ = reduced density of SF and $\rho_{r, liq.}$ = reduced density of the fluid in the liquid state.

The solubility parameter is made up of two terms. The first term, $1.25P_c^{1/2}$, is referred to as the chemical effect which is dependent on the identity of the solvent (specifically the intermolecular forces). The second term, $\rho_{r, SF} / \rho_{r, liq.}$, is a state effect. As $\rho_{r, SF} \rightarrow \rho_{r, liq.}$, a maximum value is obtained for δ .

The solubility parameter, δ , as a function of pressure is shown in Figure 4. This plot resembles that of density versus pressure (for a given fluid) (Figure 5). The δ values varies from 0 up to liquid-like values of 10 at high densities (45). The δ for gaseous carbon dioxide is essentially zero whereas the value for liquid carbon dioxide is comparable with that of a hydrocarbon like hexane ($\delta = 7.3$). This can be seen with a large increase in δ upon condensation from a vapour to liquid as occurs at -30°C . Above the critical temperature, it is possible to tune the solubility parameter continuously over a wide range with a small isothermal pressure change or a small isobaric temperature change. This ability to tune the solvent strength of a SF is its unique feature, and it can be used to selectively extract, then recover products.

Figure 4. Solubility parameter of CO₂ vs. pressure (45).
(□ : -30°C, O : 31°C, Δ : 70°C)

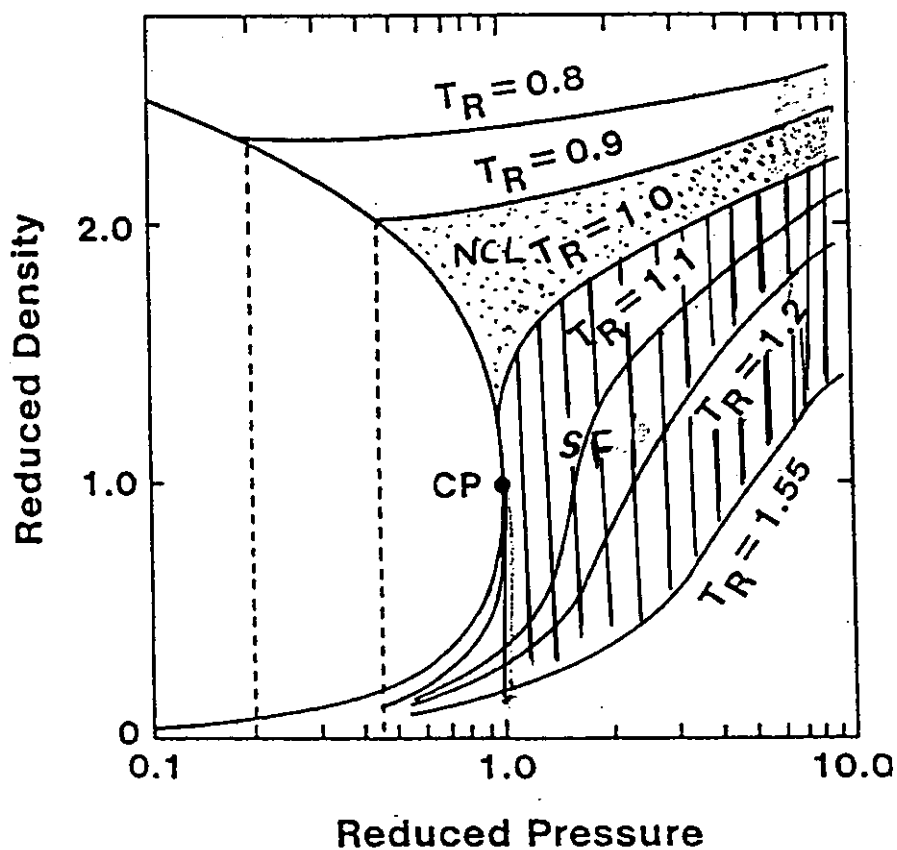


1.4.2 SOLUBILITY

The solubility of a substance in a SF is contributed to by two factors, the volatility of the substance and the solvating effect of the SF (46). The solvating effect is primarily a function of the density of the fluid (47), and since density is a function of pressure and temperature (48), these two factors can be manipulated to increase the extraction efficiency.

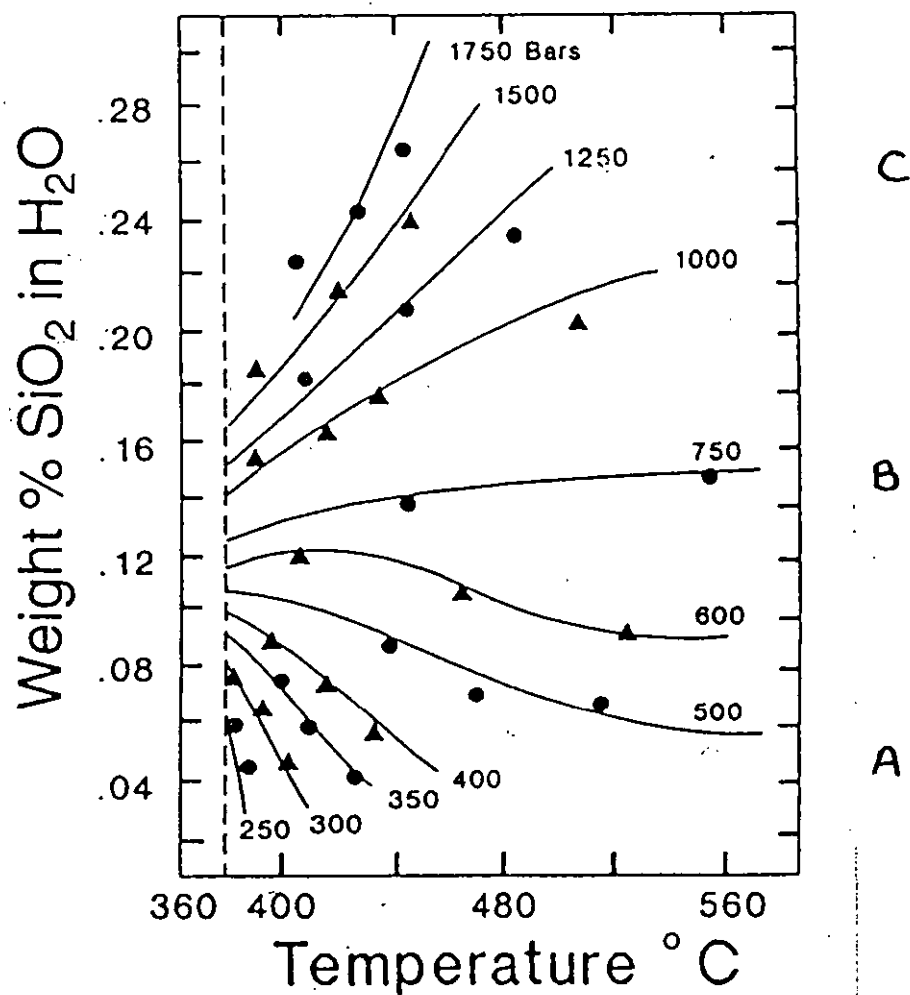
Typical relationship between density and pressure at different temperatures are shown in Figure 5 (49).

Figure 5. Pressure-density isotherms for carbon dioxide (49). T_r = reduced temperature = CO_2 fluid temperature/ CO_2 critical temperature.



From Figure 5, the region near the critical point (CP) marked SF and NCL are the regions of greatest interest. In this region, small changes in pressure at constant temperature results in large changes in the density. At very high temperatures the pressure-density isotherms become more linear. In this region, very large pressure changes are required to produce substantial density changes. Therefore, SFE are usually carried out at no more than 100°C above T_c . The general trend of the effect of pressure on density is for higher pressures (at a given temperature) to increase density and solvating power, while increasing temperature at constant pressure results in a reduction in density and hence solvent strength.

The importance of these density changes with temperature and pressure can be seen in the silicon dioxide/water system (Figure 6)(50). The two major factors controlling solubility are solute vapour pressure and solvent density. An increase in temperature at constant pressure leads to the vapour pressure of the solute increasing which tends to increase solubility and conversely the SF density decreases which tends to decrease solubility. The consequences of these two competing effect can be seen in Figure 6. At lower pressures, the solubility decreases with an increase in temperature (region A), but at higher pressures, the solubility increases with an increase in temperature (region C). At region B the two competing effects balance each other.

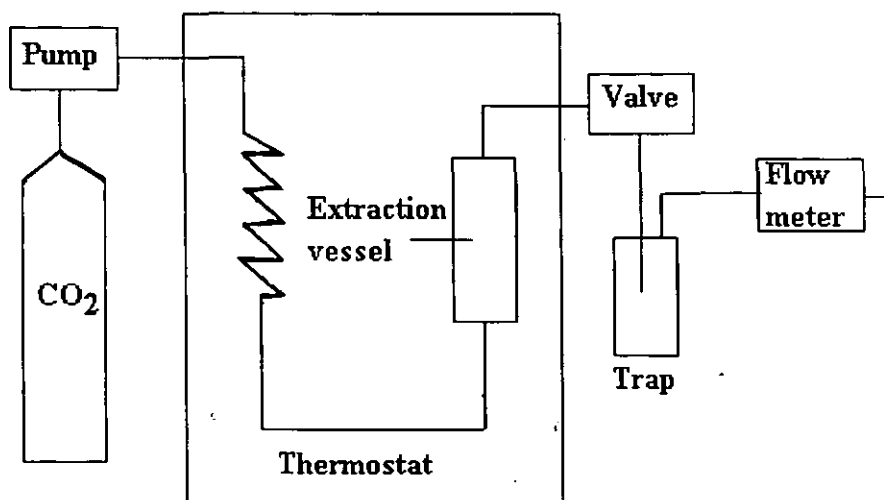
Figure 6. Solubility of SiO_2 in supercritical water (50).

1.4.3 SOLUBILITY STUDIES IN CARBON DIOXIDE

The methods used for analysing the solubilities of substances in a SF_6 solution can be grouped into four categories: gravimetric, spectrometric, chromatographic and miscellaneous (titration, radioactive trace technique)

(51). The most popular approach is gravimetric methods, followed by chromatographic methods. In the gravimetric method, solutions (solvent + solute) are trapped in a pre-weighed trapping system after being depressurised and the trap reweighed to determine the solubility of solute (Figure 7). Most chromatographic methods are modifications of the gravimetric method. In some cases the trap and flow meter are removed and a sampling valve inserted between the extraction cell and the back pressure regulator or valve. A fixed volume of the saturated solution is sampled and injected directly into a chromatograph and analysed by GC (52), HPLC (53) or SFC (54).

Figure 7. Schematic diagram of an apparatus for the gravimetric method.



The widely used methods for measuring solubilities in SFs, such as trapping and quantifying the solute from the supercritical phase (gravimetric) become less accurate at lower pressures where the solubility is low. Most studies of this type concentrated on higher pressures well above the critical pressure. Relative solubilities at lower pressures have been obtained by using UV absorption (55) for naphthalene, in CO₂, CH₄ and C₂H₄, but the extinction coefficients were thought to be changing with pressures at higher pressures.

The solubility of compounds in SFs was first examined by Francis four decades ago (56). The solubilities of 261 other substances in liquid CO₂ were reported. Nearly half of these were miscible with carbon dioxide. Despite the wealth of data obtained, Francis only briefly discussed the effect of solubility of the structure of a compound. Czubyrt *et al.* (57) studied the solubility of Carbowax 1000, Carbowax 4000, 1-octadecanol and stearic acid in SC CO₂ at 40°C in the pressure range 270-1900 atm using a high pressure gas chromatographic instrument with a flame ionisation detector. It was shown that maxima exist in all these solubility curves at between 300 and 2500 atm, depending on the solute.

Wong and Johnston (23) studied the solubility of sterols in pure CO₂ at 35°C and 200 bar. It was found that the solubilities and also selectivities of solutes in carbon dioxide are governed primarily by vapour pressures and only secondarily by solute-solvent interactions in the SF phase. An exception is strong bases such as ammonia that can react with carbon dioxide.

Chromatographic retention time can be used under some circumstances to determine the solubility of substances in SF where the chromatographic retention (capacity factor) in SFC is inversely proportional to solubility (58). Barker *et al.* (59) used this method (measurement of chromatographic retention) and vapour pressure values for obtaining values for the solubility of naphthalene and 1-methylnaphthalene in CO₂ at 308K at low pressures (in the range 3 MPa - 6.5 MPa) in a SF. Their results generally agree with the conventional method. Bartle *et al.* (60) further measured the solubilities of fluorene, phenanthrene and pyrene in carbon dioxide at four temperatures between 308.2K and 328.2K and pressures between 70 and 250 bar using the retention measurement method. The study demonstrates the feasibility of obtaining a large body of somewhat less accurate, but still reasonable, solubility data in SFs, using comparatively rapid chromatographic measurements.

Hansen and Bruno (61) measured the solubility of solid naphthalene in SC CO₂ at 55°C for pressures between 6.58 and 10.23 MPa by direct injection of saturated SF solutions into a high performance liquid chromatographic system. The chromatographic system was designed to operate at pressures and temperatures up to 34 MPa and 140°C respectively. The solubilities of xanthines, caffeine, theophylline and theobromine in SC CO₂ were measured at 313-353K and 20-35 MPa using a supercritical fluid chromatographic system (62). The measurements of solubilities of other solids are given in Table 6.

Table 6. References with solubilities of solids in SC CO₂.

Solid	T/K	Pressure (bar)	Method	Ref.
Anthracene	293-368	up to 1200	FTIR	63
Phenanthrene, 2,6-dimethylnaphthalene, 2,3-dimethylnaphthalene	308, 318, and 328	96-280	Gravimetric	64
Naphthalene	308-328	60-330	Gravimetric	65
Diphenylamine	305-310	50-225	Gravimetric	66
Cholesterol, stearic acid, α -tocopherol	313-353	80-250	Gravimetric	67
Amino acids	313	500-2000	Gravimetric	68
Linuron, methoxychlor, diclofopmethyl, diclofop 3,4-dichloroaniline and 2,4-dichlorophenoxy- acetic acid	313	200	Chromatogr.	53
Mycotoxins, pyrene and benzo[e]pyrene	308 and 338	70-140	Chromatogr.	69

1.4.4 PREDICTION OF SOLUBILITY

The solubility of an analyte in a SF is an important consideration when planning an extraction. Prediction of the equilibrium solubility of solutes in a supercritical solvent is very important for both SFE and SFC. In SFE, the analyte must first dissolve in the SF. Thus, without an understanding of the solubility phenomena, the optimisation of SFE conditions can only be done by trial and error.

Several investigators have qualitatively attempted to correlate solute molecular structure with its solubility in SF. Dandge *et al.* (70) used molecular structure to qualitatively correlate solubility of different groups of compounds. By using this approach, some general structure-carbon dioxide solubility relationships have been developed for compounds like hydrocarbons, alcohols, phenols, aldehydes, carboxylic acid, ethers, amines, esters, alcohols and nitro compounds. Among structural features which greatly influence the solubilities in SF CO₂ are: chain length, branching, number of rings, and position and type of substituents. In the case of normal alkanes, complete miscibility exists between carbon dioxide and alkanes with carbon number 12 and below. As the carbon number increases beyond 12, the miscibility continues to decrease rapidly. The branched alkanes are more soluble compared to normal alkanes. This is explained by Hildebrand (71) in terms of smaller intermolecular interactions in branched alkanes, so aiding solubility. Stahl *et al.* (72) formulated several extractions rules based on changes in solutes molecular structure to qualitatively predict the extent of the dissolution of the solutes in SC CO₂. The rules are:

- (a) Hydrocarbons and other typically lipophilic organic compounds of relatively low polarity (e.g. esters, ethers, lactones, and epoxides) can be extracted in the lower pressure range (i.e. 70-100 atm).
- (b) The introduction of strongly polar functional groups (e.g. OH and COOH) makes the extraction more difficult. Phenols with three or more OH groups cannot be extracted.
- (c) More strongly polar substances (e.g. sugars and amino acids) cannot be extracted in the range up to 400 atm.

(d) Fractionation occurs in the pressure gradient when there are sufficient differences in the starting boiling point or sublimation temperature of the substances. The fractionation effects are most marked in the range where there is a sharp rise in the density and dielectric constant of CO₂.

Quantitative predictions of solubility of solutes in SC CO₂ have been attempted by several workers (23, 64, 73, 74, 75). Statistical mechanical models, equations of state (EOS) and solution thermodynamic approaches (23, 64, 75) have been investigated to correlate phase equilibria and solubility in SFs. However, the most practical approach is that described by King and Friedrich (73) and Mitra and Wilson (74). King and Friedrich used the concept of reduced solubility parameter to quantitatively predict the solubility of solutes in a SF at a particular temperature and pressure. The reduced solubility parameter (Δ) is defined as

$$\Delta = \delta_1/\delta_2$$

where δ_1 is the solubility parameter of the fluid and δ_2 is the solubility parameter of the solute. The solubility parameter of the fluid, δ_1 may be calculated from the formula earlier (section 1.4.1) and the solubility parameter of the solute, δ_2 was calculated by King and Friedrich using Fedors group contribution method (76). Thus δ_2 can be related to molecular structure using the expression (77)

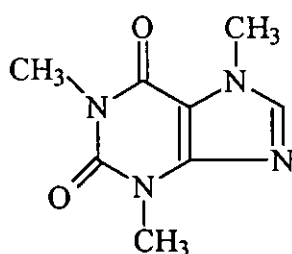
$$\delta_2 = (\sum \Delta \epsilon / \sum \Delta v)^{1/2}$$

where $\sum \Delta \epsilon$ is the sum of energy of vaporisation at a given temperature and $\sum \Delta v$ is the corresponding sum of group molar volume, which is calculated from known values of molecular weight and density. An example of the

approach using the group contribution method of Fedors is shown in Table 6 for caffeine (I) (73). Good agreement is also recorded when one compares solubility parameters computed from Fedor's method with those of obtained from other sources (23) as shown in Table 7 for sterols.

Table 6. Calculation of the solubility parameter for caffeine using the Fedor's group contribution method (73).

Group	$\Delta\epsilon$ (cal mol ⁻¹)	Δv (cm ³ mol ⁻¹)
3 CH ₃	3375	100.5
2 C =	2060	-11.0
1 CH =	1030	13.5
3 N	3000	-27.0
1 -N=	2800	5.0
2 C = O	8300	21.6
2 5-6 membered rings	500	32.0
2 conjugated double bonds	800	-4.4
	$\Sigma\Delta\epsilon = 21\,865$	$\Sigma\Delta v = 130.2$
	$\delta_2 = 12.96 \text{ cal}^{1/2} \text{ cm}^{-3/2}$	



Caffeine

Table 7. Comparison of solubility parameters calculated by two different methods for sterols.

Sterol	Solubility parameter ($\text{cal}^{1/2} \text{cm}^{-3/2}$)	
	Wong and Johnston (23)	Fedors (76)
Cholesterol	9.2	9.6
Stigmasterol	8.8	9.5
Ergosterol	9.1	9.4

Mitra and Wilson (74) developed empirical equations for predicting solubility of solutes as functions of temperature and density, as well as of temperature and pressure. The logarithm of the solubility of solute was fitted with density and temperature to a relationship of the form

$$\ln(s) = a\rho + bT + c$$

where s is the solubility of solute in mole percent (or wt %), ρ is the density in g cm^{-3} , T is the temperature in Kelvin, and a , b , and c are constants. The three constants in the equations (a , b and c) can be evaluated from known values of solubility at three different temperatures and densities (or pressures). The constant a reflects the solubility change with density at constant temperature and the constant b reflects the solubility change with temperature at constant density. The logarithm of solubility was found to correlate linearly with density and temperature. There was good agreement between the predicted and experimental values of solubilities of solutes for all the systems studied. The main advantage of this empirical approach is that the three constants can be calculated and the

solubility can be predicted over a wide range of temperature and density (or pressure) from a few experimental data points.

Another method of directly relating solute structure to retention and solubility in SFs is based on molecular connectivity indices (78). The indices are single numbers which attempt to encapsulate how atoms are interconnected in a molecule. So far this method has only been applied to a limited range of solutes.

1.4.5 SOLVATOCHROMIC PROBES IN CARBON DIOXIDE

The solvatochromic scales, which are based on shifts in the wavelength of maximum absorption for various indicators (79), are one of the most widely used and successful measures of solvent strength. The purpose of these scales is to provide a guide for choosing solvents and co-solvents to achieve desired solvent strength. Other terms which have been used to characterised solvent strength are dipole moment, dielectric constant, refractive index, solubility parameter and polarisability per volume. Solvatochromic parameters are influenced by the local solvent environment near the solute and thus describe solvent strength more effectively than bulk properties, such as solubility parameter or dielectric constant. The solvatochromic scale is defined as

$$E_T = hc/\lambda_{\max}$$

where E_T is the transition energy, h is Planck's constant, c is the speed of light and λ_{\max} is the wavelength of maximum absorption.

Hyatt (80) measured UV-Visible spectra of several solvatochromic probes in an attempt to compare liquid and SC CO₂. There was little difference between the E_T in the liquid and SC states, with CO₂ being in the same low polarity range as toluene, methylcyclohexane, decahydronaphthalene and tetrachloroethylene. This view is confirmed by Deye *et al.* (81) who used data from three probes and found liquid CO₂ to be comparable to liquid pentane.

With the use of solvatochromic probes, other non-specific forces (dispersion, dipole-induced dipole, and dipole-dipole) and specific acid-base forces have been explored in SF solvents. Hyatt (80) measured the acidity of CO₂ by measuring the shifts in the IR C=O stretching frequency of acetone and cyclohexanone. The acidity of CO₂ was insignificant as it gave the same results as non-polar non-acidic liquid solvents. However, the acidity of CO₂ does become important in the presence of strong bases such as ammonia and amines, as solid carbamates are formed. Based on shifts in the N-H stretching frequency for pyrrole, it was found that CO₂ exhibited a modest hydrogen bond basicity in the diethyl ether to ethyl acetate range (80).

Sigman *et al.* (832) determined π^* (a scale based on a linear free energy relationships) and measured the strength of the polarity and polarisability of the solvent and β (basicity) parameters for SC CO₂ as a function of density. The β values ranged from about -0.10 for gas; -0.05 for liquid and -0.08 for SC CO₂. Since all the values are essentially zero,

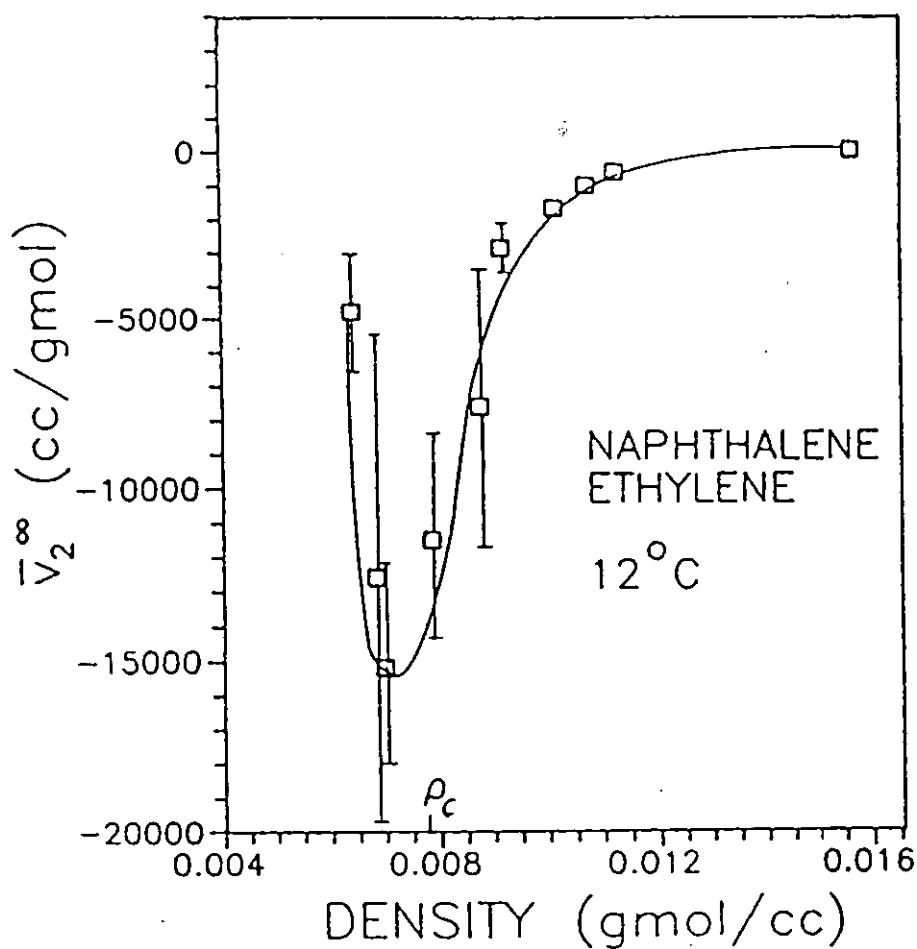
β , unlike π^* does not show any correlation with the density of the CO_2 and suggests a non-hydrogen bonding solvent.

1.4.6 CLUSTERS AND ENTRAINER EFFECTS

A unique feature of SFs is that solvent molecules condense about solutes in regions where the compressibility is large. This physical condensation is often called clustering, which contributes to the unusual behaviour of SFs.

A strong indication of the unusual behaviour in SF solutions was measurement of the partial molar volume (V^∞) at infinite dilution of several solutes in SC ethylene and CO_2 at pressures of 50 to 250 bar and temperature of 12°C to 45°C (83, 84). At high reduced pressures, where the solvents are virtually incompressible, the infinite dilution partial molar volumes were slightly positive. Very sharp negative dips in V^∞ were observed for solutes in the compressible region of the solvent, which is near the critical point. These negative values were extremely large in magnitude ($-1\,000$ to $-16\,000\text{ cm}^3\text{ g}^{-1}\text{ mol}^{-1}$) and were largest for the isotherms closest to the critical temperatures (Figure 8). The extremely large negative infinite dilution partial molar volume suggests the "condensation" of many solvent molecules when a solute molecule is added to solution. This can be envisioned as the collapse of the solvent shell about the solute or the formation of solute-solvent clusters in solution.

Figure 8. Infinite dilution partial molar volume (V^∞) of naphthalene in SC ethylene at 285 K (83).



Spectroscopic techniques such as UV-Vis and fluorescence have been used to probe solute-solvent interactions directly. Solvatochromic shifts are caused by the same types of solute-solvent intermolecular forces (i.e. dispersion, induction and dipole-dipole forces) that influence solubilities. Consequently, the values of clustering determined

spectroscopically are appropriate for considering the effect of clustering on solubilities. Several studies provided experimental evidence for the clustering phenomenon. A number of studies using spectroscopy have addressed the formation of clusters in SC solutions. Kim and Johnston (85) first suggested the link between the local density and the isothermal compressibility, showing that the local density is highest in the region of highest compressibility near the critical point. Yonker and co-workers (86) have studied the wavelength of maximum absorption of a chromophore in SFs and used that information to determine Kamlet-Taft π^* values as a function of solvent density in the SC region. Kajimoto *et al.* (87) have used both absorption and fluorescence to look at the system of N, N-dimethylamino benzonitrile, (DMABN) which forms a charge transfer (CT) complex, in addition to the normal fluorescence in SC CHF_3 . Brennecke and Eckert (88) used fluorescence spectroscopy to study intermolecular interactions (solute-solvent and solute-solute) of pyrene in pure SC CO_2 , ethylene and CHF_3 and of naphthalene, dibenzofuran and carbazole in SC CO_2 . Solute-solvent interactions was deduced from relative intensity ratios in the spectra and overall fluorescence intensities. Solute-solute interactions was revealed by the formation of excimers (excited state dimers that result in a broad structureless band at significantly lower wavelengths than normal fluorescence) in dilute solutions at concentrations as low as 5×10^{-6} mol fraction.

The addition of a small amount of a co-solvent (entrainer) to a SF can increase solubilities of certain substances from a few percent to several orders of magnitude (89 - 91). For example, the solubility of naphthalene

in a mixture of CO₂ and 3.5 mol % n-octane is 2.8 times its solubility in pure CO₂ at the same density (48) and the solubility of aminobenzoic acid increased by 620% with the addition of 3.5 mol % methanol (89). Joshi and Prausnitz (92) used thermodynamic analysis to calculate the solubilities of phenanthrene in carbon dioxide and mixtures of CO₂ and propane at 341 K, the solubilities of phenanthrene in ethylene and mixtures of ethylene and propylene at 341 K, the solubilities of benzoic acid in propane and in mixtures of propane with sulphur dioxide and propane with dimethyl ether at 349 K and the solubilities of benzoic acid in propane and mixtures of propane and ammonia at 349 K. In all cases the solubility of phenanthrene increased when a co-solvent was used. In general, the addition of co-solvent to a SF will enhance the solubility of a solute in the fluid phase as well as alter the separation factor between co-extracted solutes (93).

Spectroscopic data indicate that preferential solvation by a co-solvent contributes to the large increases. The co-solvents acetone, methanol, ethanol and n-octane were investigated by Kim and Johnston (94) using the solute phenol blue as a solvatochromic indicator. It was found that the local concentration of co-solvent near the solute exceeds the bulk value since the co-solvent interacts more strongly with the solute than does SC CO₂ (94).

Yonker *et al.* (95) and Kim and Johnston (94) used UV absorption measurements to quantify the clustering around solutes in a solute/fluid/entrainer system. The shift in the wavelength of maximum

absorption was used to determine the local composition about the solute. This vicinity was shown to be enriched with the entrainer, especially in the highly compressible region nearest the critical point.

1.5 MODEL OF EXTRACTIONS

1.5.1 MODEL NOT LIMITED BY SOLUBILITY

Early SFE studies (in process engineering) often based the extraction conditions on maximising the solubility of the target analyte. However, solubility consideration is only important in cases when the target analytes represent a large percentage of the bulk matrix and is less useful when the target analytes are present in minor and trace amounts (as in most analytical SFE) where the concentration of the analyte is well below the solubility limit. The rate of extraction is therefore not determined by the solubility, but by the mass transfer out of the matrix. A characteristic of these extractions is that the majority of the compound is removed during a short period at the beginning of the extraction but subsequently the extraction rate tails off.

Bartle *et al.* (96) developed a model called the 'hot-ball model' to fit experimental extraction results. The model is derived from the mode of the loss of heat from a sphere - hence the name. In developing the model several assumption were made:-

- (1) The particles of the matrix are considered to be all spherical (of radius r) and of the same size and that the material to be extracted is uniformly distributed within the particles at the beginning of the extraction.

- (2). The rate of flow of the SF past the particle is fast enough that the concentration of the extracted material in the fluid is always close to zero.
- (3). The compounds to be extracted are assumed to move through the matrix by a process similar to that of diffusion.

The ratio of mass, m , of extractable material that remains in the matrix sphere after extraction time, t , to that of the initial mass of extractable compound, m_o , is given by the following equation:

$$m/m_o = (6/\pi^2) \sum_{n=1}^{\infty} (1/n^2) \exp(-n^2 \pi^2 D t / r^2) \quad (1)$$

where n is an integer and D the diffusion coefficient of the compound in the material of the sphere. By substituting $\pi^2 D / r^2 = 1/t_c$ (where t_c is a characteristic time for the extraction) in the above equation and factorising the term $\exp(-t/t_c)$, the following sum of exponential decay equations was obtained:

$$m/m_o = (6/\pi^2) [\exp(-t/t_c) + (1/4)\exp(-4t/t_c) + (1/9) \exp(-9t/t_c) + \dots] \quad (2)$$

$$m/m_o = (6/\pi^2) \exp(-t/t_c) \{1 + (1/4) \exp(-3t/t_c) + (1/9) \exp(-8t/t_c) + \dots\} \quad (3)$$

Taking the natural logarithm on both sides of the equation gives

$$\ln(m/m_o) = \ln(6/\pi^2) - t/t_c + \ln\{1 + \exp(-3t/t_c) + (1/9) \exp(-8t/t_c) + \dots\} \quad (4)$$

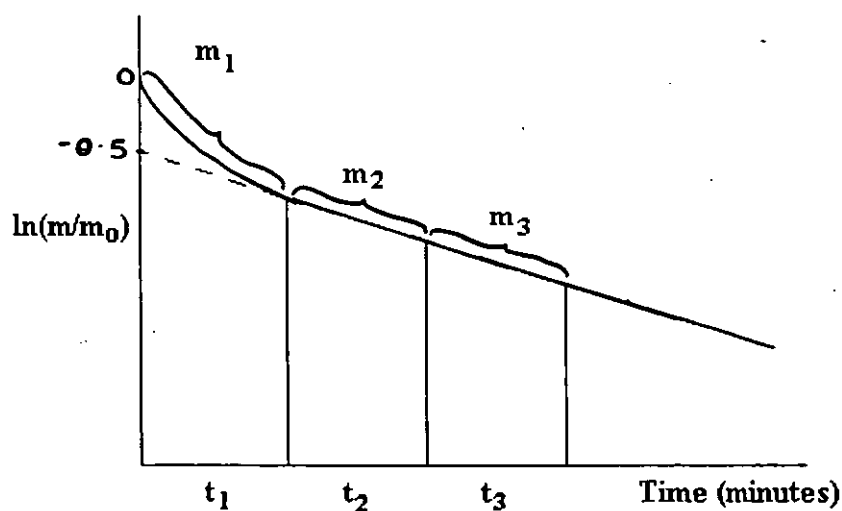
$$= -0.4977 - t/t_c + \ln\{1 + \exp(-3t/t_c) + (1/9) \exp(-8t/t_c) + \dots\} \quad (5)$$

At longer extraction times, the term in the bracket tends to zero and $\ln(m/m_o)$ is given approximately by

$$\ln(m/m_o) = -0.4977 - t/t_c \quad (6)$$

A plot of $\ln(m/m_0)$ versus time t will give a graph {of equation (5)} as in Figure 9, and the straight line portion, which is continued to the time-zero axis as a dashed line, is a plot of equation (6). The slope of the straight line portion is $-1/t_c$ and the intercept, I is -0.4977 (at $t = 0$). The theoretical curve is characterised by a rapid fall on to a linear portion, which corresponds to an extraction tail. The physical explanation of the form of the curve is that the initial portion is extraction, principally from the outer parts of the sphere, which establishes a smooth concentration profile across each sphere, peaking at the centre and falling to zero at the surface. When this has happened, the extraction becomes an exponential decay. The experimental curve gave a larger intercept and the curve does not fall as steeply from zero, and this is thought to be due to the effect of solubility limitations.

Figure 9. Theoretical curve for the hot-ball SFE model.



Bartle *et al.* (96) also developed a model for a continuous extraction from a rectangular slab of thickness, L , whose other dimensions are infinite. The ratio of the mass, m , of the amount of the compound in a section of the slab, of given area, at time t and m_0 , the amount in the same section at $t = 0$ gives

$$m/m_0 = (8/\pi^2) \sum_{n=0}^{\infty} \{1/(2n+1)^2\} \exp(-2n+1)^2 \pi^2 D t / L^2 \quad (7)$$

Going through the same procedure as for the spherical model gives the following equation

$$\ln(m/m_0) = -0.2100 - t/t_c + \ln\{1 + 1/9 \exp(-8t/t_c) + (1/25) \exp(-24t/t_c) + \dots\} \quad (8)$$

A plot of $\ln(m/m_0)$ versus time therefore again becomes linear at longer times and the approximate equation for this model at longer times becomes

$$\ln(m/m_0) = -0.2100 - t/t_c \quad (9)$$

Therefore, for an infinitely rectangular slab, the intercept, I is -0.2100 . Intercept as low as -2.0 could be found in real world samples (where the surface area to volume ratio is high) (96).

The exponential term in the equation opens up the possibility of obtaining quantitative analytical information in a shorter time than would be required for exhaustive extraction. If extraction is carried out at least as long as the initial non-linear period to obtain an extracted mass m_1 , followed by extraction over two subsequent equal time period to obtain masses m_2 and m_3 , then the total mass in the sample, m_0 , is given by

$$m_0 = m_1 + m_2^2 / (m_2 - m_3) \quad (10)$$

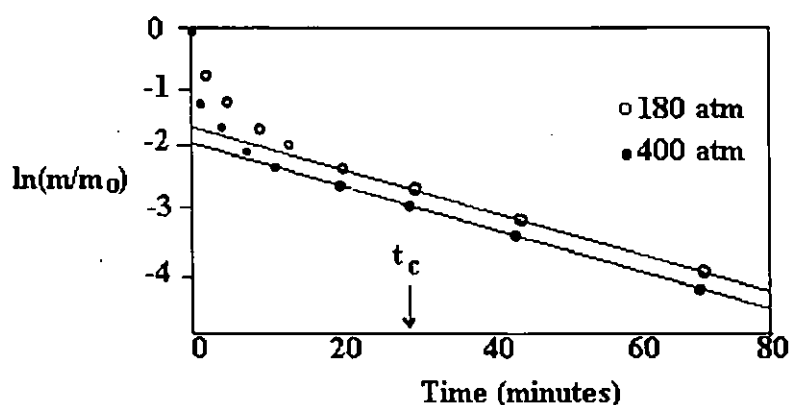
Extrapolation of this kind has been applied to the extraction of a cyclic trimer from polyethylene terephthalate (PET) films (96) and for the extraction of 2,6-di-*t*-butyl-4-methylphenol (BHT) from standard polypropylene cylinders of 3 mm in both length and diameter (97). In the first case, the difference between the total weight extracted and the weight predicted from the equation differed by -5.2% (96) and in the second case, the difference between the total weight extracted and the weight predicted from the equation differed by 3% (97).

In spite of the simplicity of the model and the neglect of the effect of solubility, a variety of real systems were found to exhibit two of the three principal characteristics of the theoretical curve. The first is that the graph curves steeply initially, but then becomes linear and secondly, the curve becomes linear after a time of approximately $0.5t_c$ if t_c is obtained from the linear portion. The third characteristic of the theoretical curve, that the intercept of the linear portion is approximately -0.5, is not shown by real systems, which showed intercepts both above and below this figure. These differences were explained in terms of the irregular shapes of the matrix particles, which lowers the intercept, limitation by solubility, which raises the intercept, and the non-uniform extractable solute distribution, which can either raise or lower the intercept.

1.5.2 EXTRACTION CONTROLLED BY SOLUBILITY AND DIFFUSION

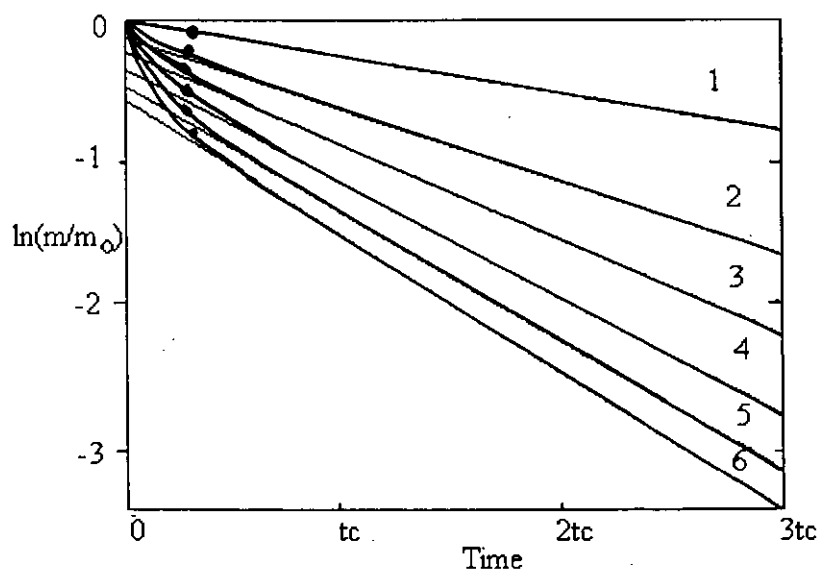
The effect of solubility limitation on the extraction rate was shown by the extraction of phenanthrene from soils at two pressures, 180 atm and 400 atm (97). At the two pressures studied, both the curves have the form of the hot ball model (Figure 10). However, at the lower pressure (180 atm) the curve falls less steeply initially and the linear portion is displaced upwards; characterised by the lowering of the intercept of the linear portion (intercept of -2 for the extraction at 400 atm and an intercept of -1.8 for the extraction at 180 atm). Similar curves have been found for the SFE of numerous analytes from environmental matrices, e.g. PAHs with molar masses from 128 to 252 (98), atrazine from soils (99) and alkyl benzenesulphonates from organic digester sludge (27). The extent of these effects increases as the pressure falls and the solubility decreases.

Figure 10. Dynamic extraction of phenanthrene by SC CO₂ from soil at two pressures at 50°C and constant flow (96).



A complete study of the effect of pressure and solubility on the kinetics of extraction has been carried out on the extraction of Irgafos 168 {tris (2,4-di-*t*-butyltriphenyl)phosphite} from ground polypropylene at 70°C and pressures of 75, 105, 175, 200 and 400 bar (100). The curves obtained mirror the predictions of the theoretical curve (Figure 11). These curves show a reduction in the steepness of the initial fall and also a reduction in the negative slope as pressure and solubility are reduced.

Figure 11. Theoretical curves for SFE, including solvation effects, for spherical matrix particles at a number of pressures, but constant flow rate. Curve 1 is at the lowest pressure and curve 5 at the highest pressure. Curve 6 is for infinite solubility (100).



Pawliszyn (101) developed a comprehensive mathematical model for a packed-tube extractor geometry to predict removal efficiency versus extraction time for SFE. The model is based on well-established theoretical relationships describing mass transfer kinetics, the chromatographic elution process and the convolution theorem. The model considers several factors that could potentially contribute to slow extraction rates, including kinetics of desorption, swelling, diffusion of analyte in the organic component of the porous matrix, and fluid/matrix distribution constants. The model can be applied to both dynamic and static/dynamic SFE techniques, and can be used to investigate analyte-matrix interaction to determine the extraction-rate limiting process. The model can be extended to predict extraction rates when other types of fluids are used to elute analytes from the packed-tube extractor.

1.6 SFE METHODOLOGIES

SFE can be carried out in two ways, viz. on-line or off-line. In the on-line method the extract is directly transferred to a coupled detection system (SFC, GC, HPLC, FTIR, NMR). In the off-line method, the extract is first collected after depressurisation and retained for subsequent analysis by a variety of appropriate techniques. The advantages and disadvantages of on-line SFE and off-line SFE are summarised in Table 8.

Table 8. Advantages and disadvantages of off-line and on-line SFE.

On-line SFE	Off-line SFE
The SFE parameters, the analyte trapping conditions and the chromatographic separation all need to be understood	Inherently simpler than on-line SFE - only extraction and analyte collection need to be considered
Sample size limited	Larger sample size can be used - better sampling reproducibility
Sample handling eliminated- risk of contamination minimised	Sample handling after extraction-risk of contamination introduced
System not available for further analysis until entire analysis completed	System available for further analysis after extraction completed -high sample throughput
Multiple analysis of extracted analytes not possible	Multiple analysis of extracted analytes possible
All extracted sample quantitatively transferred to detection system - no sample loss problem	Sample loss can be a problem after depressurisation step

1.6.1 COLLECTION OF EXTRACTS IN OFF-LINE METHOD

In SFE, after the analytes of interest have been extracted from their matrix then the next most important step is that they have to be efficiently trapped. In off-line SFE, there are basically three commonly used trapping systems. The mechanically simplest and inexpensive way is to trap the analyte in a liquid after depressurising the supercritical fluid through a restrictor. The restrictor is usually heated to prevent clogging/freezing and flow of ≤ 1 ml/min is usually employed. The majority of reports to date have utilised trapping in liquid solvents. The collection solvent has to be compatible with the modifier used (if any) and the selection of the proper

solvent polarity for the target analytes is important for achieving quantitative collection of the extracted analytes (Table 9) (102).

Table 9. SFE collection efficiencies of spiked pollutants from sand in different organic solvents. * Collection solvent at 5°C.

Test compounds	% collection efficiency in 3-ml solvent			
	Hexane	MeOH	CH ₂ Cl ₂	CH ₂ Cl ₂ (5°C)*
Phenol	43	55	77	98
2-chloroethylether	60	56	73	100
1,2-dichlorobenzene	46	58	78	100
2,4-dinitrotoluene	68	60	92	100
nitrobenzene	60	58	82	100
anthracene	79	59	88	97
2-nitrophenol	57	61	80	99
2-nitroaniline	72	57	86	98
acenaphthylene	66	60	84	99
diethylphthalate	79	60	84	99
hexachlorobenzene	79	62	86	100
benzo[a]pyrene	76	57	92	97

Lopez-Avilla *et al.* (103) used hexane as a collection solvent to trap polycyclic aromatic hydrocarbons (PAHs) and organochlorine insecticides. Flow rate was controlled by using a 60 cm piece of 50 µm fused silica. Hawthorne and Miller (104) used 2 ml of methylene chloride to trap PAHs

from urban dust, river sediment, and fly ash. A 10 cm long of 20-30 μm i.d. fused silica was used to control the flow rate of extraction. Alexandrou and Pawliszyn (105) used 1 ml of hexane to trap polychlorinated dibenzo-p-dioxins (PCDDs) and benzofurans from municipal incinerator fly ash. Nam *et al.* (106) used iced-cold hexane to trap polychlorinated organics from biological tissue samples. Langenfeld and co-workers (102) used methylene chloride to study the collection efficiencies of 66 compounds of various polarity and volatility. Porter and co-workers (107) used 15 ml of methylene chloride to trap polychlorinated biphenyls (PCBs) from sewage sludge and PAHs from marine sediment. Hawthorne *et al.* (108) used 4 ml of methylene chloride to collect essential oils from aromatic plants. A 12 cm long 32 μm i.d. fused silica was used to control the flow. Onuska and Terry (109) used 2 ml of hexane as collection solvent for the extraction of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) from sediment using a 25-30 μm i.d. linear fused-silica restrictor. McNair and Frazier (110) reported the use of methylene chloride to trap PAHs standards.

The second type of trapping system is a solid surface. The surface can be cooled by liquid nitrogen or cryogenically cooled by the expanding supercritical fluid. Glass vials (111, 112), glass beads (113), volumetric flask and round bottom flask (114, 115, 116) and stainless steel beads (38) have been used. On stainless steel beads the mechanism of trapping is by cryotrapping only (by the expanding SF). Oxide layer may be present on the stainless steel beads and there can be an appreciable adsorption of either modifier or analytes. Mulcahey and Taylor (38) studied the effect of various methanol percentages on the trapping efficiency of various polarity

substances on stainless steel trap. They found that the addition of 1% methanol (trapping via a thin layer of methanol adsorbed on the stainless steel surface) improved the recovery of volatile compounds but with greater than 2% methanol concentration there was no trapping of analytes (mechanical rinsing of the trap). Vejrosta *et al.* (117) used a two stage trapping systems to trap fluoranthene. Initially the fluoranthene was trapped onto an inner wall of a 500 μm i.d. fused silica tubing after being depressurised from a fused silica restrictor (17 μm i. d.) and then rinsed off with a minimum volume of rinsing solvent into a glass microvial.

The third type of trapping is a solid phase sorbent. Sorbent such as Tenax, C_{18} (ODS), silica gel, and solid phase extraction tube (SPE) have been used. The trap is cryogenically cooled, either by the expanding supercritical fluid or by another source. The trapped analytes are removed by rinsing with a suitable solvent and the polarity of the rinsing solvent is vital for good recovery. Since ODS contains residual silanol groups, two types of interaction can occur: sorptive interactions between the solutes and the ODS phase (dispersive forces) and sorption between the silanol groups and the analytes (H-bonding or forces stronger than dispersive). Mulcahey and Taylor (38) used a C_{18} sorbent to trap test mixture of various polarity. They studied the effect of various methanol percentages on an ODS trap and stainless steel trap. They found that with $\leq 2\%$ methanol on an ODS trap, the trap temperature need to be above the boiling point of methanol for efficient trapping and with $> 2\%$ methanol on an ODS trap, trap temperature of between 40-50°C is needed for efficient trapping. Miles *et al.* (118) used 30 μm C_{18} pellicular packing material to adsorb the extracted

components from fresh garlic, dried minced garlic and onion. Schantz (119) used C_{18} to trap PCBs from urban particulate, while Taylor and Hedrick (120) used solid phase extraction (SPE) tubes as trap for collection of phenol from water.

1.7 APPLICATIONS OF SUPERCRITICAL FLUID EXTRACTION

SFE has attracted much interest in recent years and has found applications in many areas, such as food science, pharmaceuticals, polymers and environmental. Most of the studies on the SFE of analytes are usually conducted on solid matrices. A list of typical applications where SFE has been successfully used to extract analytes from solid matrices are given in Table 10.

Many applications for SFE of aqueous systems at the analytical scales can be envisioned, such as the analysis of pesticides and herbicides from field drainage, municipal wastewater, and drugs/drug metabolites from biological fluids. However, there are studies which have demonstrated the potential of using SFE to recover analytes from water and water-based fluids (120-124). Hedrick *et al.* (120, 122) used special extraction cells (Figure 12) to ensure that the SF percolates through the water sample. The SC CO_2 enters the extraction cell through the top tube and exits into the bottom of the water sample where it bubbles through the aqueous media to extract the compound or compounds of interest. Since the CO_2 is less dense than water, the CO_2 percolates through the water

sample to the top of the cell and exits for analyte collection. This method has been applied to the extraction of di-isopropyl methylphosphonate at a concentration level of $834 \mu\text{g l}^{-1}$ to 834 mg l^{-1} (122) and also for the extraction of phenols from water (120).

SPE cartridges have also been used to trap analytes from water samples prior to SFE. This method have been applied to the extraction of sulphonyl urea herbicides at a concentration level of $50 \mu\text{g l}^{-1}$ (125) and explosives (nitrotoluenes) (126) at the $\mu\text{g l}^{-1}$ to ng l^{-1} level from water. In the latter case, a phenyl stationary phase was used to adsorb the nitrotoluenes before eluting them with CO_2 at 75°C and 400 bar.

Figure 12. Extraction cell for performing SFE of water samples (120, 122).

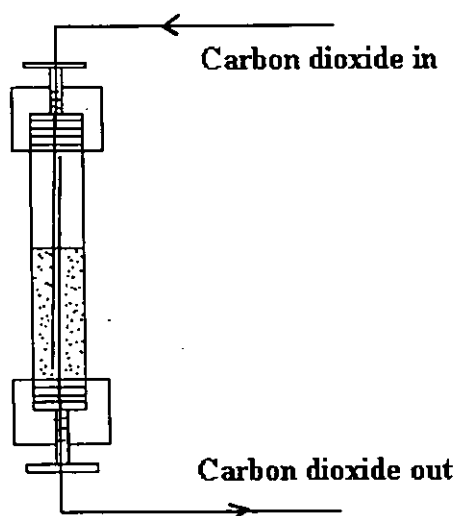


Table 10. Typical applications of SFE from solid matrices.

Analyte	Matrix	Reference
d12-lactone	milk fat	127
vitamin K ₁	infant formula	128
vitamin K ₃	rat feed	129
dioxins	cod liver	130
alachlor	lard and bovine liver	131
carbofuran	frankfurters	131
chlorpyrifos methyl	wheat kernel	132
sulphonyl urea herbicides	soybean, wheat	133
2,4-dichlorophenols	barley straw and seeds	134
vitamin E	wheat germ powder	135
lipids	onion seeds	136
hydroperoxides	peanut powder	137
cholesterol	egg yolks	138
cholesterol & lecithin	egg yolks	139
triglycerides	soybean meal	140
halogenated aromatic derivative of urea (HAU) and halogenated aromatic phenoxy derivative of alkane (HAPA)	rodent feed	141
sulpha drugs	liver and pork	142
carotene & lutein	leaf protein concentrates	143
trochothecenes mycotoxins	wheat	144
thiocarbamate pesticides	apples	145
fluazifop-P-butyl and fluazifop-P	onions	146
carbamate pesticides	muscle tissue	147
phosphorus pesticides	rice	148
nitrosamines	frankfurters	149
linuron & diuron	soil	111
halocarbons	sediment	150
chlorobenzenes	sediment	151
phenols and chlorophenols	soil	152
PCBs	sediment	119, 153, 154
PCBs	sludge	155
2,3,7,8-tetrachlorodibenzo-p-dioxins(TCDD)	sediment	109

Analyte	Matrix	Reference
dibenzo-p-dioxins, dibenzofurans	incinerator fly-ash	156
TCDD	incinerator fly-ash	153
triazines herbicides	sediment	157
triazines herbicides	soil	158
phenoxycarboxylic acids	glass wool	159
PAHs	Tenax-GC	160
PAHs	soil	161, 162, 163, 164
PAHs	urban dust, fly ash, river sediment	104
PAHs	XAD-2-resin and poly- urethane foam	114
PAHs	urban dust	165
PAHs	coal, coal tar	166
4-nitrobiphenyl, 2-nitro- fluorene, fluoranthene	XAD-4 resin	167
4-nitrophenol and parathion	soil	168
chemical warfare	soil and sediment	169
petroleum hydrocarbons	soil	170
explosives and propellants	powders and soil	171
diesel fuel	soil	172
atrazine and simazine	soil	99
fullerenes	carbon soot	173
polymer additives (Tinuvin 326, Tinuvin 770, Irganox 1010, Irgafos 168)	polypropylene pellets	174
Carbowax, 400, Triton X- 102, zinc stearate, Polywax 1000)	polystyrene cup	174
Additives (Tinuvin, BHT, Irgafos, Irganox)	polyethylene	175
Phenol	low density poly- ethylene (LDPE)	176
Additives (BHT, TPP, DNP, Tinuvin P and dimethyl phthalate)	polyvinyl chloride (PVC)	177
flame retardants	polyurethane foam	178
morphine	freeze-dried serum	179
flavone	dog plasma	180

1.8 THE PRESENT PROJECT

Since its inception in the early 80's, the use of SF₆ as an extraction medium has grown rapidly. As can be seen from Table 10, the list of substances and matrices being extracted using SFs are numerous and are on the increase. This thesis examines the use of SC CO₂ to quantitatively extract analytes from plants and environmental samples on an analytical scale. The thesis is divided into three areas. The first part of the thesis examines the use of SC CO₂ and modified SC CO₂ to quantitatively extract eugenol from pimento berries (Belize 9) followed by off-line high performance liquid chromatography (HPLC) analysis. The second part of the thesis examines the use of SC CO₂ and modified SC CO₂ to extract organochlorine insecticides from freeze dried sludge samples. Electron capture detector (ECD) was employed to analyse the extracts. The third part of the thesis examines the use of SC CO₂ and modified SC CO₂ in the extraction of a single organophosphorus insecticides, chlorpyrifos from a formulation (Dursban) and using the optimised condition for the Dursban to extract chlorpyrifos from a German standard soil. Once a quantitative extraction of the chlorpyrifos has been achieved, extraction of chlorpyrifos from other types of soils were investigated to study the effect of different soil matrixes on the recovery of spiked chlorpyrifos. The extracts were analysed off-line using gas liquid chromatography (GLC).

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CHAPTER 2

EXPERIMENTAL METHODS FOR THE EXTRACTION OF PIMENTO BERRIES

2.0 INTRODUCTION

In this chapter the experimental details and chemicals are described for the extraction of pimento berries.

2.1 CHEMICALS

2.1.1 STANDARD AND REAGENTS

Eugenol was from Sigma Chemical Company (Gillingham, Poole, U.K.), disodium hydrogen orthophosphate (AR grade) and methanol (HPLC grade) were from Fisons Scientific Apparatus (Loughborough, U.K.), sodium dihydrogen orthophosphate (SLR grade) was from East Anglia Chemicals (Hadleigh, Ipswich, U.K.) and water was purified in-house using the Millipore system. The carbon dioxide was industrial grade (99.98%) supplied by BOC Ltd. (Middlesex, U.K.). Sample of pimento berries (Belize 9) was from Tropical Products Institute (London, U.K.).

2.2 INSTRUMENTATION

2.2.1 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

The chromatographic system consisted of a Pye Unicam PU 4014 pump (Cambridge, U.K.), a Rheodyne 7125 (Cotati, California) injector valve fitted with a 20 μ l loop and a Pye Unicam PU 4025 (Cambridge, U.K.) variable wavelength UV absorbance detector operating at 278 nm. The column (100 mm x 5 mm i.d.) was packed with 5 μ m ODS-2 Spherisorb (batch number: 33/175/1, Phase Sep, Clwyd, U.K.). The column was enclosed in a circulating water jacket at 30°C using a thermostated water bath. An eluent flow rate of 1 ml min⁻¹ was maintained throughout the experiment. Analyte areas were measured using a Hewlett Packard model 3390A recording integrator. The column void volume was measured using sodium nitrate (6.34 mg ml⁻¹).

2.2.2 SUPERCRITICAL FLUID EXTRACTION (SFE)

The SFE system consisted of two JASCO (Japan Spectroscopic Company, Japan) 880-PU HPLC pumps. One of the pump heads was cooled to between -10°C and -5°C (depending on the ambient laboratory temperature) to ensure that CO₂ was pumped as a liquid. The organic modifier was added to the pressurised CO₂ using the second JASCO 880-PU pump and a dynamic mixer (Gilson 811B). Extractions were carried out in a JASCO 1 ml extraction vessel contained in a JASCO 860-CO column oven and the progress of the extractions were monitored with a JASCO 870-UV detector at 278 nm.

connected to a W & W 600 Tarkan (Switzerland) chart recorder or an ABB SE 120 chart recorder. The pressure was maintained by the JASCO back pressure regulator (BPR) and samples were collected in a 100 ml round bottom flask with a side arm and the bottom immersed in liquid nitrogen (Figure 1). The level of liquid nitrogen in the flask was kept constant by topping up the liquid nitrogen from time to time. The SFE instrumentation used is as shown in Figure 2. Figure 3a and 3b shows the SF flow path in the system.

Figure 1. SFE collection vessel for the pimento extract.

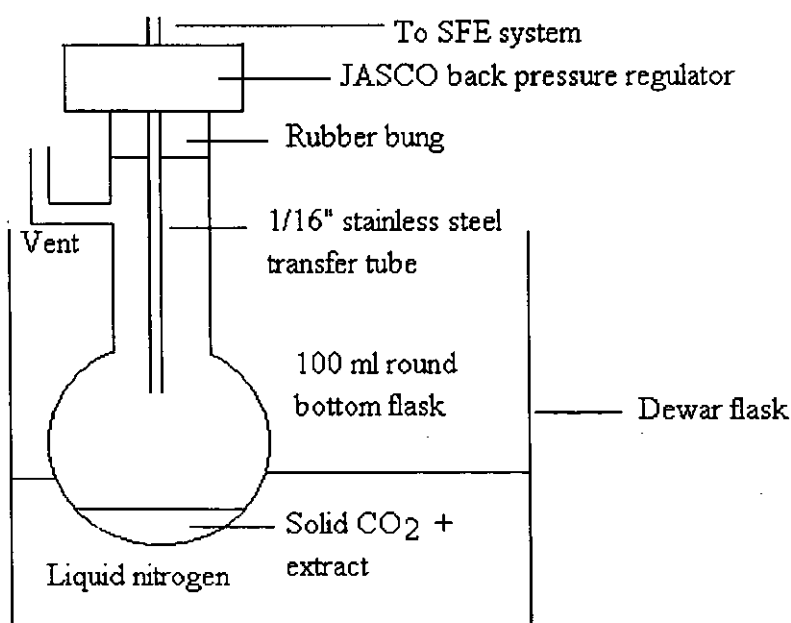


Figure 2. Schematic diagram of the JASCO SFE system used for the extraction.

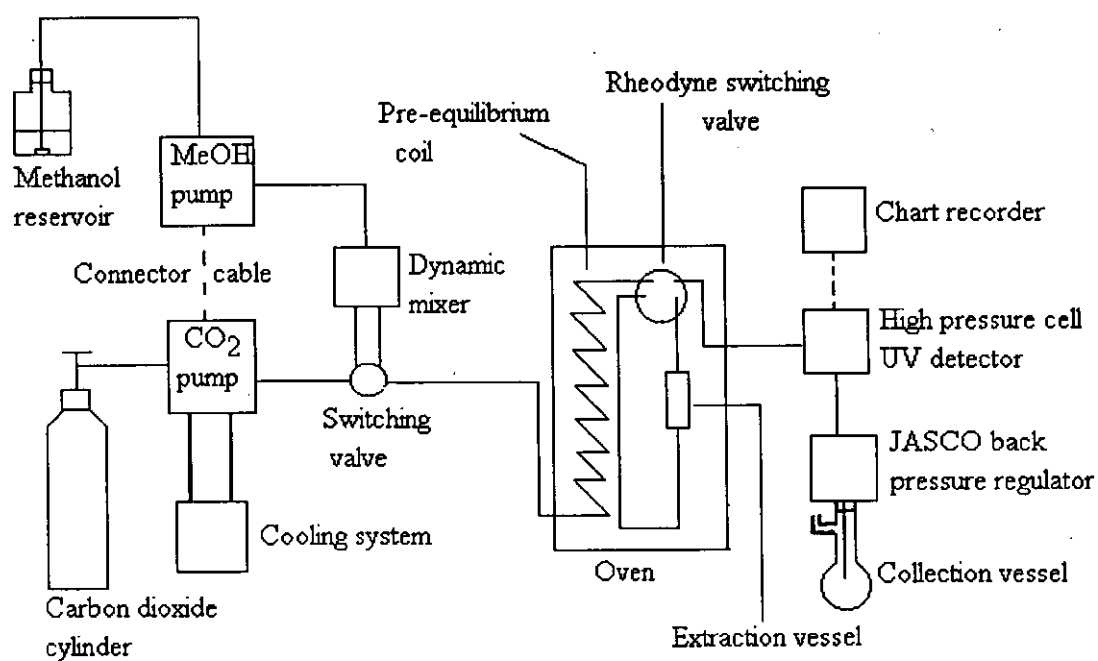
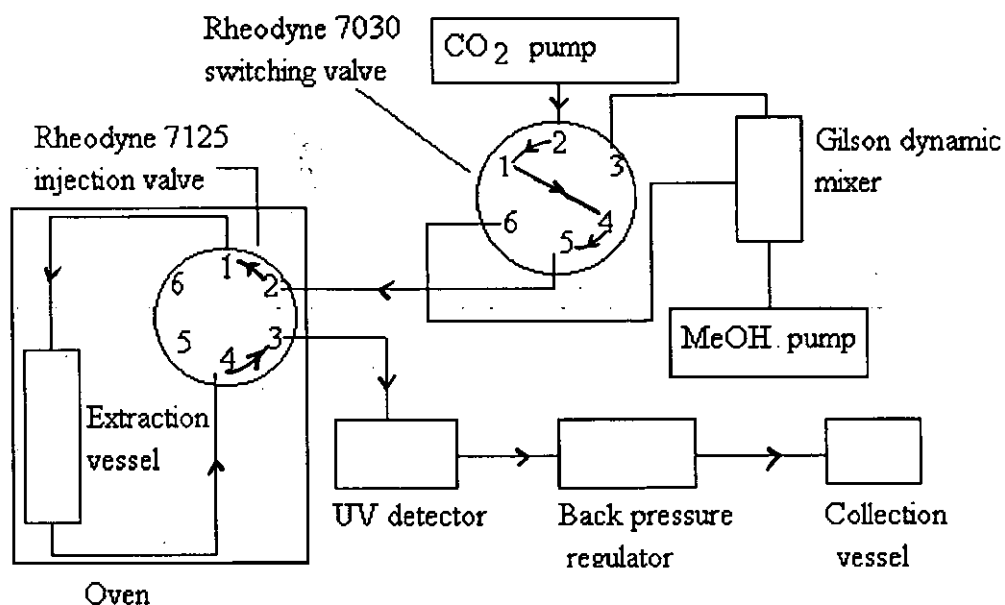
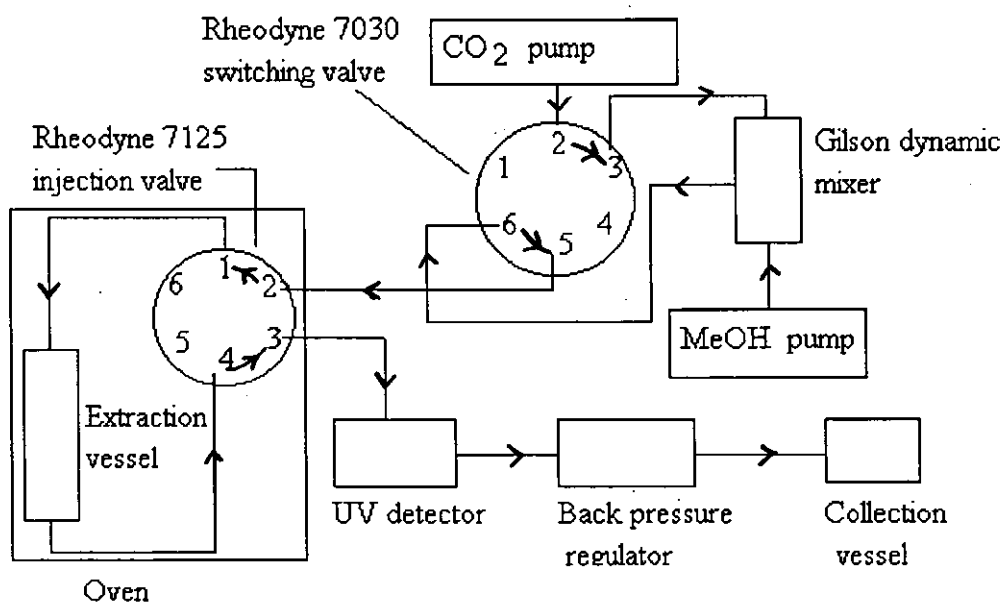


Figure 3a. SF flow path through the SFE system for CO₂ only.Figure 3b. SF flow path through the SFE system for modified CO₂.

2.3 PROCEDURE

2.3.1 PREPARATION OF ELUENT

Phosphate buffer (0.011M) pH 7.0 was prepared by dissolving sodium dihydrogen orthophosphate (1.370 g) and disodium hydrogen orthophosphate (1.580 g) in deionised water and appropriate amount of concentrated phosphoric acid added to give the needed pH (measured with a pH meter), and finally made up to 1 litre with deionised water. A methanol-phosphate buffer pH 7.0 (50:50 v/v) was used as the eluent (vacuum degassed before use).

2.3.2 SOLVENT EXTRACTION OF BELIZE 9

About 0.5 g of ground Belize 9 (ground on 1-8-1990) was accurately weighed into a sample vial and methanol (10 ml) was added. The stoppered vial was sonicated for 10 minutes and then left at room temperature overnight for the extraction to take place. After a time lapse of approximate 16 hours, 1 ml of the reddish yellow extract was pipetted into a 5 ml volumetric flask. The extract was made up to mark with methanol. 10 μ l of the extract was injected onto the HPLC column. Three replicate injections were made and the average peak area was calculated. Extractions were carried out in duplicate. The concentration of eugenol was determined by comparison with calibration of standard eugenol solution.

2.3.3 SFE OF BELIZE 9

Approximately 0.2 g of Belize 9 was accurately weighed and transferred to the JASCO 1 ml SFE extraction vessel (Japan Spectroscopy Co.). The sample was extracted when the Rheodyne valve was turned from the load to the inject position. Operating conditions were obtained by varying the flow setting on the pumps, oven and the JASCO 880-81 back pressure regulator (BPR). Pressures of between 200 -300 kg cm⁻², temperatures of between 40-80°C and methanol percentage of between 2%-20% were used for the extractions. The BPR temperature was kept at 60°C throughout the extractions. For each condition, at least triplicate extractions were carried out. The extracts were collected as solid carbon dioxide in a 100 ml round-bottom flask with the bottom immersed in liquid nitrogen (Figure 1). The solidified carbon dioxide was allowed to evaporate slowly in a refrigerator to avoid loss of eugenol. Each of the oily yellow extracts was dissolved in methanol (10 ml). A sample (10 µl) of each extract was injected onto the HPLC column. The average area of each extract was used to calculate the percentage yield and for some of the extracts appropriate dilutions were done so that the area falls within the calibration range used. The recovery of eugenol by SFE was calculated with respect to the methanolic solvent extractions.

2.3.4 COLUMN PACKING (HPLC)

The packing material (2g) was first dispersed in 10 ml acetone and sonicated for about 10 minutes before pouring the slurry into the stainless steel packing reservoir (30 cm x 8 mm i.d.). Packing was done using the

Haskel air driven fluid pump from Olin Energy System Ltd. (Burbank, California, U.S.A.) at a pressure of 6500 p.s.i. using acetone as the packing solvent. The column was then flushed with methanol for 1 hour to remove the acetone. During this time the column was not connected to the detector to avoid passing the acetone through the detector. The column was equilibrated with the eluent {methanol-pH 7 phosphate buffer (50:50 v/v)} for another hour. The column efficiency was tested with a test mixture from Phase Sep consisting of dimethyl phthalate, nitrobenzene, anisole, diphenylamine and fluorene (for reversed phase chromatography). Column efficiency, N was found to be 3637 based on the fluorene peak.

2.3.5 CALIBRATION PROCEDURE

Standard eugenol solutions were prepared by serial dilution of the stock eugenol solution (100 ml l^{-1}) in methanol. The calibration range used was from 0.30 to 0.10 ml l^{-1} eugenol standard. Samples ($10 \mu\text{l}$) of each standard solutions were injected onto the HPLC column and the average area of three replicate injections were taken. A calibration graph of area vs. eugenol concentration was plotted and the least square fit calculated. The concentration of the eugenol extracted in Belize 9 by the methanol and supercritical fluid extractions were obtained from the regression line. The regression equation was obtained using the MINITAB statistical package version 8.21 on the PC.

2.3.6 CALCULATIONS

2.3.6.1 COLUMN EFFICIENCY AND CAPACITY FACTOR

The column efficiency N was calculated using the formula

$$N = 5.54 (t_r/w_h)^2$$

where t_r is the retention time; and w_h is the peak width at half peak height.

The capacity factor (k) of eugenol from methanol extraction and SFE of Belize 9 was calculated using the formula

$$k = (t_r - t_0)/t_0$$

where t_0 is the retention time of an unretained peak (void volume). Sodium nitrate (6.34 mg ml^{-1}) was used as the void volume marker.

2.3.6.2 % YIELD OF EUGENOL

An example of calculation of % yield of eugenol in a methanolic extract.

Weight of Belize 9 used = 0.5048 g

The extract was dissolved in 10 ml methanol. 1 ml of this solution was diluted to 5 ml with methanol, i.e. sample (0.5048 g Belize 9) is in 50 ml solution. Average peak areas obtained from triplicate injections of the solution is 30919000 I.U. From the calibration graph, this area is equivalent to 0.238 ml l^{-1} .

Concentration of eugenol is 0.238 ml l^{-1} in 50 ml

Density of eugenol = 1.064 mg ml^{-1} .

Mass of eugenol in sample = $0.238 \text{ ml} \times (50 \text{ ml}/1000 \text{ ml}) \times 1.064 \text{ mg ml}^{-1}$
= 0.0127 g eugenol in sample

$$\begin{aligned}\% \text{ eugenol in sample} &= 0.0127 \text{ g eugenol} / 0.5048 \text{ g Belize 9} \times 100 \\ &= 2.52 \%\end{aligned}$$

\therefore % eugenol in the pimento (Belize 9) is 2.52 %.

2.3.6.3 CALCULATION OF SUPERCRITICAL FLUID DENSITY

Numerous equations of state for describing the pressure, volume, temperature (PVT) variations in carbon dioxide are available in the literature (2-7). For this study, the density of carbon dioxide was calculated using the SF-Solver software from ISCO which uses an equation of state according to Pitzer (6, 7). The equation of state for an ideal gas ($PV/RT=1$) can be extended for real gas by adding another term, z . This then becomes

$$PV/RT = z$$

where z is the compressibility factor.

Pitzer defined z as a truncated power series

$$z = z(0) + \omega z(1) + \dots$$

where $z(0)$, $z(1)$, are tabulated and assumed to be functions of T_r and P_r .

ω is the acentric factor, defines as $-\log P_r^{-1.00}$.

P_r is the reduced pressure, defined as P/P_c at $T_r = 0.7$

T_r is the reduced temperature, defines as T/T_c .

P_c is the critical pressure.

2.3.7 CARBON DIOXIDE FLOW RATE

Carbon dioxide flow rate (l min^{-1}) was measured using a rotameter to give a volume flow rate. However, it was more convenient to express the CO_2 flow rate as the mass flow rate (g min^{-1}), as this enabled a direct comparison with the modifier concentrations.

$$\text{CO}_2 \text{ flow rate (g min}^{-1}\text{)} = \{\text{CO}_2 \text{ flow} \times \text{RMW of CO}_2 \times P\} / R \times T \text{ of CO}_2$$

where

$$\text{RMW of CO}_2 = 44.01 \text{ g mol}^{-1}, R = 0.08205 \text{ l atm mol}^{-1}\text{K}^{-1} \text{ and } T \text{ of CO}_2 = 293 \text{ K}$$

2.3.8 MODIFIER CONCENTRATION

The modifier concentration in SFE was calculated from the mass flow rate of CO_2 and the modifier flow rate indicated on the modifier pump. Modifier mass flow rate (g min^{-1}) was calculated using the formula

$$\begin{array}{ccc} \text{modifier flow rate} & = & \text{flow rate at pump} \times \text{density of modifier at RTP} \\ (\text{g min}^{-1}) & & (\text{ml min}^{-1}) \quad (\text{g ml}^{-1}) \end{array}$$

The modifier concentration was then calculated as:

$$\begin{array}{l} \text{modifier concentration} = \frac{\text{modifier flow rate (g min}^{-1}\text{)}}{\text{CO}_2 \text{ flow rate} + \text{modifier flow rate}} \\ (\% \text{ w/w}) \quad \quad \quad (\text{g min}^{-1}) \quad (\text{g min}^{-1}) \end{array}$$

2.4 REFERENCES

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CHAPTER 3

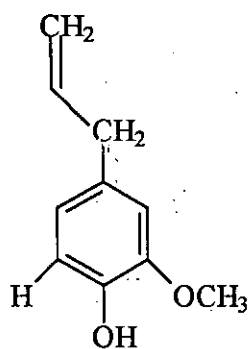
SUPERCRITICAL FLUID EXTRACTION OF PIMENTO BERRIES

3.0 INTRODUCTION

Berries of the pimento tree are the source of the important spice Allspice. The Latin name of pimento tree is *Pimenta dioica* and it belongs to the class Myrtaceae. Pimento trees are cultivated in the Carribean Islands and Central America. Dried pimento berries of commerce are 4 to 7 mm in diameter, of sub-globular shape, and reddish brown colour. They have a rough surface, caused by protuberant, minute oil glands. The odour and flavour of the berries are due to the essential (volatile) oil present, chiefly in the shells (pericarps). The dried berries contain from 3 to a little more than 4 percent of essential oils, more than 8 percent of quercetannic acid (hence the astringent taste), a soft resin with a burning taste, up to 25 percent of crude fibre, some fixed oil, proteins, about 3 percent starch, and traces of an alkaloid (1).

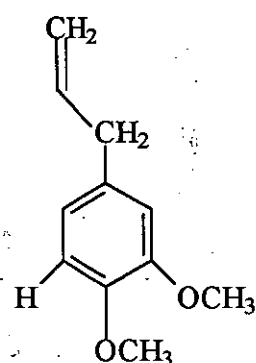
The components that have been identified in the essential oils derived from pimento berries are eugenol (the main component about 70%), methyl eugenol (about 9%), β -caryophyllene, α -phellandrene, 1,8-cineole (eucalyptol) and palmitic acid (Figure 1) (2).

Figure 1. Components of the essential oils in pimento berries (2).



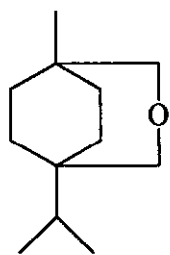
Eugenol

2-methoxy-4-(2-propenyl) phenol



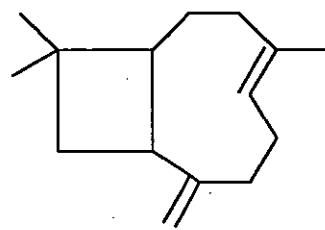
O-methyl eugenol

1,2-dimethoxy-4-(2-propenyl) benzen

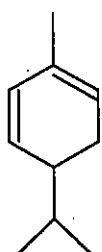


1,8-cineole

(1,8-epoxy-p-menthane)

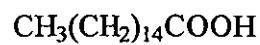


Beta-caryophyllene



alpha-phellandrene

(1-methyl-4-isopropyl-1,5-cyclohexadiene)



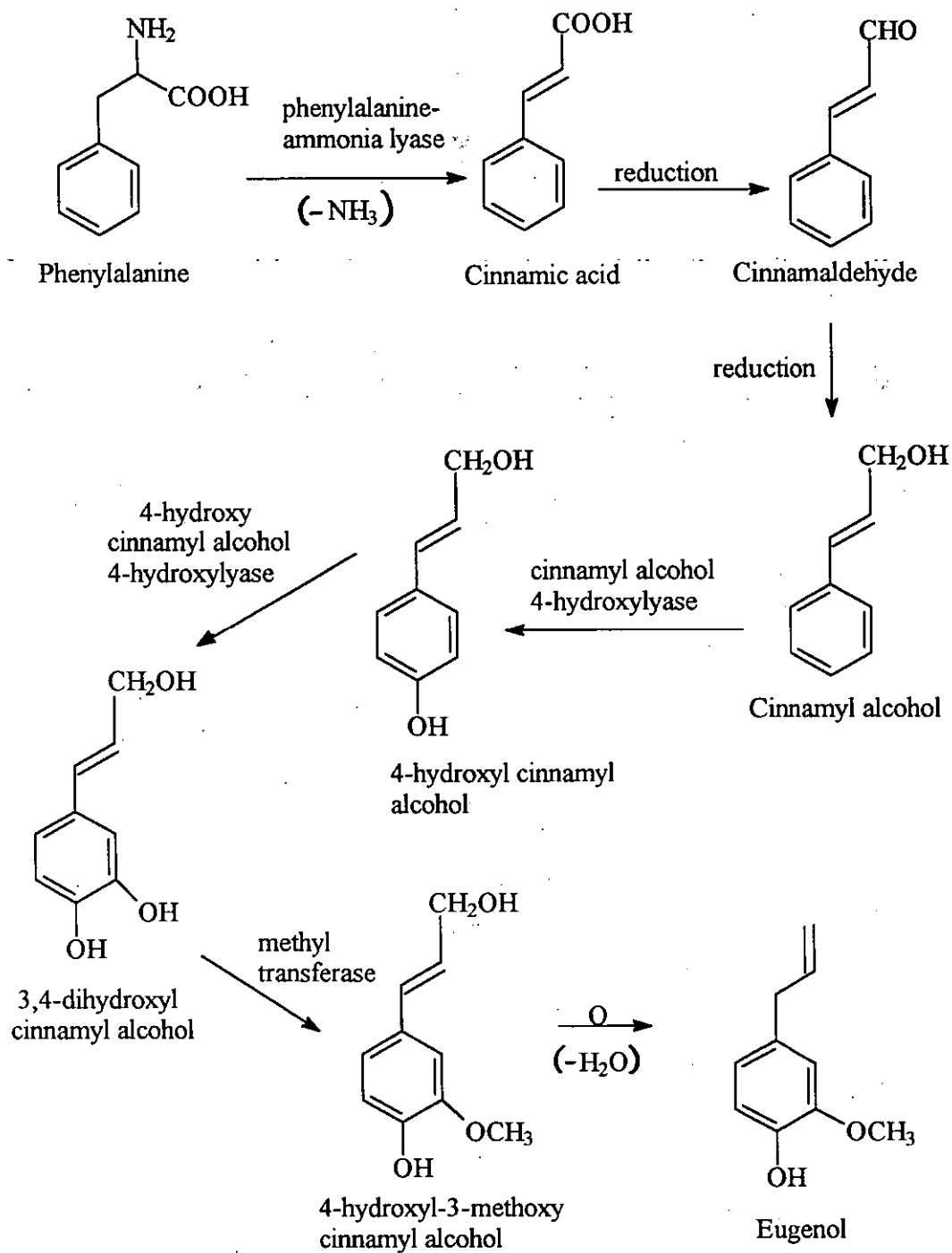
palmitic acid

(n-hexadecanoic acid)

Eugenol is a colourless to yellow or yellow-reddish liquid, darkening with age, and possessing the characteristic odour and flavour of "allspice". It is also the main constituent of several other important essential oils, e.g., oil of clove, clove stem and leaf, pimento berry and leaf, bay and cinnamon leaf (1). In smaller quantities, eugenol occurs in numerous essential oils, e.g., camphor, ylang ylang, cananga, sassafras, nutmeg, galangal, laurel, Java citronella, etc. The boiling point of eugenol is 253°C (3). It is widely used in pharmaceutical preparations, in perfumes, cosmetics, and for the scenting of soaps. It serves for the flavouring of all kinds of food products, especially meat, sausages, tables sauces, etc. The main use of eugenol, however, is as a starting material for the making of high quality vanillin (2). In the therapeutic category it is an analgesic. It is practically insoluble in water but miscible with alcohol (1-2 volume of 70% alcohol), chloroform and ether.

The biosynthesis of eugenol (and also of cinnamaldehyde and safrole) originates from phenylalanine and the following reaction sequence is assumed (Figure 2) (4):-

Figure 2. Biosynthesis of eugenol from phenylalanine in plants (4).



3.1 TRADITIONAL METHODS OF ESSENTIAL OILS EXTRACTION

Numerous methods have been used for the isolation of essential oils from plant material but the majority of essential oils have always been obtained by hydrodistillation (3). Other extraction methods include headspace analysis, volatile solvent extraction, extraction with hot fat (maceration) or cold fat (enfleurage), cold expression (3, 5, 6) and Soxhlet extraction (7).

There are three types of hydrodistillation methods:

1. Water distillation;
2. Water and steam distillation;
3. Steam distillation.

In the water distillation method, the plant material to be distilled is in direct contact with the boiling water. In the water and steam distillation method, the plant material is supported on a perforated grid or screen inserted some distance above the bottom of the still. The lower part of the still is filled with water. In this method the plant material is in contact with steam only, and not with boiling water. In the steam distillation method, live steam (frequently at pressures higher than atmospheric pressure) is introduced through open or perforated steam coils below the charge, and proceeds upward through the charge above the supporting grid.

The method of maceration and enfleurage are used for plant materials which yield no oil at all on distillation. The oil has either been

destroyed by the action of steam, or the minute quantities of oil actually distilling over are 'lost' in the large volume of distillation water from which the oil cannot be recovered. This problem applies to jasmine, violet, mimosa, hyacinth, gardenia, narcissus, acacia, tuberose and a few others. The process of maceration has been largely superseded by extraction with volatile solvents (usually petroleum ether).

3.2 SUPERCRITICAL FLUID EXTRACTION OF ESSENTIAL OILS

The range of samples for which SFE has been applied continues to broaden. SFE is a method that takes advantage of both distillation and extraction. SC fluids have several characteristics that make them suitable for extraction purposes especially for essential oils. Many SC fluids have a low critical temperature (T_c) and this enables the extraction to be carried out at a low temperature and avoids decomposition of thermally labile compounds. Artifact production and the thermal degradation of products are possible in hydrodistillation and conventional extractions (8, 9). In SFE, the concentration step is greatly simplified since the SC fluids, such as carbon dioxide, is removed from the extract by depressurisation at ambient temperature. This is very useful, especially in flavours industry, as the solvent does not impart flavour to the products.

Essential oils are usually a complex mixture containing tens and sometimes hundreds of components (3, 6). The majority of the components usually are terpenoids based on the isoprene molecule, $\text{CH}_2=\text{C}(\text{CH}_3)-$

$\text{CH}=\text{CH}_2$. The union of two or more of this isoprene units produce the mono- and sesquiterpenes (C_{10} and C_{15} respectively) and their oxygenated compounds (3). The components of the essential oils may be classified into five groups according to their differing properties:-

- (i) Hydrocarbon monoterpenes (e.g. α -phellandrene)
- (ii) Oxygenated monoterpenes (e.g. 1,8-cineole)
- (iii) Oxygenated sesquiterpenes (e.g. valeranone)
- (iv) Hydrocarbon sesquiterpenes (e.g. β -caryophyllene)
- (v) Phenylpropanoids (e.g. eugenol, methyl eugenol)

Essential oils are widely used in the flavours and fragrance industries. The composition of the essential oils is obviously one of the main factors that determine the quality of the final product and hence its retail price. The variability of the essential oil composition due to climatic and source variations makes quality-control checks a prerequisite. Variability can also arise due to the selection of the analytical technique, method of extraction and deterioration of the oil upon storage (10). Many essential oils and terpenes are known to be chemically unstable and can undergo changes such as intermolecular rearrangement, polymerisation, oxidation, hydrolysis, and thermal decomposition which can become a problem in their analysis. These changes are most rapid with very small samples, even when they are kept in the dark and at low temperatures. For instance, a gradual decomposition of zingibrene to ar-curcumene (11) on storage at 7°C , and polymerisation of myrcene (12) have been reported.

Essential oils are highly soluble in liquid and SC CO₂ (13) as they are composed of low molecular weight lipophilic substances with relatively high vapour pressures. The selective production of essential oils using SF (quantitative recovery) without the co-extraction of unwanted compounds (e.g. waxes, colouring matter, undesirable fatty acids) is a problem. However, by careful choice of pressures and temperatures, the selectivity of the SC fluids can be altered and hence the possibility of separating unwanted impurities by fractional precipitation can be used. The fractionation of the extract from caraway seeds using liquefied CO₂ (extraction at 90 bar and 20°C) indicated how a three stage precipitation could enable a good precipitation of the essential oils (14). The first separator (90 bar, 40°C) collected mainly fatty oils containing only 1% essential oils, the second separator (75 bar, 40°C) collected a mixture of essential and fatty oils (9.6%) and the third separator (30 bar, 0°C) collected over 90% of a caraway essential oil having excellent flavour.

A survey of the literature shows that SFE has gained popularity as an extraction method for essential oils. Verschuere *et al.* (15) used SFE to optimise the SFE fractionation of the essential oils (myrcene, humulene and β -caryophyllene) and bitter principles (humulones and lupulones) of hops. 99% humulene, 100% myrcene and 100% β -caryophyllene were extracted at 50°C and a CO₂ density of 0.2 g ml⁻¹. 100% lupulones were extracted at a CO₂ density of 0.7 g ml⁻¹ and 100% humulones were extracted at a CO₂ density of 0.9 g ml⁻¹. Gopalakrishnan and Narayanan (16) used SC CO₂ at 40°C and 100 bar to extract essential oils from cardamom seeds. Yield of extracts were fairly constant in the pressure range from 100 - 600

bar. Riekkola *et al.* (17) use SFE to extract essential oils from savory, rosemary and dragonhead. SFE was compared to hydrodistillation. Even though the essential oils are highly soluble in SC CO₂, the extraction rates were relatively slow with pure CO₂ (ca. 80% after 90 minutes). However, a 15 minute static extraction with methylene chloride as modifier followed by a 15 minute dynamic extraction with pure CO₂ yielded high recoveries of the essential oils and agreed well with the results of hydrodistillation performed for 4 hours. Coppella and Barton (18) evaluated the feasibility of SC CO₂ extraction of lemon oil near ambient temperature to generate equilibrium data for CO₂ with multi-component essential oil constituents (limonene, geranial and β -caryophyllene). The phase equilibrium diagram for CO₂-lemon oil resembled a typical binary CO₂-hydrocarbon system. The highest solubility level was 2.8 wt % (at 313 K) essential oils. Riekkola *et al.* (19) used on-line SFE/GC for the analysis of volatiles (α -terpinene, γ -terpinene, thymol and carvacrol) in thyme at 200 atm. and 54°C for 30 minutes. Excellent peak shapes were obtained for thyme components and the chromatograms were comparable to those obtained by GC after hydrodistillation. Huston and Ji (20) used SFE-GC/MS to evaluate this type of system for the analysis of complex natural products. They used the system for the extraction and analysis of ground clove buds. Table 1 gave a list of other works which have focused on the extraction of essential oils using SFs.

Table 1. Typical papers which have focused on extraction of essential oils using SFs.

Sample	Compounds extracted	Ref.
Caraway seeds	Carvone and terpene fractions.	21
Lemon peel	Limonene, β -pinene, γ -terpinene, geranial, terpineol, neral, citronellal, geranyl acetate, β -bisbolene, myrcene, sabinene, neryl acetate.	22
Rhizome of <i>Z. Zerumbet</i>	α -pinene, camphene, linalool, camphor, β -caryophyllene, humulene.	23
Anise	methyl-chavicol, α -terpineol, anethole.	24
<i>Origanum vulgare</i>	cineole, p-cymene, linalool, α -terpineol, terpinene-4-ol, borneol, thymol, carvacrol.	24
Rosemary	α -pinene, camphene, camphor, 1,8-cineole, borneol, bornyl acetate, humulene.	25
Chewing gum	limonene, menthone, isomenthone, menthol, carvone, $C_{15}H_{24}$ isomer.	25
Orange peel	α -pinene, β -pinene, limonene, $C_{15}H_{24}$ isomer.	25
Spruce needle	α -pinene, camphene, β -pinene, limonene, camphor, bornyl acetate.	25
Thyme	borneol, thymol, carvacrol, $C_{15}H_{26}O$ isomer.	25
Cinnamon	cinnamaldehyde, coumarin, $C_{15}H_{24}$ isomer.	25
Cedar wood	cedrene, cedrol.	25
Lime peel	α -pinene, β -pinene, limonene.	26
Eucalyptus leaves	α -pinene, 1,8-cineole, $C_{15}H_{24}$ isomer, $C_{15}H_{24}O_2$ isomer, $C_{15}H_{26}O$ isomer.	26
Lemon peel	α -pinene, β -pinene, α -terpineol, geranial, neral, nerol, geraniol, $C_{15}H_{24}$ isomer.	26
Basil	1,8-cineole, $C_{10}H_{18}O$ isomer, estragole, eugenol, $C_{15}H_{24}$ isomer.	26
Brewing hops	β -pinene, $C_{15}H_{24}$ isomer, $C_{10}H_{16}O$ isomer, $C_{15}H_{24}O$ isomer.	27
Grape fruit	Limonene, carvone.	28
Feverfew (<i>Tanacetum parthenium</i>)	camphor, parthenolide, dihydroparthenolide chrysanthanol acetate.	29
Cold-pressed orange	α -pinene, sabinene, myrcene, phellandrene, d-limonene.	30
Vetiver root	$C_{15}H_{24}$ isomer, β -vetivene, $C_{15}H_{22}$ isomer, $C_{15}H_{24}O$ isomer, khusimone, $C_{15}H_{22}O$ isomer, zizanal, zizanol, bicyclovetivenol, β -vetivone and α -vetivone.	31
Coriander fruits	Linalool, α -pinene, γ -terpinene, d-limonene, camphor, geranyl acetate, geraniol, camphene.	32

3.3 ANALYSIS OF ESSENTIAL OILS

The quality and the characteristics of an essential oil are usually established using various analytical techniques ranging from physicochemical and non-specific chemical methods, to chromatographic methods. Gas chromatography-flame ionisation detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) are supreme for the analysis of essential oils (6). Many essential oil constituents cannot be analysed by high performance liquid chromatography (HPLC) with ultraviolet (UV) detection because of the lack of chromophoric groups in compounds such as monoterpene hydrocarbons and alcohols. However, a number of essential oils contain, often as their major constituents, compounds which may be readily assayed by HPLC with UV detection. In these cases, HPLC provides a quicker analysis method and because of the selectivity of the UV detector produces a much simpler chromatogram than that obtained by gas liquid chromatography (GLC).

Essential oils which have been analysed by HPLC-UV include cinnamaldehyde and eugenol from cinnamon bark and leaves (33), bergapten from bergamot (34) and eugenol from pimento berries (35). Ross (36) used HPLC-UV to separate phenolic constituents (vanillin, methyl salicylate, eugenol and thymol) of volatile oils on Hypersil SAS column. Quantitation of eugenol has been done by Ross (33), Smith and Beck (35) and also a preliminary study carried out in this laboratory using SF CO₂ (37). GLC (38-41) have been used to determine eugenol and numerous minor constituents in the essential oils.

Cartoni and Coccioli (42) reported the analysis of essential oils from lemon, bergamot, and orange using HPLC with microbore columns and UV detection at different wavelengths. They tentatively identified the non-volatile components of the essential oils by using HPLC-MS. Enantiomer ratios of carvone in the essential oils extracted from caraway seeds and spearmint leaves were determined by liquid chromatography (LC) with UV and polarimetric detection (43). HPLC has been particularly useful in the analysis of sesquiterpene lactones, where it is claimed that GC is of limited use because the compounds are not always sufficiently volatile and may need derivatisation (44, 45).

In GLC, the relatively high temperatures used for the separation may cause problems for the analysis of some thermally unstable essential oil constituents. High injection temperatures usually employed in GLC (above 120-150°C), may dehydrate compounds like linalool (46), camphene hydrate (47) or α -terpineol (48). In such cases, the use of supercritical fluid chromatography (SFC) is very useful, though not many applications were found in the literature for essential oil analysis. GC with capillary columns was much superior for the analysis of complex mixtures such as essential oils. Manninen *et al.* (49) used capillary SFC to analyse thyme, peppermint and basil oil. They found that SFC was a useful and reliable method than capillary GC for the quantification of oxygenated main components in essential oils. Morin *et al.* (50) have separated and identified sesquiterpene hydrocarbon mixtures on a bare Spherisorb silica column with on-line FT-IR detection. These non-polar but extremely thermosensitive compounds required low-temperature rather than a low-

density experimental conditions. Burford (51) used packed column SFC (Spherisorb silica, Spherisorb ODS and PLRP-S column) to separate limonene, carvone, caryophyllene, santonin and quercetin. The silica and ODS column acted as a normal phase mode with the most non-polar analytes eluting first and the most polar last. The elution order was limonene, caryophyllene, carvone, santonin and quercetin. This was unexpected for the ODS column as the stationary phase is non-polar, so that a reverse phase elution order was expected. Schoenmakers *et al.* (52) suggested that a mixed retention mechanism exists in SFC. In SFC, the bonded phase appears to possess two retention mechanisms, one attributed to the bonded alkyl substituents, so that the stationary phase may have a greater affinity for the non-polar analytes, and the other, often referred to as the secondary retention mechanism, is due to the remaining accessible silicas surface, which absorbs the polar analytes such as santonin and carvone. On the PLRP-S column (no silanol groups) the order of elution of the essential oils was limonene, carvone, caryophyllene, santonin and quercetin. It appears that the analytes were eluting in order of volatility, possessing the same elution order as on a capillary GC column.

3.4 AIM OF WORK

The present work is a continuation of the work done earlier in this laboratory (37). The reproducibility of SFE for the extraction of eugenol from pimento berries was determined on a few of the conditions used before. Eugenol has been quantitated in cinnamon bark and leaves using HPLC-UV (33), in pimento berries using HPLC-UV (35), in cinnamon

bark using GLC-FID (41). In all these cases, the eugenol has been extracted using traditional solvent extraction. It was of interest to use SF as an extraction medium as it is faster (53) and this offers significant time saving, leaves no solvent residues, eliminates solvent disposal and provides comparable or better extraction efficiencies than Soxhlet extraction (54, 55). Preliminary work by Lawson (37) on the quantitation of eugenol from pimento berries using HPLC-UV after extraction with SC CO₂ and modified SC CO₂ with 2% methanol as an alternative extraction method has been done in this laboratory. However, the SFE recovery of the eugenol was not quantitative and it is the aim of this work to obtain a quantitative recovery of the eugenol using SC CO₂. The effect of pressure, temperature, modifier and additive were investigated in order to obtain a quantitative recovery of eugenol from the berries.

3.5 RESULTS AND DISCUSSIONS

3.5.1 PRELIMINARY WORK

3.5.1.1 SOLVENT EXTRACTION

The pimento berries used in this work comes from the Belize Island and is known as Belize 9. Methanolic extracts of Belize 9 were examined by reversed phase HPLC using an ODS-2 Spherisorb column. The chromatogram (Figure 3a) contained two major peaks. One large peak which eluted at the solvent front. A second peak at 8.70 min was identified as eugenol by comparison with an authentic sample. The peak areas of eugenol in each extract were compared with those of standard to determine the amount of eugenol in pimento berries. The UV detector gave a linear

response ($r = 0.9959$) for eugenol in the concentration range used ($0.10 - 0.30 \text{ ml l}^{-1}$). The peak at the solvent front represents polar compounds since a non-polar stationary phase and a polar mobile phase is used. Thus liquid extraction using methanol, extracted other polar compounds apart from eugenol from the berries. The chromatogram of SFE of Belize 9 (Figure 3b) give only one major peak ($t_r = 8.70 \text{ min}$) which was identified as eugenol by comparison with an authentic sample. We can conclude that SFE is more selective than the solvent extraction. The average concentration of eugenol in Belize 9 from methanol extraction was found to be 2.57%. This value was taken to represent 100% extraction by methanolic solvent extraction. This concentration was lower than those reported by Smith (35) and Lawson (37) which was 2.93% and 2.80% respectively. This lower value could probably be due to ageing of the pimento berries. In the present study the pimento berries were ground in 1990. Figure 4 is a the calibration graph of eugenol standard.

Figure 3a. HPLC chromatogram of methanol extraction of pimento berries. HPLC conditions: column, 5 μm ODS-2 Spherisorb (100 mm x 5 mm i.d.); eluent, 50:50 (v/v) methanol- phosphate buffer pH 7; eluent flow rate, 1 ml min⁻¹; column temperature, 30°C; UV detector at 278 nm.

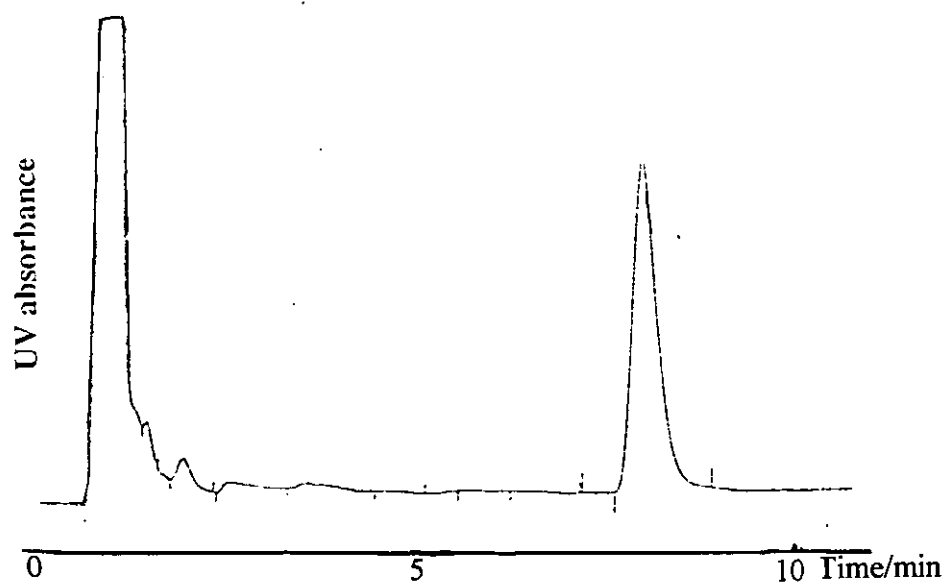


Figure 3b. HPLC chromatogram of SFE extraction of Belize 9 using CO₂ at 60°C and 200 kg cm⁻². HPLC condition as Figure 3a.

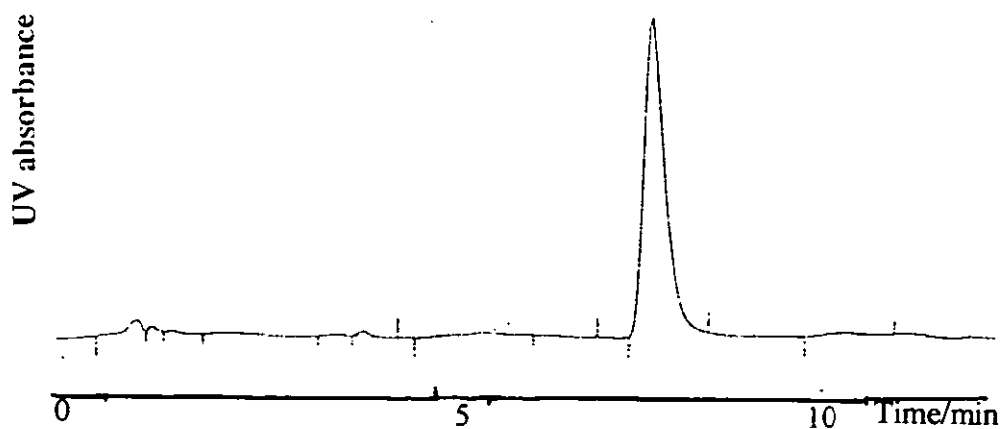
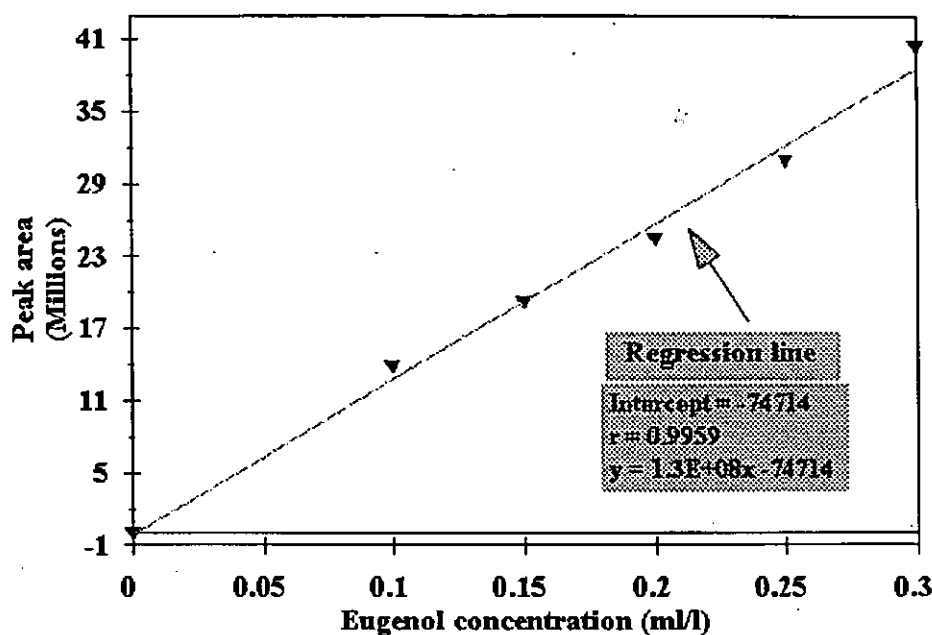


Figure 4. Calibration graph of eugenol concentration versus peak area of eugenol in integrator units (I.U.)



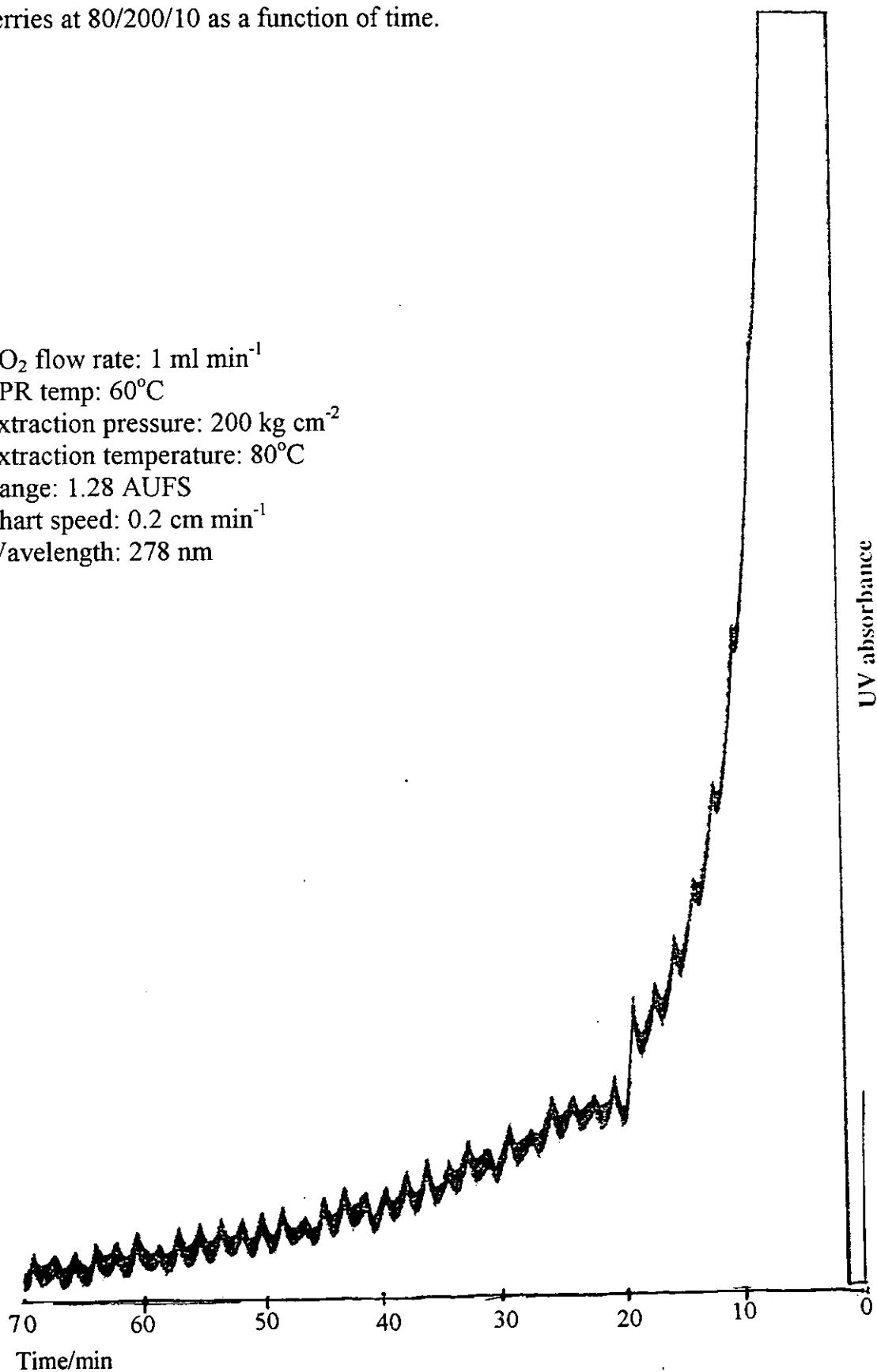
3.5.1.2 SUPERCRITICAL FLUID EXTRACTION

In the extraction of eugenol from pimento berries, shorthand notation such as 40/200/10 will be used from now onwards. This shorthand notation stands for an extraction temperature of 40°C, an extraction pressure of 200 kg cm⁻² and 10% modifier was used in the extraction. The conditions 40/200/0, 40/300/0, 60/200/0 and 40/200/2 were chosen to test the reproducibility of SFE for the extraction of eugenol from Belize 9. The condition 40/200/0 was first used to extract the eugenol.

SFE was carried out by extracting about 0.2g of ground pimento berries in a JASCO 1 ml extraction vessel which was placed in the sample loop position of the injector with the valve in the load position. After equilibration of the condition has been achieved (about 10 minutes), the valve was switched from the load to the inject position to start the extraction. A few minutes after the injector has been switched from the load to the inject position, so that SC CO₂ passes through the extraction vessel over the Belize 9, the absorbance reading shot up to its maximum value indicating maximum solubilisation of eugenol in CO₂. Each extraction was monitored by UV and was only terminated after the UV absorbance had gone back to its original absorbance prior to the extraction. As the SF and extract flows through the back pressure regulator, the fluid pressure is reduced to atmospheric pressure causing the solubility to decrease virtually to zero. The extract in the fluid precipitates and falls from the outlet port of the valve on the back pressure regulator (BPR) on to the 100 ml cooled round bottom flask collection vessel which was attached to the port outlet (Figure 1, Chapter 2). After the solidified CO₂ was allowed to evaporate, an oily yellow extract was obtained. The extraction profile was monitored with an on-line UV detector at 278 nm (Figure 5).

Figure 5. UV extraction profile of eugenol extract from Pimento berries at 80/200/10 as a function of time.

CO₂ flow rate: 1 ml min⁻¹
BPR temp: 60°C
Extraction pressure: 200 kg cm⁻²
Extraction temperature: 80°C
Range: 1.28 AUFS
Chart speed: 0.2 cm min⁻¹
Wavelength: 278 nm

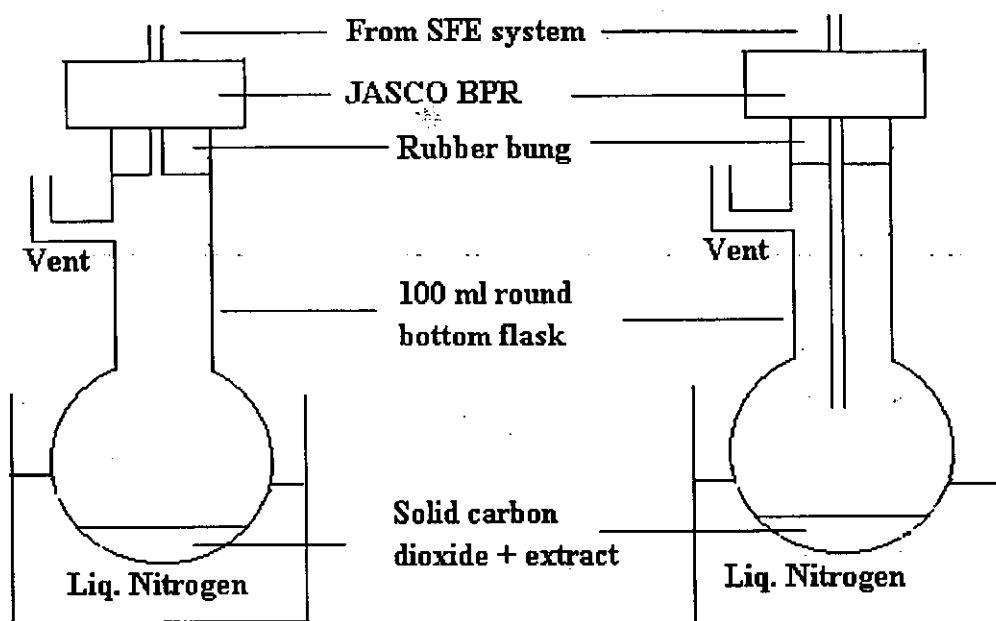


3.5.2 MODIFICATIONS TO THE JASCO SYSTEM

The extract from the SFE of pimento berries were initially collected in a nitrogen cooled round bottom flask as in Figure 6a. As the outlet of the BPR of the SFE system did not extend past the side arm of the round bottom flask (Figure 6a), it was feared that some eugenol might have been lost through the side arms before being cooled by the liquid nitrogen. Therefore a longer stainless steel tubing (3" long, 1/16" i.d.) was used. This tubing extends past the side arm of the round bottom flask (Figure 6b). It was found that when the longer metal tubing was attached to the outlet of the BPR, the recovery of eugenol was higher (66% at 40/200/0) compared to when the outlet was a short tubing (59% at 40/200/0). Even though the recovery of eugenol with the longer metal tubing outlet was not significantly different from the recovery with the short metal tubing outlet, the long metal tubing outlet was chosen for all subsequent extractions and also for extractions of chlorpyrifos (Chapter 6).

Figure 6. Collection vessel for eugenol from pimento berries by SFE.

(a) short (flush fit) metal tubing (b) 3" long metal tubing



3.5.3 EFFECT OF FLOW RATE

The effect of CO_2 flow rate on the recovery of eugenol from the berries at an extraction temperature of 40°C and a pressure of 200 kg cm^{-2} was studied at two different flow rates. It was found that a CO_2 flow rate of 2 ml min^{-1} gave a lower recovery of eugenol compared to a flow of 1 ml min^{-1} :

% Recovery of eugenol at a CO_2 flow of $1 \text{ ml min}^{-1} = 65.8$

% Recovery of eugenol at a CO_2 flow of $2 \text{ ml min}^{-1} = 39$

Percentage recovery was calculated based on the assumption that solvent extraction gave a 100% recovery. The recovery was found to decrease as

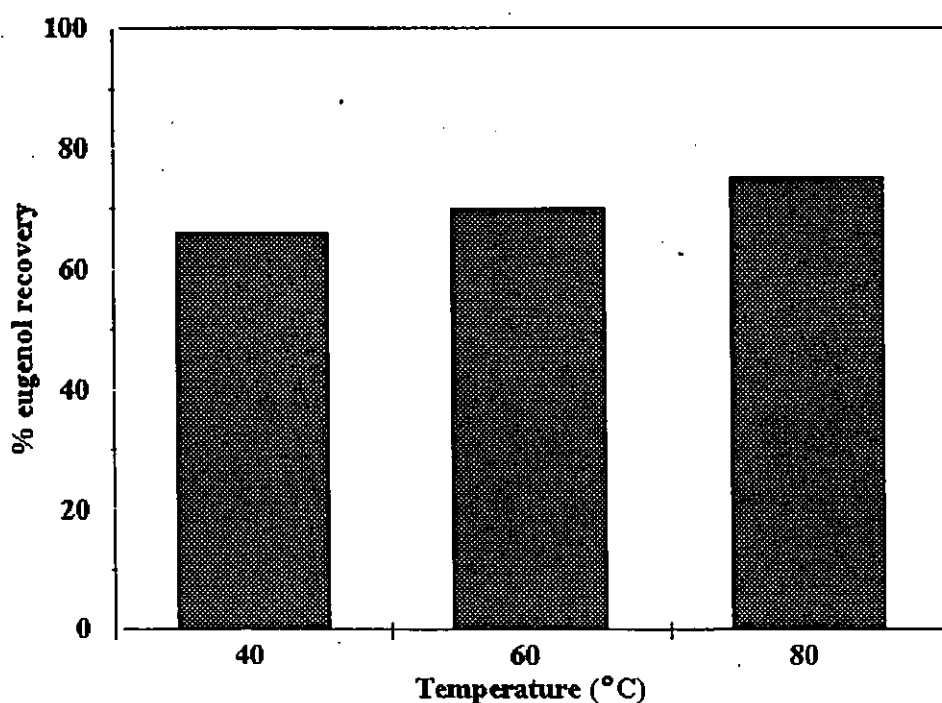
the flow rate was increased. A similar observation was noted by McNally (56) in her study on the recovery of linuron and diuron from sassafras soil. Burford (61) found that with the same collection system as at present, a CO₂ flow rate of 0.80 ml min⁻¹ was the optimum for the collection of essential oils from an α -cellulose matrix. In the present project a CO₂ flow of 1 ml min⁻¹ was employed for all subsequent extractions of eugenol from the berries.

3.5.4 EFFECT OF TEMPERATURE

The % eugenol recovered at 40/200/0 was 66% in the present studies. This was similar to the one obtained by Lawson (37). As the recovery was not quantitative, the effect of different extraction temperatures on the recovery of eugenol from the berries were carried out and the results are presented in Figure 7. The change of eugenol recovery is not sufficiently large to be significant. However, a general trend of increased eugenol recovery was observed on increasing the temperature from 40°C to 60°C and finally to 80°C even though there was a drop in the CO₂ density (from 0.846 g ml⁻¹ to 0.725 g ml⁻¹ to 0.578 g ml⁻¹ respectively). An increase in temperature might have been expected to decrease the solubility of a component in a SF rather than increase (57). This suggests that the extraction is not limited by the solubility of eugenol in the SF. The increase in recovery of eugenol with increasing extraction temperature from the berries could reflect increased volatility of eugenol. Also an increase in temperature results in an increase in the diffusivity of the substance in the matrix, and secondly a decreased viscosity of the SF

i.e. the SF can penetrate the matrix more easily. A similar observation (i.e. an increase in recovery with increased temperature) was noted by Euerby *et al.* (58) in the extraction of a corticosteroid (Tipredane INN) from a rodent diet.

Figure 7. Effect of temperatures on the recovery of eugenol at extraction pressure of 200 kg cm^{-2} .

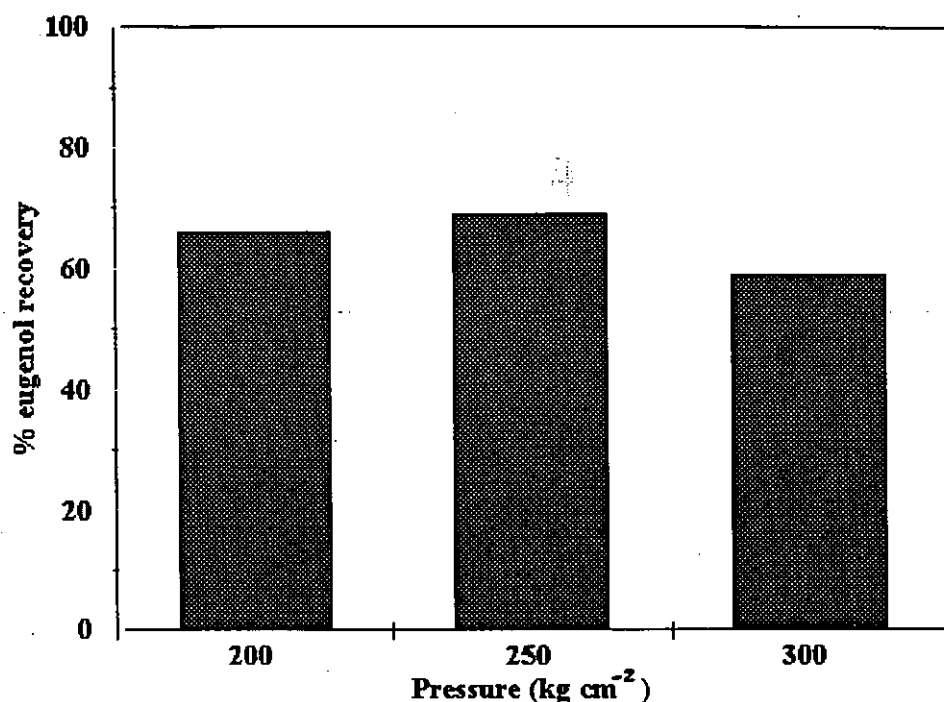


3.5.5 EFFECT OF PRESSURE

The effect of pressure on the recovery of eugenol was studied at 40°C (as the effect of higher temperature was not significant) at three different pressures (200, 250 and 300 kg cm^{-2}) (Figure 8). The density of

CO₂ at these conditions were 0.854 g ml⁻¹, 0.894 g ml⁻¹ and 0.926 g ml⁻¹ respectively. Higher pressures were not studied as the pressure limit of the SFE system is 350 kg cm⁻². The recovery obtained was lower at 40/300/0 (59%) compared to the extraction at 40/200/0 (66%). This is unexpected as increasing the pressure should increase the density of the supercritical fluid and thus the solubility of eugenol in the SF and hence the recovery. However, there is no significant difference in the variances at the 95% confidence level between these two results. This further supports the suggestion that the extraction is not limited by the solubility of eugenol in the SF. As there is no significant difference in the recovery of eugenol on increasing the pressure, a pressure of 200 kg cm⁻² was used.

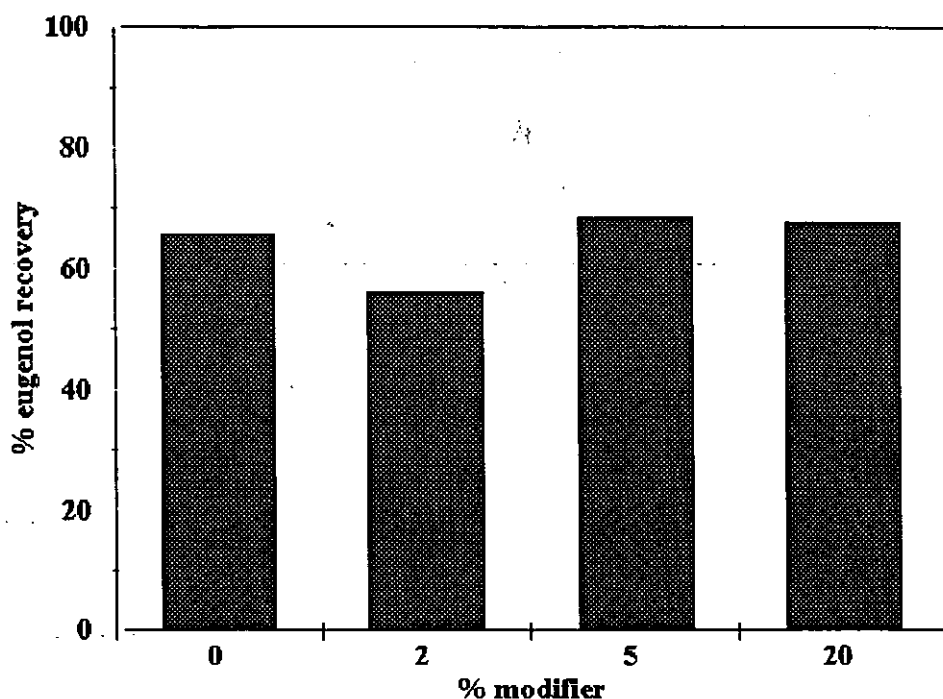
Figure 8. Effect of pressures on the recovery of eugenol at an extraction temperature of 40°C.



3.5.6 EFFECT OF MODIFIER

As the % recovery of eugenol was still not quantitative, the effect of methanol as modifier in the extraction fluid was examined. Modifiers have been known to increase the recoveries of relatively polar analytes from complex matrices (59, 60). The effect of increasing the solvent strength of SC CO₂ was first investigated using a 2% and 5% methanol modifier at 40°C and 200 kg cm⁻² and later a 20% modifier was used at this pressure and temperature (Figure 9).

Figure 9. Effect of % methanol on the % recovery of eugenol at an extraction temperature of 40°C and a pressure of 200 kg cm⁻².



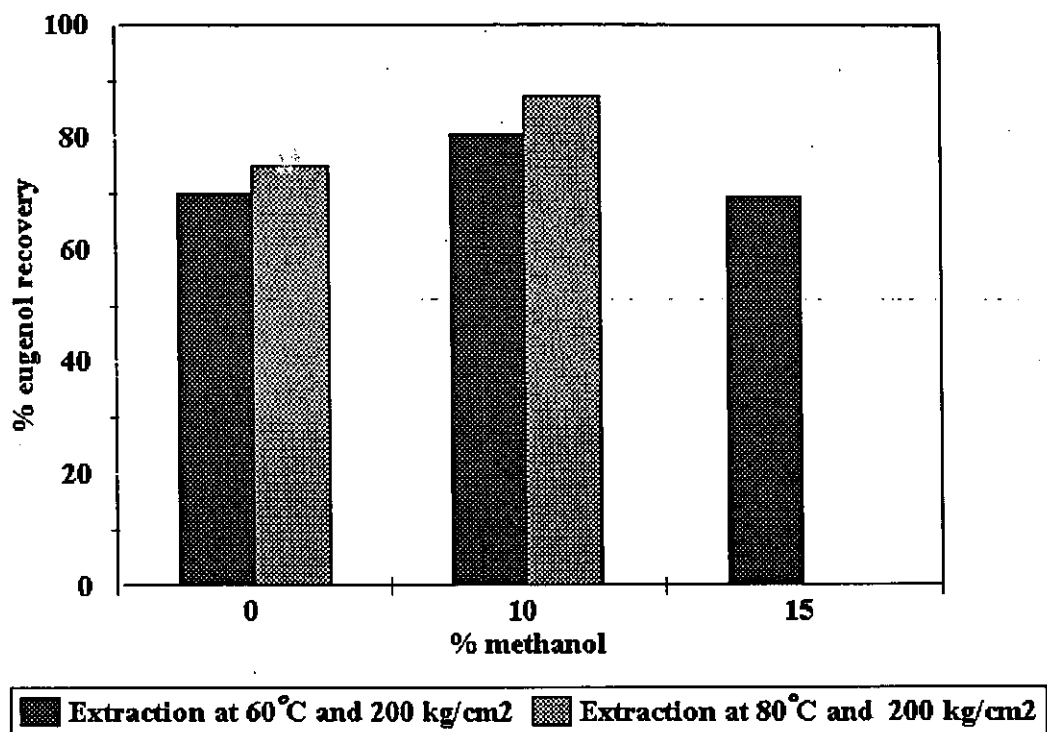
The recovery of eugenol decreased on adding 2% methanol as modifier. No explanation could be given to this observation. However, there was no significant difference in the variance (95% confidence level) of the eugenol recovery with 0% and 2% methanol. The eugenol recovery increased when the modifier concentration was further increased to 5%. The recovery was comparable to the recovery with no modifier added. This suggest that 5% modifier has no effect on the recovery of eugenol from the pimento berries. Even with higher percentages of methanol (20%) it was found that there was no effect on the recovery of eugenol at this

condition of pressure and temperature. Thus it appeared that moderate amounts of methanol were insufficient to release the eugenol from the plant material at these conditions. Higher percentages of methanol (greater than 20%) were not tried as this was deemed unsuitable for SFE. The very high proportions of methanol would resemble solvent extractions, and the advantage of using SFE would be lost.

When a higher temperature (60°C) was used at a pressure of 200 kg cm⁻² with 5% methanol the recovery was not significantly different from the yield at 40°C. However, there was an increase in the eugenol recovery on increasing the modifier concentration to 10% under these conditions (Figure 10).

When the modifier concentration was increased further to 15% there was a decrease in eugenol recovery which was comparable to the recovery without the modifier. Extractions at even higher temperature (80°C) at the same condition with 10% methanol gave an average eugenol recovery of 87% with 17% remaining unextracted (Figure 10). For some of the extraction conditions, the amount of eugenol left unextracted in the berries was determined by methanolic solvent extraction overnight at room temperature. The condition 80/200/10 gave the maximum recovery of eugenol. It was also found that the extraction time of 50 - 70 minutes did not give a complete extraction of eugenol. There was no significant difference in the amount of eugenol extracted at 50 minutes compared to one extracted at 70 minutes at the condition 80/200/10. There was always some eugenol left unextracted in the berries.

Figure 10. Effect of % methanol on the % recovery of eugenol at 60°C and 80°C and 200 kg cm⁻².

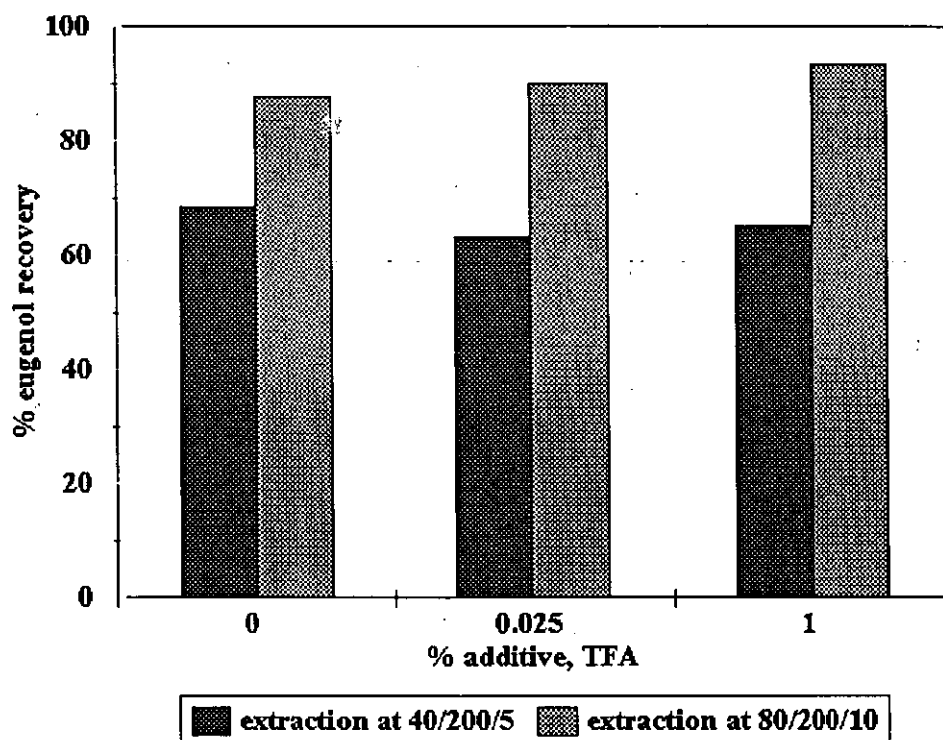


3.5.7 EFFECT OF ADDITIVE

Solvatochromic dye studies (61) suggest that very polar compounds, such as trifluoroacetic acid (TFA) when added to modifiers can significantly increase the polarity of modified mobile phases. Small concentration of this very polar compound (known as an additive) improves chromatographic peak shapes (62-65) and elute solutes that are normally retained. The mode of action of additives are not exactly known but many workers assume that additive functions by covering active sites.

Since eugenol is a phenol and might be trapped on basic sites on the plant material displacement with an acid was considered. The effect of small amount of trifluoroacetic acid in methanol on the recovery of eugenol was examined (Figure 11). With a 0.025% (v/v) trifluoroacetic acid (TFA) in methanol at the condition 40/200/5, the recovery of eugenol from the berries was not significantly different from the one without the TFA. When the TFA concentration in methanol was increased to 1% (at the same extraction condition) the recovery of eugenol was also not significantly different from the one with 0.025% TFA. There was still about 27% eugenol remain unextracted. However, at the condition 80/200/10 with 1% TFA in methanol, the recovery of eugenol was 93% (RSD 19%) with 13% remain unextracted. However, the increase in eugenol recovery with 1% TFA was not significantly different from the one with no TFA added or when 0.025% TFA was added. Similar observation was noted by Ahmad *et al.* (23) in the extraction of turmeric with 0.1% acid in methanol. The mode of action of TFA is unclear but it may have displaced the analyte from polar interactions. The TFA is to some extent capable of driving out the eugenol from the plant matrix although not very successfully.

Figure 11. The effect of TFA on the % recovery of eugenol from pimento berries.



3.6 REPRODUCIBILITY OF SUPERCRITICAL FLUID EXTRACTION

The reproducibility of SFE of eugenol from pimento berries is presented in Table 3. Triplicate extractions were carried out for each extraction conditions and for each extraction triplicate HPLC injections were carried out. The RSD of the recovery of eugenol from the berries from the present results are all less than 9% with the exception of 80/200/0 and 80/200/10 with 1% TFA.

Table 3. Standard deviation (% yield) and relative standard deviation (RSD) of SFE recovery of eugenol from pimento berries at different extraction conditions for the present results and from Lawson's work (36).
ND: Not determined.

Condition	Present results			Lawson's results		
T/P/M	Recovery	SD (% yield)	RSD (%)	Recovery	SD (% yield)	RSD (%)
40/200/0	65.8%	0.14	8.6	65.6%	0.30	16.7
40/200/2	56.0%	0.04	2.9	75.4%	0.24	11.7
40/200/5	68.5%	0.14	8.1	ND	ND	ND
40/200/20	67.6%	0.06	3.4	ND	ND	ND
60/200/0	70.0%	0.08	4.7	62.3%	0.03	1.9
60/200/10	80.6%	0.16	7.9	ND	ND	ND
60/200/15	69.4%	0.02	1.3	ND	ND	ND
80/200/0	74.9%	0.27	13.9	ND	ND	ND
80/200/10	87.5%	0.06	2.6	ND	ND	ND
40/200/0	65.8%	0.14	8.6	65.6%	0.30	16.7
40/250/0	69.0	0.02	1.3	69.4	0.20	10.4
40/300/0	59.1%	0.08	5.1	71.2%	0.03	3.6
40/200/5 (0.025% TFA)	63.1	0.03	1.9	ND	ND	ND
40/200/5 (1% TFA)	65.1	0.04	1.97	ND	ND	ND
80/200/10 (0.025% TFA)	90	0.07	3.1	ND	ND	ND
80/200/10 (1% TFA)	93.3	0.46	19.2	ND	ND	ND

3.7 CONCLUSIONS

From the experiments carried out it can be concluded that SC CO₂ modified with methanol is a suitable solvent for the extraction of eugenol from pimento berries and it is faster and more selective than liquid solvent extraction. Without the addition of TFA, the maximum recovery of eugenol was 88% at the condition 80°C, 200 kg cm⁻² and 10% methanol. When 1% TFA was added to the modifier, the % recovery of eugenol at these extraction conditions increased to 93%. Even with the addition of TFA, the increase in recovery of the eugenol was not significant and could not match direct solvent extraction using methanol.

These results together with the results on the extraction of other relatively polar constituents from plant matrices suggest that care must be taken in the interpretation of the results of these experiments as incomplete extraction may be occurring. Importantly they emphasise that extraction from complex matrices may often be incomplete even if no further analyte is being obtained with prolonged extraction or if nothing is obtained on repeating an extraction. This lack of quantification emphasises the need to compare the recovery of supercritical fluid extractions with alternative methods in any quantitative studies.

The principal advantage of SFE is that the use of a mild solvent results in a relatively clean extract which lacks polar constituents which are often present in solvent extractions. This could be an important advantage if the extracted material was to be used in further chemical or separation stages as the number of clean-up stages could be greatly reduced. The second advantage is that the need to use, evaporate and dispose of organic

solvents is greatly reduced. The third advantage is the reduction in time (1 hour for SFE compared to overnight for solvent extraction).

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CHAPTER 4

EXPERIMENTAL METHODS FOR THE EXTRACTION OF PESTICIDES

4.0 INTRODUCTION

In this chapter the experimental conditions for the extraction of organochlorine pesticides from sludge and chlorpyrifos from Dursban and soils are described. First, the experimental conditions for the extraction of organochlorine pesticides from sludge will be described followed by the extraction of chlorpyrifos from Dursban and soils.

4.1 EXPERIMENTAL

4.1.1 CHEMICALS

Dursban 5G (batch number 91RM216) and blank (as test) soil samples were from Dow-Elanco, Wantage, Oxford, U.K.. Chlorpyrifos standard (purity 99.9%) was from Riedel de Haen (Germany). HPLC grade ethyl acetate, methanol, hexane and acetone were from FSA Laboratory Supplies (Loughborough, U.K.). n-Docosane was from Sigma Chemicals Company (Gillingham, Poole, U.K.). Industrial grade carbon dioxide (99.98%), liquid nitrogen, helium, nitrogen and air were from BOC

Ltd., Middlesex, U.K.. Lindane (Laboratory reagent grade) was from BDH, Poole, Dorset, U.K..

4.1.2 STANDARD SOLUTIONS

(a) Organochlorine pesticides

Standards of OCPs, each $1 \mu\text{g ml}^{-1}$ consisting of hexachlorobenzene (HCB), p,p'-DDT, α -hexachlorocyclohexane (α -HCH), β -hexachlorocyclohexane (β -HCH), γ -hexachlorocyclohexane (lindane), p,p'-TDE (p,p'-DDD) and p,p'-DDE in a mixture of 20% hexane, 30% hexane and 50% toluene were supplied by Wessex Water plc. (Bristol, U.K.). Standards used by Wessex Water (external standard method) for calculating amount of pesticides in the sludge samples were 20 pg ml^{-1} each of the pesticides except for p,p'-DDT which was 40 pg ml^{-1} .

(b) Chlorpyrifos

Standard solution of chlorpyrifos (20 mg ml^{-1}) was prepared in ethyl acetate and standards for the calibration graphs were prepared by serial dilutions of this stock solution in acetone. n-Docosane stock solution (44 mg/ml) was prepared in ethyl acetate and used as an internal standard. Different amounts of n-docosane were used for the different calibration graphs. For the standard chlorpyrifos calibration graph range $40 - 400 \mu\text{g ml}^{-1}$, $4 - 40 \mu\text{g ml}^{-1}$ and $2 - 20 \mu\text{g ml}^{-1}$, the amount of n-docosane added was $880.8 \mu\text{g}$, $88.08 \mu\text{g}$ and $17.616 \mu\text{g}$ respectively. Different amount of n-docosane was added for each calibration graph because different sensitivity was in used in the gas chromatographic analysis.

4.1.3 SAMPLE PREPARATION

(a) Sludge

Wet sludge sample (102.2g) prepared by Wessex Water was spiked with 400 ng of HCB, α -HCH, β -HCH, lindane, p,p'-TDE (p,p'-DDD) and p,p'-DDE except for p,p'-DDT, which was spiked at 800 ng. The sludge was then freeze dried and mixed. Unspiked sludge sample (79.2 g) was also freeze dried. The freeze dried sludge samples (10.2g) were used as received and stored in the refrigerator when not in use.

(b) Dursban and soils

Dursban granules were ground in a commercial blender (Waring, California, U.S.A.) and sieved through a number 40 mesh. Standard German soil samples from Dow Elanco was sieved through a number 40 mesh. Other soil samples from Dow Elanco (Speyer 2.1, Speyer 2.2 and Speyer 2.3) were used as received. Clay soil samples (from Loughborough) was air dried in the laboratory overnight and any rubbish (sticks, pebbles, leaves etc.) removed and the remainder sieved through a number 40 mesh. Dark peat samples were bought from B and Q store (Loughborough, U.K.) and used as it was. Acid washed sand (FSA Laboratory Supplies, Loughborough, U. K.) was used as received.

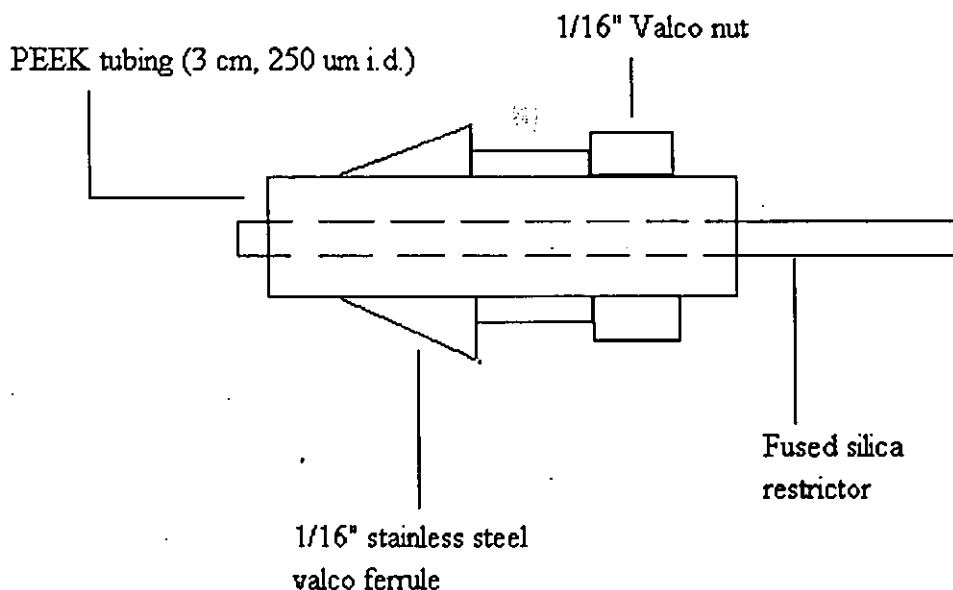
4.2 INSTRUMENTATION

4.2.1 SUPERCRITICAL FLUID EXTRACTION (SFE)

(a) Sludge

All SFE were performed using a JASCO SFE system as described in Chapter 2 in the instrumentation section of SFE except that the BPR and detector were omitted when a linear fused silica restrictor was used. A linear fused silica restrictor (10 cm long, 50 μm i.d., 150 μm o.d.) from Fisons Laboratory Supplies (Loughborough, U.K.) was used as the restrictor for the extraction of OCPs from sludge. The required pressure was achieved by varying the flow rate setting on the CO_2 pump. The fused silica restrictor was inserted through a PEEK tubing sleeve (3 cm long, 250 μm i.d.) and secured to a Valco connector using a nut and a Valco stainless steel ferrule (Figure 1). Graphite ferrules were initially tried but it was found that the ferrules burst when the pressure exceeds about 250 kg cm^{-2} . Collection in round bottom flask was also carried out to compare the efficiency of the two collection methods. When the round bottom flask was used as the collection vessel, the BPR and the UV detector were used as part of the SFE system.

Figure 1. Diagram of fused silica restrictor insertion into the PEEK tubing.



(b) Dursban and soils

The same JASCO SFE system as used in the sludge extraction was used for the extraction of chlorpyrifos from Dursban and soils. When the extracts were collected in a cooled round bottom flask, the BPR and the detector were used but omitted when the extracts were collected in a liquid. A linear fused silica restrictor (35 cm long, 40 μm i.d., 150 μm o.d.) from Composite Material (Worcester, U.K.) was used to control the extraction pressure and depressurise the SF and extract into the solvent when trapping in liquid solvent was carried out. The restrictor was inserted through a PEEK tubing as described previously. In the extraction of chlorpyrifos from soils the restrictor was heated using a cartridge heater. The top and side view of the heater is as shown in Figure 2a and 2b respectively.

Figure 2a. Top view of the heating block.

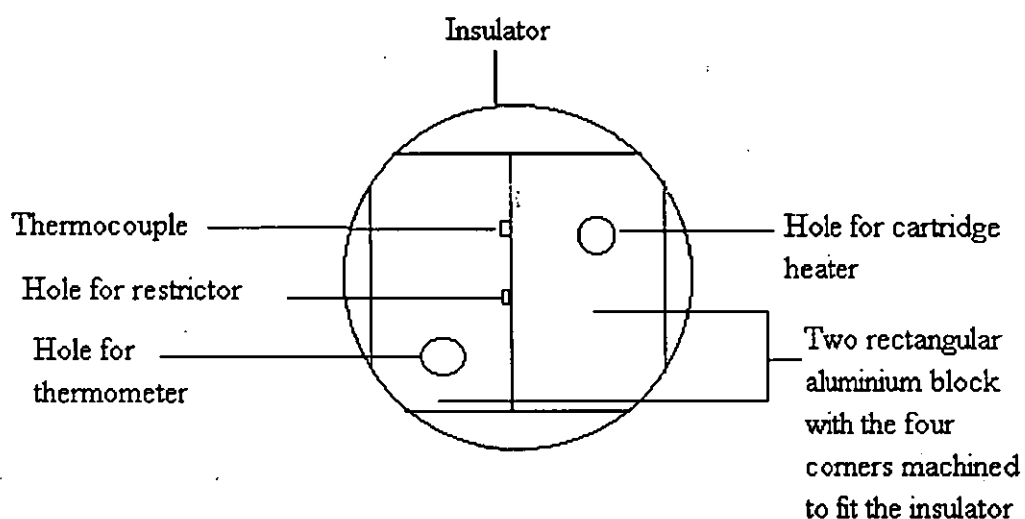
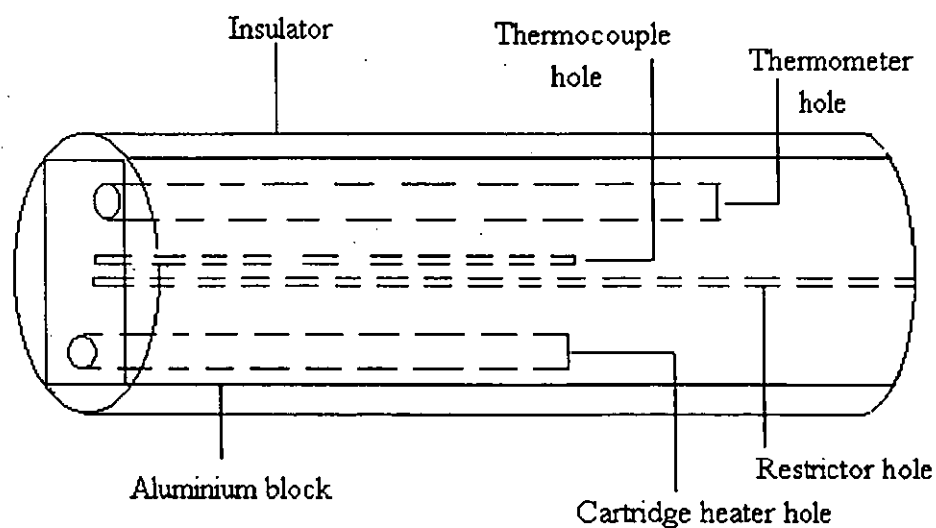


Figure 2b. Side view of the heating block.



4.2.2 HEATER DESIGN

The heater design used in the extraction was made from two rectangular aluminium blocks (each 19 mm thick by 19 mm wide and 223 mm long). These two blocks were screwed together. The four corners at the top and bottom of the screwed block were machined to fit a circular insulator (5 mm thickness, 223 mm long x 44 mm i.d.) which was made from glass reinforced epoxy resin (Tufnal Ltd., Birmingham, U.K.). Four holes were made in the aluminium block. One hole (100 mm long x 10 mm diameter) was used to fit an electric cartridge heater (200W, 62 mm long x 9 mm i.d.), a second hole (230 mm long x 1 mm diameter) to accommodate the restrictor (35 cm long x 40 μ m i.d.), a third hole (62 mm long x 5 mm i.d.) to accommodate a thermometer and a fourth hole to accommodate a thermocouple. The temperature of the block was regulated with a thermocouple and a temperature controller unit (designed in Loughborough University of Technology workshop by the technician). The heating block was positioned about 40° down from the horizontal during the course of the extraction (see Figure 3 later). About 9 cm of the restrictor end was unheated when the restrictor was inserted through the tilted heating block.

4.2.3 GAS CHROMATOGRAPHIC ANALYSIS

(a) Organochlorine pesticides

The gas chromatographic (GC) analysis were done with a Carlo Erba Fractovap series 2150 instrument consisting of a LT programmer model 220, an electrometer model 180 fitted with an electron capture detector (ECD) model 400 which was on loan from the Ministry of

Agriculture and Fisheries (MAFF). Nitrogen was used as the make up gas to the detector at a flow rate of 20 ml min^{-1} . The helium and nitrogen line was fitted with a gas purifier GF-IMS100 (SGE, Australia) and an oxytrap (Altech Associates Inc.) respectively. The split ratio for the OCPs detection was 10:1. The column used for the OCPs detection was a BP1 dimethylpolysiloxane fused silica (12m x 0.32 mm i.d., 0.25 μm film thickness from SGE, Australia). The injector and detector temperature was maintained at 175°C and 280°C respectively. The column was temperature programmed from 160°C, after a 3 min hold to 220°C at 4°C min⁻¹.

At Wessex Water a Perkin Elmer 8700 chromatograph fitted with dual column dual ⁶³Ni ECD with built-in data handling and a model AS3000 autosampler was used for the OCPs analysis. The columns used were a SPB-608 from Supelco and a DB-5 from J & W Scientific. Both the columns were 30 m long with an inner diameter of 0.25 mm and a 0.25 μm film thickness. The two columns were connected in parallel to the dual detectors via a twin hole injection ferrule. The carrier gas used was helium at a flow rate of 1 ml min^{-1} per column and the detector purge gas was nitrogen at a flow of 30 ml min^{-1} . A splitless injection mode with solvent venting after 30 second was used. 2 μl (1 μl /column) of redissolved extracted sludge samples in 1 ml hexane was injected onto the column. The injector and detector temperature were set at 270°C. The column was temperature programmed from 45°C, held for 4 min then ramped to 150°C at 15°C min⁻¹, ramped to 195°C at 5°C min⁻¹, held for 5 min then ramped to 270°C at 2°C min⁻¹ and a final hold of 1 min.

(b) Chlorpyrifos

The gas chromatographic (GC) analysis were done with a Carlo Erba Fractovap series 2150 instrument consisting of a LT programmer model 220, an electrometer model 180 and a flame ionisation detector (FID) with helium as the carrier gas (flow rate 1.17 ml/min). The flow rate of hydrogen was 24 ml min⁻¹ and air flow rate was 300 ml min⁻¹. Oven temperature was set at 230°C (actual reading 246°C) and injector temperature at 225°C. Injections were performed in the split mode with a 13:1 splitting ratio for chlorpyrifos detection for the calibration range 40 - 400 µg ml⁻¹ (sensitivity x1, x8), a split of 17:1 for the calibration range from 4 - 40 µg/ml (sensitivity x1, x2) and a split ratio of 30:1 for the calibration range 2 - 20 µg/ml (sensitivity x1, x1). The chlorpyrifos was separated on an SE 54 column (30 m long, 0.32 mm i.d. and 0.25µm film thickness) from Alltech Associates Applied Science Ltd. (Carnforth, Lancashire, U.K.). Samples (1µl) were injected using the cold needle injection technique with a 5µl syringe (Scientific Glass Engineering, Australia). The chromatograms were recorded on an Olivetti made computer via a 2600 P.E. Nelson Interface and a software from Nelson.

4.3 PROCEDURE**4.3.1 COLLECTION OF EXTRACTS****(a) OCPs**

Extracted analytes were either trapped in a flask cooled by liquid nitrogen after the BPR (Figure 1 in Chapter 2) or depressurised through a

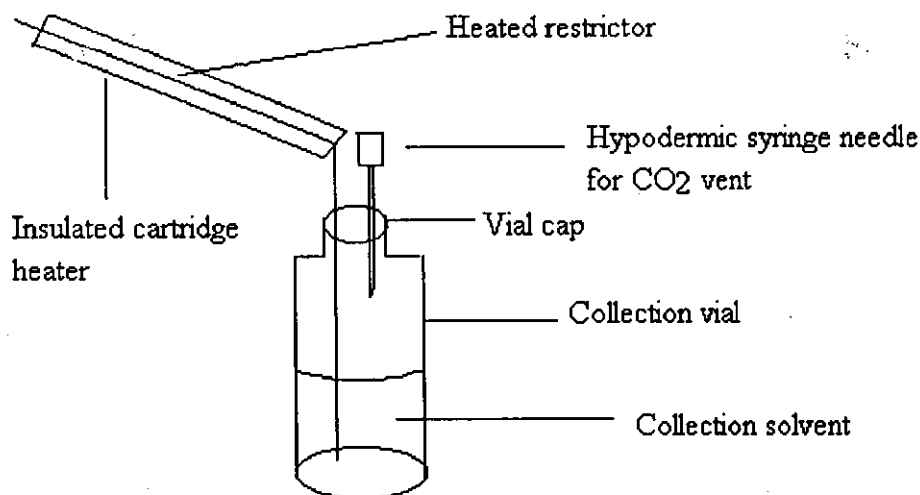
fused silica capillary (10 cm long, 50 μm i.d., 150 μm o.d.) into a vial measuring 40 mm height x 10 mm i.d. neck containing hexane (3 ml).

(b) Chlorpyrifos

Two types of trapping chlorpyrifos were evaluated. Initially a 100 ml round bottom flask (RBF) with a side arm (Figure 1 of Chapter 2) cooled in liquid nitrogen was used for the direct collection of extract of chlorpyrifos from Dursban and from the standard soil. The solidified CO_2 was allowed to evaporate slowly in the refrigerator and the extract reconstitute in methanol.

Secondly, a linear fused-silica capillary restrictor (40 μm i.d., 35 cm long) was used to control the extraction pressure and to depressurise the SF and extract into the solvent. Chlorpyrifos collection was carried out in a 40 mm x 10 mm i.d. neck vial containing 3 ml methanol or acetone (solvent height 15 mm) capped with the vial top. The extracts were either reconstituted in methanol or acetone depending on the collection solvent. Two holes were made in the vial top, one for dipping the restrictor into the solvent and one for a hypodermic syringe needle serving as a vent for the CO_2 (Figure 3). The collection vial was free standing in air. The restrictor needed to be replaced after two or three extractions as the part that dipped into the solvent becomes brittle. This problem has also been observed by several others.

Figure 3. Collection vessel used in the trapping of chlorpyrifos from Dursban and soil.



4.3.2 SUPERCRITICAL FLUID EXTRACTION OF SLUDGE, DURSBAN GRANULES AND SOILS

4.3.2.1 ORGANOCHLORINE PESTICIDES FROM SLUDGE

Approximately 0.4g (accurately weighed) of the spiked freeze dried sludge samples were extracted at various temperature, pressures and methanol percentage. Progress of the extraction was monitored at 254 nm for the OCPs. Samples were assayed using FID and ECD at Loughborough University and sent to Wessex Water for assayed by ECD. Extracted sludge samples that were sent to Wessex Water were evaporated by blowing with a gentle stream of nitrogen to dryness.

4.3.2.2 CHLORPYRIFOS FROM DURSBAN GRANULES AND SOILS

(i) Dursban granules

Approximately 0.5g (accurately weighed) of Dursban granules were extracted dynamically at a temperature of 40°C and a pressure of 150 kg cm⁻² with carbon dioxide containing various percentages of methanol.

(ii) Soils

About 1g (accurately weighed) of sieved German standard soil (passed through a number 40 mesh) spiked at concentration levels ranging from 10 - 1000 µg was extracted dynamically using the optimum conditions found from the extraction of Dursban granules (40°C, 150 kg cm⁻² and 10% methanol). For the German standard soils and Dursban granules a 1 ml JASCO extraction vessel was used but later on this vessel was replaced by a 1 ml extraction vessel from Keystone Scientific (Bellafonte, U.S.A.). Two slip-free connectors (Keystone Scientific, Inc., Bellafonte, U.S.A.) were connected to the extraction vessel (top and bottom of the EV). About 0.8 g (accurately weighed) of the Speyer 2.1, Speyer 2.2, Speyer 2.3 and clay were used and for peat about 0.5 g (accurately weighed) was used. In some cases, solvent extraction with methanol was carried out on the German standard soil after SFE to determine the exhaustiveness of the SFE.

4.3.3 SOLVENT EXTRACTION OF DURSBAN GRANULES

About 0.5 g of Dursban granules was accurately weighed and extracted with 10 ml methanol (initially sonicated for 3 minutes and then

static extraction) at room temperature for approximately 24 hours. Duplicate extractions were carried out. The extract was carefully pipetted out into a volumetric flask and appropriate dilutions carried out. *n*-Docosane (880.8 μg) was added as internal standard. Percentage yield of chlorpyrifos from Dursban was calculated from a calibration graph of standard chlorpyrifos. Recovery of solvent extraction was assumed to be 100 %. Solvent extraction for soil was similar to the solvent extraction of the Dursban granules.

4.3.4 SOXHLET EXTRACTIONS

Soxhlet extraction was carried out on three soils (standard, clay and peat) according to the Environmental Protection Agency (EPA) Method 3540 (1). About 10 g of spiked soil (1 mg g⁻¹) was mixed with 10g anhydrous sodium sulphate and extracted with 300 ml of 1:1 v/v acetone/dichloromethane for 16 hours using a Soxhlet apparatus. The extract was passed through a sodium sulphate drying column, evaporated to dryness using a rotary evaporator (Buchi, Switzerland) and reconstituted with acetone (25 ml). Appropriate dilution was carried out and *n*-docosane was added as an internal standard (880.8 μg). Soxhlet extraction were carried out in duplicate.

4.3.5 SOIL pH DETERMINATION

The soil pH was determined according to standard reference method (2). Distilled water (5 ml) was added to air dried soil (5 g). The mixture

was mixed thoroughly with a glass rod for 5 seconds and allowed to stand for 10 minutes. The soil pH in water (pH_w) was recorded using a calibrated Corning pH meter 140 (R. W. Jennings and Co. Ltd., Nottingham, U.K.) after the soil suspension was stirred by swirling the electrodes slightly.

4.3.6 CARBON CONTENT OF SOIL

The organic carbon content in the soils was determined according to the Walkley and Black method (3). Approximately 1 g of the soil was accurately weighed and transferred to a 500 ml conical flask. 10 ml of 0.1667M potassium dichromate was run into the flask and 20 ml concentrated sulphuric acid added. The mixture was shaken for 1 minute and allowed to stand for 30 minutes on an asbestos mat away from heat and draught. 250 ml distilled water and 2 ml of N-phenyl anthranilic acid (indicator) was added. The solution was then titrated with 1M ferrous sulphate solution and the end point occurs when the colour changes from purple to green. A blank determination was also carried out.

The total carbon, hydrogen and nitrogen content in the soils were determined using a Perkin Elmer 2400 CHN analyser. Duplicate samples were used.

4.3.7 CALIBRATION GRAPHS

(a) Organochlorine pesticides

Quantitation of OCPs from sludge was done using the external standard method (at Wessex Water). The components were tentatively identified by comparisons of their retention times with those obtained by a single point calibration of individual OCPs standard. On detector 1 quantitation was based on peak height and on detector 2 quantitation was based on peak area.

(b) Chlorpyrifos

Quantitation of chlorpyrifos from Dursban and soil was performed using the internal standard method. A five point linear calibration curve plotting concentrations and area ratio of chlorpyrifos (A) to n-docosane (A_S) as an internal standard were performed. The average of three replicate injections of the standards and extracted chlorpyrifos were taken. Calibration graphs of standard were from 40 to 400 $\mu\text{g ml}^{-1}$ for the quantitation of chlorpyrifos from Dursban and for the 0.1% ($\sim 1000 \mu\text{g/g}$) chlorpyrifos spiking level (extract diluted 5x) from soils, 4 to 40 $\mu\text{g ml}^{-1}$ for the 0.01% ($\sim 100 \mu\text{g/g}$) spiking level (extract diluted 5x) and 2 to 20 $\mu\text{g ml}^{-1}$ for the 0.001% ($\sim 10 \mu\text{g/g}$) spiking level (extract undiluted). Concentration of chlorpyrifos in the extracted analytes was calculated using the regression analysis on the MINITAB statistical package version 8.21 on the PC. Table 1 is a calibration data of chlorpyrifos standards on capillary GC.

Table 1. Calibration data for the various chlorpyrifos concentration range used.

Concentration range ($\mu\text{g ml}^{-1}$)	Regression equation	Correlation coefficient (r)
40 - 400	$A/A_s = 0.0016 \text{ conc.} - 0.0014$	0.9998
4 - 40	$A/A_s = 0.0146 \text{ conc.} + 0.0083$	0.9989
2 - 20	$A/A_s = 0.05944 \text{ conc.} + 0.0826$	0.9936

4.3.8 CALCULATIONS

4.3.8.1 % RECOVERY OF CHLORPYRIFOS

(i) The % recovery of chlorpyrifos from soil was calculated using the formula

$$\% \text{ Recovery} = (\text{measured chlorpyrifos level} / \text{spiking level}) \times 100$$

where

$$\text{measured chlorpyrifos level} = (\text{chlorpyrifos conc./soil wt.}) \times \text{dil. factor}$$

and

$$\text{spiking level} = \text{spiked chlorpyrifos weight} / \text{weight of soil}$$

(ii) The percentage recovery of chlorpyrifos from SFE from the Dursban granules was calculated using the formula

$$\% \text{ Recovery} = (\text{yield from SFE} / \text{yield from solvent extraction}) \times 100$$

(Solvent extraction recovery was assumed to be 100%).

4.3.8.2 ORGANIC CARBON CONTENT

The percentage of carbon in the soil was calculated using the formula

$$\% \text{ C} = \{(v_1 - v_2) / w\} \times 0.300 \times f$$

where v_1 = volume (in ml) of ferrous sulphate (M) required for blank titration

v_2 = volume (in ml) of ferrous sulphate required in the titration of excess potassium dichromate

w = weight of soil taken in grams

f = recovery factor ($f=1.3$ for soil samples)

4.3.8.3 THE AMOUNT OF OCPs IN 0.1 μL OF THE INJECTED VOLUME OF EXTRACT ONTO THE GC COLUMN

Weight of sludge used for extraction = 0.1731g

Given: 10.2g of freeze-dried sludge which has been spiked with 800 ng DDT - (a) and 400 ng of DDE, DDD, HCB, lindane, α -HCH, β -HCH and dieldrin - (b)

\therefore in 0.1731g of the sludge there is $(0.1731\text{g}/10.2\text{g}) \times 400 \text{ ng}$ of (b)
 $= 6.79 \text{ ng}$ of (b)

and $(0.1731\text{g}/10.2\text{g}) \times 800 \text{ ng DDT} = 13.58 \text{ ng DDT}$

This extract was dissolved in 2 ml hexane

\therefore the concentration of the extract = $6.79 \text{ ng of (b)}/2000 \mu\text{l of (b)}$
 and $13.58 \text{ ng}/2000 \mu\text{l of DDT}$.

The volume used for injection onto the GC column was 0.1 μl .

\therefore the amount of DDT in this 0.1 μl volume is $(0.1 \mu\text{l}/2\,000 \mu\text{l}) \times 6.79 \text{ ng}$
 $= 3.4 \times 10^{-4} \text{ ng (0.34 pg)}$ and
 the other seven OCPs is $(0.1 \mu\text{l}/2\,000 \mu\text{l}) \times 13.58 \text{ ng}$
 $= 6.8 \times 10^{-4} \text{ ng (0.68 pg)}$

4.3.8.4 LIMIT OF DETECTION ON GC SYSTEM

The regression output for the calibration graph in the concentration range 2-20 $\mu\text{g ml}^{-1}$ is as follows:

Constant = 0.0826	Standard error of y estimate = 0.0554
x coefficient = 0.0594	Standard error of x coefficient = 0.0030
Degrees of freedom = 5	$r^2 = 0.9872$ (i.e. $r = 0.9936$)

The limit of detection is defined as the analyte concentration giving a signal equal to the blank, y_B , plus 3 standard deviation of the blank, s_B ,

$$\text{i.e. } y = y_B + 3s_B \quad (1)$$

where $y_B = 0.0826$ and $s_B = 0.0554$

The value of y at the limit of detection is

$$\begin{aligned}
 y &= 0.0826 + 3 \times 0.0554 \\
 &= 0.2488
 \end{aligned}$$

The equation for the regression line is $y = 0.0594x + 0.0826$ (2)

substituting $y = 0.2488$ into the regression equation (2) gives

$$0.2488 = 0.0594x + 0.0826$$

$$\therefore x = 2.8 \mu\text{g ml}^{-1}$$

Limit of detection is $2.8 \mu\text{g ml}^{-1}$.

4.4 REFERENCES

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CHAPTER 5

SUPERCritical FLUID EXTRACTION OF ORGANOCHLORINE PESTICIDES FROM SLUDGE SAMPLES

5.0 INTRODUCTION TO PESTICIDES

Pesticides are chemicals designed to combat the attacks of various pests on agricultural and horticultural crops. They are biologically active molecules deliberately introduced into the environment to control pests, disease or weeds. They fall into three major classes: insecticides, fungicides and herbicides (or weed killers) (1). There are also rodenticides (for control of vertebrate pests), nematicides (to kill microscopic eelworms), molluscicides (to kill slugs and snails), and acaricides (to kill mites).

Pesticides may also be divided into two main types, namely contact or non-systemic pesticides and systemic pesticides. Contact or surface pesticides do not appreciably penetrate plant tissues and are consequently not transported, or translocated within the plant vascular system. Systemic pesticides can effectively penetrate the plant cuticle and move through the plant cuticle and vascular system. Many of the most recent pesticides are systemic in character, e.g. hexaconazole and benomyl. Systemic pesticides are also sometimes termed plant chemotherapeutants and can not only

protect the plant from fungal attack but also cure or inhibit an established infection.

It has been estimated that the production from each of the major manufacturing areas in 1987 were valued at the following amounts (2):

Western Europe	\$5670 million
Far East	\$4835 million
United States	\$4465 million

In 1981, the world consumption was 44.2% herbicides, 28.8% insecticides, and 20.9% fungicides, the remaining 6.1% for growth regulators and miscellaneous agrochemicals (1). In the United Kingdom, the pesticides usage for 1986 is given in Table 1.

Table 1. Pesticides usage in the United Kingdom for 1986 (3).

Product type	Value £m	Tonnage of active ingredient	% of total tonnage
Herbicides	190.3	17 122	64.6
Insecticides	31.7	1 090	4.1
Fungicides	96.5	5 549	20.9
Seed treatment products	13.8	189	0.7
Plant growth regulators	8.8	2 081	7.8
Other pesticides	9.4	488	1.9
	355.3	26 519	

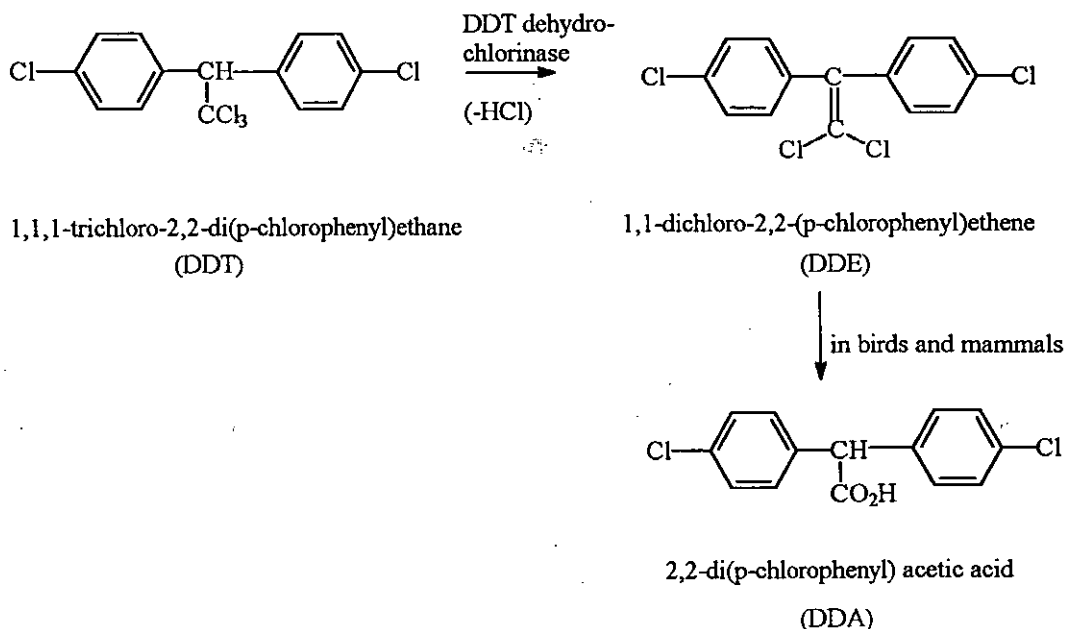
The four main groups of insecticides are organophosphates, carbamates, pyrethroids and organochlorines. In this laboratory selected organochlorine pesticides were studied and the aim of this work was to selectively extract these organochlorine pesticides (OCPs) from sludge samples using supercritical fluid extraction (SFE).

5.1 ORGANOCHLORINE PESTICIDES

The most important group of the organochlorine pesticides (OCPs) is 1,1,1-trichloro-2,2-di(p-chloro-phenyl)ethane, also termed dichlorodiphenyltrichloroethane, or DDT. Only the p,p' isomer has significant insecticidal activity. The mammalian toxicity LD₅₀ (oral) to rats is 150 mg kg⁻¹ (2). DDT kills a wide variety of insects (including domestic insects and mosquitoes) but is not effective against mites. DDT appears to alter the permeability of the axonal membrane by delaying the closing of some of the sodium channels (2). It therefore acts as a nerve poison by upsetting the sodium balance in nerve membranes.

The metabolism of DDT occurs by a number of pathways, but the most important appears to be the dehydrochlorination of DDT to give dichlorodiphenylethylene, DDE by the non-microsomal enzyme DDT-dehydrochlorinase (Figure 1). DDE is a highly persistent metabolite and therefore is a major environmental pollutant. In birds and mammals it is further slowly metabolised to the carboxylic acid DDA [2,2-di(p-chlorophenyl) acetic acid] which is sufficiently water soluble to be excreted as amino acid conjugates (Figure 1) (4).

Figure 1. Metabolism of DDT (4).



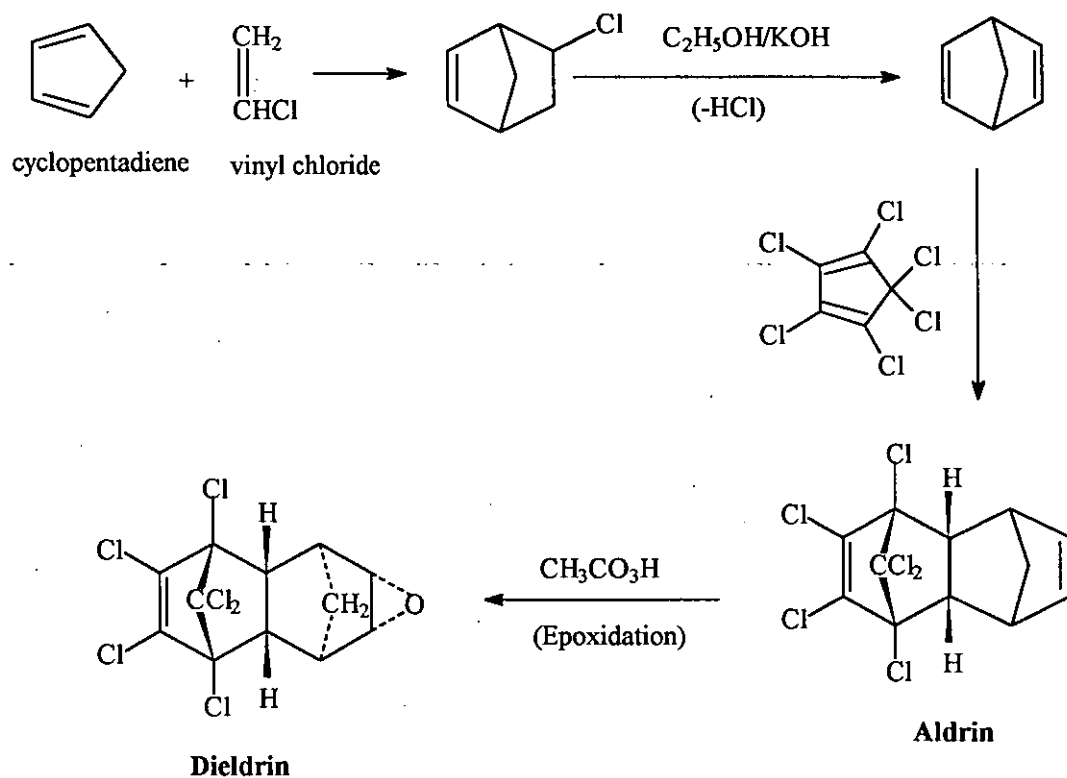
The discovery of the insecticidal properties of DDT stimulated the search for analogous organochlorine compounds. Only a few of the many hundreds of the synthesised compounds had sufficient activity and were sufficiently cheap for commercial exploitation. One example is 1,1-dichloro-2,2-di(p-chlorophenyl)ethane, known as DDD. DDD has a much lower mammalian toxicity than DDT. The LD₅₀ value (oral) to rats is 5 000 mg kg⁻¹. DDD has found use on food crops since it is appreciably less toxic than DDT.

Hexachlorocyclohexanes (HCH) are another class of organochlorine pesticides. HCH is prepared by treatment of benzene with chlorine under the influence of ultraviolet light without a catalyst. Theoretically HCH can

exists as eight different isomers. However, only five isomers (α , β , γ , δ and ϵ) are found in the crude products and only the γ -isomer or lindane has powerful insecticidal properties (5). Lindane has a similar insecticidal spectrum to DDT, but its physical properties are more suitable than those of DDT for use as a soil insecticide because of its greater volatility and water solubility (6). Lindane rapidly penetrates the insect cuticle. Like DDT, lindane probably kills insects by bringing about a sodium-potassium imbalance in nerve membranes. The LD_{50} value (oral) to rats is 76 mg kg^{-1} for lindane, 177 mg kg^{-1} for α -HCH, and 1000 mg kg^{-1} for δ -HCH (7).

The cyclodiene insecticides make up another group of organochlorine pesticides. This group of cyclodiene pesticides are synthesised from the Diels-Alder reaction (2). The best known members are aldrin and dieldrin, named after Diels and Alder, the discoverers of the diene synthesis. Both are chemically very stable. They were the most active general contact insecticides until the introduction of the synthetic pyrethroids. The synthesis of dieldrin is shown in Figure 2. Like DDT they are highly lipophilic and persistent. They are excellent soil insecticides against wire-worms and are the best compounds for termite control (8). Dieldrin is remarkably effective against ectoparasites such as blowflies, lice and ticks. The LD_{50} (oral) value to rats is 38.3 mg kg^{-1} for dieldrin and aldrin (7). It is much more toxic than DDT or lindane. The mode of action of the cyclodienes appears to be due an excessive release of acetylcholine which result in loss of nerve co-ordination.

Figure 2. Synthesis of aldrin and dieldrin (2).



Hexachlorobenzene (HCB) is a simple chlorinated aromatic compound which is used as a fungicide. The mode of action does not seem to be known. It is used to control *Tilletia caries* on wheat. The LD_{50} for rat (oral) is $3\,500\text{ mg kg}^{-1}$ (7).

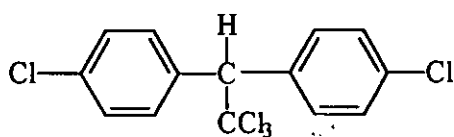
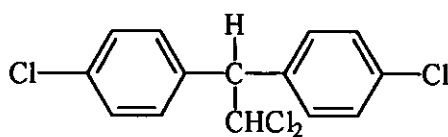
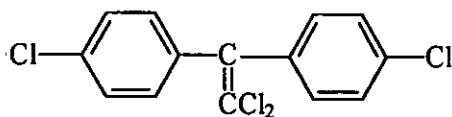
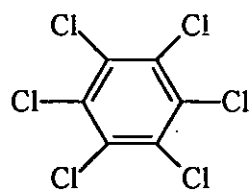
Organochlorine pesticides are very stable substances which are very slowly chemically or biologically degraded. Also this class of compounds are very mobile and thus they adhere to dust particles and are blown around in the dusts of the world. The above mentioned insecticides have been

used and most are still used, except for DDT which is now banned for agricultural use in most developed countries. The United States of America banned its use in 1972 and Britain in 1984 (2). However, DDT is still important in the control of insect vectors, e.g. malaria-carrying mosquito in the Far East as it is the cheapest available pesticides. Dieldrin, which is a persistent OCP, was revoked in 1989 (2, 9). The HCB was banned in the United Kingdom in 1975 (2). As a result of continued use of these insecticides in agriculture and horticulture, appreciable amounts accumulate in the environment. Since most of the above mentioned insecticides are harmful to man, animals, plants and the environment, it is important to monitor their residues in samples such as soils, plants, food, air and other matrices such as sludge (which is further used to enrich soils). Table 2 gives the molecular weight, $\log K_{ow}$ (logarithm of the partition coefficient in n-octanol-water system) (10) and melting point (7) of the pesticides. The OCPs are defined as fat-soluble compounds since its $\log K_{ow} > 5$ (10). The isomers of HCH have a $\log K_{ow}$ value in the range 3 - 4 but they are also defined as fat-soluble compounds. The structure of the OCPs used in the present study are shown (Figure 3).

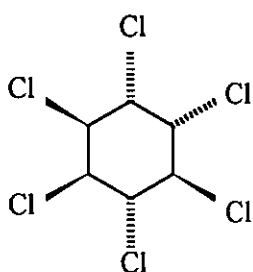
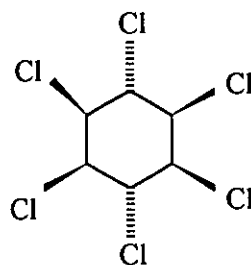
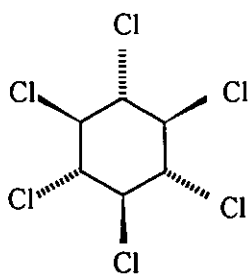
Table 2. Molecular weight, log K_{ow} (10) and melting point (7) of organochlorine pesticides used in the work.

Compounds	Molecular weight	Log K_{ow}	m.pt.(°C)
Dieldrin	380.9	4.54	143-144
α -HCH	290.8	3.81	156-161
β -HCH	290.8	3.80	297-312
γ -HCH	290.8	3.72	113-115
DDT	354.5	6.38	107-109
DDE	318.0	5.69	88-90
DDD	320.0	6.22	109-111
HCB	284.8	5.50	227-229

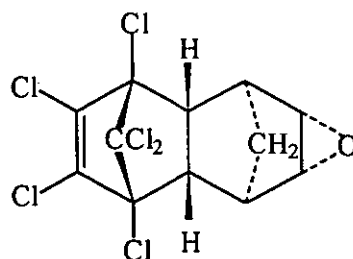
Figure 3. Structures of the OCPs and metabolites studied in this work.

1,1,1-trichloro-2,2-di(p-chlorophenyl)ethane
(DDT)1,1-dichloro-2,2-di(p-chlorophenyl)ethane
(DDD)1,1-dichloro-2,2-di(p-chlorophenyl)ethene
(DDE)

Hexachlorobenzene (HCB)

alpha-hexachlorocyclohexane
(alpha-HCH)gamma-hexachlorocyclohexane
(gamma-HCH)

beta-hexachlorocyclohexane (beta-HCH)



Dieldrin

5.2 INTRODUCTION TO SLUDGE

Sewage sludge is the residue collected after treatment of the contents of urban drainage system. The bulk of its content derives mainly from human wastes, but there are significant contributions from discharges to sewers of industrial effluents and animal or vegetable processing wastes as well as from run-off storm water from roads and other paved areas. Some sludge is chemically inert but organic sludge often has an offensive smell and needs^a special form of treatment. The composition of sludge depends both on the nature of the initial pollution of the water and the treatment processes to which that water has been submitted, whether physical, physical-chemical or biological.

In addition to organic waste material, sludge therefore contains traces of many of the contaminating substances used in our modern society. Sludge can be classified as:

- (a) **hydrophilic organic sludge** - all types of sludge resulting from the biological treatment of wastewater from municipal waste, agrifood industry, textile industry and organic chemical industry are included in this category.
- (b) **hydrophilic inorganic sludge** - contains metal hydroxides formed during the physical-chemical treatment process as a result of the precipitation of metallic ions present in the raw water (Al, Fe, Zn, Cr) or due to the use of organic flocculants.

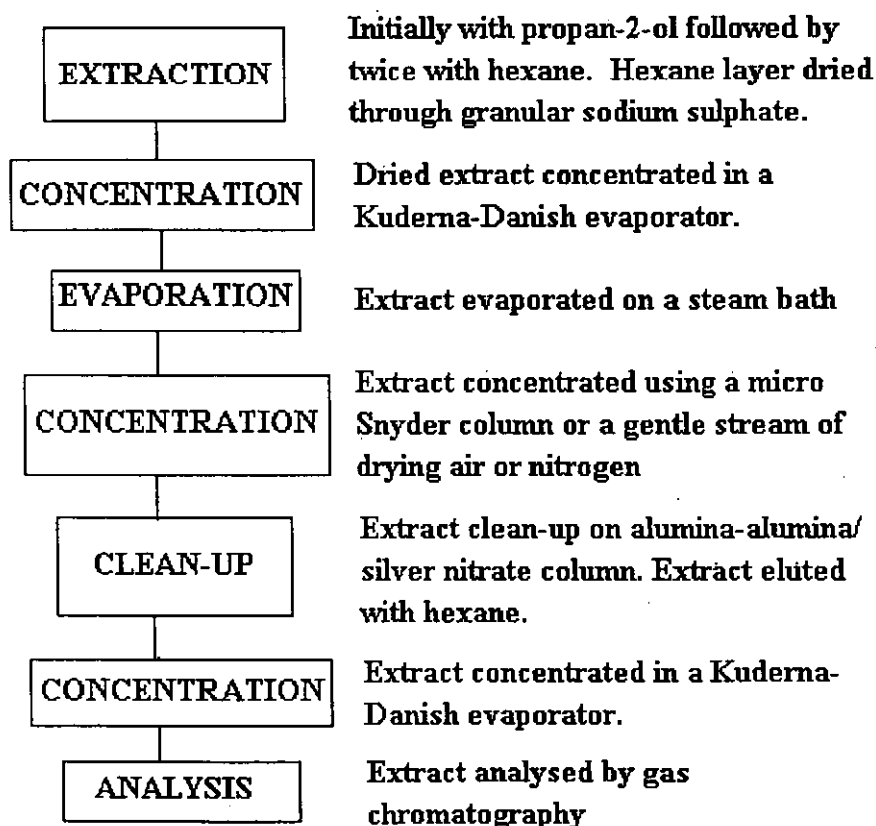
- (c) **oily sludge** - presence of small quantities of mineral (or animal) oils or fats. Wastewater from refineries, from engineering work and from cold rolling mills are included in this category.
- (d) **hydrophobic inorganic sludge** - contains preponderant amount of particulate matter with a low amount of bound water.
- (e) **hydrophilic-hydrophobic inorganic sludge** - comprise mainly hydrophobic substances, but contains substantial amount of hydrophilic substances (often metallic hydroxides).
- (f) **fibrous sludge** - wastewater from paper mills, paper pulp mills and cardboard mills fall in this category.

The value of sludge for agricultural use lies more in the humic matter it provides and in the improvement of water-retention properties of the soil than in its nutritive content alone. The main potential risk in using sludge for agriculture is the heavy metals that are present. The most common dangerous cations are zinc, cadmium, copper, nickel, chromium and mercury. Besides these heavy metals, pesticides and residues are also dangerous. It is therefore necessary to monitor and control the concentrations in sludge so as to preserve the yield of crops and to ensure that animal or human health is not put at risk through the food chain.

5.3 CONVENTIONAL METHOD OF PESTICIDE EXTRACTION FROM SLUDGE SAMPLES

In the method described in the "Blue Book" produced by the Standing Committee of Analysts (SCA), organochlorine pesticides in sludge samples are extracted using liquid extraction method with hexane as the extracting solvent (11) (Figure 4). As can be seen from the figure, the sample preparation step involves several steps i.e., extraction, drying, concentration, evaporation, concentration and clean-up before the analysis. They are therefore tedious and time consuming.

Figure 4. Conventional liquid extraction of OCPs from sludge sample (11).



5.4 EXTRACTIONS FROM SLUDGE SAMPLES USING SUPERCRITICAL FLUID

There is an increasing use of supercritical fluids, especially carbon dioxide for the extraction of chemical pollutants from various matrices. SFE with ion-pair extraction has been used by Field *et al.* (12) to extract anionic surfactants from sewage sludge. Secondary alkanesulphonate (SAS) and linear alkyl benzenesulphonate (LAS) surfactants were quantitatively (>90%) extracted from sewage sludges as ion pairs with tetrabutylammonium chloride using SC carbon dioxide at 80°C and 400 atm with a 5 min static extraction followed by a 10 min dynamic extraction before being analysed by GC/MS. Hawthorne *et al.* (13) extracted linear alkylbenzenesulphonates (LAS) from municipal wastewater treatment sludge using SC carbon dioxide with 40 mol% methanol for 30 minutes at 380 bar and 125°C. The extract was collected in ethanol (5ml) and analysed by HPLC with fluorescence detection.

Polycyclic aromatic hydrocarbons (PAHs) at the µg/g level have also been extracted from petroleum sludge by Hawthorne and co-workers (14). It was found that extraction with difluorochloromethane (Freon-22) for 40 minutes at 100°C and 40 MPa was much more efficient than methylene chloride sonication for 18 hours. Porter *et al.* (15) extracted polychlorinated biphenyls (PCBs) from sewage sludge. Extracts were cleaned up by passing through a clean-up column of 1:1 sulphuric acid silica gel and 10% silver nitrate silica gel and washed with hexane and then concentrated before analysis. David and co-workers (16) applied SFE to extract PCBs from a certified sewage sludge sample (CRM 392, BCR,

Brussels, Belgium) using the optimised extraction conditions for PCBs from spiked sediment sample. They found that the recovery was not quantitative. This illustrates that SFE conditions successfully applied to spiked samples cannot be transferred to real samples. In real samples, there is a fixation effect of the solutes into the matrix. A literature search on the Science Citation Index (from 1981 to August, 1994) on the Bath Information System (BIDS) showed that no SFE method has been used to extract OCPs from sewage sludge.

5.5 AIM OF PROJECT

As the conventional method of OCPs extraction from sludge is tedious, time consuming and uses hazardous solvents, an alternative extraction method which is selective and fast, and uses less toxic organic chemicals, is needed to extract organochlorine pesticides from sludge matrix. This would be useful for routine work. A method using SFE was examined for the extraction of organochlorine pesticides from municipal waste water treatment sludge samples.

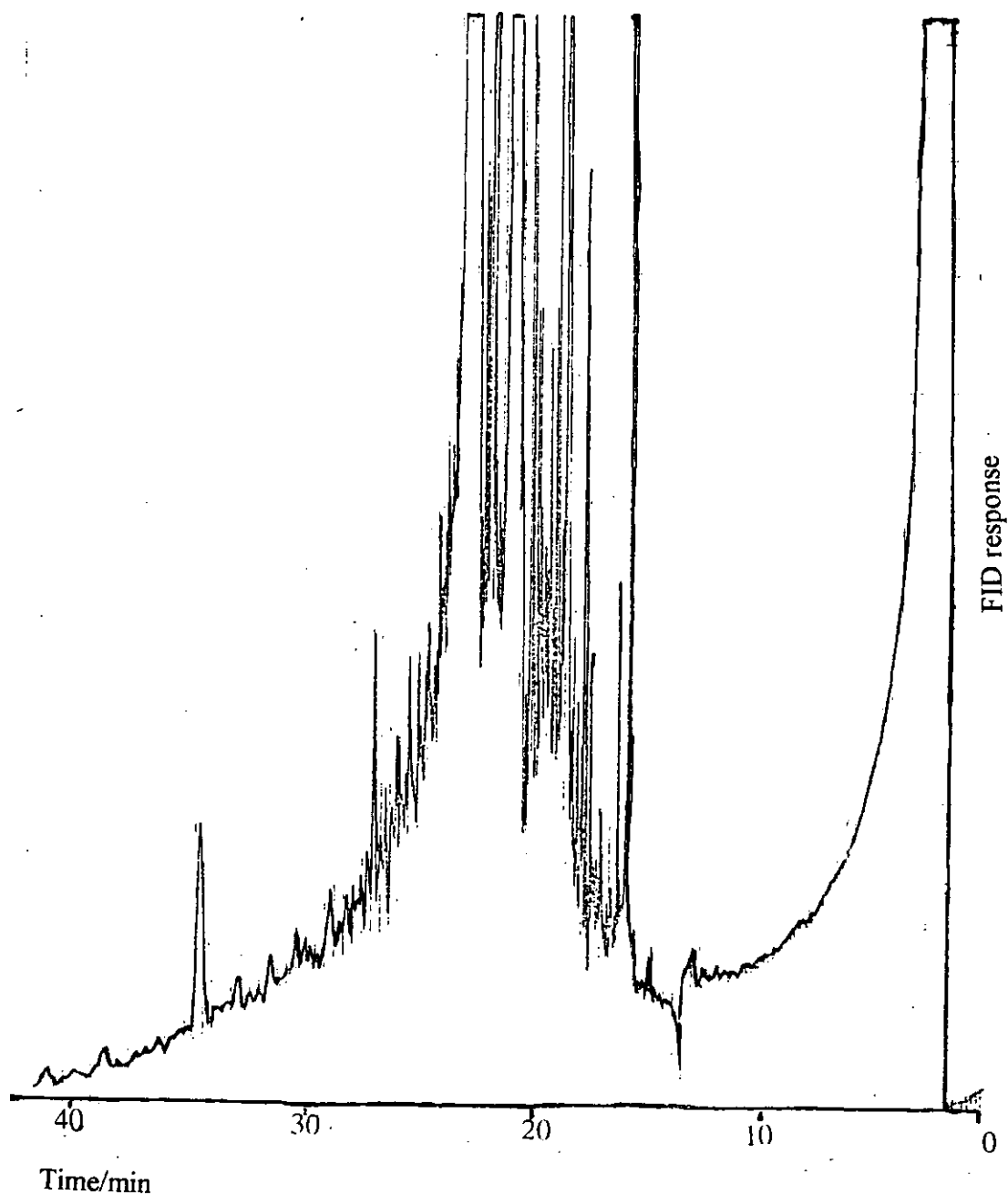
In this chapter, SC carbon dioxide and modified SC carbon dioxide were used to extract spiked OCPs from lyophilised sludge samples. The intention was to determine if selectivity could be achieved in the extraction and interferences from coextractives could be reduced. Most of the previous work in this area (17, 18) has studied residues from soil samples and relatively clean backgrounds were obtained. Two types of trapping system (liquid trapping and solid trapping on cryogenically cooled solid

surface) were evaluated for the extraction of organochlorine pesticides from sludge samples.

5.6 EXTRACTION WITH ASSAY USING FLAME IONISATION DETECTION ANALYSIS

The initial aim of the study was to try to determine the applicability of SFE for real environmental sample such as sewage sludges. Initially an unspiked sludge sample (blank) was extracted at 40°C and a pressure of 200 kg cm⁻² with collection of extract in hexane after being depressurised through a pressure restrictor and the extract was analysed by a flame ionisation detector (FID). The chromatogram showed that many interference peaks were observed (Figure 5). These peaks are most probably from hydrocarbons. This is not surprising as the FID detector is not selective. Therefore a more selective detector, an electron capture detector (ECD) was substituted for the FID.

Figure 5. GC chromatogram of an unspiked sludge sample at an extraction temperature of 40°C and a pressure of 200 kg cm⁻². GC conditions: Column, BP1 (12m x 0.22 mm i.d., 0.25 µm film thickness); oven temp, initially at 84°C, hold for 6 min, then ramp to 250°C at 10°C min⁻¹ and hold at 250°C for 15 min; injector temperature, 200°C; split ratio, 10:1; detector, FID; He flow, 1 ml min⁻¹.



5.7 EXTRACTION WITH ASSAY USING ELECTRON CAPTURE DETECTION (ECD) ANALYSIS

Initially time was spent to find the best conditions to separate the eight standard OCPs (Figure 3) and detection on the ECD. An isothermal run at 156°C separated all the eight standard OCPs studied but the later eluting peaks were broad. The best temperature program was an initial set temperature of 150°C (actual temperature 164°C), a 3 min hold and then ramped to 220°C at the rate of 4°C min⁻¹ (Figure 6). Since no individual OCPs (except for lindane) were available, the 'rough' identity of the peaks was compared with reported chromatogram (chromatogram 1550) from chromatography brochure (19) as the elution order should almost be identical (on a BP-1 non-polar column elution is in order of increasing boiling point). The identity of lindane was confirmed by running a solution of lindane standard in hexane.

Two peaks at 4.66 min and 8.72 min were detected in the chromatogram of an unspiked sludge sample at an extraction pressure of 200 kg cm⁻² and a temperature of 40°C (Figure 7). Comparison of the retention time of these two peaks with the retention time of the peaks from the OCPs standard chromatogram suggests that these two peaks could be lindane and HCB peak respectively. As the sludge sample was not spiked with any OCPs, no peaks should have been observed.

Figure 6. GC chromatogram of OCPs standard on BP1 column. GC conditions: oven temperature, initially 164°C, hold for 3 min, then ramp to 220°C at 4°C min⁻¹; detector, ECD; detector temperature, 280°C; injector temperature, 175°C. Peaks: 1. α -HCH. 2. β -HCH. 3. γ -HCH (lindane). 4. HCB. 5. DDE. 6. Dieldrin. 7. DDD. 8. DDT.

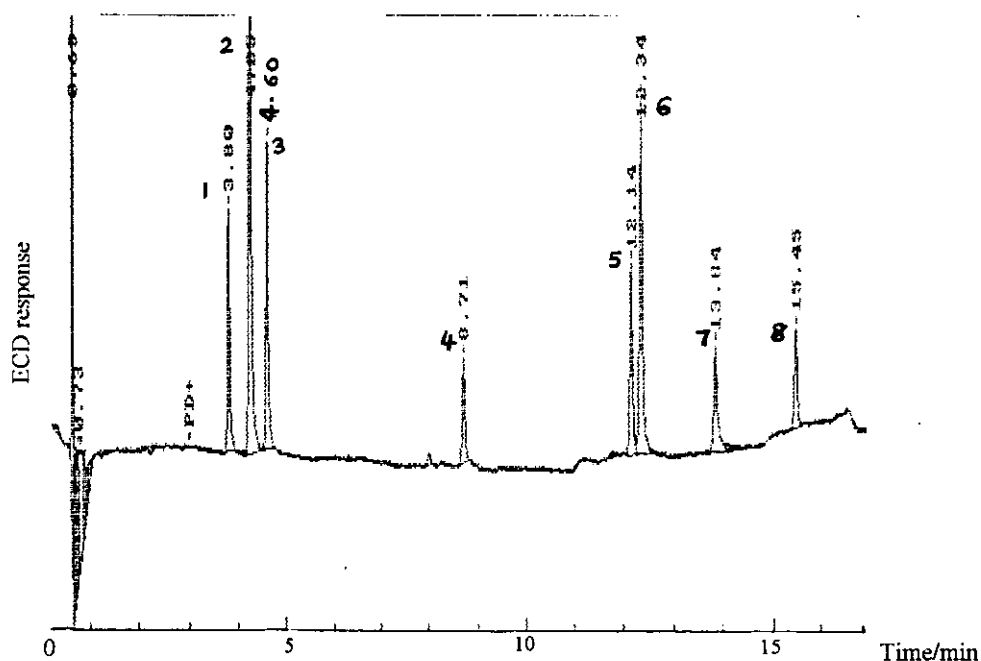


Figure 7. GC chromatogram of an unspiked sludge sample at an extraction temperature of 40°C and a pressure of 200 kg cm⁻² with collection of extract in hexane. GC conditions as in Figure 6.

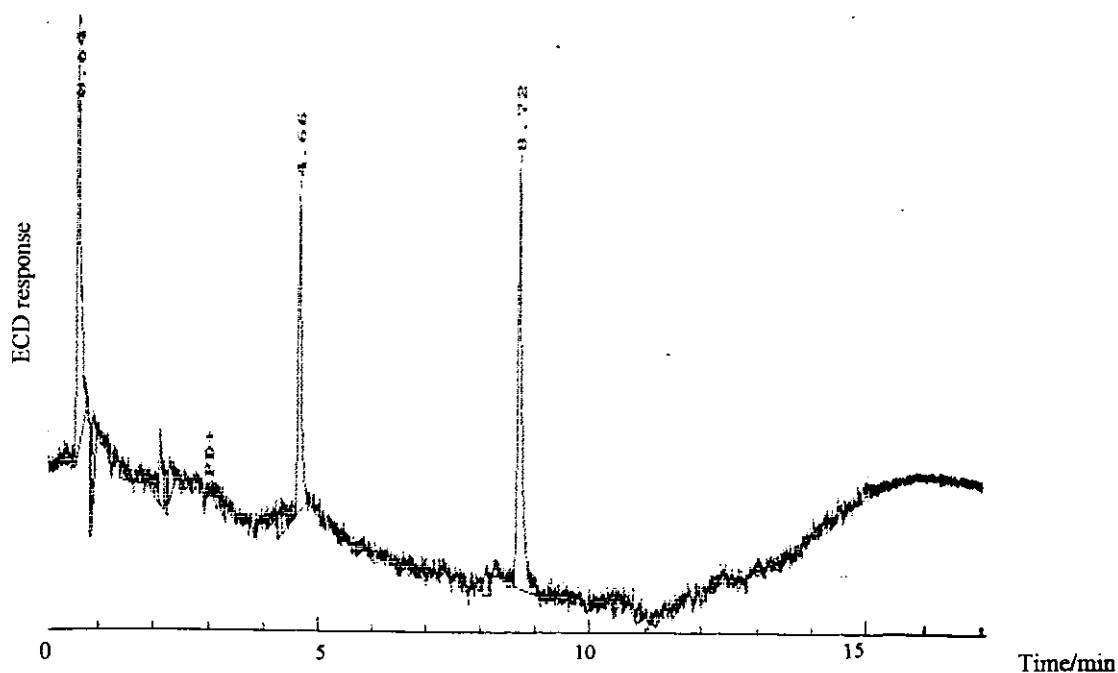
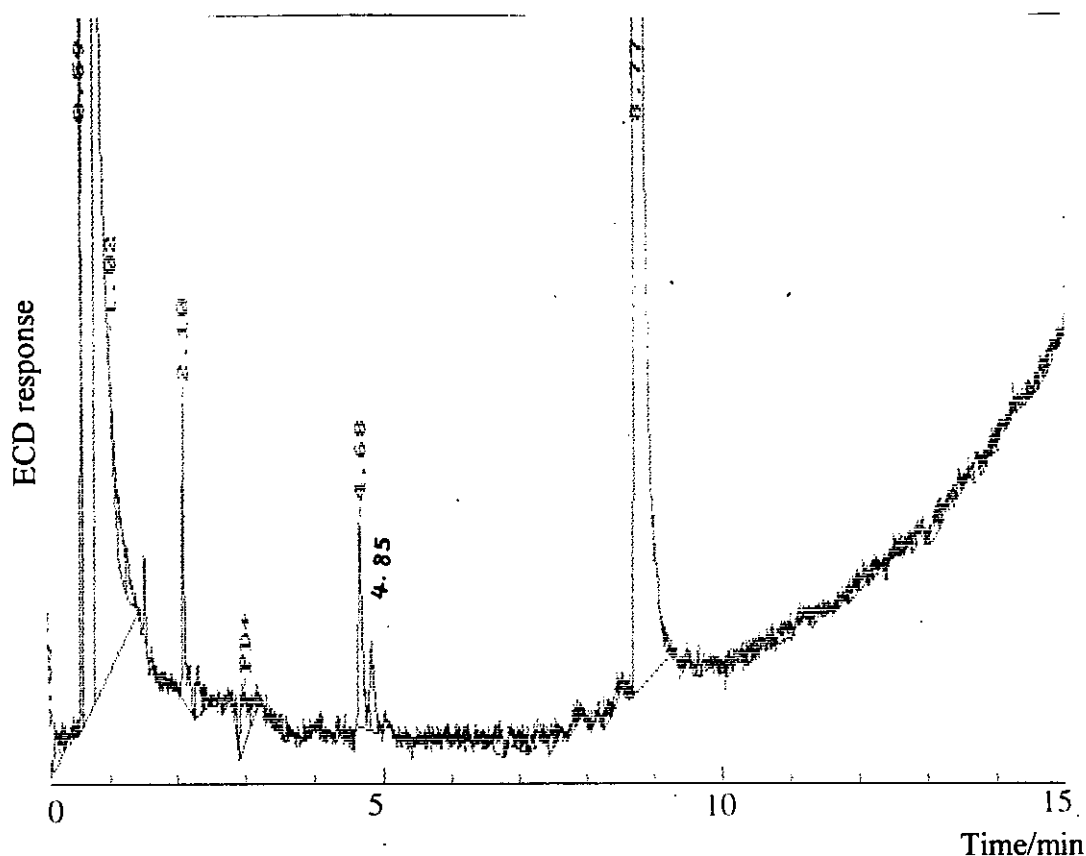


Figure 8. GC chromatogram of spiked sludge sample at an extraction temperature of 40°C and a pressure of 200 kg cm⁻² with collection of extract in hexane. GC conditions as in Figure 6.



Two peaks were also observed in the chromatogram of a spiked sludge sample at the same extraction condition [Figure 8]. The peaks appeared at 4.68 min and 8.77 min. The peak at 8.77 min is too big to be a HCB peak from the spiked standard OCPs in hexane. Therefore this peak must be an impurity peak, either from hexane or from carbon dioxide. Also the peak at 4.68 min could not be a lindane peak as the other two HCHs were not detected. This peak must also be an impurity peak either from hexane or from carbon dioxide or from the blank sludge. The peak at 4.68 min is actually an impurity peak from the hexane used as the collection

solvent (tentatively identified from the retention time of hexane solution run) and the peak at 8.77 min does not come from the carbon dioxide used as the extracting fluid. Thus this peak must be from the sludge itself.

The chromatogram of the spiked sludge (Figure 3) shows that either the standard OCPs were not extracted or not detected. The freeze-dried sludge sample has been spiked with 400 ng of the standard OCPs except for DDT which has been spiked at 800 ng. Calculation of the amount of OCPs in the used spiked sludge sample (see Chapter 4) showed that the concentration of the OCPs in 0.1 μ l of the injected volume is about 0.68 pg of DDT and 0.34 pg of the other seven OCPs (assuming 100% extraction and trapping). The limit of detection (L.O.D.) {3 x S/N ratio} for the standard OCPs in hexane are all above 1 pg (except for lindane - below 1 pg). L.O.D. for DDT, DDE and α -HCH is about 4 pg, 10 pg for DDD and HCB, about 2 pg for dieldrin and 1 pg for β -BHC. Thus most likely the OCPs were not detected and the two peaks observed in the chromatograms earlier confirms that they are not OCPs. A larger sample of sludge (calculated to be about 0.4g) were therefore used for subsequent extraction.

5.7.1 PROBLEMS ENCOUNTERED

After using the BP 1 column for some time, there was a severe rise in baseline when the temperature of the oven was programmed. This suggests that either there is a bleed in the column or the ECD is contaminated. Even after conditioning the column overnight at 250°C and cleaning the collector electrode there was no improvement. With nothing

injected through the column the same problem was observed. The BP1 column was substituted with a BP 5 column. However there was problems with this column as well. A negative baseline drift was observed as the temperature was increased and it took a long time for the baseline to stabilise after each temperature programming. A negative baseline drift was also observed with nothing injected through the column. This suggests that there is a carrier gas leak in the system. However, no leaks were detected in any part of the system. With both columns, the baseline produced was cyclic. This cyclic baseline was found to match the time it takes the injector light to go on and off. The injector light should be blinking all the time indicating that it is regulating the injector temperature (a fault in the injector light or heater). The ECD was then fitted on to a Carlo Erba Vega 6000 Series gas chromatograph which houses a BP1 column. Even at very low reference current the frequency reading was very high. Leaving it for a few days to allow the system to stabilise was of no help. Cleaning the collector electrode (sonicating in toluene followed by hexane and allowing it to dry before assembling) was of no help as well. Changing the carrier gas from helium to argon with 10% methane failed as well. The high background current could be due to the proximity of the GC system to the NMR laboratory where quantities of deuteriochloroform were continually being used. So the extracted sludge samples were sent to Wessex Water for analysis. All sludge extracts were blow dry with a gentle purge of nitrogen before sending them to Wessex Water. At Wessex water the dried extracts were reconstituted with hexane (1 ml) and 2 μ l (1 μ l/column assuming a 50:50 split between columns) was injected onto the

dual column dual detector GC system. From this point onwards all results ~~were~~ analysed at Wessex Water using an ECD.

5.8 RESULTS FROM WESSEX WATER

The chromatogram of a blank sample of sludge extracted at a temperature of 40°C and a pressure of 300 kg cm⁻² shows numerous co-extractive peaks were observed even with the selective ECD (Figure 9a). Figure 9b is a chromatogram of the standard OCPs.

5.8.1 EFFECT OF TEMPERATURE

The aim of the experiment was to find the optimum conditions for the extraction of the spiked OCPs from sewage sludge. The variables that were varied are temperatures, pressures and percentage modifier. First the effect of different temperatures (40 - 80°C) on the recovery of the OCPs after a 30 min extraction time were studied (Table 3). As a dual column (Supelco SPB 608 and J & W Scientific DB 5) dual ECD detector were used, then the compounds have to be detected on both columns and detectors for confirmation. The peaks were tentatively identified by comparison with the standard OCPs. Quantitation of the OCPs was done using the external standard method using a single calibration standard (20 pg/μl of all the OCPs except for DDT which was at 40 pg/μl).

Figure 9a. GC chromatogram of an unspiked sludge sample at an extraction temperature of 40°C and a pressure of 300 kg cm⁻² with collection in nitrogen cooled flask. GC conditions: column, Supelco SPB 608 and J & W Scientific DB 5 (both 30m x 0.25 mm i.d. and 0.25 mm film thickness); injection, splitless with solvent venting after 30s; injection volume, 2 ml volume; oven temperature, initially 45°C, hold for 4 min, then ramp to 150°C at 15°C min⁻¹, ramp to 195°C at 5°C min⁻¹ and hold for 5 min, then ramp to 270°C at 2°C min⁻¹ and a final hold of 1 min; detector, ECD; detector temperature 270°C and injector temperature, 270°C.

Figure 9b. GC chromatogram of a standard solution of OCPs. GC conditions as in Figure 9a.

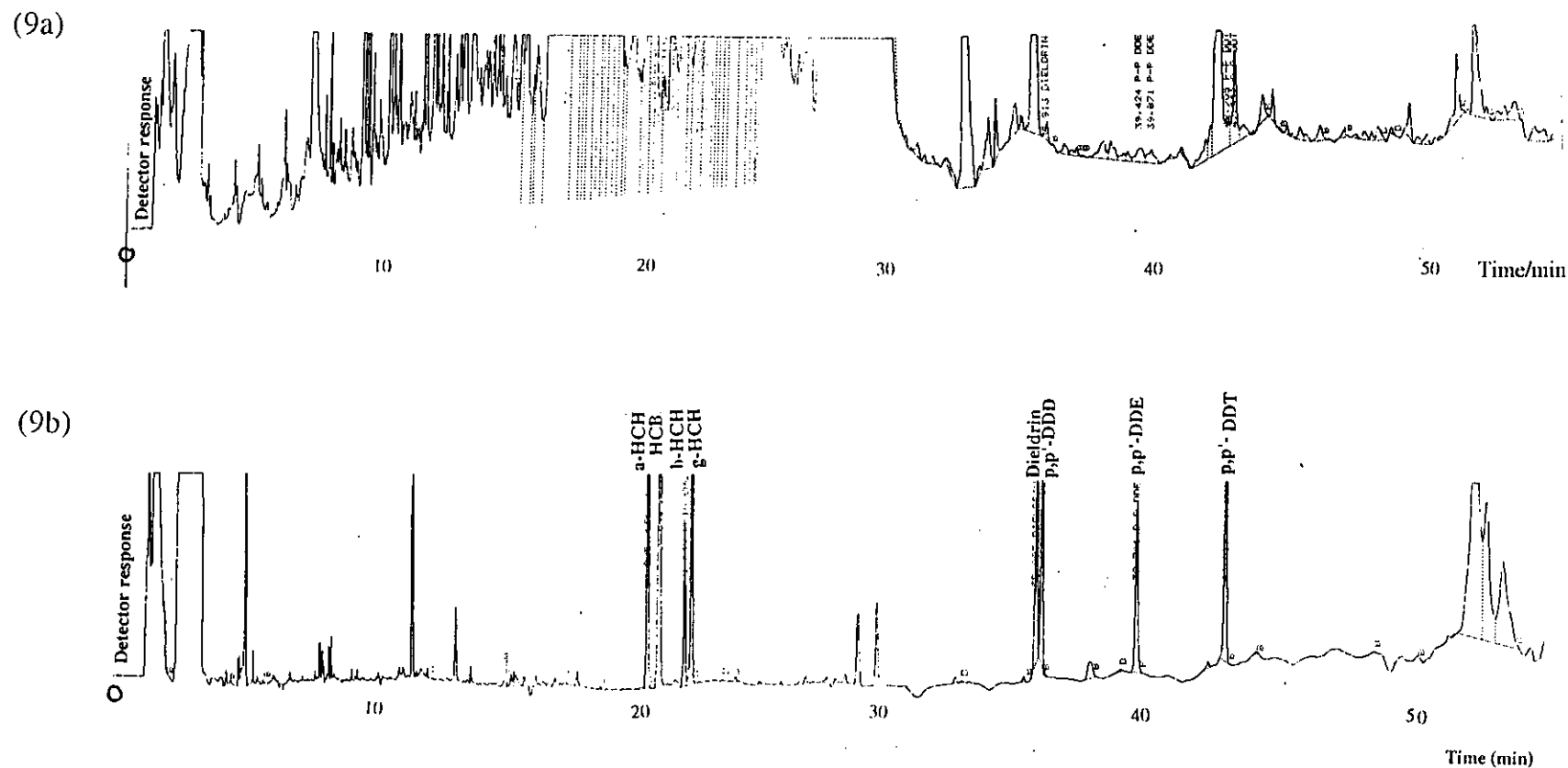


Table 3. Effect of temperature on % recovery of pesticides with trapping in hexane. 30 minutes extraction time.

~ : over integration

? : too big a value - unlikely to be the compound

- : not detected

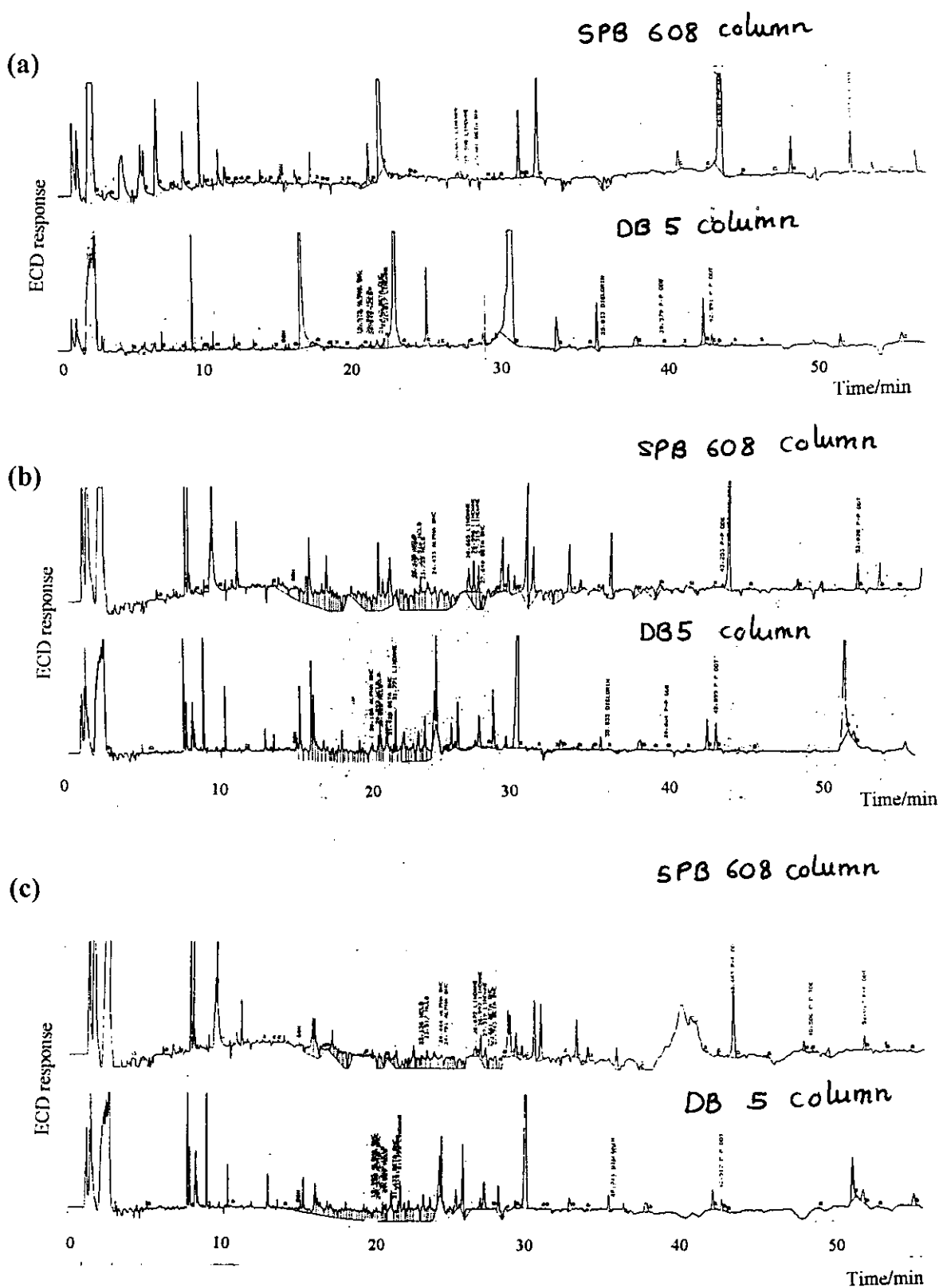
* 80/100/0 is a shorthand for extraction temperature at 80°C, extraction pressure at 100 kg cm⁻² and 0% modifier respectively.

col.1 is a Supelco 608 column

col.2 is a J & W Scientific DB 5 column

% Recovery						
	40/100/0		60/100/0		80/100/0	
Sample	col.1	col.2	col.1	col.2	col.1	col.2
DDT	-	-	59	59	26	34
DDD	-	-	-	-	-	-
DDE	-	-	-	-	-	-
Dieldrin	-	-	-	-	-	-
HCB	-	-	26~	69~	17	62
α-HCH	-	-	62~	132~	16	116?
β-HCH	-	-	53~	93~	31	190?
Lindane	20	15	24	108~	46	40

Figure 10. Gas chromatogram of spiked sludge sample extracted at an extraction temperature of (a) 40°C and a pressure of 100 kg cm⁻², (b) 60°C and a pressure of 100 kg cm⁻² and (c) 80°C and a pressure of 100 kg cm⁻² with collection in liquid hexane. GC conditions as in Figure 9a.



At an extraction temperature of 40°C and an extraction pressure of 100 kg cm⁻² only lindane was recovered (Figure 10a). As the extraction temperature was increased to 60°C more of the pesticides were recovered (Figure 10b). Only DDD and dieldrin were not recovered. Some of the peaks were over-integrated as a result of inappropriate assignment of the baseline. At 80°C less DDT, HCB, α -HCH and β -HCH were recovered (Figure 10c). More lindane was recovered at this temperature. DDD, dieldrin and DDE were not recovered at the three temperatures. The DDE, dieldrin and DDD were either not extracted (unlikely) or not trapped (more likely).

5.8.2 EFFECT OF PRESSURE

(a) Trapping in liquid hexane

Apart from temperature, pressure is the next most important parameter that can affect the recovery of analyte extraction. The solvent strength of a supercritical fluid (SF) is directly related to its density (20) and the solvating ability of a particular SF can easily be modified by changing the extraction pressure. An increase in pressure at a constant temperature should increase the extraction strength of CO₂. The effect of sequential increases in pressure on the recovery of the pesticides at 40°C was examined (Table 4). The density of the CO₂ at a temperature of 40°C and a pressure of 100 kg cm⁻², 200 kg cm⁻² and 300 kg cm⁻² are 0.626 g ml⁻¹, 0.849 g ml⁻¹ and 0.931 g ml⁻¹, respectively. At a pressure of 100 kg cm⁻² only lindane was recovered (Figure 11a). The chromatogram was

rather 'clean' and as the pressure was sequentially increased to 200 kg cm⁻² only DDT was recovered (Figure 11b). The chromatogram was also 'clean'. However, as the pressure was increased further to 300 kg cm⁻² more interference peaks were extracted as well. This time DDT, HCB, α -HCH and lindane were recovered (Figure 11c). Even at 300 kg cm⁻² the total recovery of each pesticides were all less than 30% except for lindane.

Table 4. Effect of sequential increase in pressure on the % recovery of pesticides extraction from spiked sludge sample for a 30 minutes extraction time with collection in hexane.

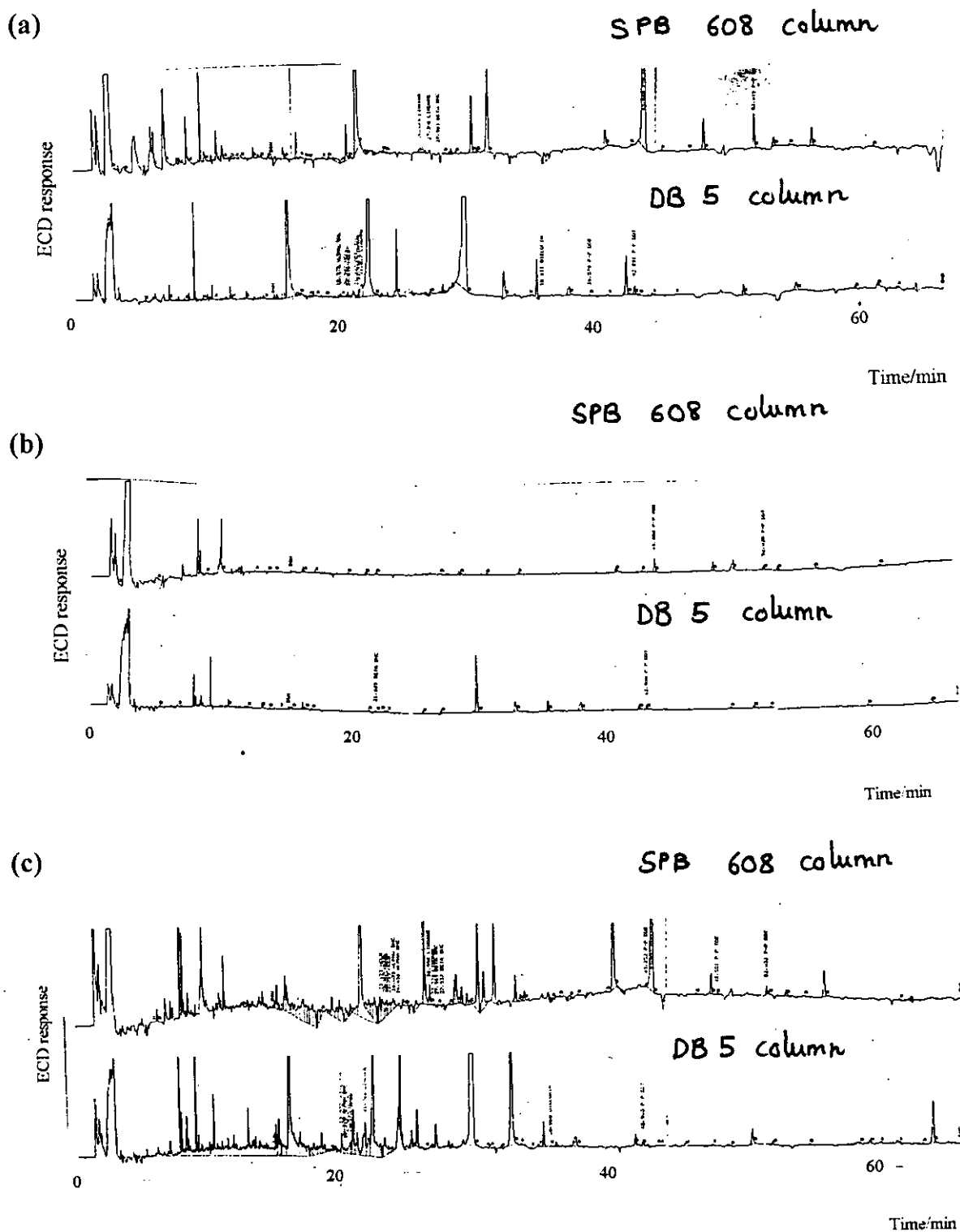
- : not detected

col.1 is a Supelco SPB 608 column

col.2 is a J & W Scientific DB 5 column

	% Recovery						Total % recovery	
	40/100/0		40/200/0		40/300/0			
Sample	col.1	col.2	col.1	col.2	col.1	col.2	col.1	col.2
DDT	-	-	12	12	16	19	28	31
DDE	-	-	-	-	-	-	-	-
DDD	-	-	-	-	-	-	-	-
Dieldrin	-	-	-	-	-	-	-	-
HCB	-	-	-	-	12	19	12	19
α -HCH	-	-	-	-	8	7	8	7
β -HCH	-	-	-	-	-	-	-	-
γ -HCH	20	15	-	-	44	38	64	53

Figure 11. Gas chromatogram of spiked sludge sample extracted at (a). 40/100/0 (b). 40/200/0 and (c). 40/300/0 with trapping in liquid hexane. GC conditions as in Figure 9.



(b). Trapping in nitrogen cooled flask

As the recovery of the OCPs were rather low, trapping in a round bottom flask cooled by liquid nitrogen was tried to see if more of the analytes could be trapped. The cooled flask method has been found to be efficient for trapping essential oils from a cellulose matrix (21). The effect of sequential increases in pressure on the recovery of the pesticides with collection in a cooled round bottom flask was carried out (Table 5). As the pressure was increased from 100 kg cm⁻² to 300 kg cm⁻² more interfering peaks were extracted as well (Figure 12a - 12c). However, more of the pesticides were trapped in the cooled flask compared to trapping in hexane. In the case of liquid trapping, the extracted analytes could have been purged out from the collection solvent by the fast flow of CO₂ upon depressurisation or they are not trapped effectively by the collection solvent (as will be seen later on in Chapter 6). The nitrogen cooled flask was therefore used for subsequent extractions.

Table 5. Effect of sequential increases in pressure on the extraction of OCPs from spiked sludge sample with collection of extracts in a flask cooled by liquid nitrogen.

~ : over integration

- : not detected

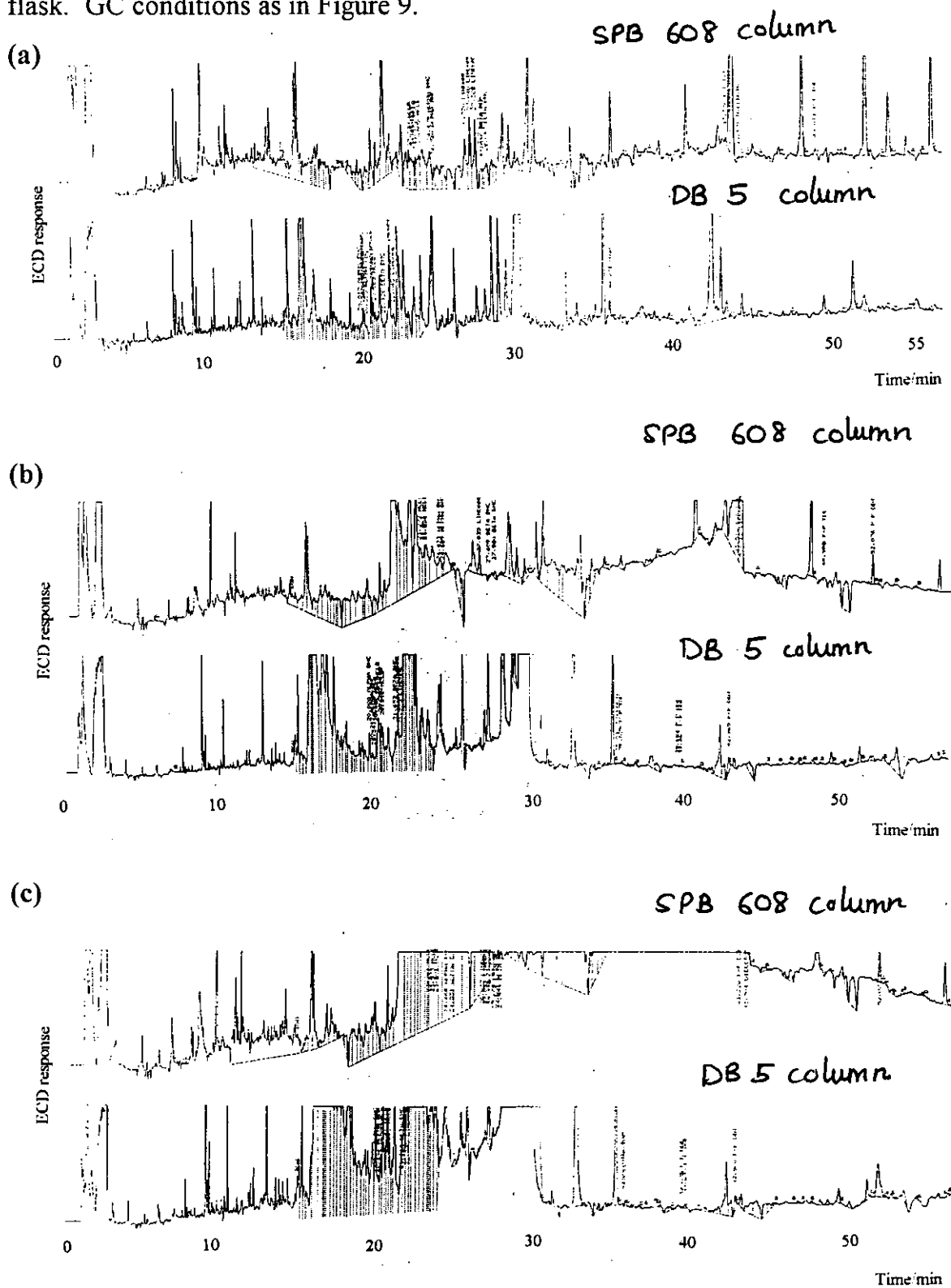
: many interferences, inappropriate baseline assignment

col. 1 is a Supelco 608 column

col.2 is a J & W Scientific DB 5 column

	% Recovery						Total % recovery	
	40/100/0		40/200/0		40/300/0			
Sample	col.1	col.2	col.1	col.2	col.1	col.1	col.1	col.2
DDT	-	-	26	70~	26	80~	52	150~
DDE	5	17	16	26	-	-	21	43
DDD	15	12	-	-	-	-	15	12
Dieldrin	-	-	-	-	-	-	-	-
HCB	37	27	38~	54~	#	#~	75	81
α -HCH	-	-	62	42	#	#	62	42
β -HCH	6	83~	94~	12	#	#	100~	95~
γ -HCH	-	-	18	70~	#	#	18	70

Figure 12. Gas chromatogram of spiked sludge sample extracted at (a). 40/100/0 (b). 40/200/0 and (c). 40/300/0 with trapping in nitrogen cooled flask. GC conditions as in Figure 9.



5.8.3 EFFECT OF METHANOL AS MODIFIER

As methanol is known to increase the recovery of analytes (22 -24) the use of methanol as modifier with trapping of extracts in nitrogen cooled flask was used to see if more of the analytes could be extracted. Methanol was chosen as the modifier of choice from a similar extraction of OCPs from spiked sediment (22). Initially the effect of 5% methanol on the recovery of the OCPs with sequential increase in pressure from spiked sludge was carried out (Table 6). The density of the modified SC CO₂ at a temperature of 40°C and pressure of 100 kg cm⁻², 200 kg cm⁻² and 300 kg cm⁻² are 0.782 g ml⁻¹, 0.877 g ml⁻¹ and 0.928 g ml⁻¹, respectively. At a pressure of 100 kg cm⁻² the peaks were overscale suggesting that more contaminants/inteferents were extracted. At 200 kg cm⁻² five out of the eight peaks were detected (after contaminants were extracted). However, DDD, DDE and dieldrin were still not detected and even at 300 kg cm⁻² these three peaks were not detected as well. The average total recovery for lindane is 53%, β-HCH is 63%, 20% for α-HCH, 26% for DDT and about 60% for HCB. When the methanol concentration was increased to 10%, a similar chromatogram to the extraction with 5% methanol were produced. As the pressure was increased, a 'cleaner' chromatogram was produced. DDD, DDE and dieldrin were not detected at all at the three extraction pressures. It is obvious that with just SFE as a sample preparation method, accurate quantitation of the pesticides is not possible as there were many interfering peaks or co-extractives.

Table 6. Effect of 5% methanol as modifier with a sequential increase in pressure on the extraction of spiked OCPs from sludge sample with collection of extracts in flask cooled by liquid nitrogen.

~ : over-integration

#: overscale

-: not detected

col. 1 is a Supelco 608 column

col.2 is a J & W Scientific DB 5 column

	% Recovery						Total % recovery	
	40/100/5		40/200/5		40/300/5			
Sample	col.1	col.2	col.1	col.2	col.1	col.1	col.1	col.2
DDT	#	#	7	12	12	21	19	33
DDE	#	#	-	-	-	-	-	-
DDD	#	#	-	-	-	-	-	-
Dieldrin	#	#	-	-	-	-	-	-
HCB	#	#	53	60	9	42~	62	102~
α -HCH	#	#	10	37	8	8	18	45
β -HCH	#	#	60	33	29	30	89	63
γ -HCH	#	#	37	29	29	21	58	48

5.9 CONCLUSIONS

SFE of OCPs from sludge failed to selectively extract the analytes and reduced interferences from co-extractives. All the chromatograms of the extracts showed that many interfering peaks/contaminants were extracted as well. With carbon dioxide alone little was extracted. However, if methanol was added to the extraction fluid there appeared to be an increase in the extraction of the pesticides but the changes were largely obscured by a significant increase in the background material that was obtained. Even with the use of a selective detector and a simultaneous dual column separation the response from the additional components completely swamped the OCPs and often interfering peaks were present which co-eluted on one of the two columns with the components of the test mixture. These interferences made it very difficult to determine the efficiency of the extraction procedure as often accurate quantification could not be obtained. However, it appears that recoveries were relatively low. It was not clear whether this was due to the conditions for extraction or the methods of trapping the analyte.

Because problems in recovery and trapping were encountered, subsequent work in the next chapter examined the extraction of organophosphorus pesticide chlorpyrifos from a formulation as a less complex model followed by chlorpyrifos extraction from soils.

5.10 REFERENCES

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CHAPTER 6

SUPERCRITICAL FLUID EXTRACTION OF CHLORPYRIFOS FROM DURBAN GRANULES AND SOILS

6.0 INTRODUCTION

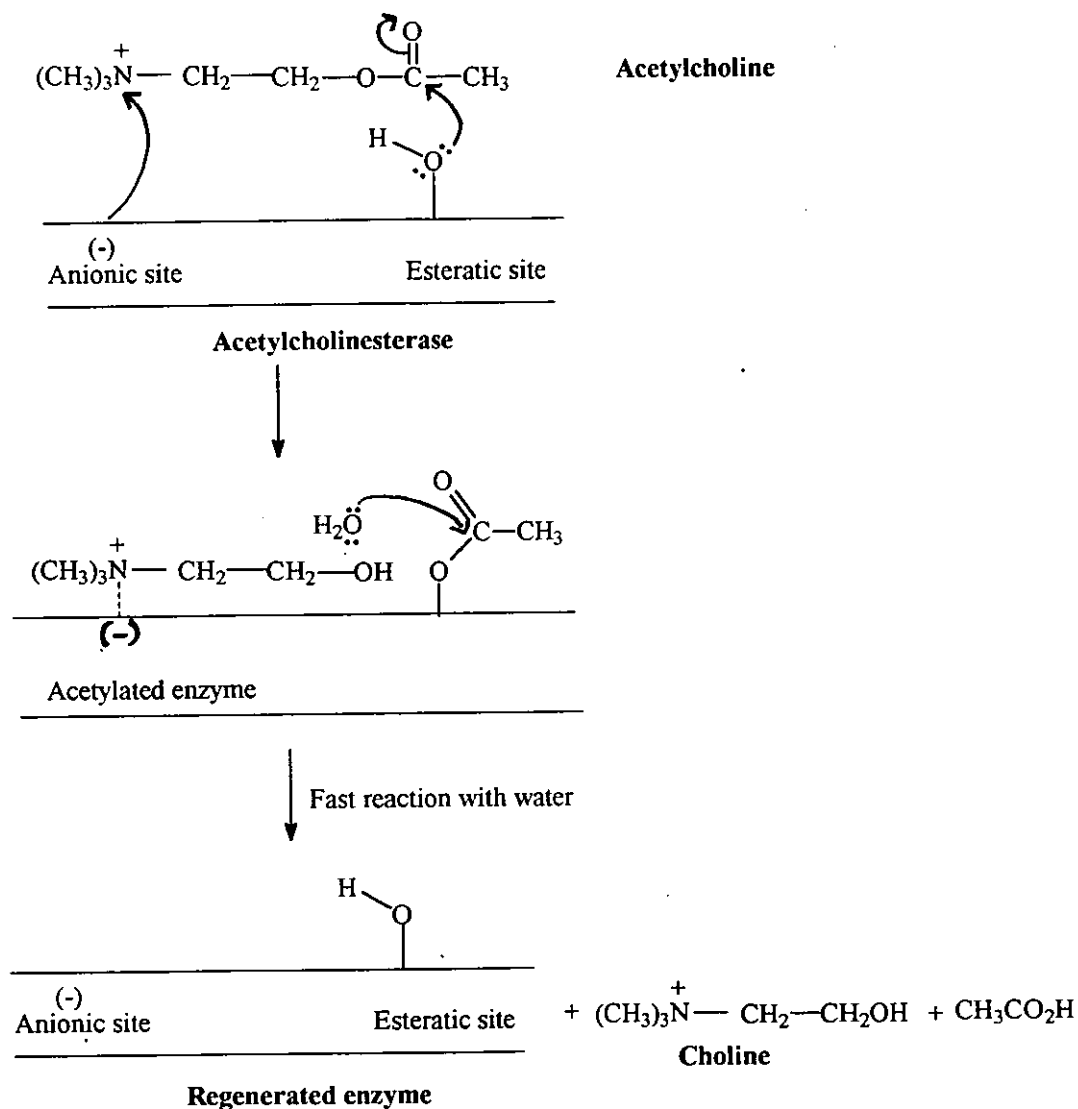
Soil forms an environmental reservoir, which is continually polluted by pesticides used for agricultural purposes. As a consequence the determination of residual amounts of agricultural products in soils is of regulatory and environmental significance. A wide range of these chemicals are either applied directly to the soil or reach it from foliage drip-off or spray drift (1, 2).

The assays of these compounds have been the subject of numerous studies and most are based on conventional extraction methods (Soxhlet extraction, liquid extraction) which are tedious and time consuming. For the past ten years or so, the use of supercritical fluid (SF) as alternative extraction method for the extraction of pesticides from various matrices has been explored with success (3, 4, 5).

6.1 ORGANOPHOSPHORUS INSECTICIDES

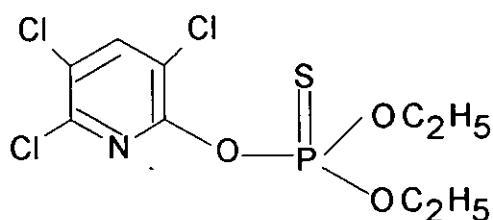
The organophosphorus insecticides (OPPs) are the most widely used in agriculture after the organochlorine insecticides. The OPPs inhibit the action of several enzymes, but the major action *in vivo* is against the enzyme acetylcholinesterase (7, 8). This controls the hydrolysis of acetylcholine generated at nerve junctions into choline (Figure 1). In the absence of effective acetylcholinesterase, the liberated acetylcholine accumulates and prevents the smooth transmission of nerve impulses across to synaptic gap at nerve junctions. This causes loss of muscular coordination, convulsions, and ultimately death. Unlike the organochlorine pesticides, the OPPs are less toxic to vertebrates and are non-persistent. The OPPs are usually degraded in soil within 2-4 weeks of application, e.g. diazinon, dichlorvos, dimethoate, malathion, parathion, and phorate (9). Some OPPs, like mevinphos are degraded after 24 hours of application (9). Therefore the OPPs are more suitable for agricultural use compared to organochlorine insecticides.

Figure 1. Schematic mode of action of OPPs - normal enzymic hydrolysis of acetylcholine to choline (9).



6.1.1 CHLORPYRIFOS

Chlorpyrifos (I) is among six of the OPPs formulated specifically as granules for application to soil to control pests feeding. The other five OPPs are chlorfenviphos, diazinon, disulfoton, fonofos and phorate (10). Chlorpyrifos belongs to the class of heterocyclic organothiophosphorus insecticides. It has a wide spectrum of activity, by contact, ingestion and vapour action (11). It is moderately persistent and retains its activity in soil for 60 - 120 days, before it is finally degraded to 3,5,6-trichloro-2-pyridinol (11). Chlorpyrifos is a comparatively safe insecticide; the mammalian toxicity LD₅₀ (oral) to rats is 160 mg/kg (11) and it is readily detoxified in animals. Chlorpyrifos is a white granular crystal-like material with a melting point of 42 - 43.5°C and has a vapour pressure of 1.87×10^{-5} mmHg at 20°C (12). The octanol-water partition (K_{ow}) for chlorpyrifos is 128 825 ($\log K_{ow} = 5.11$) (13). It is classified as a fat soluble compound (14). It has the odour of mild mercaptan.



Chlorpyrifos

{O,O-diethyl - O - (3,5,6-trichloro-2-pyridinyl) phosphorothioate}

6.1.2 CONVENTIONAL METHODS FOR EXTRACTION OF PESTICIDES FROM SOIL

Conventional methods for extracting pesticides from soil usually involve an initial solvent extraction followed by a clean-up procedure and concentration of solvent. Extraction using apolar solvents followed by Rotavapour (15, 16) or Kuderna-Danish (17, 18) concentration to small volume is a usual way of achieving quantitative evaluation of pesticides in soils. All neutral pesticide analytes can be isolated from solids by extracting them with 1:1 acetone/dichloromethane (v/v) using a Soxhlet apparatus (EPA Method 3540) or by using an ultrasonic apparatus with 1:1 (v/v) acetone/dichloromethane (EPA Method 3550) (19). Once the solid samples have been extracted, it is almost always necessary to remove co-extracted matrix constituents before they can be analysed. Gel permeation chromatography (GPC), adsorption chromatography, thin layer chromatography (TLC) or Florisil column (Method 3620) are some of the clean-up methods used.

Parathion, an organophosphorus pesticides (20) was extracted from soil with acetone and then partitioned into water. The aqueous layer was then extracted with dichloromethane and cleaned on a Sep-Pak C₁₈ column. Extracts were determined by GC fitted with a thermionic detector. However this process is lengthy and time consuming (not desirable for routine work) and involves the use of toxic organic solvents.

6.1.3 EXTRACTION AND ANALYSIS OF CHLORPYRIFOS

Chlorpyrifos residues have been evaluated in many substrates (21-25). The analytical procedures generally adopted for the determinations consisted of extraction with solvent, water partition, and clean-up on a chromatographic column followed by gas chromatographic determination with electron capture detection {ECD} (22-24), flame photometric detection (FPD) (21) or thermionic detection (TID) (25).

Inman *et al.* (26) extracted chlorpyrifos and its metabolite, 3,5,6-trichloro-2-pyridinol from peppermint hay using liquid extraction. The chlorpyrifos residues were cleaned up using a silica gel column and were quantitated by phosphorus-specific gas chromatography. The metabolite was separated from the extraction solvent by liquid-liquid partitioning with aqueous sodium carbonate, followed by chromatography on acid alumina and analysed by gas chromatography with electron capture detector. These methods are sensitive to 0.02 ppm in hay for chlorpyrifos and for the metabolite. Maini and Collina (27) used sweep co-distillation method, developed by Storherr and Watts (28) for some organophosphate pesticide residues, for the extraction and clean-up of chlorpyrifos insecticide residues in lettuce, carrot, sugar beet, (leaves and roots), potato, apple, onion, cauliflower, orange (peel and pulp), corn grain, and agricultural soil. The mean recovery on all substrates at the 0.1 ppm level was 90.19% \pm 5.71% RSD. This method was much quicker than extraction, partition, and Florisil clean-up method (29). Bowman and Beroza (21) determined chlorpyrifos and its oxygen analogue in corn and grass using a flame photometric detector. The recoveries of chlorpyrifos and its oxygen analogue in the 0.1 - 5 ppm range were 96-99% and 85-90% respectively.

Rice and Dishburger (22) determined chlorpyrifos in water and silt. Residues as low as 0.0001 ppm in water and 0.005 ppm in silt were determined by gas chromatography (GC) using a nonpolar column and ECD. Recoveries of chlorpyrifos from water and silt averaged 92% and 83% respectively. Hunt *et al.* (24) analysed chlorpyrifos in turkey and chicken tissues after extraction of the chlorpyrifos by petroleum ether and aliquots were analysed without prior clean-up by GC using an ECD. Residues as low as 0.05 ppm could be detected. Recoveries of chlorpyrifos from tissues ranged from 72-99%. Petrova and Andreev (30) extracted Dursban (chlorpyrifos) along with Basudin from soil and water samples. A 1:1 mixture of acetone with 0.05M CaCl_2 was used to extract the pesticides and the extract transferred to an acetone-hexane system. The extracted chlorpyrifos was determined using GLC. Rao *et al.* (31) extracted Dursban residues from soil with acetone. In this method there was an initial extraction step, drying step, another extraction step with hexane, a clean-up step and a concentration step before the detection step.

6.2 AIM OF PROJECT

The widespread use of agricultural chemicals, with more than 1000 pesticides (32) in common use, demands efficient and practical analytical methods for the analysis and assessment of these hazardous substances in the environment. There is particular interest in the monitoring of pesticide residues in soil. The use of SFE for the determination of pesticides has been demonstrated for urea herbicides (33, 34), carbamate pesticide (35),

chlorinated insecticides (36-38), triazines (39), and phenoxyacetic esters (40).

Since the traditional methods for extraction of pesticide are laborious and time consuming, it is of interest to use SFE as a method of extraction of chlorpyrifos from soil to see the effectiveness of this method and compare the efficiency to Soxhlet extraction. Initially the study will concentrate on determining the best conditions that will quantitatively extract chlorpyrifos from a chlorpyrifos formulation (Dursban granules). The efficiency of SFE of chlorpyrifos from Dursban will be compared to room temperature methanol extraction. The optimum conditions from the Dursban extraction will be then used to extract spiked chlorpyrifos from a range of soils (German standard soil, clay, dark peat, Speyer 2.1, Speyer 2.2 and Speyer 2.3) and also to see the effect of these different soil matrices on the percentage recovery of spiked chlorpyrifos. Two types of trapping (solid and liquid trapping) will be evaluated.

However, despite their widespread use, there are only a few previous SFE studies of the organophosphorus insecticides. Ethion from grapes (41), disulfoton and tolclufos methyl from soil (42), chlorpyrifos from grass (43) and from green onions, lettuce, strawberries, oranges and alfalfa (44), dichlorvos, diazinon, ronnel, parathion, methidathion, tetrachlovinphos (45) from soils, diazinon, disulfoton, dimethoate, malathion, parathion, carbofenthion, azinphos methyl and coumaphos from soil (46) and fenithrothion, esfenvalerate and diniconazole from soil (47) have all been studied.

Even though spiked samples may not always represent the extractability of "real world" samples (48), spiked samples are a good way of testing the collection efficiency. Matrix reference materials (MRMs) can then be used to evaluate the efficiency of the extraction (49). However, MRMs are not nearly so readily available as certified reference materials (CRMs) (50) and this justifies the use of spiked soil samples.

6.3 CHLORPYRIFOS EXTRACTION FROM DURSBAN GRANULES

6.3.1 PREVIOUS WORKS

In order to establish suitable conditions for the quantitative extraction of spiked chlorpyrifos from soil, it was decided to examine Dursban granules which have been sprayed with chlorpyrifos. A previous trial study of Dursban granules in this laboratory obtained a maximum recovery of chlorpyrifos of about 70% using SC CO₂, at a temperature of 40°C, a pressure of 150 kg cm⁻² and 5% methanol as modifier (51). The shorthand notation T/P/M which stands for extraction temperature, extraction pressure and % modifier, respectively will be used from here onward (eg. 40/150/5 means extraction temperature of 40°C, extraction pressure of 150 kg cm⁻² and % modifier of 5%). The samples were collected in nitrogen cooled round bottom flask. However, the reproducibility obtained was poor (RSD 21%). This variation was ascribed to sample inhomogeneity resulting from the spraying process and the small and possibly unrepresentative samples that were taken (0.05 g). Final step was therefore to carry out a more detailed study to increase the recovery.

The chlorpyrifos extracts were analysed by GC and the peak identified by comparison with an authentic sample of chlorpyrifos standard. The concentrations of chlorpyrifos in the extracted sample (Dursban granules and spiked soils) were obtained from a calibration graph of chlorpyrifos standard versus area ratio of chlorpyrifos:n-docosane (internal standard) using the regression line and least square fit calculated using the MINITAB statistical package version 8.0 on the PC (Figure 2 - for Dursban extraction and spiked soil extraction at 0.1% spiking level). The reproducibility of GC injections of the area of chlorpyrifos and area of internal standard, n-docosane as measured by the area ratio of chlorpyrifos to n-docosane was determined for the chlorpyrifos standard in the 40 - 400 $\mu\text{g ml}^{-1}$ range (Table 1) The reproducibility of the GC injections for the area ratio of chlorpyrifos :n-docosane were all less than 3%.

Figure 2. Calibration graph of chlorpyrifos concentration (40-400 $\mu\text{g ml}^{-1}$) versus area ratio of chlorpyrifos : n-docosane .

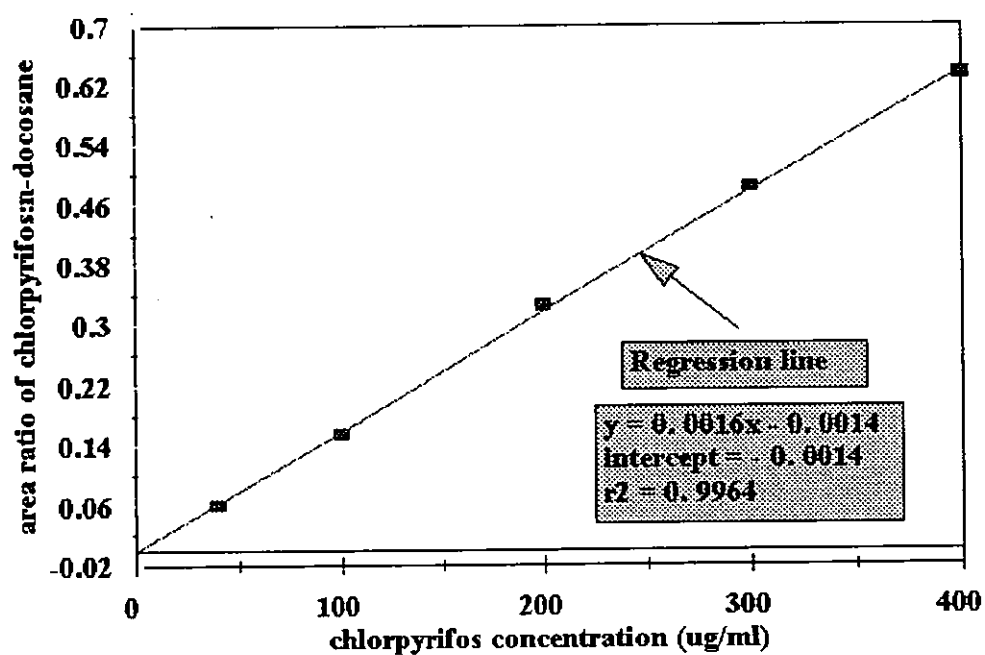


Table 1. Reproducibility of GC separations of chlorpyrifos standards in the range 40 - 400 $\mu\text{g ml}^{-1}$.

I. S. is internal standard (n-docosane).

area ratio = chlorpyrifos area/I.S. area.

Regression equation : $y = 0.0016x - 0.0014$; $r = 0.9982$; intercept = - 0.0014. (see Figure 2).

Std. conc.	Chlorpyrifos area (A)	I.S.area (A _s)	Area ratio (A/A _s)	Average A/A _s	RSD (%)
40 $\mu\text{g ml}^{-1}$	1080	18216	0.0593		
	1124	19430	0.0578	0.0588	1.4
	1060	17888	0.0592		
100 $\mu\text{g ml}^{-1}$	3104	20104	0.1543		
	2990	19342	0.1546	0.1551	0.71
	3123	19965	0.1564		
200 $\mu\text{g ml}^{-1}$	6763	21303	0.3175		
	6289	18805	0.3349	0.3251	2.6
	6613	20446	0.3234		
300 $\mu\text{g ml}^{-1}$	9281	19606	0.4734		
	8896	18200	0.4888	0.4817	1.6
	9427	19524	0.4828		
400 $\mu\text{g ml}^{-1}$	12276	19489	0.6299		
	11197	17529	0.6388	0.6334	0.76
	12011	19024	0.6314		

Solvent extraction of a 0.5 g sample of Dursban granules gave a 4.5% yield (RSDs 3.5%). This value lies in the range reported by the manufacturer. The granules had been sprayed with 4-6% chlorpyrifos. These extraction yield was assumed to represent 100% recovery and the SFE recoveries for the Dursban extraction were calculated relative to this value.

When the extractions were repeated under the same SFE conditions used previously (51), an average chlorpyrifos recovery of 43% (RSDs 8%) was obtained. In the current experiments the extraction time had been extended from 15 to 40 minutes, until the baseline of the UV detector at 225 nm returned to zero. The UV extraction profile of the SFE of the Dursban granules was recorded during the course of the extraction (Figure 3). With longer extraction time more of the chlorpyrifos have been lost after the depressurization of the supercritical fluid CO₂ into the cooled round bottom flask because it had been blown out of the extraction vessel by the flow of modified carbon dioxide. The gas chromatogram of the Dursban extract contained three peaks (Figure 4). The first peak is the solvent peak, the second peak is the chlorpyrifos peak and the third peak is the internal standard peak, n-docosane. The identity of the chlorpyrifos peak in the extract was made by comparison with an authentic sample of chlorpyrifos (Figure 5).

Figure 3. UV profile of chlorpyrifos extraction from Dursban as a function of time.

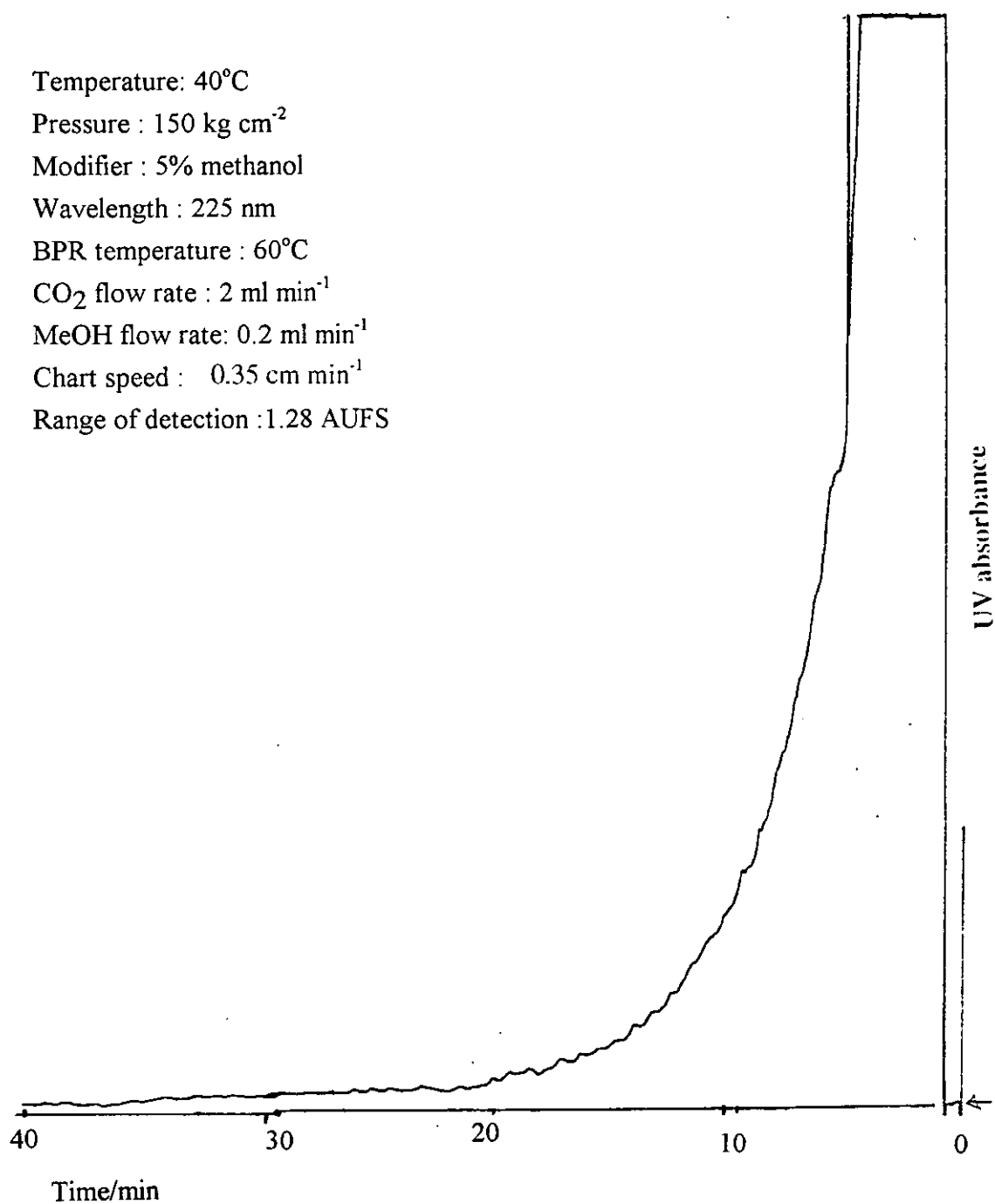


Figure 4. Gas chromatogram of Dursban extract at 40/150/10. GC conditions:- Column, SE 54 (30m x 0.32 mm i.d., 0.25 μ m film thickness); injector, 225°C; oven temperature, 230°C; He flow, 1 ml min⁻¹; split ratio, 13/1. Peaks: 1. Solvent peak. 2. Chlorpyrifos 3. n-Docosane.

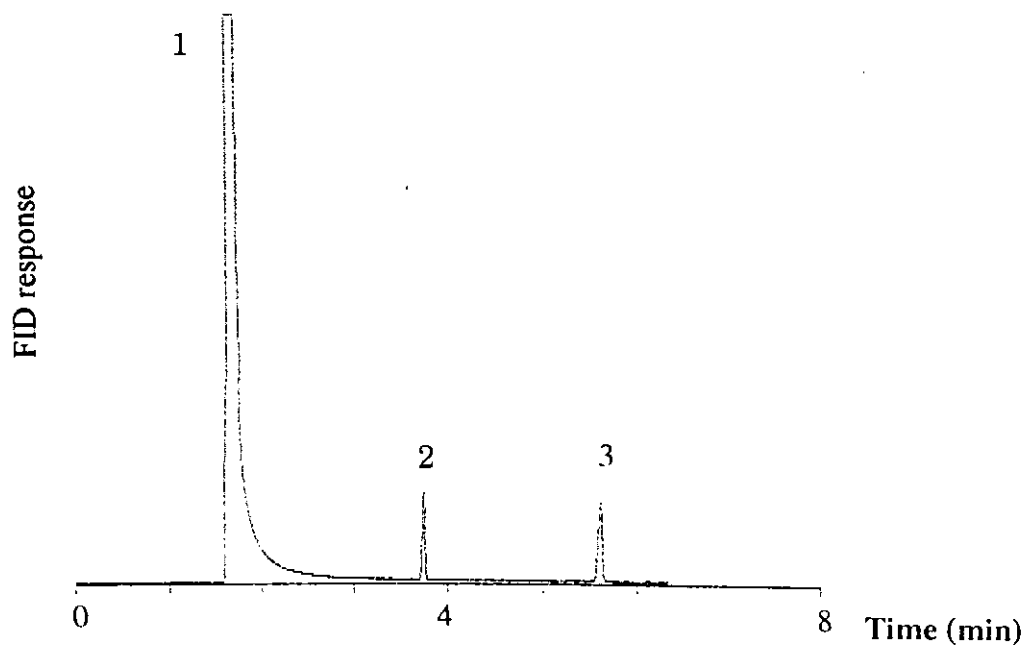
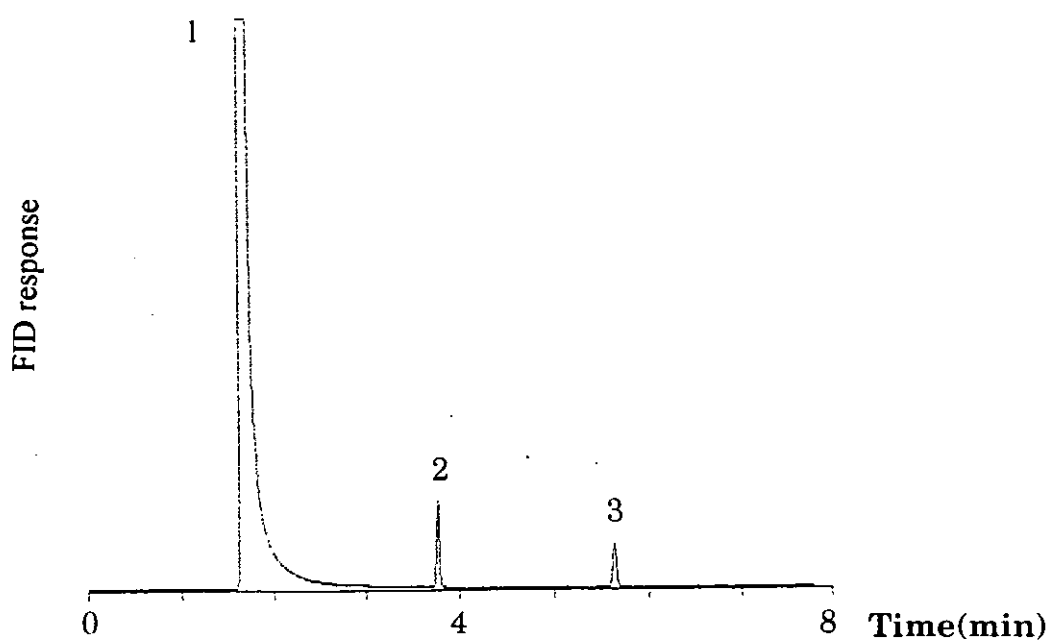


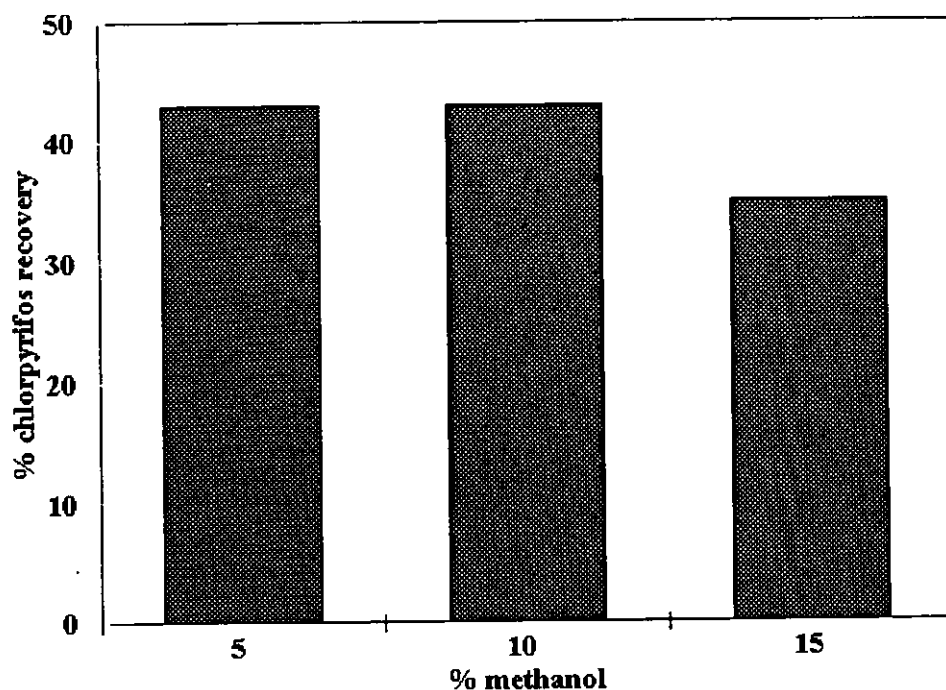
Figure 5. Gas chromatogram of 1 mg ml⁻¹ standard chlorpyrifos solution. Conditions as in Figure 4.



6.3.2 EFFECT OF MODIFIER

As the recovery of chlorpyrifos from the Dursban granules was not quantitative with 5% methanol as modifier, the effect of different methanol percentages was examined (Figure 6). Modifiers may not only induce changes in the nature of the solvent but also influence the matrix. Matrix swelling resulting from the interaction of the modifier and the matrix has also been proposed as a predominate interaction in SFE (52). It has been well documented in chromatographic studies (53, 54) that polar modifiers may deactivate adsorption sites on polar matrices such as silica based packing materials. Methanol was chosen as modifier based on a similar extraction of spiked chlorpyrifos methyl from wheat kernel substrate by Campbell and co-workers (55) and chlorpyrifos from grass (43). In the present study no significant difference in recovery was observed on increasing the methanol from 5% to 10% but when 15% was used the recovery dropped to 35% (Figure 6). The reason for this drop is unclear. This suggests that between 5%-10% methanol is the optimum modifier concentration. Deye *et al.* (56) found out that about 5% of a polar modifier was needed before an appreciable increase in solvent strength was discernible.

Figure 6. Effect of methanol concentration on the percentage recovery of chlorpyrifos from unground Dursban granules at 40°C and a CO₂ flow rate of 2 ml min⁻¹.



6.3.3 EFFECT OF PARTICLE SIZE

As sample preparation method may have a direct influence on the extraction yield and hence the recovery, the effect of grinding the Dursban granules were investigated. Homogenising the matrix to increase the surface area could also greatly aid the mass transfer step. Grinding and sieving are techniques that can be utilised to increase the surface area and hence improve diffusion through the matrix (57). Study on the recovery of Tinuvin-326 (58) demonstrated the great advantage in extraction rates achieved by grinding samples (originally 5 mm) to sample sizes of < 0.6

mm and 0.6 - 1.2 mm. It was found that with the 5 mm beads more than 80 hours were needed to achieve quantitative recovery of the Tinuvin-326, with the 0.6 - 1.2 mm sample size, 95% Tinuvin-326 recovery was achieved in 250 minutes and for the < 0.6 mm sample size it took only 45 minutes for 95% recovery of the Tinuvin-326. In this work it was found that grinding the granules significantly increased the recovery of chlorpyrifos and reduced the RSD (Table 2). The recovery of chlorpyrifos almost doubled when the Dursban granules were ground. Thus in future studies the Dursban granules was used as a ground powder.

Table 2. Effect of grinding Dursban granules on the recovery of chlorpyrifos at 40°C and 150 kg cm⁻² and a CO₂ flow of 2 ml min⁻¹.

%MeOH	Unground		Ground	
	%Recovery	Average % recovery \pm RSD	%Recovery	Average % recovery \pm RSD
5	39		59	
	45	43 \pm 8.0 (n = 3)	64	62 \pm 5.8 (n = 2)
	45			
10	41		70	
	48	43 \pm 10.1 (n = 3)	73	73 \pm 3.5 (n = 3)
	40		77	
15	37		66	
	33	35 \pm 7.0 (n = 2)	71	68 \pm 3.7 (n = 2)

6.3.4 EFFECT OF FLOW RATE

In previous studies (51) the effect of CO₂ flow rate (at 2, 4 and 6 ml min⁻¹) on the recovery of chlorpyrifos was studied. It was found that the recovery decreased with increasing flow rate. A similar observation was noted by McNally (34) in the study of the extraction of linuron and diuron from a sandy loam soil. Since the flow rate used (2 ml min⁻¹) has not been optimised, lower flow rates of between 0.5 ml min⁻¹ - 1.5 ml min⁻¹ at an extraction temperature of 40°C and an extraction pressure of 150 kg cm⁻² were used to find the optimum flow. It was found that a flow of 1 ml min⁻¹ was the optimum (Figure 7). The effect of 5%, 10% and 15% methanol as modifiers were examined with a total flow rate of 1 ml min⁻¹ CO₂ on the recovery of chlorpyrifos from ground Dursban granules (Figure 8). The highest recovery (90%) of chlorpyrifos was obtained with 10% methanol. These conditions (temperature 40°C, extraction pressure 150 kg cm⁻², CO₂ flow 1 ml min⁻¹ and 10% methanol) were therefore used for the extraction of chlorpyrifos from spiked soil.

Figure 7. Effect of flow rate on the recovery of chlorpyrifos from ground Dursban granules. Single extractions at 40°C, 150 kg cm⁻² and 10% methanol.

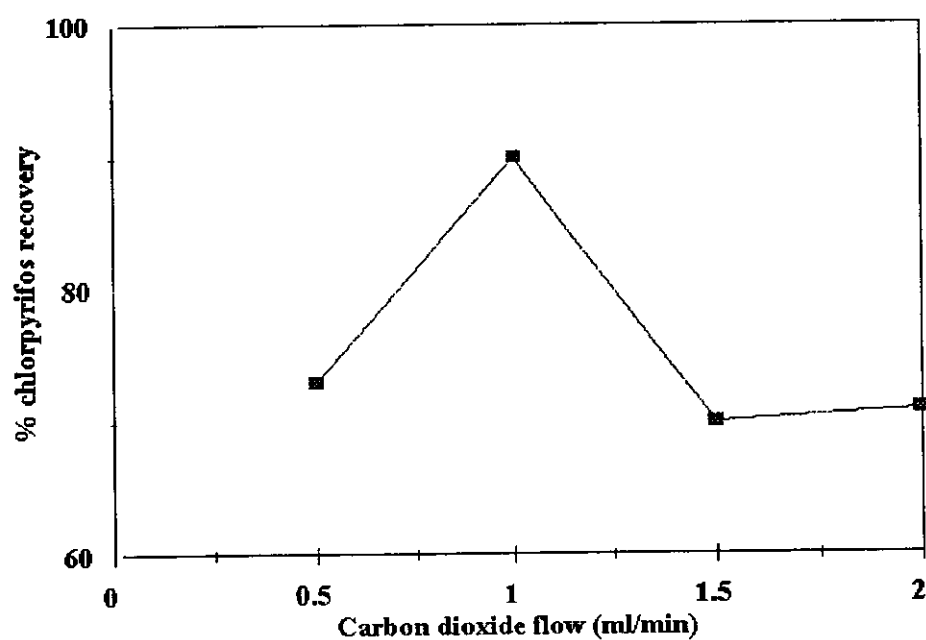
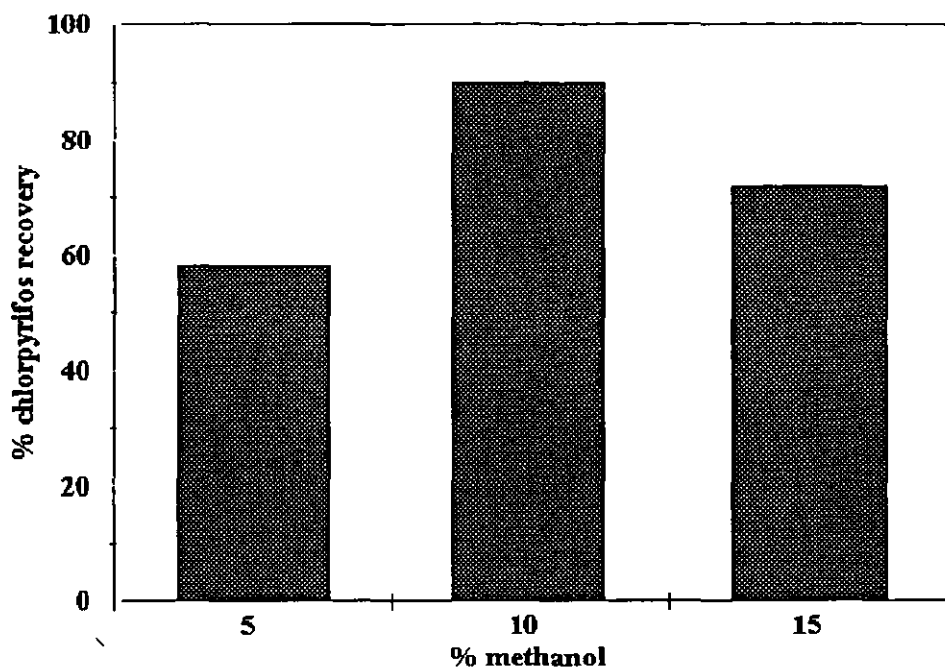


Figure 8. Percentage recovery of chlorpyrifos from ground Dursabn granules at 40°C, 150 kg cm⁻² and a CO₂ flow rate of 1 ml min⁻¹ at various methanol percentages. Collection in flask cooled by liquid nitrogen.



6.4 EXTRACTION OF SPIKED CHLORPYRIFOS FROM SOIL

6.4.1 COLLECTION IN FLASK COOLED BY LIQUID NITROGEN

The optimum extraction conditions from the Dursban samples were applied to the extraction of chlorpyrifos in acetone which had been spiked at 1000 µg/g level to a German standard soil. The recovery of chlorpyrifos with collection in a round bottom flask cooled by liquid nitrogen was only 57%.

Subsequent solvent extraction of the soil with methanol after SFE showed that about 15% chlorpyrifos remained unextracted. Although a low pressure and mild conditions were sufficient to recover the chlorpyrifos from Dursban granules, it appeared that higher pressure (250 kg cm⁻²) might be needed to recover the chlorpyrifos from spiked soil samples, presumably due to increased matrix associations. Therefore pressures higher than 150 kg cm⁻² were examined.

When the pressure was increased to 250 kg cm⁻², the recovery of chlorpyrifos increased to 74% (Figure 9). Chromatographic analysis of a solvent extract with methanol of the soil after SFE showed no detectable chlorpyrifos peak suggesting that the extraction had removed all the analyte. In all these extractions the spiked soil was extracted immediately after spiking without allowing the solvent used to apply the analyte to evaporate, as it was found that there was a significant reduction in recovery when the solvent was allowed to evaporate (for 2 hours) at room temperature (Figure 9). The spiking solvent acetone may have thus acted as a modifier and affected the extraction. The extraction profile of chlorpyrifos from the German standard soil at 40°C, 250 kg cm⁻² and 10% methanol (shorthand 40/250/10) is shown in Figure 10.

As only 74% of the spiked chlorpyrifos was recovered and the solvent extraction on the extracted sample showed no detectable residual chlorpyrifos, then the non-quantitative recovery must be attributed to poor solute trapping. Previous workers have suggested that the most likely loss of analyte is through aerosol formation (i.e. formation of liquid droplets of

the carrier solvent at the point of expansion (59) which can be formed by the rapid expansion of supercritical fluid solutions through the pressure restrictors (60). Aerosol particles in the 0.01 - 0.02 μm range are formed when SF solutions expand through pressure restrictors (60). The use of a polar modifier with a high critical temperature, such as methanol, in carbon dioxide is reported to substantially decreased aerosol formation (60). This can be ascribed to the formation of sizable liquid-methanol droplets during expansion, which likely contain or scavenge many of the analyte molecules and have larger deposition efficiencies due to their size and liquid character. In previous work (51) in this laboratory, a 19% recovery of chlorpyrifos from Dursban was obtained when no methanol was used compared to a 58% recovery of chlorpyrifos when 5% methanol was used (for both cases extraction was conducted at 40°C and 150 kg cm⁻² and collection in an empty vial).

Figure 9. Effect of allowing spiking solvent to evaporate on the percentage recovery of spiked chlorpyrifos from German standard soil samples at 40°C, 10% methanol and various pressures and CO₂ flow of 1 ml min⁻¹. Extracts were collected in a round bottom flask cooled by liquid nitrogen.

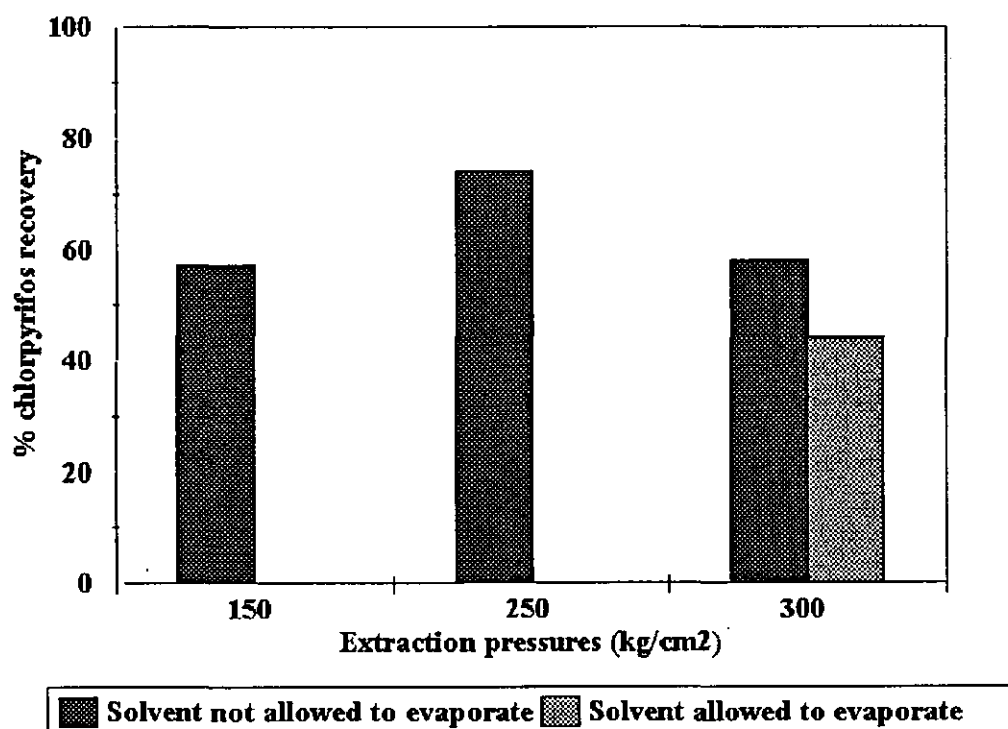
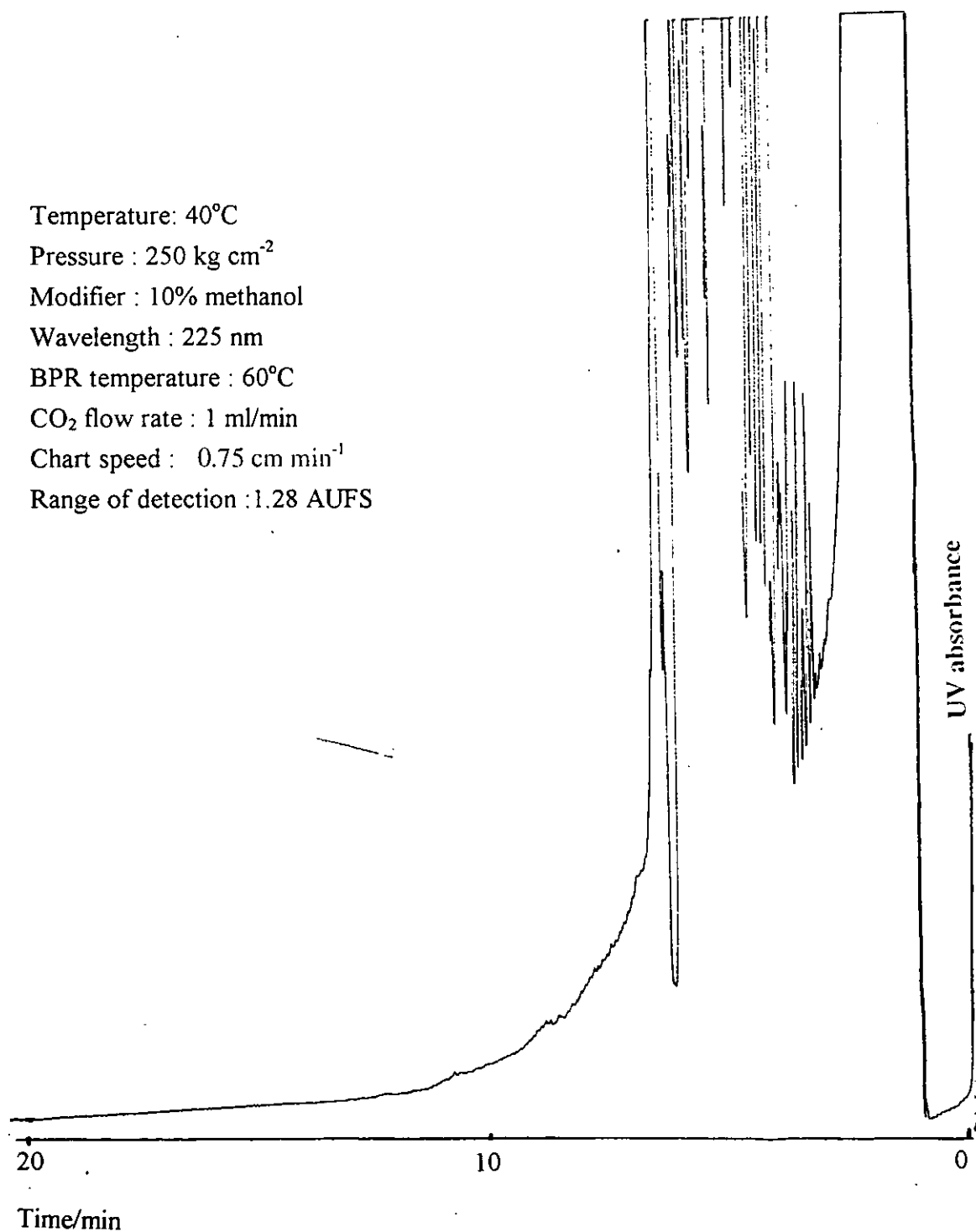


Figure 10. UV extraction profile of chlorpyrifos from a German standard soil at 40/250/10 as a function of time.



6.4.2 COLLECTION IN LIQUID

In an attempt to improve the collection efficiency using an inexpensive and simple way, bubbling into a liquid (methanol) was tried to trap the analyte after the depressurisation step.

When a liquid trapping was used the JASCO back pressure regulator was replaced with a linear fused silica capillary restrictor and the UV detector was omitted. A linear fused-silica capillary of different lengths and 40 μm i. d. was attached directly after the extraction vessel to control the extraction pressure and to direct the supercritical CO_2 and extract into the collection solvent. With this kind of restrictor, the flow rate has to be changed to change the back pressure because the back pressure is produced only by flow resistance. In this work the restrictor used was 40 μm i.d. and the pressure used was 250 kg cm^{-2} . The flow of CO_2 required to produce a pressure of 250 kg cm^{-2} with a 35 cm long restrictor was obtained by changing the flow rate on the CO_2 pump and noting the resulting CO_2 pressure reading on the pump an hour later (when the pressure remains constant). A graph of CO_2 flow vs. resulting pressure was plotted (Figure 11) ($r^2 = 0.9994$, intercept = -58.4). The CO_2 flow required to produce the required extraction pressure was obtained from the regression equation of the graph produced. A CO_2 flow of 1 ml min^{-1} at 40/250/10 with a restrictor length of 35 cm produces a pressure of 250 kg cm^{-2} (Figure 11). The same procedure was carried out when a 15 cm ($y = .155x - 6.3$; $r^2 = 0.9989$; intercept = -6.3) and 50 cm ($y = 360x - 41.4$; $r^2 = 0.9984$, intercept = -41.4) long restrictor were used. Extraction at 40/250/10 with a CO_2 flow of 1 ml min^{-1} for 20 minutes gave an average (3 assays) chlorpyrifos

solvent methanol gave similar recovery (about 70%) with about 30% chlorpyrifos was not trapped by both trapping method.

Table 3. % Recovery of chlorpyrifos with different trapping methods. Collection solvent volume is 3 ml. Value in bracket is the flow rate of CO₂. All triplicate extractions except for 0.80 ml min⁻¹ CO₂ flow where only a single extraction was carried out.

Trapping method	Solvent	% Recovery \pm RSD	% unextracted
Cooled flask	-	74 \pm 5 (1.0 ml min ⁻¹)	None
Solvent	Methanol	73 * (0.8 ml min ⁻¹)	0
Solvent	Methanol	65 \pm 6 (1.0 ml min ⁻¹)	5
Solvent	Methanol	62 \pm 1 (1.5 ml min ⁻¹)	8

It should be noted here that in the course of SFE using the linear fused silica restrictor, the restrictor needed to be replaced after every two or three extractions as it became brittle and broke. The location of the break in the fused silica restrictor was random. This problem has also been observed by several other workers (36, 61-64). This is attributed to the increased instability of glass and fused silica in the presence of polar solvents like water and methanol (65). Restrictor breakage with CHClF₂ (Freon-22) proved to be the worst for the SFs so far encountered (64). Burford *et al.* (66) found that by securing the fused silica restrictor inside a 1/16 inch outside diameter stainless steel tube with an epoxy resin

eliminated the problem of restrictor breakage due to polar fluids. ISCO Inc. (67) produces unbreakable stainless steel capillary restrictors of 300 μ m o.d. which are pre-assembled and calibrated to yield nominal flow rate of 1, 1.5 and 2 ml min⁻¹ at 5000 p.s.i. and 80°C.

It should be mentioned that a loaned ISCO SFE 2300 system was also used in the extraction of spiked chlorpyrifos from soil. A 50 μ m i.d., 30 cm long restrictor (as provided by the company) was used to depressurise the SF and extract into methanol (3 ml). This system works in the constant pressure mode while the JASCO system was used in the constant flow mode. The capacity of the ISCO extraction vessel was 2.5 ml while the JASCO one was 1 ml. The recovery of spiked chlorpyrifos from German standard soil at 40°C and 150 kg cm⁻² was quantitative irrespective of the percentage modifier used (Table 4). Time did not permit further work on this system to be carried out. Surprisingly, although nominally similar to the JASCO system the result was effectively quantitative.

Table 4. % recovery of spiked chlorpyrifos from soil using ISCO SFE 2300 system (260D syringe pump for CO₂ and 100D syringe pump for methanol) at 40°C and 150 kg cm⁻² and various methanol percentage.

Condition	% chlorpyrifos extracted
40/150/2	104 ± 4.6 (n = 3)
40/150/5	97 ± 4.5 (n = 3)
40/150/10	104 ± 6.7 (n = 3)
40/150/12.5	99 ± 5.0 (n = 3)

6.5 PURGING EXPERIMENT

To investigate whether losses of analytes occur during SFE because they are purged out of the collection solvent by the high flow rate of gaseous carbon dioxide or because they failed to partition into the collection solvent, the effect of solvent purging was investigated. The effect of collection solvent height (or depth) on the trapping efficiencies of the chlorpyrifos was also tested as the solvent height can possibly affect the collection efficiencies because the analytes need a certain amount of time after the depressurisation step to diffuse into the collection solvent. A greater solvent height should permit longer solvent-analyte contact and thus increase the chances that the analyte will be trapped in the collection solvent.

Chlorpyrifos standard (1000 µg) was spiked into a collection vial containing methanol (3 ml) and modified carbon dioxide (10% methanol)

was allowed to bubble through the solution for 30 minutes at 40/250/10 but without any sample in the extraction vessel at various flow rates (Table 5). At 0.60 ml min^{-1} there is about 4% loss and at 1 ml min^{-1} (measured gaseous CO_2 flow approximately 430 ml min^{-1}) there is about 7% loss. At an even higher flow rate of 1.7 ml min^{-1} there is about 10% loss of the chlorpyrifos. This demonstrates that excessively high flow rates may result in lower overall recoveries of analyte because of purging losses. The same observation was noted by Langenfeld and co-workers (68).

The effect of different collection solvent height (depth) was investigated by using three vials of different dimension to give a solvent height of 7 mm, 15 mm and 50 mm (with 3 ml collection solvent in each). The same losses was observed from a collection solvent depth of 7 mm compared to a depth of 15 mm and 50 mm (at 1 ml min^{-1} flow) (Table 5). This indicates that the mass transfer of the analyte from the gaseous carbon dioxide into the collection solvent is very fast, and the collection solvent height is not as important as might be expected. This shows that solvent depth of between 7 - 50 mm could be used without any significant loss of the chlorpyrifos.

Table 5. Effect of methanol - modified carbon dioxide flow rate on recovery of chlorpyrifos (1000 μg) from spiked collection solvent (methanol). Value in bracket is the resulting solvent height for 3 ml methanol. Triplicate purging experiments except for CO_2 flow of 0.60 ml min^{-1} (four).

- a. Solvent height in a 75 mm x 24 mm i.d. collection vial.
- b. Solvent height in a 40 mm x 10 mm i.d. collection vial.
- c. Solvent height in a 104 mm x 11 mm i.d. collection vial (JASCO vial).

CO_2 flow rate (ml/min)	% Recovery	Average % recovery \pm RSD
	94.1	
	99.4	
0.60 (7 mm) ^a	95.6	95.7 \pm 2.7
	93.7	
	94.1	
1.0 (7 mm) ^a	93.2	92.6 \pm 1.2
	90.6	
	94.2	
1.0 (15 mm) ^b	95.8	94.4 \pm 1.2
	93.4	
	94.4	
1.0 (50 mm) ^c	91.2	93.1 \pm 1.8
	93.6	
	92.3	
1.7 (7 mm) ^a	84.1	89.6 \pm 5.3
	92.3	

A flow rate of 1 ml min^{-1} was chosen even though there were slightly more losses at this flow rate compared to one at 0.60 ml min^{-1} because the length of restrictor that need to be used to obtain a pressure of 250 kg cm^{-2} with a $40 \text{ }\mu\text{m}$ i.d. is rather long (about 55 cm). For subsequent extraction, a CO_2 flow rate of 1 ml min^{-1} and methanol flow rate of 0.1 ml min^{-1} was used with a collection solvent height of 15 mm.

6.6 COLLECTION SOLVENT TEMPERATURE

Linear fused silica restrictors used to control extraction pressure and direct the flow of SF and extract into the collection solvent can often plugged especially when the sample matrices contain high concentrations of water or extractable matrix components (68). Water plugging occurs because the expanding SF rapidly cools the restrictor tip (and the collection solvent) below the freezing point of water (see Figure 12 later), thus causing the small amounts of water that are extracted to form an ice plug at the restrictor tip. It has been observed that samples which contain more than ca. 1% water require heating to prevent ice formation from plugging the restrictor (68). The same observation was noted in this laboratory when extracting soil samples that contain more than 1% water. The water content of the soil sample was determined by heating the sample overnight in an oven at 100°C and the weight loss is taken to be equivalent to the water weight.

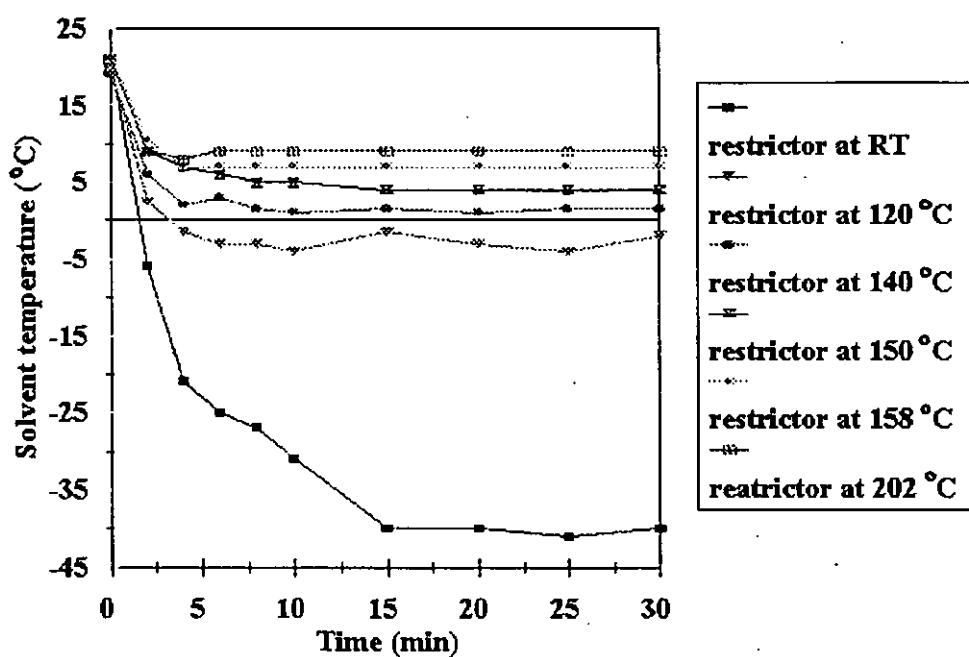
Since the depressurisation occurs at the tip and inside the restrictor (68, 69), the reduction of the extraction fluid density within the restrictor as

the pressure drops can cause a decrease in the solubility of the analyte. The decrease in analyte solubility, combined with the Joule-Thomson cooling effect of the expanding extraction fluid at the restrictor exit, produces a subcritical solvent which may lead to precipitation of the analyte and, ultimately plugging the inside of the restrictor. In order to prevent such plugging the restrictor may be heated to counteract the Joule-Thomson cooling and increase solubility of analyte.

The cooling effect on the collection solvent that results from the depressurisation of the expanding carbon dioxide with and without restrictor heating was recorded for 30 minutes by taking the temperature of the solvent at certain time intervals (Figure 12). Without any heating the temperature of the collection solvent (methanol) rapidly drops to -21°C , then slowly approaches -40°C . The collection solvent becomes so cold that restrictor plugging was observed. Warming the collection solvent in a beaker of warm water did not successfully overcome restrictor plugging. Intermittent flow of CO_2 was still observed. In order to overcome restrictor plugging, a similar linear restrictor design employed by Burford and co-workers (70), was used at a temperature of 120°C to heat the restrictor (Figure 2a and 2b in Chapter 4). This design used an aluminium heating block to heat the restrictor. When the restrictor was heated at 120°C , the flow rate has to be reduced from 1 ml min^{-1} to $0.70\text{ ml min}^{-1}\text{ CO}_2$ to maintain the extraction pressure at 250 kg cm^{-2} ($40\text{ }\mu\text{m i.d.}$, 35 cm long). The collection solvent was cooled for 5 minutes (resulting solvent temperature was about -2°C) before beginning all the extractions. When the restrictor was heated at 120°C the loss of chlorpyrifos was not

significant as demonstrated by the purging experiment (96.6% chlorpyrifos recovery with 4.6% RSD) and the percentage recovery of chlorpyrifos from the German standard soil investigated at three different spiking levels (>90%) (see Figure 16 later).

Figure 12. SFE collection solvent temperature with and without restrictor heating during SFE.



6.7 COLLECTION SOLVENT

As the collection efficiency of chlorpyrifos using methanol was not quantitative, acetone was then tried. It has been shown that the collection efficiency of the analyte depends on the solubility of the analyte in the collection solvent (68). The better trapping of chlorpyrifos in acetone

compared to trapping in methanol is demonstrated with the extraction of spiked chlorpyrifos from sand (Figure 13). Sand was chosen as it has been proposed as a non-sorptive matrix (71) and the trapping efficiency of the solvent could be evaluated. With a 10 minutes extraction and collection in acetone the trapping of chlorpyrifos was quantitative (95%) whereas only about 80% chlorpyrifos was trapped in methanol. This can be explained if we look at the solubility of carbon dioxide in acetone and methanol and also the solubility of chlorpyrifos in acetone and methanol. The solubility of chlorpyrifos at 25°C in methanol is 0.43 mg/mg and the solubility in acetone is 6.5 mg/mg (11). Chlorpyrifos is about 15x more soluble in acetone than in methanol. Carbon dioxide is twice as soluble in acetone than in methanol (Table 6) (72). The combined effect of better solubility of chlorpyrifos in acetone and better solubility of carbon dioxide in acetone could account for the better trapping efficiency of the chlorpyrifos in acetone as the SC CO₂ and entrained analyte (chlorpyrifos) is depressurised into the collection solvent, acetone. Also very small bubbles were observed at the restrictor outlet when acetone was used as the collection solvent and the restrictor heated at 120°C. The lower CO₂ flow rate (0.70 ml min⁻¹ compared to 1 ml min⁻¹ with methanol as collection solvent) and the increased viscosity of the cooled solvent possibly reduces the size of the bubbles which increases the solute-solvent contact time (73). Thus in subsequent extractions, acetone was used as the collection solvent. The extraction conditions used to carry out the SFE are extraction temperature of 40°C, extraction pressure of 250 kg cm⁻², 10% methanol as modifier, restrictor heating at 120°C and 3 ml acetone (cooled for 5 minutes before beginning extraction) as collection solvent.

Figure 13. Trapping of spiked chlorpyrifos from sand (extraction at 40/250/10) with collection in methanol (3 ml) and acetone (3 ml).

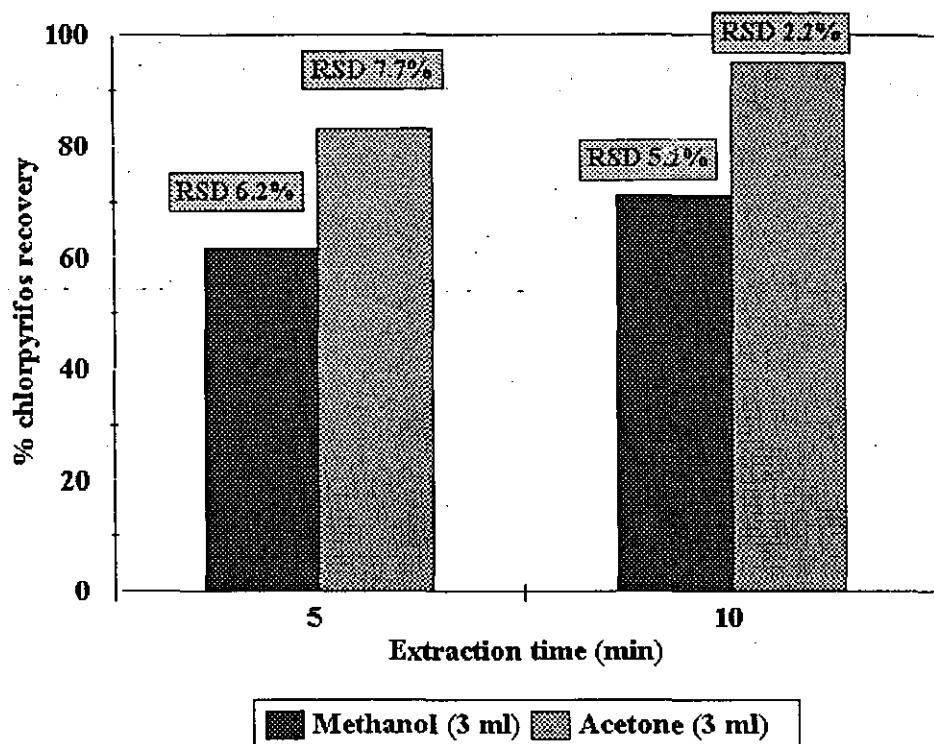


Table 6. Solubility of carbon dioxide in acetone and methanol at various solvent temperatures (72).

Solvent	cm ³ CO ₂ dissolved per cm ³ of solvent							
	18°C	20°C	22°C	24°C	26°C	28°C	30°C	32°C
Acetone	-	6.98	6.76	6.55	6.22	5.88	5.49	5.08
Methanol	3.63	3.57	3.51	3.44	3.37	3.28	3.19	3.09

compared to trapping in methanol is demonstrated with the extraction of spiked chlorpyrifos from sand (Figure 13). Sand was chosen as it has been proposed as a non-sorptive matrix (71) and the trapping efficiency of the solvent could be evaluated. With a 10 minutes extraction and collection in acetone the trapping of chlorpyrifos was quantitative (95%) whereas only about 80% chlorpyrifos was trapped in methanol. This can be explained if we look at the solubility of carbon dioxide in acetone and methanol and also the solubility of chlorpyrifos in acetone and methanol. The solubility of chlorpyrifos at 25°C in methanol is 0.43 mg/mg and the solubility in acetone is 6.5 mg/mg (11). Chlorpyrifos is about 15x more soluble in acetone than in methanol. Carbon dioxide is twice as soluble in acetone than in methanol (Table 6) (72). The combined effect of better solubility of chlorpyrifos in acetone and better solubility of carbon dioxide in acetone could account for the better trapping efficiency of the chlorpyrifos in acetone as the SC CO₂ and entrained analyte (chlorpyrifos) is depressurised into the collection solvent, acetone. Also very small bubbles were observed at the restrictor outlet when acetone was used as the collection solvent and the restrictor heated at 120°C. The lower CO₂ flow rate (0.70 ml min⁻¹ compared to 1 ml min⁻¹ with methanol as collection solvent) and the increased viscosity of the cooled solvent possibly reduces the size of the bubbles which increases the solute-solvent contact time (73). Thus in subsequent extractions, acetone was used as the collection solvent. The extraction conditions used to carry out the SFE are extraction temperature of 40°C, extraction pressure of 250 kg cm⁻², 10% methanol as modifier, restrictor heating at 120°C and 3 ml acetone (cooled for 5 minutes before beginning extraction) as collection solvent.

Figure 14. Calibration graph of chlorpyrifos concentration versus area ratio of chlorpyrifos:n-docosane (for 0.01% spiking level).

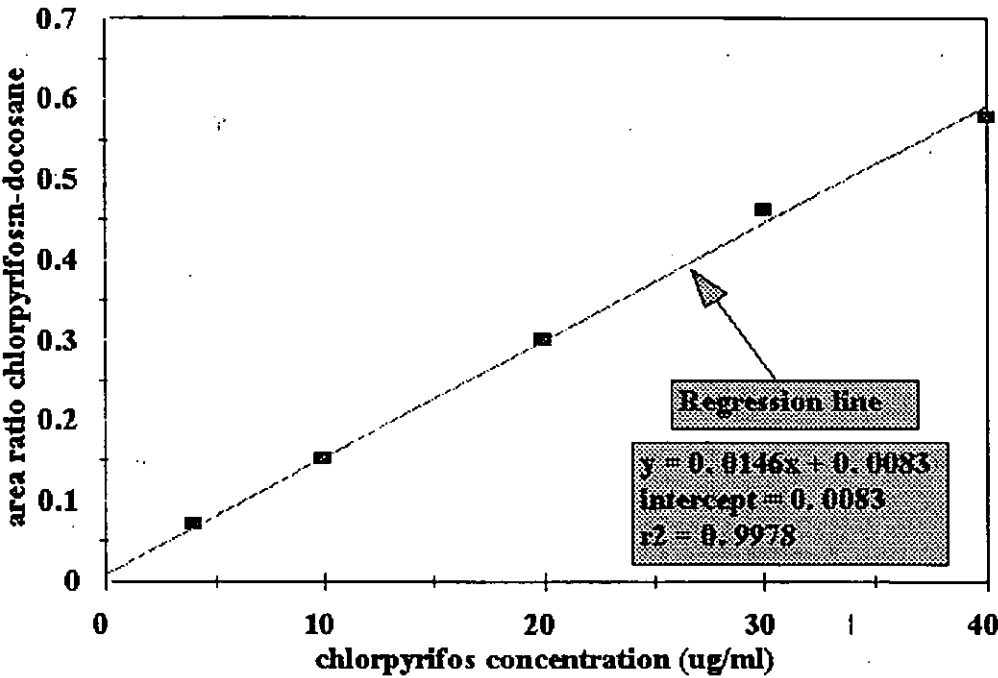


Figure 15. Calibration graph of chlorpyrifos concentration versus area ratio of chlorpyrifos:n-docosane (for 0.001% spiking level).

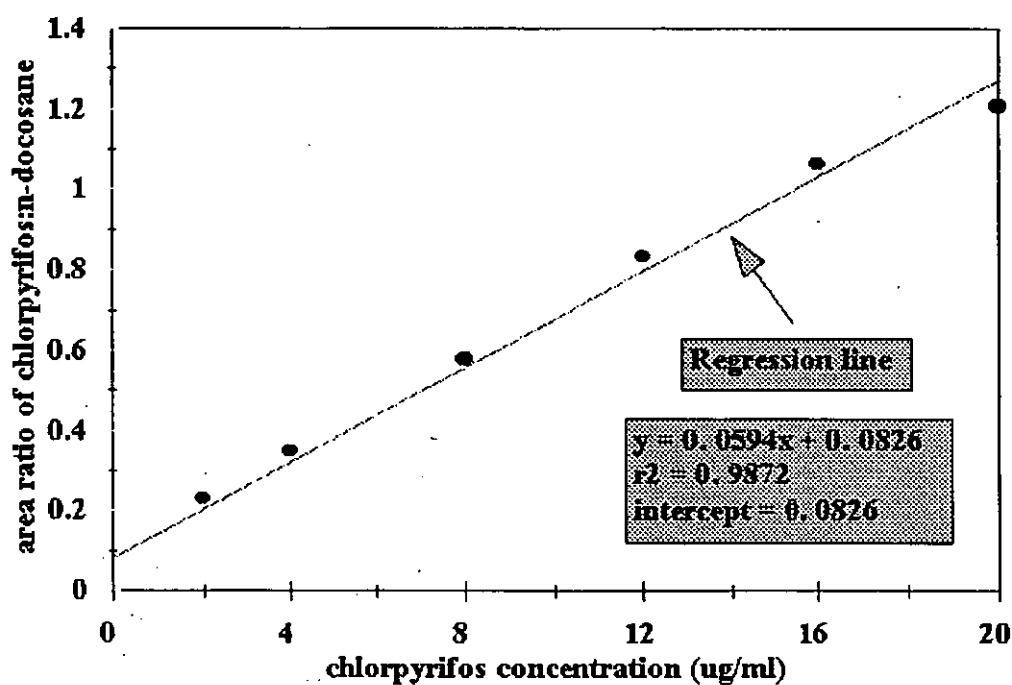
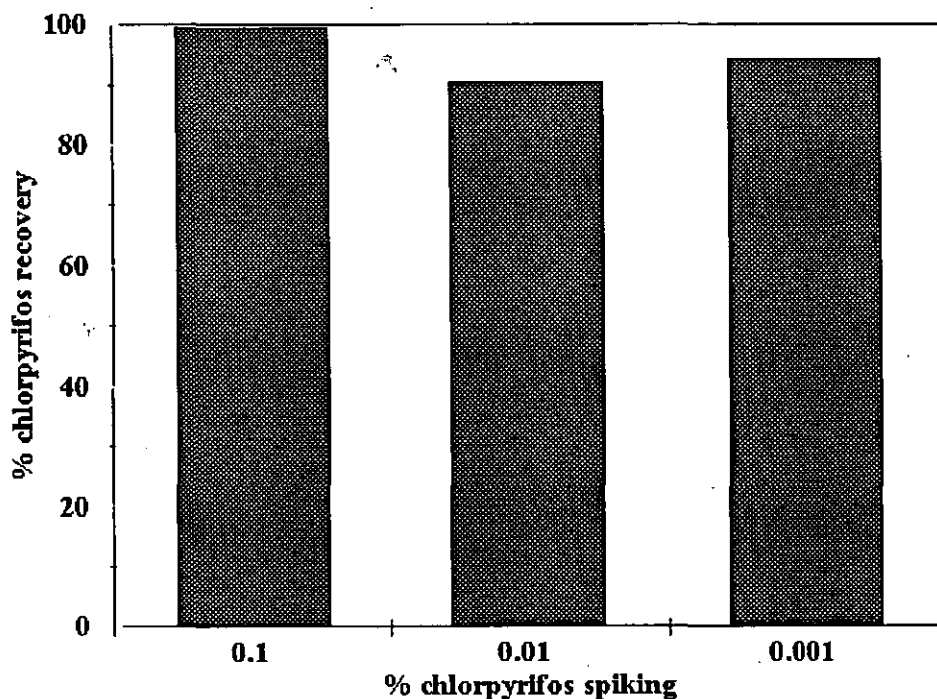


Figure 16. % recovery of spiked chlorpyrifos from a German standard soil at different spiking levels at 40/250/10 with trapping in 3 ml acetone and restrictor heating at 120°C.



6.9 EXTRACTION OF CHLORPYRIFOS FROM OTHER TYPES OF SOILS.

Once it was demonstrated that the trapping procedure was effective for trapping spiked chlorpyrifos at different levels from the German standard soil, the effect of different soil matrices on the percentage recovery of different spiking levels of chlorpyrifos was investigated. The characteristics of the soils used in the work as determined in the laboratory are given in Table 7. The % water in the different soils was determined by drying the soil overnight in the oven at 100°C. The weight loss is taken to

be equivalent to the water content. The organic carbon content of the soils was determined according to the Walkley and Black method (74). Peat has the highest carbon and water content. The others have less than 5% carbon content. The soil characteristics as determined by Dow Elanco are given in Table 8 (75).

Table 7. % water, pH_w, % total carbon, % organic carbon, % total nitrogen as determined in the laboratory for the type of soils used.

a, b - single determination. c, d, e, f - duplicate determinations.

Soil sample	% water ^a	pH _w ^b	% organic carbon ^c	% total carbon ^d	% total nitrogen ^e	% total hydrogen ^f
German std.	6.70	7.33	1.93	2.49	0.18	0.92
Clay	4.94	7.50	3.37	3.68	0.25	0.65
Speyer 2.1	0.28	5.97	0.60	0.84	0.02	0.01
Speyer 2.2	0.97	5.35	2.48	2.31	0.15	0.17
Speyer 2.3	0.86	6.24	1.04	1.22	0.06	0.03
Dark peat	61.1	3.58	16.5	33.77	0.62	6.18

Table 8. Soil properties as provided by Dow-Elanco Ltd. (75).

a. Values are in milliequivalents per 100 grams.

b. pH in 0.01M CaCl₂.

ND:-Not determined.

Soil property	German std.	Speyer 2.1	Speyer 2.2	Speyer 2.3
% Organic carbon	3.4	0.62	2.32	1.22
cation exchange ^a	9	5.0	10.9	10.2
pH ^b	6.4	5.9	5.6	6.4
% sand	86	88.4	81.2	60.9
% silt	8	9.8	13.4	29.6
% clay	6	1.9	5.5	9.5
Soil type	sand	sand	loamy sand	sandy loam
% Total nitrogen	ND	0.08	0.23	0.15
pore volume (g/1000ml)	ND	1 410	1 233	1289

The results of the effect of different soil matrices on the recovery of chlorpyrifos at three different spiking levels are given in Figure 17. There are no effect of soil matrices on the recovery of spiked chlorpyrifos. A nearly quantitative recovery of the chlorpyrifos was obtained from all the soils. However, for the peat sample, when a dry sample (dried at room temperature) was used the recovery dropped to 75% (0.001% spiking level). As peat has a high organic carbon content the spiked chlorpyrifos may have interacted more strongly with adsorptive sites in the matrix. With wet peat, the water may have acted as a modifier and was able to

displace the chlorpyrifos from the adsorption sites. Water has been known to increase the recovery of polar analytes (47, 76). It can swell the soil matrix (77) and expose small internal cavities, allowing the SF better access to adsorbed analytes.

As the spiked chlorpyrifos was applied to the soil and extracted immediately after spiking, the chlorpyrifos may have just covered the outer surfaces of the soil matrix (on labile sites) as opposed to native chlorpyrifos in soil where a significant fraction of the analyte may have migrated to resistant sites (60, 78, 79) in the matrix after a period of time and thus not enabling the extracting fluid access to the analyte. More vigorous condition will be needed to extract the analyte. The effect of ageing of chlorpyrifos on clay for a different amount of time before extraction was examined (Table 9). From the table, after ageing for 49 days the recovery has dropped to about 80%. This shows that if the sample is left to age for a longer period of time more and more of the spiked analyte fraction will migrate to remote or stronger binding sites. However, from a practical point of view, equilibration periods of days or months are unacceptable as an unknown percentage of the analyte can be lost due to evaporation and/or degradation (49). Also it is experimentally impossible to simulate or reproduce the environmental conditions that occur during the deposition of pollutants in real-world samples.

Figure 17. % Recovery of spiked chlorpyrifos from different types of soils at three different spiking levels. Extraction at 40/250/10. Triplicate extractions except for Speyer 2.2 at 0.001% (duplicate) and Speyer 2.3 at 0.001% (single).

- a. German standard

b. Speyer 2.1

c. Speyer 2.2

d. Speyer 2.3

e. Clay

f. Peat (wet)

g. Peat (dry)

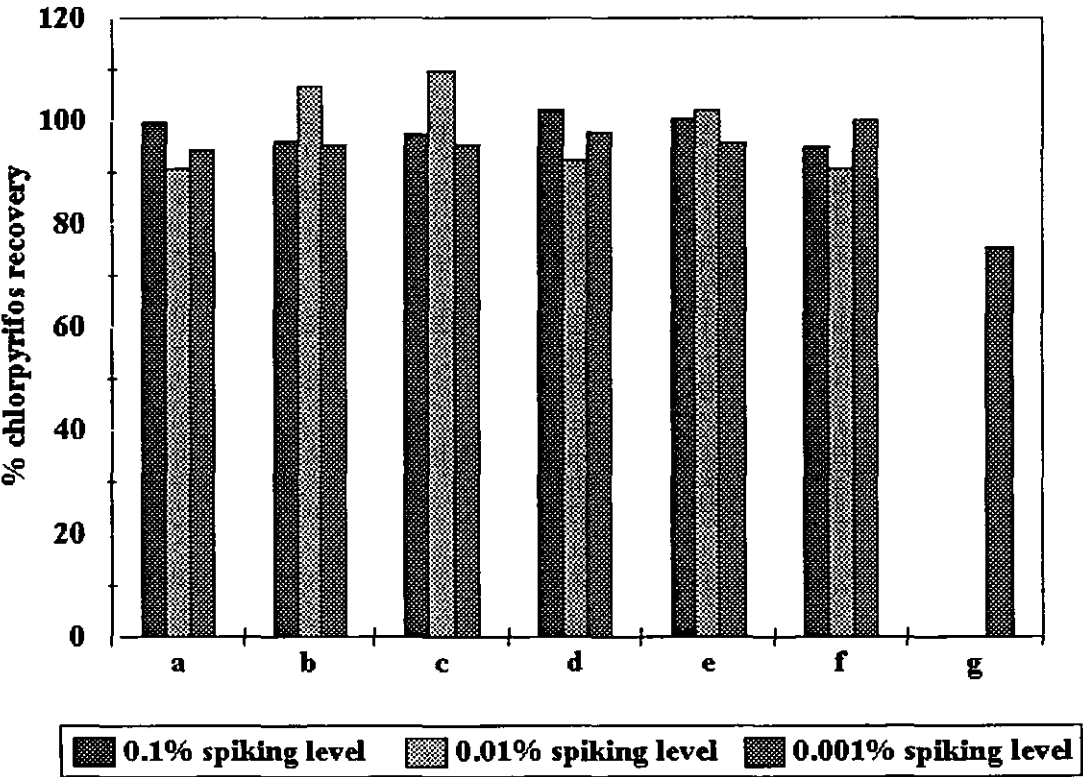


Table 9. % recovery of spiked chlorpyrifos from fresh and aged clay samples at 0.1% level. Triplicate extractions at 40/250/10 except for clay aged for 30 days (single extraction) and clay aged for 49 days (duplicate extractions).

Spiking level 0.1%			
Sample	Ageing period	% recovery	Average % recovery \pm RSD
Clay	None	100.6	
	(Fresh)	100.6	100.4 ± 0.3
		100.1	
Clay	17-19 hr.	100.8	
		102.3	103.3 ± 3.1
		106.8	
Clay	30 days	88.6	88.6
		-	
Clay	49 days	81.8	
		81.4	81.6 ± 0.3

6.10 COMPARISON OF SOXHLET EXTRACTION AND SUPERCRITICAL FLUID EXTRACTION IN THE EXTRACTION OF SPIKED CHLORPYRIFOS FROM SOILS.

Three soil samples representing soil with high carbon content (peat) and low carbon content (clay and German standard soil) were chosen to carry out a comparison between Soxhlet extraction (according to EPA Method 3540) (19) and SFE at the 0.1% spiking level (Table 10). The same spiking procedure was used for all the extractions (SFE and Soxhlet). The percentage recovery between the two methods were comparable but the SFE method only took 30 minutes whereas the Soxhlet extraction took 16 hours to perform. For the German standard soil, there was a significant difference in the mean of the chlorpyrifos recoveries by the two methods. The % recovery of chlorpyrifos was significantly higher by the SFE method. However, there was no significant difference in the precision of the two methods for the German standard soil. The RSDs of both methods for all the three soils was also comparable. However, the SFE method used less solvent and this is important as there is an emphasis on extraction methods that use smaller volume or less hazardous solvents.

Table 10. Comparison of percentage recovery of spiked chlorpyrifos from three types of soil by SFE (at 40/250/10, 3 ml acetone as collection solvent, restrictor heated at 120°C, extraction time 30 minutes, CO₂ flow of 0.70 ml min⁻¹) and Soxhlet extraction (according to EPA Method 3540) (19).

0.1% spiking level

	Soxhlet		SFE	
Sample	% Recovery	Av. % R. ± RSD	% Recovery	Av. % R. ± RSD
Standard	81.4		96.4	
	83.6	82.5 ± 1.9	104.2	99.6 ± 4.1
			98.2	
Clay	94.0		100.6	
	92.7	93.4 ± 1.8	100.6	100.4 ± 0.3
			100.1	
Peat(Wet)	92.6		92.8	
	90.2	91.4 ± 1.9	97.8	95 ± 2.7
			94.3	

6.11 MODEL FOR SUPERCRITICAL FLUID EXTRACTION

For a further insight into the extraction mechanism, a series of timed extractions of freshly spiked and aged clay soil (57 days ageing) at 0.1% level were carried out. Although no data was obtained on the solubility of chlorpyrifos in SC CO₂ and modified SC CO₂, the chlorpyrifos was present in minor amounts (1000 µg) as is the case for all environmental samples and it is well below the solubility limit. Using the conditions 40/250/10

timed extractions of freshly spiked and aged clay at 5 minutes intervals were taken to obtain the extraction profile (Figure 18). A single extraction was carried out for each time interval and a new sample was used for each of the time interval. The chlorpyrifos from the freshly spiked clay sample was quantitatively extracted (90%) after 5 minutes of extraction whereas only about 40% chlorpyrifos was extracted from the aged clay sample within the same time. It would appear that the chlorpyrifos is only distributed at or near the surface of the soil in the freshly spiked soil sample. At 10 minutes extraction, only 80% chlorpyrifos was extracted from the aged clay sample. Effectively there was no change in the chlorpyrifos recovery from the aged clay soil after 10 minutes. It would appear that some of the chlorpyrifos have migrated to more resistant sites but most are distributed at or near the surface. The UV extraction profile for the freshly spiked and aged clay sample were recorded (Figure 19). The spiked chlorpyrifos seem to be extracted faster from the unaged clay sample compared to the aged clay sample.

Figure 18. Extraction profile of freshly spiked clay soil and aged clay soil at 40/250/10 and a CO₂ flow rate of 0.70 ml min⁻¹ and methanol flow rate of 0.07 ml min⁻¹.

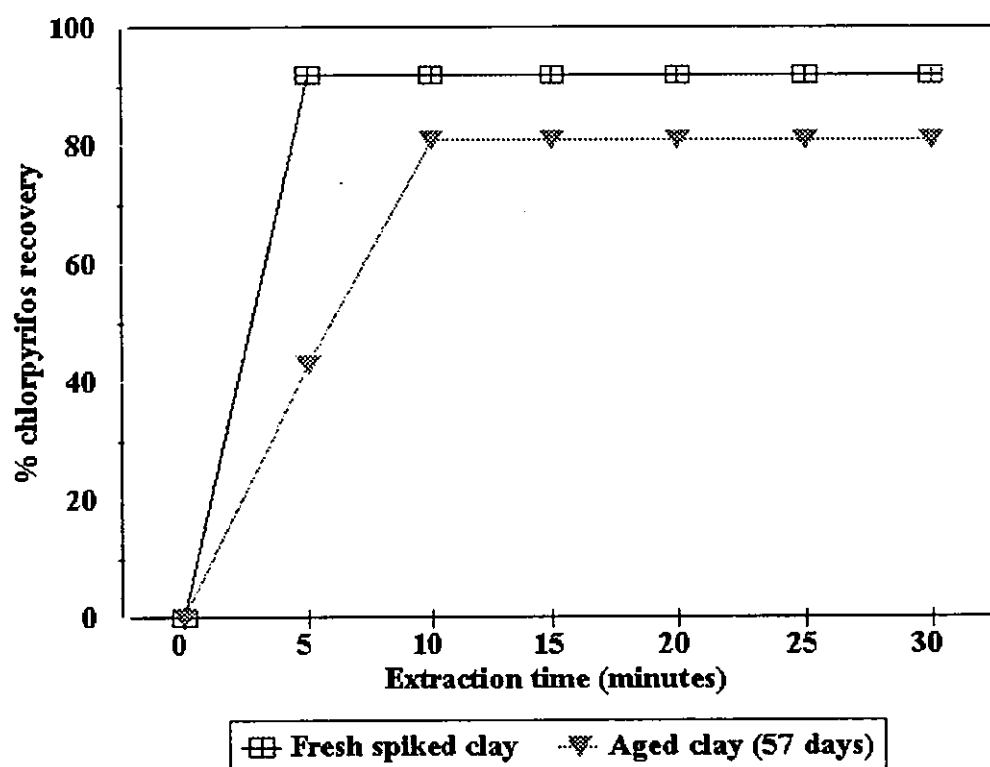
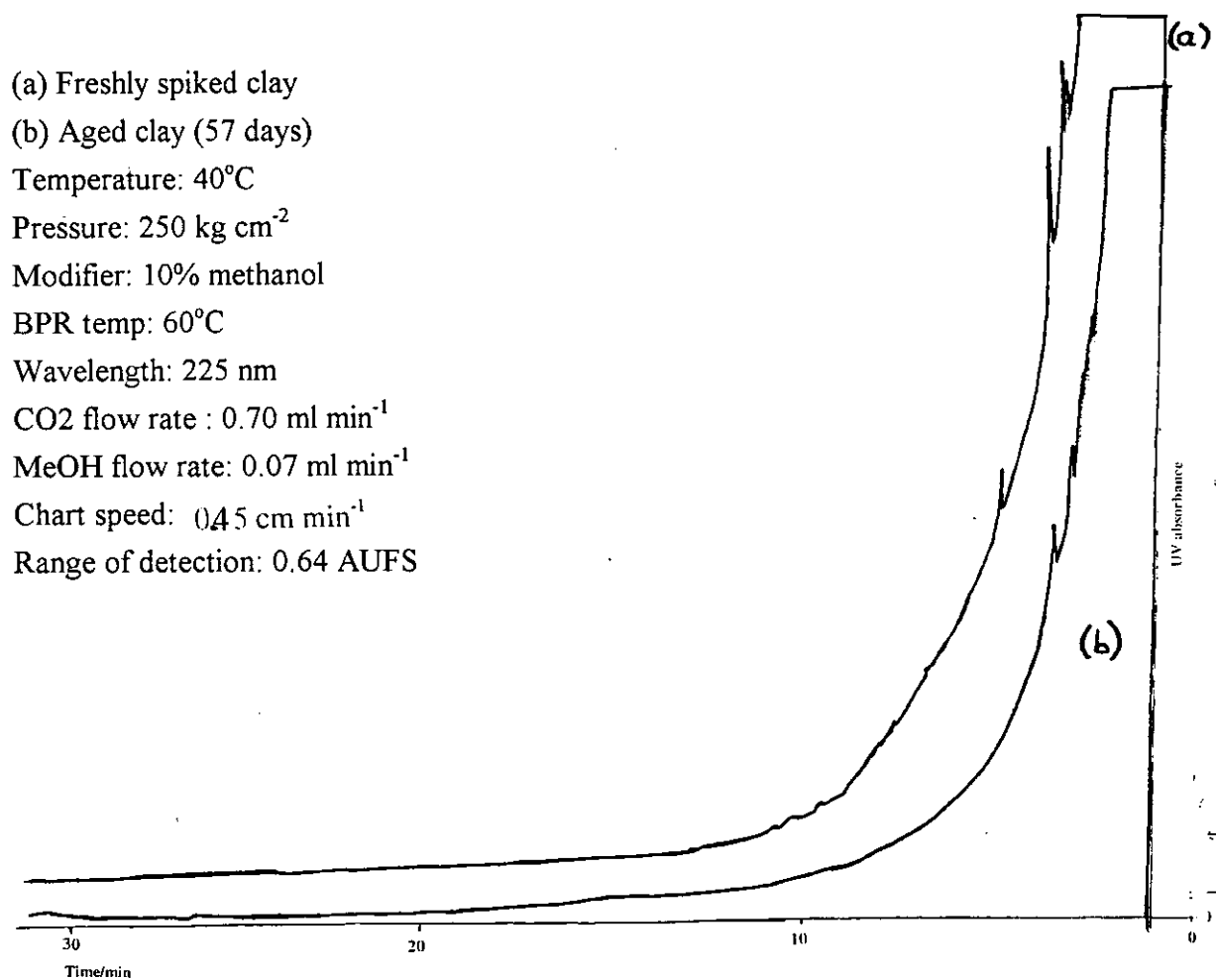


Figure 19. UV extraction profile of freshly spiked and aged clay soil (57 days) as a function of time.



6.12 REPRODUCIBILITY OF SUPERCRITICAL FLUID EXTRACTIONS

In any analytical method, the reproducibility of the proposed method need to be examined. In this work the reproducibility of SFE for the Dursban extracts and soil extracts were examined. Triplicate SFE were carried out for the Dursban and soil samples, unless otherwise stated. All the extracts were sonicated for about two minutes (after the collection step) before injecting into the GC system. Triplicate GC injections were carried out for each extract. It has been demonstrated that the reproducibility of GC injections are better after the soluble carbon dioxide is driven away from the extract (80).

The reproducibility of SFE of the effect of methanol concentration on the percentage recovery of chlorpyrifos from unground Dursban at 40°C and 150 kg cm⁻² and a total flow rate of 2 ml min⁻¹ (see Table 1 before) and the percentage recovery of chlorpyrifos from ground Dursban at 40°C and 150 kg cm⁻² at a total flow rate of 1 ml min⁻¹ were measured (Table 11). The RSDs measurements on the unground Dursban granules were greater (from 6-10%) compared to the RSDs from the ground granules (about 4%). Thus, grinding the granules produces a more homogeneous sample and this was reflected in the smaller RSDs (Table 11).

Table 11. Reproducibility of SFE data.

Effect of methanol on the percentage recovery of chlorpyrifos from ground Dursban granules at a total flow rate of 1 ml min^{-1} with collection in flask cooled by liquid nitrogen.

T/P/M is shorthand for extraction temperature, extraction pressure and % modifier (methanol), respectively.

T/P/M	Average % recovery \pm RSD
40/150/5	58 ± 4 (n = 3)
40/150/10	91 ± 3 (n = 3)
40/150/15	72 ± 4 (n = 3)

The reproducibility of SFE for the recovery of spiked chlorpyrifos from a German standard soil was determined (Table 12). The RSDs measurements from an unsieved soil was very large but when sieved the RSDs was much smaller (7% and less). This can be attributed to the homogeneous sample produced on sieving. The reproducibility of SFE of spiked chlorpyrifos at 40/250/10 at three different spiking levels with collection in 3 ml acetone and restrictor heating at 120°C were measured (Tables 13, 14 and 15). Also shown are the extraction data of chlorpyrifos from Dursban and spiked chlorpyrifos from sand at 0.1% spiking level (Table 13) using the same condition as for the soils. A quantitative recovery of chlorpyrifos were also obtained from the Dursban and sand. The RSDs of the recovery of chlorpyrifos from these matrices were between 2-8% except for Speyer 2.3 at 0.01% spiking level (12% RSDs).

Table 12. Reproducibility of SFE.

Percentage recovery of chlorpyrifos from spiked (0.1% level) German standard soil samples. Extraction carried out immediately after spiking the chlorpyrifos. Extract collected in flask cooled by liquid nitrogen.

- a. Unsieved soil.
- b. Sieved German standard soil through a no. 40 mesh
- c. Sample solvent allowed to evaporate for 2 hours at RT after chlorpyrifos spiking before extraction.

T/P/M	% Recovery \pm RSD
40/150/10	57 ± 30 (n = 3) ^a
40/150/10	57 ± 7 (n = 3) ^b
40/250/10	74 ± 5 (n = 3) ^b
40/300/10	58 ± 2 (n = 2) ^b
40/300/10	44 ± 5 (n = 2) ^c

Table 13. Reproducibility of SFE of spiked chlorpyrifos (0.1% level) from different types of soils. Triplicate extractions at 40/250/10 on each type of soil with trapping in 3 ml acetone and restrictor heating at 120°C.

Spiking level 0.1%		
Sample	% Recovery	Average % recovery \pm RSD
Standard	96.4	
	98.2	99.6 \pm 4.1
	104.2	
Speyer 2.1	94.2	
	97.8	96.1 \pm 1.8
	96.2	
Speyer 2.2	99.6	
	96.6	97.2 \pm 2.2
	95.4	
Speyer 2.3	100.5	
	98.8	102.0 \pm 4.2
	106.8	
Clay	100.6	
	100.6	100.4 \pm 0.3
	100.1	
Peat (wet)	92.8	
	97.8	95 \pm 2.7
	94.3	
Sand (10 min ext.)	104.8	
	103.2	101.6 \pm 4.2
	96.8	
Dursban	89.5	
	102.2	95.2 \pm 6.8
	93.8	

Table 14. Reproducibility of SFE of spiked chlorpyrifos (0.01% level) from different types of soils. Triplicate extractions at 40/250/10 with trapping in 3 ml acetone and restrictor heating at 120°C for each type of soil except for Speyer 2.1 (four).

Spiking level 0.01%		
Sample	% Recovery	Average % recovery \pm RSD
German standard	94.2	
	88.1	90.6 \pm 3.5
	89.6	
Speyer 2.1	94.8	
	109.9	
	109.4	106.7 \pm 7.5
	112.6	
Speyer 2.2	109.1	
	106.4	109.6 \pm 3.2
	113.3	
Speyer 2.3	105.2	
	84.2	92.2 \pm 12.3
	87.2	
Clay	109.6	
	98.3	102.1 \pm 6.4
	98.3	
Peat (wet)	104.8	
	99.4	90.6 \pm 3.5
	101.9	

Table 15. Reproducibility of SFE of spiked chlorpyrifos (0.001% level) from different types of soils. Triplicate extractions at 40/250/10 with trapping in 3 ml acetone and restrictor heating at 120°C for each type of soil except for Speyer 2.2 (duplicate extractions) and Speyer 2.3 (single extraction).

Spiking level 0.001%		
Sample	% Recovery	Average % recovery \pm RSD
German standard	90.4	
	100.1	94.4 \pm 4.1
	92.8	
Speyer 2.1	97.5	
	97.7	95.4 \pm 3.9
	91.1	
Speyer 2.2	98.4	
	92.0	95.2 \pm 4.8
Speyer 2.3	97.6	97.6
Clay	97.0	
	96.3	95.5 \pm 2.1
	100.1	
Peat (wet)	93.5	
	98.9	100.0 \pm 7.1
	107.6	
Peat (dry)	79.9	
	76.7	75.3 \pm 7.3
	69.2	

6.13 CONCLUSIONS

Quantitative recovery of chlorpyrifos (90%) was obtained from ground Dursban granules at an extraction temperature of 40°C, extraction pressure of 150 kg cm⁻², 10% methanol, a CO₂ flow rate of 1 ml min⁻¹ and with trapping in a flask cooled by liquid nitrogen.

In the SFE of chlorpyrifos from soil, trapping the analyte after the extraction step seems to be the main problem. Trapping in a flask cooled by liquid nitrogen only trapped about 70% of the extracted chlorpyrifos. Solvent extraction on the soil residue after SFE showed that the chlorpyrifos extraction was completed. Trapping in methanol also trapped about 70% of the chlorpyrifos but trapping in acetone quantitatively trapped the chlorpyrifos (>90%). This was attributed to the better solubility of the extracted chlorpyrifos and expanding carbon dioxide in acetone. Quantitative extraction of chlorpyrifos from soil was obtained at a temperature of 40°C, pressure of 250 kg cm⁻², 10% methanol as modifier with collection of extract in acetone and restrictor heating at 120°C.

Since the trapping depends on the analyte properties, different analytes may need different conditions to give the optimum extraction and trapping. A knowledge of the solubility of the analyte and the solubility of the supercritical fluid in the trapping solvent may help one to choose an initial trapping solvent.

Extraction of spiked chlorpyrifos from soil using SFE was comparable to Soxhlet extraction. However, SFE was much faster than Soxhlet extraction and it uses less amounts of non-hazardous solvent.

Heating the linear fused silica restrictor at 120°C with a cartridge heater overcomes the problem of restrictor plugging for samples which contain more than 1% water. Quantitative trapping of the chlorpyrifos was obtained from all the soil matrices at the three spiking level even when the restrictor was heated at this temperature.

The reproducibility of GC injections and SFE of the Dursban and soils respectively were better than 3% RSD and 7% RSD respectively. Grinding the Dursban granules and sieving the soil produced a more homogeneous sample and it reduced the RSDs of the recovery of chlorpyrifos from Dursban and soils.

Spiked chlorpyrifos gave quantitative recovery regardless of the soil matrix. Spiking sample onto a heterogeneous matrix such as soil may not be a valid way of quantitating real-world samples as the distribution of analyte/s in spiked samples may not represent the actual distribution of analyte in real world samples. In real world samples the analyte/s are more strongly retained. Longer equilibration times allow spiked analyte/s to migrate to remote or stronger binding sites in the matrix so that the spiked analyte/s become more and more resistant to extraction. However, the use of spiked samples is important to test the efficiency of a given method and a good way of evaluating trapping/collection efficiency. There is therefore

a need to match standards and samples and it is here that matrix reference materials (MRMs) can be used to determine the efficiency of a procedure. However, as mentioned earlier MRMs are not so readily available as certified reference materials (CRMs). As for the sample under study no CRMs was available and this justifies the use of spiked samples.

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CHAPTER 7

CONCLUSIONS

Off-line SFE has been used to quantitatively extract eugenol from pimento berries and chlorpyrifos from soils using methanol modified- CO_2 . The extraction efficiency of chlorpyrifos from soils by SFE is comparable to Soxhlet extraction, but the SFE method is faster and use less solvent.

The soil matrices studied seemed to have little influence on the recovery of spiked chlorpyrifos when CO_2 -modified with methanol was used. However, the spiked chlorpyrifos recovery was less after the soil samples has been aged for 39-57 days. Some of the spiked chlorpyrifos have migrated to resistant sites in the soil but majority are distributed at or near the surface.

The study demonstrated that for SFE to be successful, the analytes must be efficiently collected from the SF after the depressurisation step, as most often low recoveries was attributed to inefficient extraction of the analyte from the matrix. Collection efficiency can be evaluated by extracting analyte from an unretentive matrix, such as sand. Once the collection efficiency has been established, then study of spike samples onto the matrix of interest can be conducted to obtain the optimised extraction

conditions. The proposed extraction method can then be evaluated by extracting certified reference materials (CRMs).

LIST OF PRESENTATIONS

1. Smith, R. M. and Wan Ibrahim, W. A., **Supercritical Fluid Extraction of Pimento Berries** presented at the 4th International Symposium on Supercritical Fluid Chromatography and Extraction, Cincinnati, Ohio, USA, May 20 - 22, 1992.
2. Smith, R. M. and Wan Ibrahim, W. A., **The Reproducibility and Efficiency of Extraction of Pesticides from Environmental Samples by Supercritical Fluid Extraction** presented at the 16th International Symposium on Capillary Chromatography, Riva del Garda, Italy, May 24 - 26, 1993.
3. Wan Ibrahim, W. A. and Smith, R. M., **Supercritical Fluid Extraction as a Sample Preparation Method for Pesticides and Plant Analysis** presented at the 6th Symposium on Handling of Environmental and Biological Samples in Chromatography, Guildford, Surrey, July 19 - 21, 1993.
4. Smith, R. M. and Wan Ibrahim, W. A., **Supercritical Fluid Extraction of Chlorpyrifos from Soils** presented at the 5th International Symposium on Supercritical Fluid Chromatography and Extraction, Baltimore, Delaware, USA, January 11 - 14, 1994.

