Scott, John Charles

PART I. SUPERCRITICAL FLUID EXTRACTION OF ORGANICS FROM URBAN AEROSOL. PART II. N-NITROSOMOPHOLINE AND OTHER VOLATILE N-NITROSAMINES IN SNUFF TOBACCO. PART III. N-NITROSOPROLINE, AN INDICATOR FOR N-NITROSATION OF AMINES IN PROCESSED TOBACCO

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PART III. N-NITROSOPROLINE, AN INDICATOR FOR N-NITROSATION OF AMINES IN PROCESSED TOBACCO

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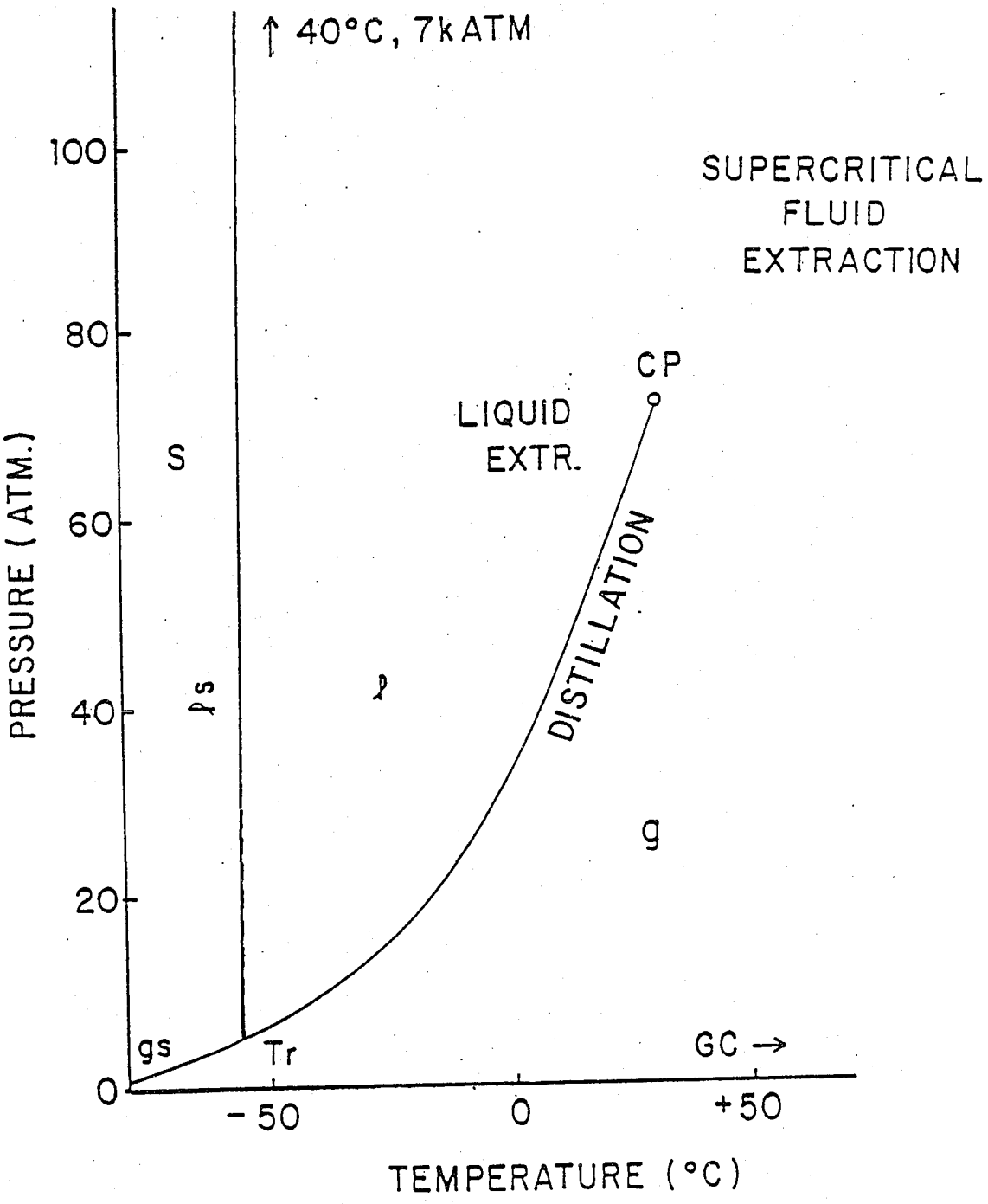
INTRODUCTION

It might be argued that there are not three states of matter (solid, liquid, and gas) but four, the fourth state being the supercritical state. A compound is in its supercritical state if it is in the region above its critical temperature (Tc) and pressure (Pc). Figure 1 shows the p-t phase diagram of carbon dioxide: area s represents the ranges of pressure and temperature in which carbon dioxide is a solid, l those for liquid carbon dioxide, and g those for gaseous carbon dioxide. Curves represent the points where two phases coexist. The carbon dioxide vapor pressure curve l-g starts at the triple point Tr (T^{tr} = -56.6°C, and p^{tr} = 5.12 atm (1)) and ends at the critical point CP (T^{cp} = 31.1°C, p^{cp} = 72.9 atm, density cp = 0.468 g/cm³ (1)). The melting pressure curve l-s starts at the triple point and rises steeply with increasing temperatures and pressures ($(dT/dP)^{ls}_{tr}$ = 45.9 atm/°C (1)).

In Figure 1 the approximate ranges are marked where separation processes can be effected. Distillation of gases from carbon dioxide will normally be possible near the vapor pressure curve l-g. For liquid carbon dioxide extraction pressures and temperatures defining the liquid range l must be reached. In gas chromatography (GC), carbon dioxide can be used as an ordinary mobile phase in the gaseous region g

Figure 1. The P-T phase diagram for carbon dioxide



at temperatures above ambient and at pressures up to 10 atmospheres. Supercritical carbon dioxide extractions and supercritical carbon dioxide chromatography are limited to the region above Tc and Pc.

Table 1 compares certain properties of the gas, liquid, and supercritical fluid (SCF) phases. The density of a SCF is of the same order of magnitude as a liquid and is 2-3 orders of magnitude greater than that of a gas. The greater the density, the greater the number of solute-solvent interactions per unit volume. The viscosity, a measure of the ability of a substance to flow, of a SCF is of the same order of magnitude as a gas and is two orders of magnitude smaller than a liquid. The diffusivity of a supercritical fluid is three orders of magnitude less than a gas and two orders of magnitude greater than a liquid. The higher diffusivity of SCF relative to liquids results in faster mass transfer and will therefore result in shorter interphase equilibration times than occur in liquids (2). It seems apparent that a SCF can be used in extractions.

A SCF as an extraction medium has certain advantages over normal extraction solvents. Because of the relatively low temperatures used, heat-sensitive components may be extracted without thermal decomposition. This advantage is shared with batch liquid extraction (e.g. separatory funnels) but not with Soxhlet extraction (3). The comparatively low viscosity for a given density of a SCF imparts to the solvent

excellent powers of penetration into a porous solid structure. This presumably accounts for the increased extraction efficiency over liquid solvents (4).

Table 1

Physical Properties of Typical Fluids

Property	Gas	Supercritical Fluid	Liquid
Diffusivity (cm^2/sec)	10^{-1}	2×10^{-4}	5×10^{-6}
Viscosity (g/cm sec)	10^{-4}	2×10^{-4}	10^{-2}
Density (g/ml)	10^{-3}	0.6	1.0

SCF have the further advantage that solvent strength varies with SCF density; compounds can be selectively dissolved by simply varying the temperature or pressure of the fluid and thus its density (5).

In addition, the extracted material may be easily recovered from the supercritical fluid since relatively small changes in temperature or pressure result in considerable changes in solubility. Essentially complete separation of supercritical fluid and solute with high solvent recovery can be accomplished by isothermal decompression or isobaric heating. This is a distinct advantage over most types of liquid extraction, where considerable energy may be expended

to evaporate the solvent. The Kerr-McGee Refining Corporation has operated a semicommercial plant (750 barrels/day) for de-asphalting residuum oils (6). In this plant supercritical solvent recovery resulted in a utility savings of 50% over the usual evaporation method of solvent recovery.

The range of solvents which can be used in the SC state is vast. Table 2 lists some of the compounds that can be used in SCF extractions. As can be seen, there is a wide range of critical constants, dipole moments, and polarizeabilities. If one SCF does not adequately extract a particular compound, a second might, or the addition to the first of a second SCF (entrainer) may improve the extraction capacity (7).

The addition of an entrainer can change the properties of a SCF. The use of mixed solvents in Soxhlet extraction is documented (8). Certain precautions must be followed in deciding which mixed solvent system should be used for Soxhlet extraction. Knowledge of possible azeotropes is a must. It is possible that a low boiling azeotrope will be formed and will collect in the top of the Soxhlet apparatus. If the volume of the azeotrope is enough to fill the reservoir at the top of the Soxhlet apparatus and thus allow it to be returned to the boiling flask, there is no problem. However, if the volume of the azeotrope is not enough to fill the top reservoir, the temperature of the solvent in the boiling flask will increase until it boils. It will remain at this

Table 2

Critical Properties of Several Gases

GAS	T_c, °C	P_c, psi	d_c, g/cc	Dipole Moment 10^{-18} esu	Polarizability 10^{-25} cc
Ar	-122	706	0.531	0	16.2
CH_4	- 82	673	.162	0	26.0
C_2H_4	9.2	735	.227	0	0
$CClF_3$	28.8	573	.58	0	0
CO_2	31.1	1070	.468	0	26.5
CHF_3	33	691	.516	1.60	0
N_2O	36.5	1050	.457	0.14	30.0
CH_3F	44.6	853	.300	1.81	0
SF_6	45.6	545	.752	0	0
HCl	51.4	1200	.42	1.05	26.3
C_3H_8	96.8	617	.220	0	62.9
H_2S	100.4	1310	.349	0.93	36.8
CCl_2F_2	111.5	582	.555	0.505	0
$C_2H_4F_2$	113.5	652	.365	2.24	0
NH_3	132.3	1640	.235	1.46	22.6
CH_3Cl	143.1	969	.353	1.86	45.6
Cl_2	144	1120	.573	0	46.1
SO_2	157.5	1140	.524	1.62	0
CH_3CHO	188	804	.262	2.72	0
C_5H_{12}	196.6	486	.232	0	99.5

temperature until the top reservoir is filled. As the lower boiling azeotrope is returned to the boiling flask, it will vigorously boil. This superheated vapor will rise rapidly and even the best condenser will not prevent the vapor from escaping. This problem does not exist in SCF extractions.

A financial savings results from the fact that since the extract can be easily removed from supercritical fluids, the fluid can be reused in the same extraction. Since supercritical fluids may be easily recovered, as mentioned previously, an additional savings in recovery costs is obtained. For extractions requiring a high degree of solvent purity, it is often easier and cheaper to purify a gas to a greater extent than it is for a liquid.

For extractions of food substances, it is essential that the solvent be completely removed from the food. Carbon dioxide, in addition to being neither flammable nor toxic, does not react with the sample in any way and is quantitatively recovered (9). In one experiment a sample of coffee beans was extracted with supercritical radioactively-labelled carbon dioxide (10). The beans were subsequently analyzed and no trace of radioactivity was found, indicating that from this point of view supercritical carbon dioxide is an ideal choice of an extraction solvent for foods.

Supercritical fluid extraction is not a new idea. The fact that compressed gases can dissolve solids was first demonstrated experimentally by Hanney and Hogarth (11) in

1879, who dissolved potassium iodide in supercritical ethanol and then precipitated the salt by reducing the pressure. In the early 1950's, Ziegler and Gellert (12) discovered the "Aufbau" reaction of triethylaluminum with ethylene under pressure at ca. 100°C. This reaction today forms the basis of the industrial preparation of long-chain primary alcohols for conversions into biodegradable detergents. This reaction was carried out on a laboratory scale. Triethylaluminum was placed in an autoclave and pressurized with ethylene at room temperature. To determine the amount of ethylene present, the autoclave was weighed. In one case it was found that the autoclave was overfull, and heating to 100°C would have resulted in too high a pressure. It seemed inadvisable to simply vent the autoclave since it was feared that traces of the spontaneously inflammable triethylaluminum might be carried over and ignite the gas. The gas was therefore vented through a cold trap, where condensation of $Al(Et)_3$ was indeed observed. It was assumed that this had been carried over either in the form of droplets or that it had crept up the sides of the autoclave. At the time, this phenomenon did not receive further attention.

A systematic investigation of the ability of pressurized ethylene to transport high-boiling material was first undertaken in 1962 as a result of a difference of opinion with a licensee concerning the quality of the product of the "Aufbau" reaction (10). It was very quickly realized that a

general principle has been discovered for the separation of substances using supercritical fluids.

Since the early 1960s, supercritical fluid extraction on the process scale has been actively developed. Details of many applications are not readily available since these are competitive industrial processes. There are excellent reviews on the subject of supercritical fluid extraction (13-14) and the following is but a brief description of some of the major applications in various fields.

Food

The advantages of carbon dioxide, which is unobjectionable from the health point of view, over organic solvents make it appear an ideal extraction agent. It is ubiquitous in nature, e.g. in the air, and dissolves in all water--fresh, rain, and sea, in addition to some ground or mineral waters. Carbon dioxide is produced when organic substances ferment, e.g. in many manufacturing processes in the food industry, such as the production of beer and wine, the preparation of bread, etc. Moreover, it is used to artificially carbonate water. Being an "inert" gas, even in the supercritical state, carbon dioxide does not react in any way with the food constituents (9). This is also the reason for its use for quick-freezing foods in the liquid and solid state. The Food and Drug Administration classifies carbon dioxide as Generally Recognized As Safe (GRAS)(15). A food additive classified as GRAS is considered safe even under high concentrations. In Germany, carbon dioxide along with air, nitrogen, and distilled

or demineralized water, is not considered a foreign substance.

In addition to its solvent properties, carbon dioxide has the advantages of being neither flammable nor toxic, and not exerting a corrosive effect in combination with moisture on the materials used in processing natural products (stainless steel or plastics). It is also inexpensive and readily available in large quantities and high purity. For these reasons, natural products have so far been processed predominantly with carbon dioxide.

The removal of caffeine from tea and coffee is an example of an important food processing application. Many patents exist for the removal of caffeine using urganic solvents (10, 16-18). In a typical procedure (16), the aroma substances are first removed from the tea or coffee by means of petroleum ether. The tea or coffee is then moistened, passed through an ammonia solution in order to separate the caffeine salts, and the the caffeine is extracted by means of solvents such as trichloroethylene. After the tea has been dried, the aroma substances originally removed by means of petroleum ether are restored to the tea. In this process, care must be taken to remove all of the solvent.

The decaffeination of tea and coffee using supercritical carbon dioxide has also been demonstrated (10,18). It can be carried out in many ways. In the first variation, presoaked green coffee beans are treated in a pressure vessel with continuously recycled carbon dioxide at 160-220 atmospheres. The caffeine is removed from the beans into the

supercritical carbon dioxide and is carried out of the pressure vessel into a washing tower where it is washed out of the carbon dioxide with water at 70-90°C. After 10 hours all of the caffeine is in the wash water which is then degassed and the caffeine is recovered by distillation. The caffeine content in the bean is decreased from an initial value lying between 3% and 0.7% to a value as low as 0.02% (10). Coffee with a caffeine value of less than 0.08% is considered by the Food and Drug Administration to be decaffeinated (10). The separation can be carried out with the help of active charcoal instead of water. However, in this case the caffeine must then be extracted from the charcoal.

Another variation has certain advantages. The pressure vessel is filled with a mixture of coffee beans and activated charcoal pellets whose diameters are smaller than those of the coffee beans. A carbon dioxide pressure of 220 atmospheres and 90°C is used (10). The caffeine is extracted by the supercritical carbon dioxide and then is absorbed from the SCF phase onto the activated charcoal without the need for recycling. The required degree of decaffeination is reached after five hours. The mixture is then separated into its components by passing over a vibrating sieve.

Another important application of supercritical carbon dioxide to the food industry is in processing of spices. O. Vitzthum et al. have patented supercritical extraction processes with carbon dioxide for the extraction of hops

(5); cocoa butter and aroma components from cocoa beans (19); piperine from pepper (5); and aroma constituents from black tea (20).

The extraction of oils is another area where the use of supercritical fluids is ideal. Zosel (5) has extracted oil from soybean flakes, corn, and bones, using propane, ethane, carbon dioxide, or nitrous oxide. Zosel has also shown that supercritical fluids can be used to deodorize plant oils (soybean, palm, and peanut) with simultaneous removal of free fatty acids and hydrogenation with a carbon dioxide/hydrogen mixture (5).

Another important application of SCF extraction to the food industry is in processing of spices. The important constituents of spices are their aroma and flavor components. Spices have a number of disadvantages, which have led to the use of spice extracts. The advantages of spice extracts include (9): the constituents are better utilized; the resulting products have a longer shelf life; greater uniformity is achieved because of the possibilities of standardization; and the products are sterile. The best extract is a preparation that has aroma, flavor, and all the characteristics (i.e. hotness) of the original spice, and after dilution reconstitutes the characteristics of the starting material (21).

Many spices, including piperine from black pepper, capsaicine from chilies, and nutmeg butter from nutmeg, have

been extracted using supercritical carbon dioxide (9,18). When supercritical carbon dioxide is used to extract ground black peppers, ground chilies, and ground nutmegs, the degree of extraction was 98%, 97%, and 98% respectively (9).

The extraction and deodorization of oils is another area where the use of supercritical fluids is ideal. There has been much work done of the extraction of oil from cocoa, coconuts, corn, olives, peanuts, soybeans, sunflowers, etc. (22-24).

Petroleum Products and Coal

One of the earliest industrial applications of supercritical fluid extraction was that of Katz and Whaley (5). Natural gas at pressures above 68 atm and temperatures up to 200°C were used to separate liquid hydrocarbon mixtures.

An important area in the petroleum industry is the de-asphalting of petroleum. This process has been accomplished by the use of supercritical carbon dioxide (25-26), methane (27-28), ethylene (29), ethane (25-26), propane (30), n-pentane (31-33), toluene (34-38), and isopropanol (39). In a typical example, the Kerr-McGee Refining Corporation has operated a semi-commercial de-asphalting plant (750 barrels/day) since 1975 (6). In this plant, the supercritical fluid recovery resulted in a utility savings of 50% over the usual solvent recovery method of evaporation.

Kerr-McGee also developed a supercritical fluid de-ashing process to separate ash from liquefied coal at pilot

plant levels (163 kg/hr) (40-41). Here, the supercritical fluid is used to remove coal liquefaction product from vacuum still bottoms leaving insoluble coal and mineral matter as a dry flowable ash concentrate. The coal components dissolved in the supercritical fluid can be fractioned (oils, asphaltenes, and multifunctional compounds) to lighter products and a heavier molten low-ash (<0.09% ash) fluid which can be solidified to the product coal. It is estimated that this process coupled with a conventional solvent refining plant will be more cost-effective than similarly sized filtration plants.

Tobacco

Tobacco is a multibillion dollar industry (42). The sale of "mild" tobacco (low in nicotine) has risen over the last twenty years. Natural tobacco of this type, good aroma and low in nicotine, is not available in large quantities.

Nicotine can react with the nitrite in the tobacco to form N-nitrosamines; specifically nicotine reacts to form N'-nitrosonornicotine which has been proven to be carcinogenic in mice, rats and hamsters (43-45). Approximately 60mg nicotine orally is estimated to be lethal to most adults (46). Nicotine has a very high acute toxicity and acts with the speed of cyanide, producing death in minutes (47). Because of this, or the public market for "mild" tobacco, attempts have been made to remove nicotine from

tobacco (46). Since tobacco treated with organic solvents often acquires a rubbery structure, special processes have been suggested to avoid this effect. Supercritical fluid extraction avoids this and has only one side effect, expansion of the tobacco (9).

Nicotine has been extracted using many supercritical fluids (48-49), using different processes. The following description is typical of a multistage extraction process. In the first stage the aroma producing compounds are removed from the starting material. The aroma compounds are either isolated as an extract or used to impregnate a previously denicotinized batch by simply allowing the aroma-carrying supercritical fluid to expand into the denicotinized batch. The moisture content of the de-aromatized tobacco is then increased to approximately 25%. It has been found experimentally that more nicotine is extracted if the water content in the tobacco is raised from an initial value of ≈ 10% to ≈ 25% (9). The nicotine is then removed in an isobaric and isothermal recycling operation. Selective absorbents are used to isolate the nicotine. Regeneration of these absorbents produces nicotine as a by-product. Finally, the aroma is replaced as previously described.

When the treated tobacco is discharged from the extraction chamber, an expansion of its fibers is observed as a result of the removal of gas residue from the vegetable tissue. The appearance of the tobacco remains unchanged or

may change only slightly to a lighter or darker color. The nicotine content of the tobacco is reduced by 94.7% and that of the mainstream smoke by 94.8% (9).

Miscellaneous Applications

Supercritical fluids can be used in almost every application involving extractions. An example of a pharmaceutical application is the supercritical fluid extraction of camomile leaves using carbon dioxide or nitrous oxide at pressures at least 1.2 times the critical pressure and temperatures less than 50°C (48,50). The products selectively extracted are bisabolol, its oxides, proazulenes, coumarins, and fragrant components while polysaccharides, acids, and flavenoids are not extracted. Other pharmaceutical applications include the supercritical fluid extraction of: crude opium for codeine, thebaine, narcotine and papaverine (48-49, 51); ground tablets for aspirin, phenacetin and caffeine (31,52); vitamin-oil mixtures for cholesterol, vitamins D3, K3, A and E (53-54), and Cinchona bark for quinine (48-49).

Other applications include the supercritical fluid extraction of: wool grease for lanoline (55-56); sea water for pure water (5); dye mixtures for fat orange, sudan red, sudan blue, ceres red, ceres orange, ceres green, sudan yellow and sudan orange (57), and argillaceous rock for bitumens (58).

THERMODYNAMICS OF EXTRACTION WITH SUPERCRITICAL FLUID

The earliest studies of solutions composed of a pure solid and a supercritical fluid were based on empirical equations of state, such as those of van der Waals (59), Keyes (60), and Beattie (61). One approach that has been used to account for the solubility of a solid in a supercritical fluid uses the virial expansion (62-63).

$$\frac{PV}{nRT} = 1 + \sum_{\ell=2} \frac{J_\ell n^{\ell-1}}{V^{\ell-1}} \qquad (1)$$

where P is the pressure, V the volume, n the number of moles, R the ideal gas constant, T the absolute temperature, and J_ℓ is the ℓ-th virial coefficient of the mixture. J_ℓ is a function of temperature and composition only (64). In general,

$$J_2 = \sum_{\alpha} \sum_{\beta} \chi_\alpha \chi_\beta B_{\alpha\beta} \qquad (2)$$

$$J_3 = \sum_{\alpha} \sum_{\beta} \sum_{\gamma} \chi_\alpha \chi_\beta \chi_\gamma \, C_{\alpha\beta\gamma} \qquad (3)$$

where α, β, γ... are associated with components 1,2,3... in the multicomponent mixture. By convention, 1 refers to solvent and 2 to solute for two component systems, such as were studied here. The pure component second virial coefficients are functions only of temperature, and represent

the specific interactions between two molecules of that component. Third virial coefficients represent simultaneous interactions among two molecules of one component and one molecule of the other component, e.g. C_{112} would represent the simultaneous collision of two molecules of solvent gas and one of solute.

The chemical potential of component 2 in a binary gas mixture is given (63) by the equation

$$\mu_2(\text{gas}) = \mu_2^+(\text{gas}) + RT_{IN}\left(\frac{nRT}{V}\right) + RT\sum_{\ell=2} \frac{\ell}{\ell-1}\frac{J_\ell^2 n^{\ell-1}}{V^{\ell-1}} \quad (4)$$

where μ_2^+ (gas) is the standard chemical potential of component 2 and is a function only of temperature, and where

$$J_2^2 = \sum_\alpha \chi_\alpha B_{2\alpha} \quad (5)$$

$$J_3^2 = \sum_\alpha \sum_\beta \chi_\alpha \chi_\beta C_{2\alpha\beta} \quad (6)$$

The chemical potential of component 2 in the solid phase is given by

$$\mu_2^+(\text{solid}) = \mu_2^+(\text{solid}) + Pv_2^s \quad (7)$$

where μ_2^+ (solid) is the standard chemical potential of

component 2 in the solid phase, and v_2^s is the molar volume of solid component 2. Terms in v_2^s express the direct effect of hydrostatic pressure on the chemical potential of the solid. There are two assumptions made in the derivation of equation 7: (1) no gas dissolves in the solid and (2) the solid is incompressible. In such a case, for dilute mixtures, e.g. in the limit of $\chi_2 << \chi_1$, it can be shown that

$$\ln(X_2/X_2^i) = \frac{v_2^s - 2B_{12}}{v} + \frac{v_2^s B_{11} - \frac{3}{2}C_{112}}{v^2} + \frac{v_2^s C_{111} - \frac{4}{3}D_{1112}}{v^3} + \ldots \quad (8)$$

where v is the molar volume of the mixture, X_2 is the mole fraction of component 2 in the supercritical fluid, X_2^i is the expected mole fraction of component 2 in the supercritical fluid assuming ideal behavior and is only a function of temperature. The right hand side of this equation is an expansion in gas density and tries to account for the enrichment of the vapor phase in component 2 over that of the saturated vapor of the pure component.

The terms with the virial coefficients B_{12}, C_{112}, D_{1112}, etc. represent the simultaneous interactions of 1, 2, or 3 molecules of component 1 respectively with one molecule of component 2. The coefficient B_{12} is often large and negative (65). It is this term which reflects the greatest part of the enhancement in solubility; two body collisions are more likely than three or more body collisions at moderate pressures.

Equation 8 has been used since the 1930s. Poynting (66) first showed that hydrostatic pressure would increase the activity (i.e. chemical potential of a solid by the amount shown in Eq. 7, and for many years the "Poynting effect" was invoked to explain the increased volatility of solids in gases with increasing pressure. However, the molar volumes of most solids are small, and the enhancement caused by this effect alone is only a small part of the total effect (65). The terms in Eq. 8 containing the virial coefficients B_{12}, etc. were added independently by Perkins (67) in 1937 (second coefficient only), by Robin, Vodar, and Bergeon (68-71) in 1951-1953 (second coefficient only), and by Ewald, Jepson, and Rowlinson (72) in 1953 (complete expansion).

Like all virial expansions this equation converges satisfactorily only at low and moderate gas densities. In practice it can usually be used to gas densities up to 10 moles/liter (i.e. or less than ¼ the critical density) with virial coefficients up to the third (65). The coefficients can be approximated from the limited knowledge we have of the relevant intermolecular forces. Solubilities can then be calculated and compared directly with the experimental data.

The second virial coefficient B_{11} can be calculated from the Lennard-Jones 6-12 potential energy function by using the following expressions:

$$B_{11} = b_o B^* \qquad (9)$$

$$T^* = T(k/\varepsilon) \qquad (10)$$

where T* is the reduced temperature, k the Boltzman constant, ε the minimum in the potential curve, and b_o the hard-sphere value of $2\pi N_o \sigma^3/3$. N_o is Avogadro's number ($6.023\ 10^{23} \times mole^{-1}$) and σ is the separation between two molecules where their combined potential energy is zero. The values of b_o and ε/k for carbon dioxide are equal to 113.9 cc/mole and 189°K respectively (73). B* can be found in published tables relating B* to T* (74).

Other models to explain the increased solubility of a solid in a supercritical fluid have been presented. One theory by Mackay and Paulatis (75) treated the SCF as an expanded liquid. Equation 11 was used to correlate the solubilities of several solute-SCF systems.

$$\chi_2 = \frac{f_2^{os}(P_c) \exp \frac{v_2^c (P-P_c)}{RT}}{\gamma_2^{\infty}(P_c) f_2^{OL}(P_c) \exp \int_{P_c}^{P\infty} \frac{\bar{v}_2^{\infty}\, dP}{RT}} \qquad (11)$$

In eq. 11, $f_2^{os}(P_c)$ is the fugacity of pure solid solute at the temperature T and the critical pressure (P_c) of the solvent, v_2^c is the molar volume of the solute, $\gamma_2^{\infty}(P_c)$ is the activity coefficient at infinite dilution, $f_2^{OL}(P_c)$ is the fugacity of the pure "liquid" solute determined at the

temperature T and the critical pressure, and $\bar{v}_2^{\infty}$ is the partial molar volume of the solute at infinite dilution.

To determine the partial molar volume at infinite dilution in eq. 11, the Redlich-Kwong equation of state (76) and the mixing rules given by Chueh and Prausnitz (77) were used. The mixing rules contain an adjustable binary parameter k_{12}. Values for k_{12} and the infinite-dilutions activity coefficient are obtained by fitting the experimental solubility data in eq. 8. These best-fit values are then used to determine the mole fractions at various pressures.

This theory used the critical pressure for the reference state, and assumed that γ_2 and $\bar{v}_2$ could be considered to be at infinite dilution. Another theory which does not use solute critical properties was proposed by Johnston and Eckert (78) and used the Carnahan-Starling-van der Waals (CS-VDW) equation of state (79). This gave rise to the following equation

$$x_2 = \frac{P_2^{sat} \phi_2^{sat} \exp \frac{v_2 (P - P_2^{sat})}{RT}}{\phi_2 P} \qquad (12)$$

where P_2^{sat} is the vapor pressure of the solute assuming ideal conditions, ϕ_2^{sat} the fugacity coefficient of the solute, and is unity for nonvolatile solids, and v_2 the molar volume.

To use eq. 12 one must solve for ϕ_2. This was accomplished by optimizing a_{12} using a linear least square for each

choice of b, until the optimal value of b was found, using the following equations:

$$P = RT \frac{\xi}{b_o} \frac{(1+\xi+\xi^2+\xi^3)}{(1-\xi)^3} - \frac{a}{v^2} \tag{13}$$

$$\ln(\phi_2 Z) = \frac{3\xi^3-9\xi^2+8\xi}{(1-\xi)^3} - \frac{2(\chi_1 a_{12}+\chi_2 a_{22})}{RTv} \tag{14}$$

where ξ is a dimensionless density (b/v), b a hard-sphere parameter, a an attraction parameter, and Z the compressibility factor.

The three theories mentioned above may be used to fit experimental solubility data points to an equation, but each may have limitations. In the virial expansion, the virial coefficients obtained may have no physical meaning. In the expanded liquid theory, the infinite-dilution activity coefficients may have no physical meaning. Also in the CS-VDW model, the fugacity coefficient obtained may have no physical meaning.

EXPERIMENTAL

Chemicals and Supplies

Benz(a)anthracene, phenanthrene, and pyrene were purchased from Eastman Organic Chemicals. Acridine, anthracene, coronene, fluoranthene, fluorene, naphthalene and phenanthridine were obtained from Aldrich Chemical Co. The liquified carbon dioxide was supplied by the Linde Division of Union Carbide and was bone dry grade. A polycyclic aromatic hydrocarbon (PAH) standard mixture containing 16 compounds was a Quality Assurance Sample supplied by the Environmental Monitoring Standards Laboratory (Cincinnati, Ohio) and originated from Supelco. The PAH standard contained: acenaphthene (1.0mg/ml); fluoranthene (0.2mg/ml); naphthalene (1.0mg/ml); benz(a)anthracene (0.1mg/ml); benzo(a)pyrene (0.1mg/ml); benzo(b)fluoranthene (0.2mg/ml; benzo(k)fluoranthene (0.1mg/ml); chrysene (0.1mg/ml); acenaphthylene (2.0mg/ml); anthracene (0.1mg/ml); benzo(ghi) perylene (0.2mg/ml); fluorene (0.2mg/ml); phenanthrene (0.1mg/ml); dibenzo(a,h)anthracene (0.2mg/ml); indeno(1,2,3-c,d)pyrene (0.1mg/ml); and pyrene (0.1mg/ml).

The high volume air filters were supplied through the courtesy of Ben Nathanson of the New York City Department of Environmental Protection. The high volume samples are placed at various sites around New York City. Air is pulled through the 8½ x 11" glass fiber filter at a rate of approximately 40-50 cubic feet per minute. Approximately 2000 cubic

meters of air is sampled by this method during a 24 hour period. The hi-volume air filter is removed and stored at a low temperature until weighed, extracted and analyzed.

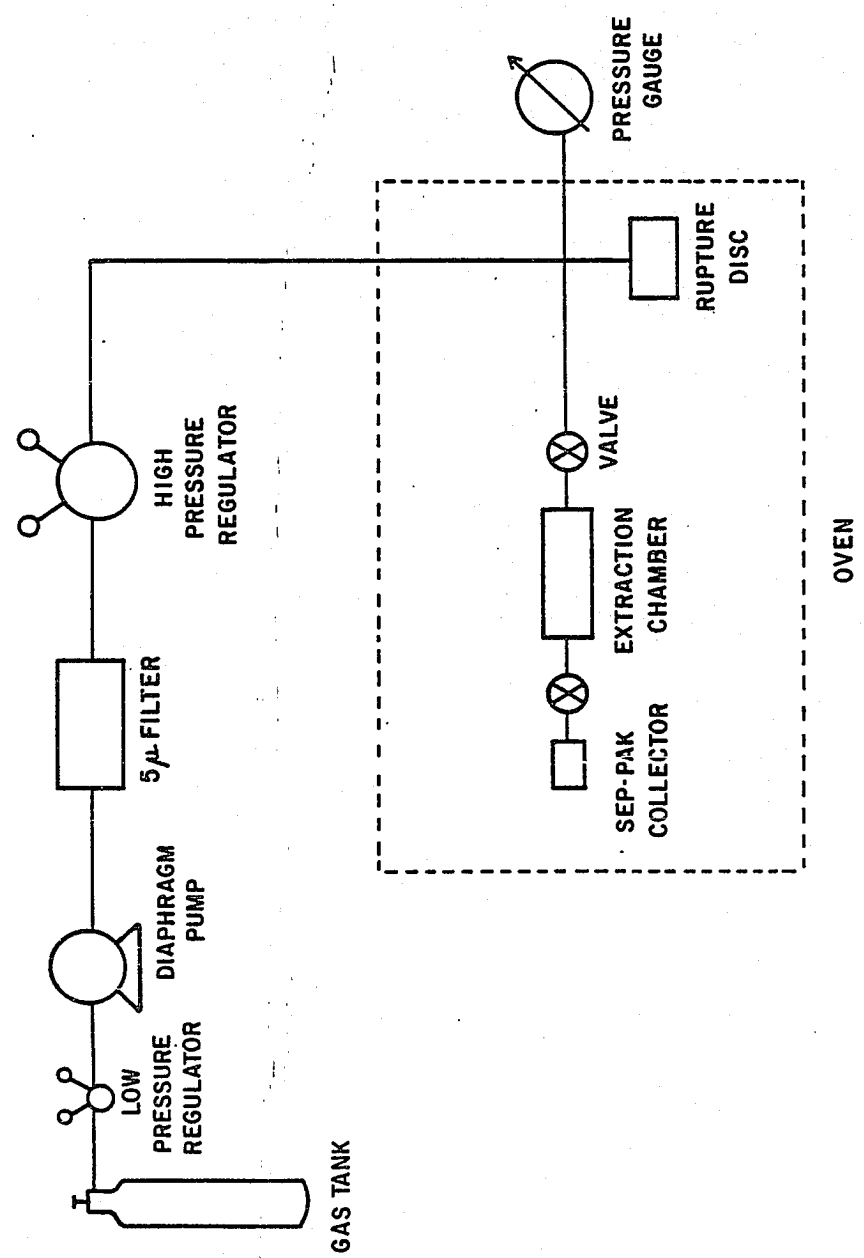
The 25cm x 25cm, 500 micron silica gel thin layer chromatographic plates were purchased from Analtech. Acetonitrile, benzene, cyclohexane and methanol were obtained from Fisher Scientific Co. All were of HPLC grade.

Apparatus

Figure 2 presents a schematic diagram of the SCF extraction apparatus used. The carbon dioxide, supplied through a single stage tank regulator, was filtered through an Autoclave Engineers (AE) 5 micron cup-type line filter (AE model #CXF4-5). The gas was then pressurized by a motor-diven single-end diaphragm compressor (American Instrument Company (now Superpressure), model #J 46-13411) with a maximum output pressure of 11,000 psi (749 atm). The pressure of the system was controlled by a Circle Seal internally dome-loaded line regulator (model #GD 80A). From this point, the apparatus was thermostated in a Labline Instruments Imperial II circulating radiant heat oven and was held at temperatures in the range of 35-60°C. Temperature control to ±1°C can be maintained.

The extraction chamber could be any size or shape depending on the sample matrix. In this study, a 10cm x 0.8cm i.d. chamber with a volume of approximately 5ml was used. The chamber can be opened easily for rapid sample changes. Care must be taken to prevent scratches on the sealing surfaces

Figure 2. A Schematic of the Supercritical Fluid Extraction Apparatus Used in this Study



of the chamber in order to prevent leaks.

All of the ¼ inch connecting tubing (AE model #15-081), valves (AE model #20-SV 4081) and attachments (tee AE model #CTX 4440, coupling AE model #20F41666) were 316 stainless steel. They are rated on capable of withstanding pressures up to 1360 atmospheres. A rupture disc rated at 1160 atm. was incorporated into the equipment to prevent accidental over-pressurization.

To perform a SCF extraction, the extraction chamber was disassembled, cleaned with cyclohexane, and dried. The sample was then placed into the extraction chamber. Glass wool, pre-rinsed with cyclohexane and dried, was placed into the ends of the extraction chamber to prevent movement of the sample. The extraction chamber was reassembled. With all the valves open, carbon dioxide under very low pressure (<3 atm) flowed through the extraction chamber and vented into the atmosphere. This was done to remove any air present in the extraction chamber. The valves were then closed. The system before the extraction chamber was pressurized with carbon dioxide until the desired pressure was obtained by adjusting the high pressure regulator. Then the valve prior to the extraction chamber was opened fully and the entire system was pressurized. Once the pressure and temperature stabilized, the valve after the extraction chamber was partially opened. The supercritical carbon dioxide containing the extract passed through this second valve and was rapidly depressurized.

The extract was collected on a Waters C-18 Sep-Pak collector. The Sep-Pak was threaded onto the 1/8-inch male end of a male/male reducing union (Swagelok Model #400-6-2). Copper wire was tightened around the thread and no carbon dioxide leak was detected.

To determine solubility, carbon dioxide (now containing no extract) passed through a small diameter Teflon tube and into an inverted 500ml volumetric flask containing water that was pre-saturated with carbon dioxide. Carbon dioxide was allowed to flow until exactly 500ml was collected in the volumetric flask. The valves were then closed. If the amount of carbon dioxide that was used in an extraction was not needed (e.g. extractions of air filters), the carbon dioxide flowing through the Sep-Pak collector was vented to the atmosphere.

The collector was rinsed with two 5 ml aliquots of cyclohexane. The cyclohexane extract was reduced to about 0.5 ml in a rotary evaporator in a water bath at 40°C.

If the extraction was performed on a pure compound (e.g. the determinations of solubilities), a Shimadzu GC-Mini 2 Gas Chromatograph with a flame ionization detector, set up for capillary columns, was used. Either a 30 meter fused silica open tubular column with the DB-5 bonded phase or a 25 meter glass capillary column wall-coated with OV-101 was used. Helium at 1 ml/min was used as carrier gas and injection and detector temperatures were 200°C and 250°C

respectively. Column temperature was programmed to give a peak well resolved from the solvent and a retention time not greater than 10 minutes. For example, the temperature program for naphthalene was an initial 4 minute 50°C isothermal period followed by temperature programming at 2°C per minute to 100°C. Quantitation was done by direct comparison of peak area determined by measuring height and width at half height of the sample with a previously injected standard.

If the cyclohexane extract contained more than one compound, as in the extraction of air filter samples, the extract was first cleaned up by streaking the entire extract on a 25cm x 25cm silica gel (500 micron) TLC plate. The plate was developed using cyclohexane/benzene (1.5/1) (v/v) as solvent. Fluoranthene and coronene were also spotted on the plate to show the upper and lower limits of the PAH fraction. The PAH fraction was then carefully scraped off and sonicated in methanol for 30 minutes. The methanol solution was filtered, the filtrate rinsed with additional methanol, and reduced to 0.5ml in a rotary evaporator. The methanol was quantitatively transferred to a micro-vial and reduced to 0.1ml using a stream of nitrogen. The methanol extract was analyzed by using a Waters Liquid Chromatograph equipped with two 6000 A Solvent Delivery Pumps, fixed wavelength (254nm) 440 U.V. Absorbance Detector, a 660 Solvent Programmer, and an E.M. LiChrosorb RP-18, 10 micrometer, 250mm x 4.0mm column at ambient temperature. A gradient elution program

of 40/60 acetonitrile/water to 100/0 over 50 minutes was used at a flow rate of 1.0ml/min. Quantitation was performed by direct comparison of peak areas, determined by measuring peak height and width at half height, of the samples with the peak areas of a previously injected standard containing 16 PAHs. The concentrations of the compounds in the standard ranged from 0.1 to 2.0 mg/ml.

Collector Efficiency

To determine the Sep-Pak collection efficiency, exactly 20.0mg of naphthalene was placed inside the extraction chamber. One end of a 1 meter x 1/8 inch i.d. Teflon tube was connected to the Sep-Pak collector and the other end was placed into an erlenmeyer flask containing 150ml of cyclohexane. This allowed the carbon dioxide to bubble through the cyclohexane. The extraction chamber was heated to 45°C. The pressure of the carbon dioxide inside the system was increased to 272 atm. Carbon dioxide was allowed to pass through the system at a rate of approximately 100ml of carbon dioxide (equivalent at ambient conditions) per minute for 60 minutes.

The extraction chamber, collector, and teflon tube were each rinsed with cyclohexane. Each of the cyclohexane extracts, together with the erlenmeyer flask of cyclohexane, was reduced to 1.0ml in a rotary evaporator with a water bath of 40°C. Each reduced extract was analyzed for naphthalene by capillary gas chromatography as discussed previously.

In a second experiment, 10mg of fluoranthene was placed inside the extraction chamber. The fluoranthene was extracted as above. The extraction chamber, collector, and Teflon tube were rinsed with cyclohexane and together with the flask of cyclohexane were reduced to 1.0ml and analyzed for fluoranthene by capillary gas chromatography as described before.

Supercritical Fluid Extraction Equilibration Time

To determine the SCF extraction equilibration time, approximately 2 grams of fluorene were placed inside the extraction chamber. The apparatus was maintained at 45°C and the pressure of the carbon dioxide was increased to 136 atm. Immediately, 500ml (at STP) of carbon dioxide was passed through the collector. The collector was rinsed with two 5ml aliquots of cyclohexane and the extract was analyzed for fluorene by capillary gas chromatography as described before. The apparatus was then disassembled, cleaned with cyclohexane, dried and reassembled. Another 2 grams of fluorene was added into the extraction chamber. The system was again pressurized to 136 atm. In the second extraction, 5 minutes were allowed to pass before the carbon dioxide was passed through the collector. In the third and fourth extractions, 10 and 20 minutes respectively passed before the carbon dioxide was allowed to flow through the collector. The temperature was held constant at 45°C for all extractions.

Effect of Particle Size on Solubility

To determine the effect of particle size on solubility, approximately 2 grams of anthracene were placed inside the extraction chamber at 45°C. The average area of anthracene crystals was large, of the order of $4mm^2$.

The carbon dioxide pressure was increased to 340 atm and held constant for 5 minutes, after which 500ml (at STP) of carbon dioxide was passed through the extraction chamber into the collector. In a second extraction, the anthracene was finely ground before being placed into the extraction chamber. Again the carbon dioxide pressure was raised to 340 atm and kept constant for 5 min before the carbon dioxide was passed through the system.

Both extracts were analyzed by capillary gas chromatography to determine the amount of anthracene extracted.

Determination of Solubility

To determine solubility of a compound in supercritical carbon dioxide, approximately 1-2 grams were placed into the extraction chamber. This high amount (1-2 grams) was chosen in order to ensure saturation of the supercritical carbon dioxide in the extraction chamber. The carbon dioxide pressure was increased to the test value and was held constant for at least 5 minutes. Exactly 500ml (at STP) of carbon dioxide passed through the collector. The collector was then rinsed with two 5ml aliquots of cyclohexane. The extract was analyzed by capillary gas chromatography for

that particular compound as described above. Three different temperatures in the 35-65°C range were studied for each compound. At each temperature, 5 pressures ranging from 136 atm to 408 atm were studied. For each compound, 15 different temperature and pressure combinations were studied. For each combination at least two extractions were performed. At some combinations three extractions were performed.

Solubilities are expressed in grams of solute in one liter of supercritical carbon dioxide (at that particular temperature and pressure). Another useful term in which to express the amount of solute that dissolves in a supercritical fluid is the enhancement, E, defined as

$$E = X_2 P / P_2^{sat} \qquad (15)$$

where X_2 is the mole fraction of the solute in the supercritical fluid, P is the system pressure, and P_2^{sat} is the vapor pressure of the solute under ideal conditions. The vapor pressure of the various compounds were found in published tables (80-83).

Supercritical Carbon Dioxide Extraction of a Synthetic PAH Standard and Blank

One hundred microliters of a synthetic PAH standard containing 16 compounds were streaked using a microsyringe onto a blank glass fiber filter and the solvent was allowed to evaporate. The filter was then placed into the extraction

chamber and preheated to 45°C. The carbon dioxide pressure was increased to 340 atm. Carbon dioxide passed through the collector at a flow rate of approximately 100ml (at STP) per minute for 30 minutes. The collector was rinsed with cyclohexane and reduced to about 0.5ml in a rotary evaporator.

The cyclohexane extract was purified using silica gel TLC and quantitated using reverse phase HPLC as discussed above.

Extraction of PAHs from Air Filters

Supercritical:

One half of a high volume air filter was shredded and placed into the extraction chamber. The system was heated to 45°C and the carbon dioxide passed through the collector at a rate of 100ml (at STP) per minute for 30 minutes. The collector was rinsed with cyclohexane. The extract was purified by silica gel TLC and quantitated by reverse phase HPLC as described before.

Soxhlet:

The other half of the high volume air filter was extracted using 150ml of cyclohexane in a soxhlet extraction apparatus for 7 hours. The extract was reduced in volume on a rotary evaporator, purified by silica gel TLC and quantitated by reverse phase HPLC as discussed before.

RESULTS

Collector Efficiency

Since no naphthalene was found in the extraction chamber and the apparatus is a closed system, it was assumed that all of the naphthalene must have passed through the collector. 3.9mg of naphthalene was found in the Waters C-18 Sep-Pak collector, only 19.5% of the total. When the plastic tube connecting the collector to a flask of cyclohexane was rinsed with cyclohexane and analyzed, 0.6mg or 3% of the original naphthalene was found. The cyclohexane in the flask through which the carbon dioxide passed was analyzed and 2.7mg or 13.5% of the original naphthalene was found. Thus of the initial 20mg of naphthalene in the extraction chamber only 7.2mg or 36% was found. Table 3 summarizes the results. This leaves 12.8mg or 64% unaccounted for. Because of the relatively high sublimation pressure (0.15 torr at 35°C), most was presumably lost to the environment. This assumption seemed correct since there was a noticeable odor of naphthalene present.

In a second experiment, 10mg of fluoranthene was placed in the extraction chamber. When the collector was analyzed, 9.6mg of fluoranthene was found. Table 4 summarizes the results. Analysis of the other components of the apparatus failed to show any fluoranthene. The collector was

successful in capturing 96% of the fluoranthene that passed through it but only 19.5% of the naphthalene. The C-18 Sep-Pak collectors are therefore efficient for compounds with sublimation pressure less than or equal to that of fluoranthene.

Table 3

Collector Efficiency for Naphthalene

at 272 atm and 45°C

Sample	Amount Found	Percentage of Total
Extraction Chamber	0.0 mg	0.0 %
Sep-Pak Collector	3.9 mg	19.5 %
Plastic Tube	0.6 mg	3.0 %
Cyclohexane (flask)	2.7 mg	13.5 %
Total	7.2 mg	36.0 %
Unaccounted for	12.8 mg	64.0 %

Table 4

Collector Efficiency for Fluoranthene

at 272 atm and 45°C

Sample	Amount Found	Percentage of Total
Extraction Chamber	0	0
Sep-Pak Collector	9.6 mg	96 %
Plastic Tube	0	0
Cyclohexane (flask)	0	0
Total	9.6	96 %
Unaccounted for	0.4	4 %

Equilibration Time

At 0 minutes delay time, 3.03mg of fluorene were extracted. Equilibration times of 5, 10, and 20 minutes resulted in extractions of an identical quantity, 3.11mg. It is concluded that equilibrium is attained in 5 minutes or less. Thus a 5 minute equilibrium time was used in all subsequent extractions.

Effect of Particle Size on Solubility

Anthracene was chosen because it can be obtained in very large crystals, which would give the greatest difference in surface area between the ground and non-ground solute. When the non-ground anthracene was extracted, 0.569mg was recovered. For the finely ground anthracene, extraction of 0.597mg resulted. Thus in the case of anthracene, a 5% solubility difference was obtained. In all of the solubility studies the solid solute was finely ground before being tested.

In the extraction of the high-volume air filters, all of the particles deposited on the filters are very small. Therefore, this effect of particle size does not pertain to the extraction of the air filters.

Solubility of Various Polycyclic Aromatic Hydrocarbons in Supercritical Carbon Dioxide

Tables 5-10 show solubility data for a number of pure organic solids in supercritical carbon dioxide. The ranges

of temperatures and pressures were 35°C-65°C and 136-408 atm respectively. In the tables, the solubility expressed in mole fraction, ln (X_2/X_2^i) milligrams of solid solute extracted per 500ml (at STP) carbon dioxide, grams of solute per liter of carbon dioxide (SCF), and enhancement.

The enhancement, as discussed before, is a measure of the increase in solubility of the solute in the SCF as compared to the standard state. The enhancement ranges from 10^3 for fluoranthene (55°C, 136 atm) to 5×10^8 for benz(a)-anthracene (37°C, 408 atm). For each compound, as expected, as the temperature increases at constant pressure, the enhancement decreases. At constant temperature, the enhancement increases linearly as pressure increases. This is consistent with enhancement data in the literature (78).

Acridine, with its basic nitrogen, has similar enhancement values as phenanthrene and does not show an addition enhancement due to its basicity. More work is needed to be done in order to see if there is an additional enhancement of basic compound in the acidic carbon dioxide.

The solubility values of these compounds in supercritical carbon dioxide (35°C, 408 atm, density = 0.89g/l) are 10-25 times greater than in water at 25°C (84-88). It is interesting to note that when a log (solubility in SCF) is plotted against solubility in water, a straight line with a correlation coefficient of 0.989 is obtained. A similar correlation is obtained when log solubility in SCF is

Table 5

Solubility of Fluorene in Supercritical Carbon Dioxide

T	P	Mole Fraction	IN(X_2/X_2^i)	mg Ext.	g/l	E
35°C	136	1.94×10^{-3}	6.91	8.48	6.84	1.36×10^{5}
	204	3.48×10^{-3}	7.50	15.2	13.5	3.67×10^{5}
	272	4.44×10^{-3}	7.74	19.4	18.0	6.24×10^{5}
	340	5.42×10^{-3}	7.94	23.7	22.8	9.53×10^{5}
	408	5.86×10^{-3}	8.02	25.6	25.4	1.24×10^{6}
45°C	136	1.26×10^{-3}	4.93	5.51	3.80	1.88×10^{4}
	204	2.13×10^{-3}	5.46	9.31	7.51	4.78×10^{4}
	272	2.72×10^{-3}	5.70	11.9	9.99	8.14×10^{4}
	340	3.12×10^{-3}	5.84	13.6	12.2	9.65×10^{4}
	408	3.32×10^{-3}	5.90	14.5	13.6	1.24×10^{5}
55°C	136	1.39×10^{-3}	3.80	6.08	3.43	6.06×10^{3}
	204	2.12×10^{-3}	4.22	9.27	6.71	1.39×10^{4}
	272	2.46×10^{-3}	4.37	10.8	8.34	2.15×10^{4}
	340	2.75×10^{-3}	4.48	12.0	10.1	3.00×10^{4}
	408	2.92×10^{-3}	4.54	12.8	11.2	3.82×10^{4}

Table 6

Solubility of Fluoranthene in Supercritical Carbon Dioxide

T	P	Mole Fraction	$\ln(X_2/X_2^i)$	mg Ext.	g/l	E
35°C	136	2.59×10^{-4}	5.08	1.13	0.913	2.18×10^{4}
	204	3.43×10^{-4}	5.36	1.50	1.33	4.34×10^{4}
	272	4.58×10^{-4}	5.65	2.00	1.85	7.74×10^{4}
	340	5.77×10^{-4}	5.88	2.52	2.42	1.22×10^{5}
	408	7.23×10^{-4}	6.11	3.16	3.13	1.83×10^{5}
47°C	136	2.56×10^{-4}	3.32	1.12	0.751	3.76×10^{3}
	204	3.06×10^{-4}	3.50	1.34	1.05	6.75×10^{3}
	272	3.34×10^{-4}	3.59	1.46	1.20	9.83×10^{3}
	340	3.66×10^{-4}	3.68	1.60	1.42	1.34×10^{4}
	408	4.05×10^{-4}	3.78	1.77	1.64	1.79×10^{4}
55°C	136	2.62×10^{-4}	2.00	1.15	0.647	1.00×10^{3}
	204	3.18×10^{-4}	2.19	1.39	1.01	1.83×10^{3}
	272	3.72×10^{-4}	2.35	1.63	1.26	2.86×10^{3}
	340	4.32×10^{-4}	2.50	1.89	1.59	4.13×10^{3}
	408	4.78×10^{-4}	2.60	2.09	1.83	5.50×10^{3}

Table 7

Solubility of Acridine in Supercritical Carbon Dioxide

T	P	Mole Fraction	$\ln(X_2/X_2^i)$	mg Ext.	g/l	E
38°C	136	1.41×10^{-4}	6.10	0.545	0.423	6.05×10^{4}
	204	3.93×10^{-4}	7.12	1.52	1.31	2.53×10^{5}
	272	7.06×10^{-4}	7.71	2.73	2.48	6.05×10^{5}
	340	1.05×10^{-3}	8.11	4.08	3.85	1.13×10^{6}
	408	1.24×10^{-3}	8.27	4.80	4.66	1.59×10^{6}
48°C	136	3.31×10^{-4}	5.37	1.28	0.850	2.92×10^{4}
	204	6.28×10^{-4}	6.01	2.43	1.90	8.32×10^{4}
	272	8.58×10^{-4}	6.32	3.32	2.70	1.51×10^{5}
	340	1.18×10^{-3}	6.64	4.55	3.99	2.61×10^{5}
	408	1.14×10^{-3}	6.82	5.45	5.05	3.03×10^{5}
65°C	136	8.16×10^{-4}	4.22	3.16	1.41	9.25×10^{3}
	204	1.04×10^{-3}	4.46	4.02	2.60	1.76×10^{4}
	272	1.24×10^{-3}	4.64	4.80	3.50	2.82×10^{4}
	340	1.43×10^{-3}	4.78	5.53	4.32	4.05×10^{4}
	408	1.46×10^{-3}	4.80	5.65	4.63	4.96×10^{4}

Table 8

Solubility of Pyrene in Supercritical Carbon Dioxide

T	P	Mole Fraction	$\ln(X_2/X_2^i)$	mg Ext.	g/l	E
35°C	136	6.82×10^{-4}	11.7	2.98	2.40	1.66×10^{7}
	204	7.78×10^{-4}	11.8	3.40	3.01	2.84×10^{7}
	272	8.58×10^{-4}	11.9	3.75	3.47	4.17×10^{7}
	340	8.67×10^{-4}	12.0	3.79	3.64	5.27×10^{7}
	408	8.97×10^{-4}	12.0	3.92	3.88	6.54×10^{7}
47°C	136	7.30×10^{-4}	10.0	3.19	2.14	2.95×10^{6}
	204	7.84×10^{-4}	10.1	3.43	2.70	4.96×10^{6}
	272	8.15×10^{-4}	10.1	3.56	2.92	6.88×10^{6}
	340	8.47×10^{-4}	10.2	3.70	3.28	8.95×10^{6}
	408	8.65×10^{-4}	10.2	3.78	3.50	1.10×10^{7}
60°C	136	2.44×10^{-4}	7.10	1.07	0.53	1.64×10^{5}
	204	3.31×10^{-4}	7.41	1.45	0.99	3.36×10^{5}
	272	4.53×10^{-4}	7.72	1.98	1.50	6.13×10^{5}
	340	6.30×10^{-4}	8.05	2.75	2.22	1.07×10^{6}
	408	9.06×10^{-4}	8.41	3.96	3.36	4.42×10^{6}

Table 9

Solubility of Phenanthrene in Supercritical Carbon Dioxide

T	P	Mole Fraction	$\ln(X_2/X_2^i)$	mg Ext.	g/l	E
35°C	136	1.25×10^{-3}	7.65	5.46	4.41	2.86×10^{5}
	204	1.74×10^{-3}	7.98	7.61	6.73	5.97×10^{5}
	272	1.96×10^{-3}	8.10	8.57	7.93	8.96×10^{5}
	340	2.09×10^{-3}	8.17	9.14	8.78	1.19×10^{6}
	408	2.09×10^{-3}	8.17	9.14	9.04	1.43×10^{6}
45°C	136	1.18×10^{-3}	6.02	5.16	3.56	5.57×10^{4}
	204	1.90×10^{-3}	6.49	8.30	6.70	1.35×10^{5}
	272	2.34×10^{-3}	6.70	10.2	8.59	2.21×10^{5}
	340	2.64×10^{-3}	6.82	11.5	10.3	3.11×10^{5}
	408	2.86×10^{-3}	6.90	12.5	11.7	4.05×10^{5}
58°C	136	1.47×10^{-3}	4.64	6.43	3.38	1.51×10^{4}
	204	2.28×10^{-3}	5.08	9.97	7.02	3.27×10^{4}
	272	2.90×10^{-3}	5.32	12.7	9.75	5.55×10^{4}
	340	3.33×10^{-3}	5.46	14.6	11.8	7.97×10^{4}
	408	3.54×10^{-3}	5.52	15.5	13.3	1.02×10^{5}

Table 10

Solubility of Benz(a)anthracene in Supercritical Carbon Dioxide

T	P	Mole Fraction	$\ln(X_2/X_2^i)$	mg Ext.	g/l	E
37°C	136	3.18×10^{-6}	13.3	1.39×10^{-2}	1.09×10^{-2}	7.94×10^{7}
	204	5.84×10^{-6}	13.9	2.55×10^{-2}	2.22×10^{-2}	2.18×10^{8}
	272	6.36×10^{-6}	14.0	2.78×10^{-2}	2.53×10^{-2}	3.18×10^{8}
	340	6.45×10^{-6}	14.0	2.82×10^{-2}	2.66×10^{-2}	4.03×10^{8}
	408	6.54×10^{-6}	14.0	2.86×10^{-2}	2.80×10^{-2}	4.91×10^{8}
45°C	136	6.20×10^{-6}	12.6	2.71×10^{-2}	1.87×10^{-2}	4.04×10^{7}
	204	7.50×10^{-6}	12.8	3.28×10^{-2}	2.64×10^{-2}	7.32×10^{7}
	272	7.50×10^{-6}	12.8	3.28×10^{-2}	2.75×10^{-2}	9.76×10^{7}
	340	7.50×10^{-6}	12.8	3.28×10^{-2}	2.93×10^{-2}	1.22×10^{8}
	408	7.57×10^{-6}	12.8	3.31×10^{-2}	3.09×10^{-2}	1.47×10^{8}
55°C	136	7.31×10^{-6}	11.2	3.20×10^{-2}	1.81×10^{-2}	9.95×10^{6}
	204	8.08×10^{-6}	11.3	3.53×10^{-2}	2.56×10^{-2}	1.64×10^{7}
	272	8.93×10^{-6}	11.4	3.90×10^{-2}	3.03×10^{-2}	2.43×10^{7}
	340	9.14×10^{-6}	11.4	3.99×10^{-2}	3.36×10^{-2}	3.11×10^{7}
	408	10.1×10^{-6}	11.5	4.41×10^{-2}	3.87×10^{-2}	4.12×10^{7}

plotted against molecular weight, with a correlation coefficient of 0.960. This shows that if the solubilities in a SCF of some compounds in a similar class are known, other compounds in that class can be predicted.

Most of the solubility values determined in this study are of the order of milligrams extracted per 500 ml (at STP). This is much greater than the amounts found on high volume air filters (on the order of micrograms). In one compound, benz(a)anthracene, approximately 10-50 micrograms were extracted per 500 ml carbon dioxide (at STP). In the extraction of the high volume air filters, approximately 3000ml (at STP) of carbon dioxide were used. This was more than enough to extract quantitatively all of the PAH's extracted.

Comparison of Solubility Data with Literature Solubility Data

Although numerous applications of supercritical fluid extraction have appeared in the literature, there are very few that determine quantitatively the solubility of solids over a range of temperatures and pressures (78,89-95). In one of these studies (93), the solubility values of naphthalene are used as "standard" values. Naphthalene, in the apparatus used in this study, cannot be extracted quantitatively and therefore cannot be used for comparison. Of the other papers only one has a hydrocarbon-supercritical fluid system identical to one in this study (89). The solubility of phenanthrene in supercritical carbon dioxide was carried out by Johnston in the temperature and pressure range of 30.0-70.0°C and 79.8-409.1 atmospheres respectively. In this study, the temperature and pressure ranges were 35.0-58.0°C and 136-408 atmospheres respectively. To determine the solubility of a solid in a supercritical solvent using the method used by Johnston, a solid solute was packed into the 15cm by 0.79cm ID column or saturator in a constant temperature bath. The solvent gas was compressed into the reservoir and bled into the system at a regulated pressure (spring-loaded regulator), at a flow rate controlled by a 0.157cm orifice micrometering valve. The solvent reached bath temperature in an

immersed heat exchange coil and then equilibrated with the solute. Next, the fluid was flashed to ambient pressure and the solute collected in a cold trap. The volume of gas flow was measured with a calibrated wet test meter. A sufficient amount of solid was initially collected in a waste trap until the saturator equilibrated with respect to composition, temperature, and pressure. Then a tared trap was substituted. The solubility was determined from the weight gain in the trap. Figure 3 shows a schematic of their apparatus.

Figure 4 shows a plot of solubility as a function of pressure at various temperatures. Each curve is for the specified temperature and the black figures (circles, triangles, and squares) represent data of Johnston (89) and the white figures are data from this study. As can be seen, at pressures greater than 260 atm, there seems to be a high degree of agreement between the two studies. At pressures below 260 atm, the solubilities reported by Johnston are consistently lower than the data from this study. The data of Johnston were interpolated to give mole fractions at pressures of 272, 340 and 408 atm. Isobaric curves of 10^4 × mole fraction versus temperature were plotted and are shown in Figure 5. The values at 30°C were omitted since they correspond to a subcritical state. The three curves have high correlation coefficients of 0.964, 0.997, and 0.983 for pressures of 272, 340, and 408 atmospheres respectively. This shows that the data obtained in this study are in good agreement with those of Johnston (89).

Figure 3. The Schematic of the Supercritical fluid Apparatus Used by Johnston and Eckert (150).

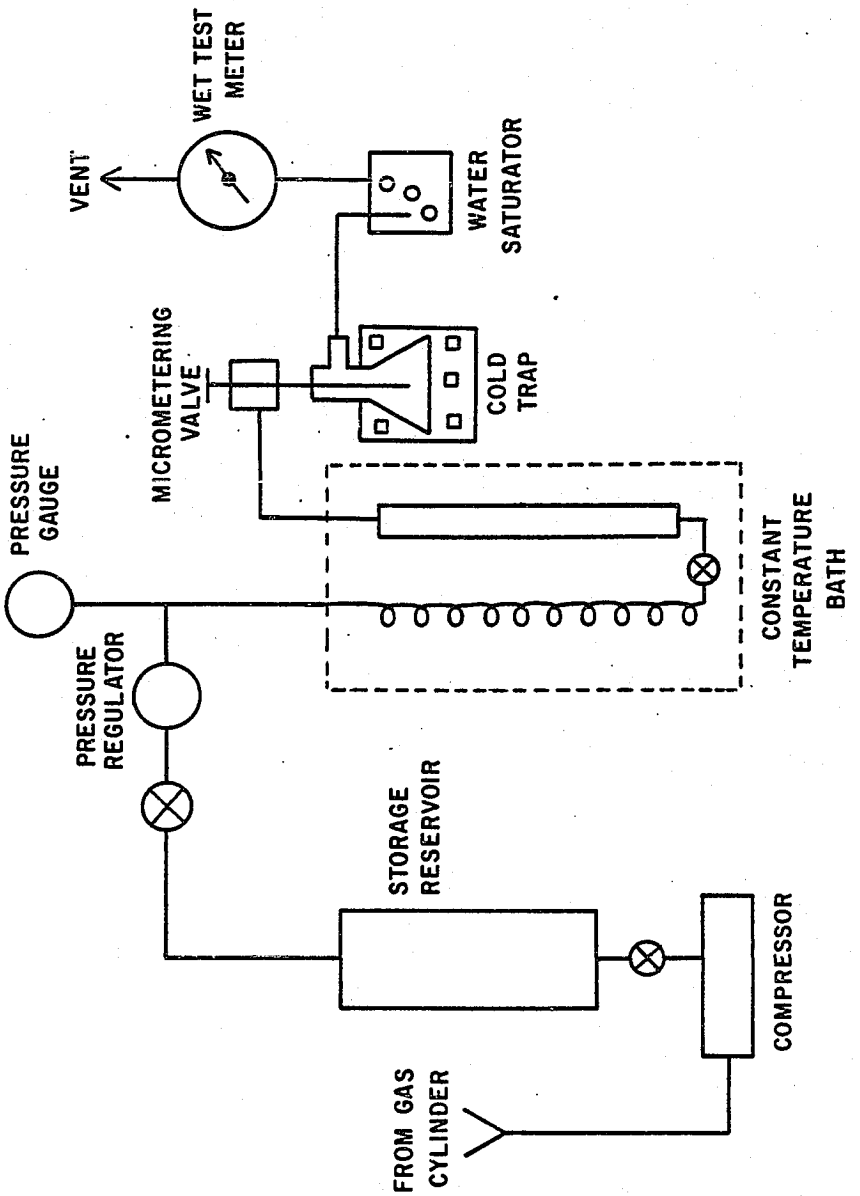


Figure 4. 10^4 × Mole Fraction vs. Pressure at Constant Temperatures of Phenanthrene in Supercritical Carbon Dioxide.

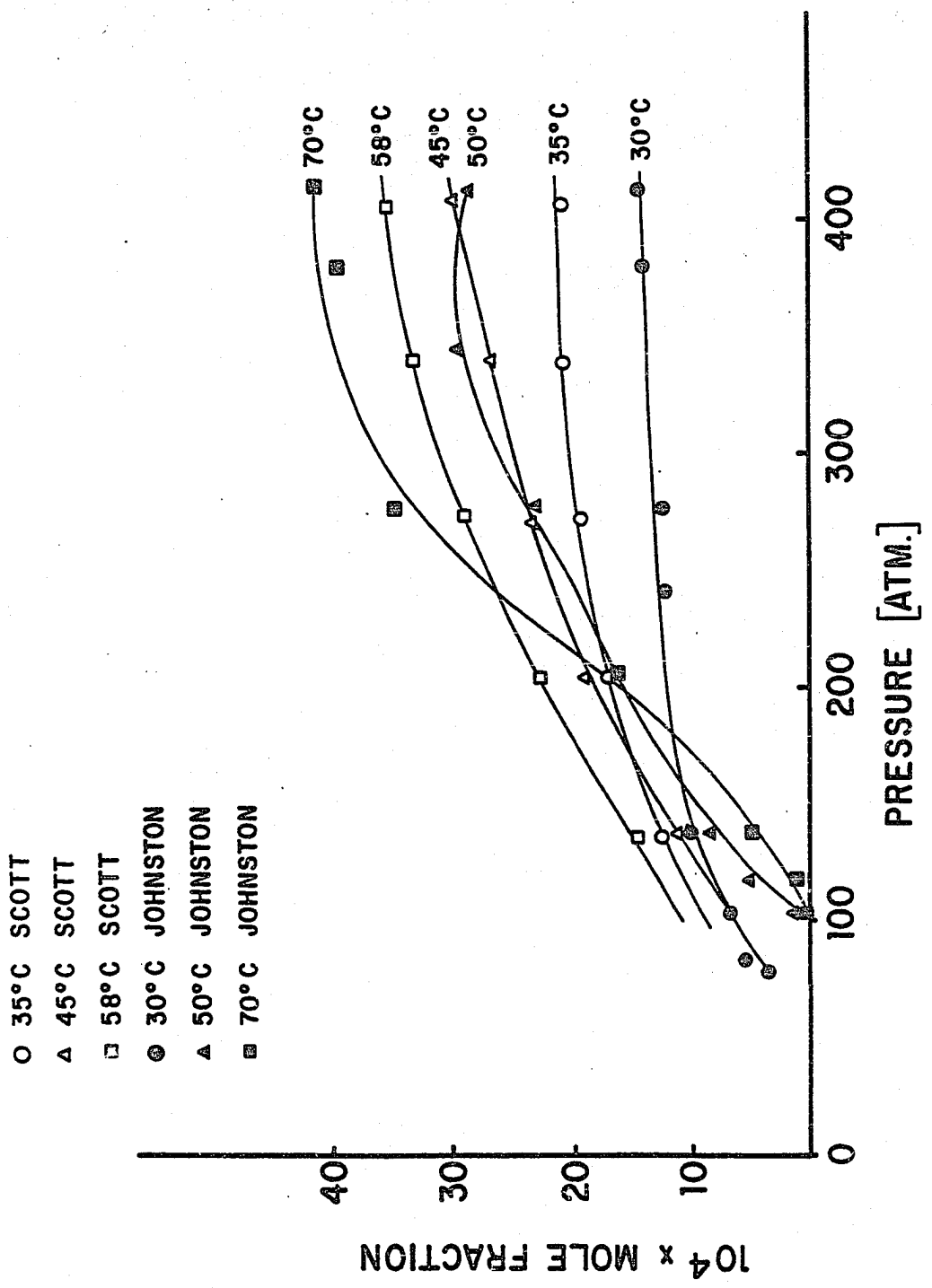
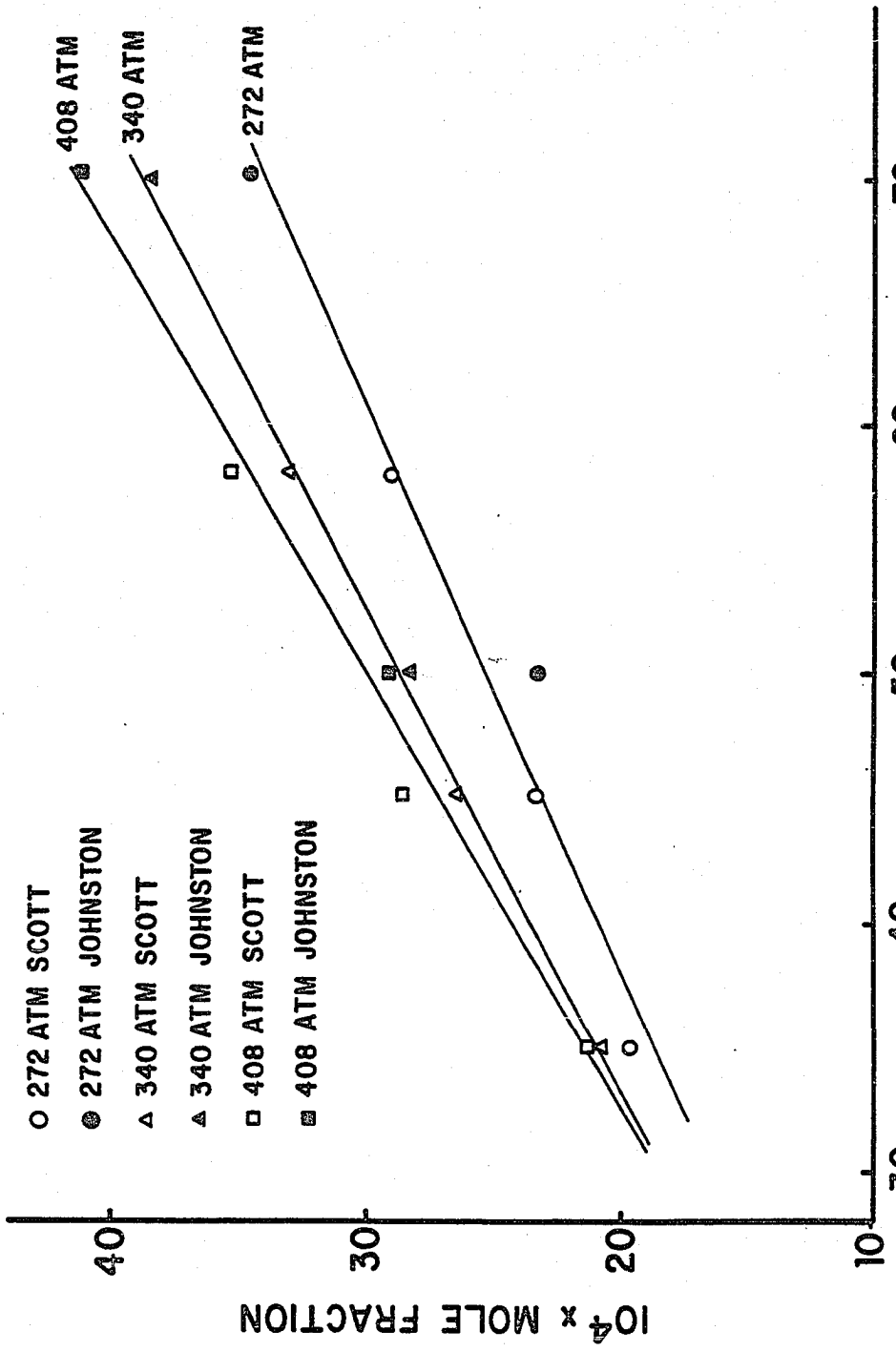


Figure 5. 10^4 × Mole Fraction vs. Temperature at Constant Pressure of Phenanthrene in Supercritical Carbon Dioxide



Interpretation of Results in Terms of Thermodynamics

As discussed earlier, Equation 8 is applicable to gas densities up to about 10 moles/liter (65). In this study, densities up to 20.2 moles/liter were used. Therefore, the virial coefficients obtained from Equation 8 may have reduced physical meaning and may be limited to correlating solubilities for a particular SCF/solid solute system.

To derive virial coefficients from Equation 8, only the first two terms on the right hand side are used. The equation is multipled through by v to give

$$v\ln(\chi_2/\chi_2^i) = v_2^s - 2B_{12} + \frac{v_2^s B_{11} - \frac{3}{2}C_{112}}{v} \qquad (16)$$

For each of the three temperatures studied, the five values of $v\ln(\chi_2/\chi_2^i)$ are plotted against 1/v. A least squares analysis is then performed and the equation of a straight line is generated with a slope of $v_2^s B_{11} - \frac{3}{2}C_{112}$ and a y-intercept of $v_2^s - 2B_{12}$. The values of correlation coefficients of these straight lines range from 0.837 to 0.997 with an average of 0.972 and a median of 0.990. The slope and y-intercept were solved for C_{112} and B_{12} respectively and are summarized in Table 11. These values of B_{12} and C_{112} are used to calculate $\ln(\chi_2/\chi_2^i)$, which are summarized in Tables

Table 11

B_{12} and C_{112} Values for Various Compounds Calculated from Solubility Data

Compound	T °C	B_{12}(cc/mole)	C_{112}((cc/mole)^2)
Fluoranthene	35	-45.0	-15800
	47	-95.0	- 6800
	55	- 1.50	-11300
Acridine	38	-23.0	-22600
	48	- 155	- 8970
	65	- 172	- 1940
Fluorene	35	- 190	-16300
	45	- 161	-17100
	55	- 162	-19100
Phenanthrene	35	- 275	-27800
	45	- 214	-24500
	58	- 265	-26400
Pyrene	35	- 531	+ 9650
	47	- 531	+14700
	60	- 419	+11400
Benz(a)anthracene	37	- 591	+ 9250
	45	- 676	+19400
	55	- 681	+25100

12-17. There is good agreement between the experimental values of $\ln(\chi_2/\chi_2^i)$ and those calculated by using the first two right side terms of Equation 8, i.e. the data are self-consistent.

Extraction of Standard PAH Mixture and Blank

A blank high-volume air filter was placed into the extraction chamber and extracted. The HPLC chromatogram showed no traces of any PAH and it was concluded that the extraction system was free from any co-eluting impurity deriving from the apparatus or the work-up.

In an experiment to determine the percent recovery of various PAH's, a known amount of a synthetic PAH standard was spiked on a blank 6 inch by 1 inch glass fiber filter. The results of three trials are summarized in Table 18. With the exception of naphthalene, compounds with two aromatic rings (but $>C_{10}$) (fluorene, acenaphthalene, and acenaphthylene) had an average percent recovery of 97.7%. Naphthalene had an average percent recovery of 38%. Compounds with three aromatic rings (phenanthrene, anthracene, and fluoranthene) had an average percent recovery of 98.5%; four aromatic rings (pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, and benz(a)anthracene), 98.5%; and five aromatic rings (benz(a)pyrene, dibenzo(a,h)anthracene, benzo(ghi)perylene, and indeno(1,2,3-c,d)pyrene), 92.0%. This experiment demonstrates that supercritical carbon dioxide can be used effectively as a quantitative extraction method for

Table 12

Experimental and Calculated Values of $\ln(\chi_2/\chi_2^i)$ for Acridine

T°C	Pressure (atm)	$\ln(\chi_2/\chi_2^i)$		
		Exp.	1 Term (Eq.8)	2 Terms (Eq.8)
38	136	6.10	6.47	6.10
38	204	7.12	7.20	7.12
38	272	7.71	7.59	7.69
38	340	8.11	7.88	8.13
38	408	8.27	8.10	8.45
48	136	5.37	5.10	5.35
48	204	6.01	6.01	6.06
48	272	6.32	6.27	6.24
48	340	6.64	6.76	6.57
48	408	6.82	7.13	6.82
65	136	4.42	2.83	4.29
65	204	4.46	4.07	4.81
65	272	4.64	4.61	4.79
65	340	4.78	4.93	4.67
65	408	4.80	5.17	4.55

Table 13

Experimental and Calculated Values of

$\ln(\chi_2/\chi_2^i)$ for Phenanthrene

T°C	Pressure (atm)	$\ln(\chi_2/\chi_2^i)$		
		Exp.	1 Term (Eq.8)	2 Terms (Eq.8)
35	136	7.65	7.09	7.67
35	204	7.98	7.77	7.94
35	272	8.10	8.14	8.08
35	340	8.17	8.45	8.16
35	408	8.17	8.70	8.21
45	136	6.02	5.53	6.02
45	204	6.49	6.47	6.53
45	272	6.70	6.73	6.64
45	340	6.82	7.15	6.82
45	408	6.90	7.49	6.92
58	136	4.64	3.80	4.58
58	204	5.08	5.08	5.23
58	272	5.32	5.55	5.36
58	340	5.46	5.87	5.41
58	408	5.52	6.22	5.43

Table 14

Experimental and Calculated Values of $\ln(\chi_2/\chi_2^i)$ for Benz(a)anthracene

T°C	Pressure (atm)	$\ln(\chi_2/\chi_2^i)$		
		Exp.	1 Term (Eq.8)	2 Terms (Eq.8)
37	136	13.3	12.2	13.4
37	204	13.9	13.5	13.8
37	272	14.0	14.1	13.9
37	340	14.0	14.6	14.0
37	408	14.0	15.2	14.1
45	136	12.6	10.2	12.5
45	204	12.8	11.9	12.9
45	272	12.8	12.4	12.9
45	340	12.8	13.2	12.8
45	408	12.8	13.8	12.7
55	136	11.2	8.05	11.0
55	204	11.3	10.3	11.6
55	272	11.4	11.0	11.6
55	340	11.4	12.0	11.4
55	408	11.5	12.5	11.1

Table 15

Experimental and Calculated Values of $\ln(\chi_2/\chi_2^i)$ for Pyrene

T°C	Pressure (atm)	$\ln(\chi_2/\chi_2^i)$		
		Exp.	1 Term (Eq.8)	2 Terms (Eq.8)
35	136	11.7	10.5	11.7
35	204	11.8	11.5	11.9
35	272	11.9	12.1	12.0
35	340	12.0	12.5	12.0
35	408	12.0	12.9	12.0
47	136	10.0	8.41	9.91
47	204	10.1	9.87	10.2
47	272	10.1	10.3	10.2
47	340	10.2	11.1	10.2
47	408	10.2	11.6	10.1
60	136	7.10	5.18	7.03
60	204	7.41	7.02	7.94
60	272	7.72	7.76	8.06
60	340	8.05	8.27	8.09
60	408	8.41	8.69	8.07

Table 16

Experimental and Calculated Values of $\ln(\chi_2/\chi_2^i)$ for Fluorene

T°C	Pressure (atm)	$\ln(\chi_2/\chi_2^i)$		
		Exp.	1 Term (Eq.8)	2 Terms (Eq.8)
35	136	6.91	6.74	6.95
35	204	7.50	7.38	7.45
35	272	7.74	7.73	7.71
35	340	7.94	8.02	7.92
35	408	8.02	8.26	8.08
45	136	4.93	4.63	4.95
45	204	5.46	5.42	5.48
45	272	5.70	5.64	5.61
45	340	5.84	5.99	5.82
45	408	5.90	6.28	5.96
55	136	3.80	3.24	3.78
55	204	4.22	4.16	4.27
55	272	4.37	4.45	4.38
55	340	4.48	4.82	4.47
55	408	4.54	5.04	4.50

Table 17

Experimental and Calculated Values of $\ln(\chi_2/\chi_2^i)$ for Fluoranthene

T°C	Pressure (atm)	$\ln(\chi_2/\chi_2^i)$		
		Exp.	1 Term (Eq.8)	2 Terms (Eq.8)
35	136	5.08	4.96	4.82
35	204	5.36	5.44	5.36
35	272	5.65	5.70	5.65
35	340	5.88	5.91	5.89
35	408	6.11	6.09	6.10
47	136	3.32	2.86	3.29
47	204	3.50	3.36	3.55
47	272	3.59	3.50	3.60
47	340	3.68	3.77	3.69
47	408	3.78	3.95	3.74
55	136	2.00	1.69	1.95
55	204	2.19	2.17	2.29
55	272	2.35	2.32	2.38
55	340	2.50	2.52	2.48
55	408	2.60	2.63	2.53

Table 18

Supercritical CO_2 Extraction of Synthetic PAH Standard

#	Compound	Percentage Recovered			
		1	2	3	Avg.
4	Naphthalene	35.3	41.0	37.7	38.0
5	Acenaphthylene	95.4	96.3	96.0	95.9
6	Acenaphthene +				
	Fluorene	99.0	103	101	101
7	Phenanthrene	98.4	98.9	98.9	98.7
8	Anthracene	99.4	101	100	100
9	Fluoranthene	98.7	99.5	99.4	99.2
10	Pyrene	98.4	99.1	98.9	98.8
11	Chrysene +				
	Benz(a)anthracene	96.4	101	99.8	99.1
12	Benz(b)fluoranthene +				
12a	Benz(k)fluoranthene	93.0	96.8	96.2	95.3
13	Benz(a)pyrene	94.8	96.0	96.4	95.7
14	Dibenzo(a,h)anthracene	92.0	93.1	93.0	92.7
15	Benzo(ghi)perylene	90.1	90.5	90.4	90.3
16	Indeno(1,2,3-c,d)pyrene	88.9	90.0	89.2	89.4

PAHs less volatile than naphthalene.

Comparison of Supercritical Fluid Extraction and Soxhlet Extraction

Three hi-vol air filters, exposed at unknown locations, were analyzed for PAHs. Figure 6 shows a HPLC chromatogram of the PAH standard. Figure 7 represents a HPLC chromatogram of one half of sample #03195 which was extracted with supercritical carbon dioxide. The cyclohexane Soxhlet extract of the other half of sample #03195 is shown in Figure 8. Quantitation was performed by direct comparison of the peak areas of the sample with a previously injected standard. The values are tabulated in Table 19. The ratios of the amount extracted by supercritical carbon dioxide to the amount extracted by soxhlet extraction vary from 0.25 to 1.56.

Two other filters (#27207 and #30224) were extracted by both supercritical carbon dioxide and soxhlet using cyclohexane. The results are summarized in Tables 20 and 21. In filter #27207, the ratios of the amount extracted by supercritical carbon dioxide to the amount extracted by soxhlet range from 0.15 to 2.67. The ratios in filter #30224 range from 0.16 to 1.62. When all three filters are averaged, the range in the ration of SCFE/Soxhlet ranges from 0.15 to 2.67.

The differences in the amounts of PAH extracted by supercritical carbon dioxide as compared to cyclohexane may be caused by co-eluting impurities extracted by the SCF but not by the cyclohexane. Since a blank filter was run, impurities

Figure 6. An HPLC Trace of a Synthetic Polynuclear Aromatic Hydrocarbon Mixture.

Chromatographic conditions: Acetonitrile/water 40/60, 1.0 ml/min., 10µl injection, 1.2% acetonitrile/min. gradient. Column: E.M. LiChrosorb RP-18 (25 cm., 4.0 mm id., 10µm). Peaks: (1) unknown ; (2) unknown ; (3) unknown ; (4) naphthalene ; (5) acenaphthylene ; (6) acenaphthene + fluorene ; (7) phenanthrene ; (8) anthracene ; (9) fluoranthene ; (10) pyrene ; (11) benz(a)anthracene + chrysene ; (12) benz(b)fluoranthene ; (12a) benz(k)fluoranthene ; (13) benz(a)pyrene ; (14) dibenzo(a,h)anthracene ; (15) benzo(ghi)perlyene ; (16) indeno(1,2,3-c,d)pyrene.

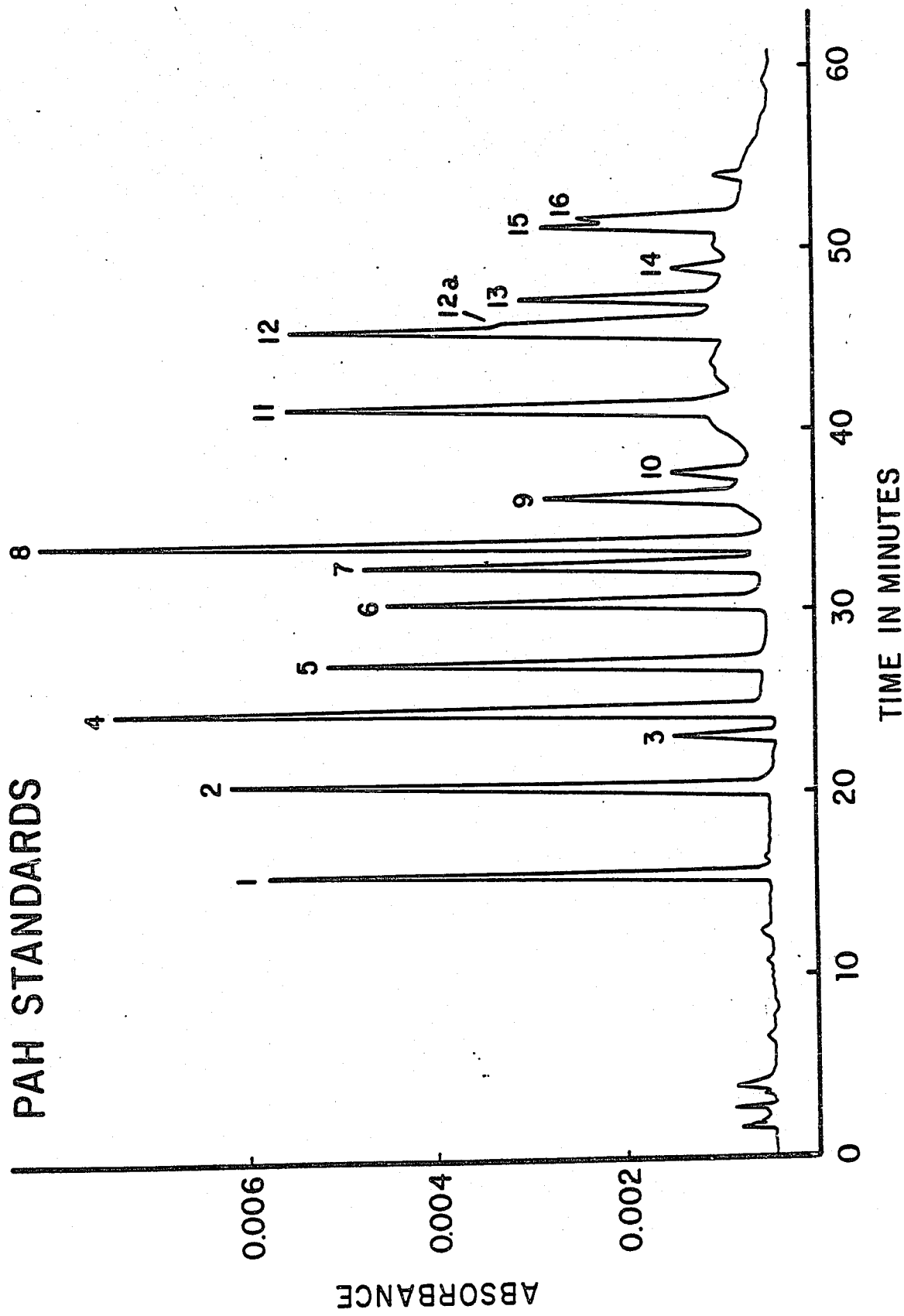


Figure 7. An HPLC Chromatogram of Sample #03195 that was Extracted with Supercritical Carbon Dioxide.
Chromatographic conditions and peak numbers same as in Figure 6.

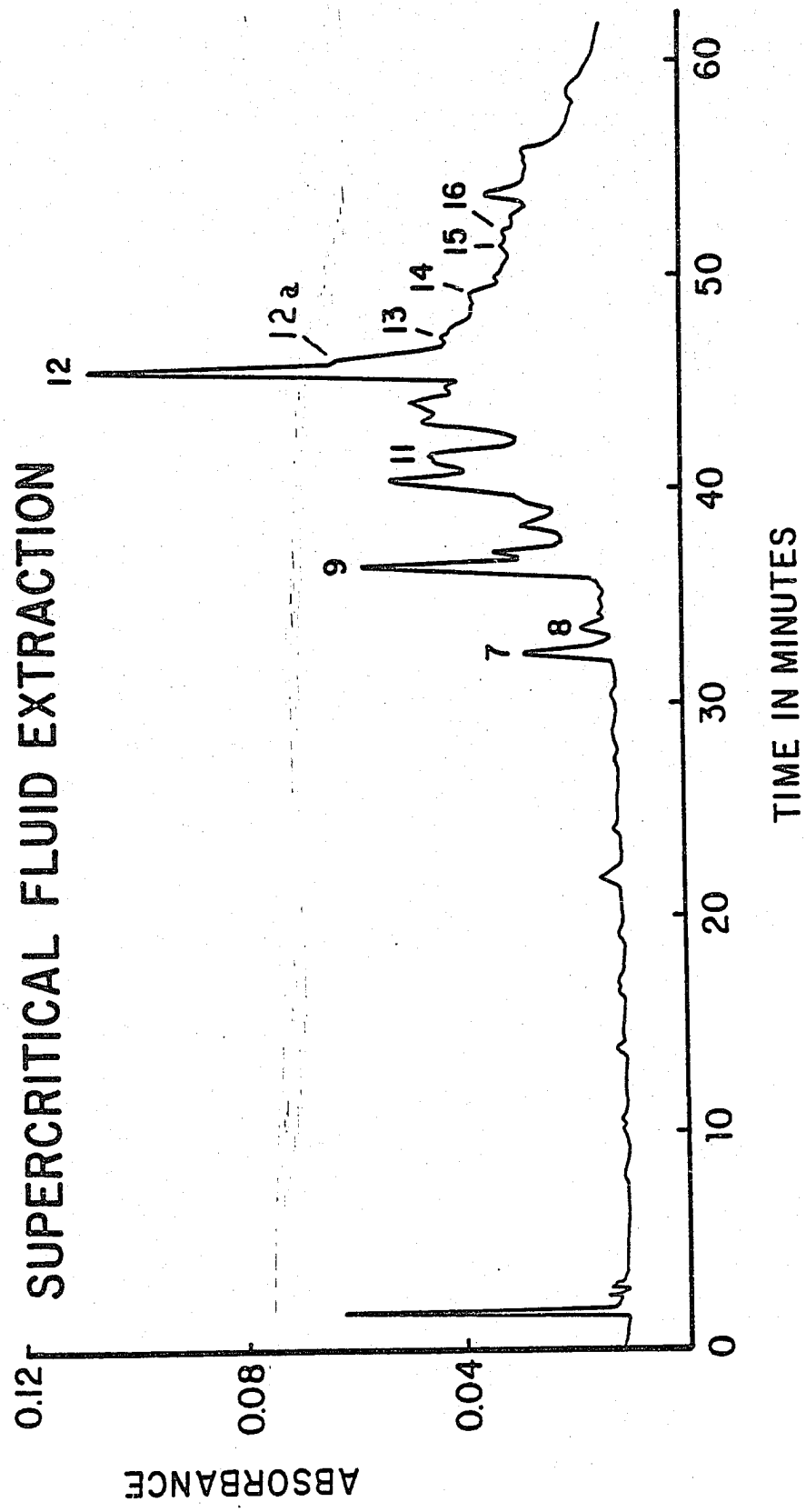


Figure 8. An HPLC Chromatogram of Sample #03195 that was Extracted by Soxhlet with Cyclohexane. Chromatographic conditions and peak numbers same as in Figure 6.

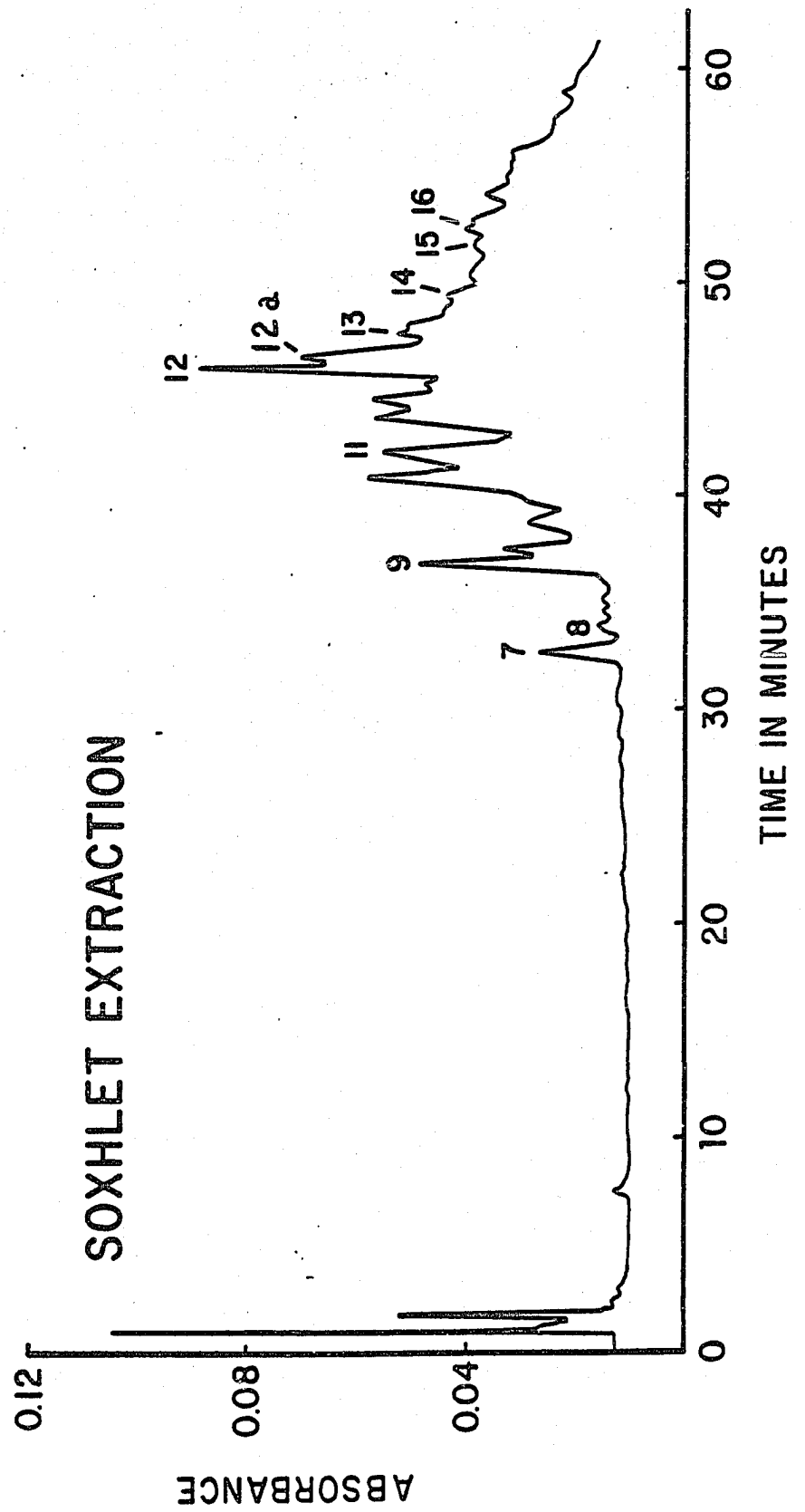


Table 19

Comparison of Extraction of Air Filter #03195
Using Both Supercritical Carbon Dioxide
and Soxhlet with Cyclohexane

Compound	SF Extracted	Soxhlet Extracted	Ratios SF/Sox
Phenanthrene	37.0 ng	33.4 ng	1.11
Anthracene	6.2	4.1	1.50
Fluoranthene	362	269	1.35
Chrysene + Benz(a)anthracene	50.8	84.8	0.60
Benz(b)fluoranthene	290	186	1.56
Benz(a)pyrene	9.4	30.2	0.31
Dibenzo(a,h)anthracene	28.6	114	0.25
Benzo(ghi)perylene	17.1	34.2	0.50
Indeno(1,2,3-c,d)-pyrene	2.0	3.0	0.67

Table 20

Comparison of Extractions of Air Filter #27207 Using Both Supercritical Carbon Dioxide and Soxhlet with Cyclohexane

Compound	SF Extracted	Soxhlet Extracted	Ratios SF/Sox
Phenanthrene	40.8 ng	55.5 ng	0.73
Anthracene	1.5	10.2	0.15
Fluoranthene	400	385	1.04
Pyrene	400	150	2.67
Chrysene + Benz(a)anthracene	220	152	1.44
Benz(b)fluoranthene	294	121	2.43
Benz(a)pyrene	3.8	9.5	0.40
Dibenzo(a,h)anthracene	71.4	64.4	1.11
Benzo(ghi)perylene	17.1	28.6	0.60
Indeno(1,2,3-c,d)pyrene	10.0	25.1	0.40

Table 21

Comparison of Extraction of Air Filter #30224 Using Both Supercritical Carbon Dioxide and Soxhlet with Cyclohexane

Compound	SF Extracted	Soxhlet Extracted	Ratios SF/Sox
Phenanthrene	19.5 ng	80.5 ng	0.24
Fluoranthene	385	365	1.05
Pyrene	200	500	0.40
Chrysene + Benz(a)anthracene	135	135	1.00
Benz(b)fluoranthene	20.6	130	0.16
Benz(a)pyrene	37.7	60.4	0.62
Benzo(ghi)perylene	343	686	0.50
Indeno(1,2,3-c,d)pyrene	30	150	0.20

from the SCF extraction apparatus was rules out. Unknown compounds may have been extracted at different percent recoveries (SCF compared to soxhlet) and are co-eluting with known peaks. Co-eluting peaks give rise to the interference of determining peak areas. When two adjacent peaks are not well resolved, there is an uncertainty in each peak area. This uncertainty increases as the concentrations and/or resolution decrease. Another possible reason for the discrepancy may be that the air filter is not uniform. This could arise in the course of air sampling when air that contains a greater amount of certain PAHs falls upon part of the high volume air filter. If this were the case, it would account for the discrepancies of the amount of PAH extracted.

Overall, this experiment demonstrates that supercritical carbon dioxide extraction is comparable to soxhlet extraction for PAHs from high-volume air filters. Using the apparatus in Figure 2, the extraction can be performed in 30 minutes. The soxhlet extraction required 7 hours. Therefore, not only were the results comparable, but the SCF extraction was performed in only a fraction of the time needed for the soxhlet extraction. The apparatus could easily be modified to include many extraction chambers rather than just one. This will enable one to extract many more high-volume air filters per day than are currently being extracted by soxhlet.

Determination of PAHs from High-Volume Air Filters

Two high-volume air filter samples from Frost Street, in the Greenpoint section of Brooklyn, and two high-volume air filter samples from an unknown location on the side of the Long Island Expressway (LIE) were taken on June 21 and 22, 1982 and April 1 and 2, 1982, respectively. They were extracted with supercritical carbon dioxide and analyzed by HPLC as described before.

The results are summarized in Tables 22-23. As can be seen, there is a great day to day variation. This variation can be attributed in part to the weather. Wind speed direction, precipitation, and temperature can all have an effect on the amount of PAHs in the air (96). The differences between sites are primarily caused by differences in the neighborhood. The PAH content at the site by the LIE is caused mainly by traffic while the Frost Street sample is caused by mixed sources including residential furnaces.

Table 22

Amount of PAHs Extracted with Supercritical Carbon Dioxide from Two Frost Street Samples Collected on June 21 and 22, 1982

Compound	June 21	June 22
Phenanthrene	90.5 ng	24.0 ng
Anthracene	8.2	2.1
Fluoranthene	269	50.0
Pyrene	25.0	15.0
Chrysene + Benz(a)anthracene	33.8	6.8
Benz(b)fluoranthene	31.0	65.6
Benzo(ghi)perylene	68.6	--
Indeno(1,2,3-c,d)pyrene	10.0	--

Table 23

Amounts of PAHs Extracted with Supercritical Carbon Dioxide from Two Long Island Expressway Samples Collected on April 1 and 2, 1982

Compound	April 1	April 2
Phenanthrene	30.1 ng	13.5 ng
Anthracene	16.4	7.2
Fluoranthene	38.5	423
Pyrene	--	3775
Chrysene +		
Benz(a)anthracene	98.4	372
Benz(b)fluoranthene	--	552
Benz(a)pyrene	--	7.5
Dibenzo(a,h)anthracene	--	114
Benzo(ghi)perylene	--	206
Indeno(1,2,3-c,d)pyrene	--	171

CONCLUSION

This study was conducted in order to determine if supercritical carbon dioxide is capable of quantitatively extracting organics from high-volume air pollution filters. The study was also undertaken to determine the solubility of various compounds in supercritical carbon dioxide at different temperatures and pressures.

A working apparatus was set up and various collectors were considered. A C-18 Waters Sep-pak was chosen as an inexpensive and easily removable collector. It was determined that the sep-pak collector failed to quantitatively trap naphthalene, but it was effective in trapping compounds with a vapor pressure less than or equal to that of fluoranthene. The sep-pak collector was shown to be reproducible and was easily removed. Half of the various high-volume air pollution filters were extracted using supercritical carbon dioxide and compared with their corresponding other halves which were Soxhlet extracted in cyclohexane for seven hours. All samples were purified by silica-TLC and quantitated by reverse phase HPLC. There was a very high correlation between soxhlet extraction and supercritical carbon dioxide extraction. It was shown that supercritical carbon dioxide extractions can be used as a replacement for soxhlet extractions.

The solubility of various organics in supercritical

carbon dioxide was also determined. Solubility data from this study was compared with data found in the literature (89). There was a very high correlation between the two studies and it was assumed that the apparatus used in this study was capable of producing similar solubility data as found in the literature.

In summary, it was determined supercritical carbon dioxide can be used in extractions of organics from high-volume air pollution filters. This technique enables one to extract more filters since the time required to perform a supercritical carbon dioxide extraction is only a fraction of the time required for a soxhlet extraction. This study has shown that supercritical carbon dioxide can be used to replace soxhlet extractions.

More work needs to be done on supercritical fluid extractions. The solubility of organic compounds from various classes need to be determined. Once this is accomplished, the questions of theory and thermodynamics may be answered. Then a study of mixed supercritical fluids needs to be undertaken. The use of supercritical fluids as an extraction medium is a major advancement in the field of analytical chemistry. As a greater understanding of supercritical fluid is realized, supercritical fluids will emerge as the leading technique of extraction.

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Part II. N-Nitrosomorpholine and Other Volatile N-Nitrosamines in Snuff Tobacco

INTRODUCTION

Snuff use has been suggested as a smokeless alternative to cigarettes (1-3). Although snuff dipping has not been demonstrated to represent a risk factor for lung cancer, its association with increased risks for cancer of the oral cavity and cancer of the pharynx has been established (4,5). Snuff has induced lesions of the oral mucosa in rats (6) and it has been found to contain relatively high levels of carcinogenic, tobacco-specific N-nitrosamines (TSNA*; 1-80 ppm). These compounds are formed during tobacco processing and, most likely also during chewing of tobacco, as indicated by the presence of up to 0.9µg/ml TSNA in the saliva of snuff dippers (7,8). U.S. snuff tobaccos also contained up to 7 ppm of the carcinogenic N-nitrosodiethanolamine (NDELA; 9).

Further analytical investigations revealed that snuff tobaccos also contain volatile carcinogenic N-nitrosamines (VNA) including N-nitrosomorpholine (NMOR). This is the subject of this study.

MATERIALS AND METHODS

Apparatus

For the analyses of the VNA in a concentrate from snuff, we employed a modified GC-TEA system and gas-chromatographic conditions described previously (9,10). Mass spectral analyses were performed on a Hewlett-Packard Model 5710-5980 instrument.

Snuff

Commercial U.S. snuff products were purchased in 1981 in New York and in Tennessee; Swedish snuff originated from Stockholm. A recently introduced snuff brand (USA V) was purchased in North Carolina. All snuff samples were stored in a cold room (3°C) and were opened only immediately before the analyses.

Reagents

The reference mixture of seven VNA including NMOR was purchased from Thermo Electron Corp., Waltham, MA, USA. 2,6-Dimethylmorpholine was obtained as a *cis*- and *trans*-mixture from Aldrich Chemical Co., Inc., Milwaukee, WI, USA. Its *cis*-isomer (~65%) was isolated and purified by preparative GC (12mm o.d. × 4m stainless steel column, packed with 10% UC-98 on Chromosorb WAW DMCS; column temp. 150°C). The purified *cis*-isomer was nitrosated with sodium nitrite and 0.1 *N* acetic acid (pH 4). The resulting N-nitroso-cis-2,6-dimethyl-

morpholine (NDMMOR) was purified by column chromatography with dichloromethane (DCM) on basic alumina (Woelm; act II-III). The purity of NDMMOR was then ascertained by GC-TEA.

NMOR-U-^{14}C was prepared by nitrosation of morpholine-U-^{14}C (24.7μCi/μM; New England Nuclear, Waltham, MA, USA) with $NaNO_2$ and 0.1 N acetic acid at pH 4. The reaction mixture was extracted with DCM and purified by column chromatography on basic alumina (Woelm; act. II-III). The radiopurity of NMOR-U-^{14}C was ascertained by repeated column chromatography until the specific activity was stable.

Methanol, acetone, ascorbic acid and Celite 545 were obtained from Fisher Scientific Co., Springfield, NJ. DCM was freshly redistilled over an excess of ascorbic acid assuring that the solvent was free of VNA. All other solvents and agents were free of VNA according to GC-TEA analysis.

Snuff Analysis for VNA

Twenty grams of snuff were extracted for 2 hrs. under magnetic stirring with 150 ml citrate phosphate buffer (pH 4.5) containing 20 mM ascorbic acid and [^{14}C]NMOR (63,000 dpm = 0.143μg) as an internal standard. The mixture was filtered through washed Celite 545 and the filtrate was extracted 4 times with 150ml DCM. The combined DCM-layers were dried (Na_2SO_4) and reduced to about 5 ml. The VNA concentrate was chromatographed on a column of 65g basic alumina (Woelm; act. II-III) with DCM. The resulting 50ml fractions

were monitored for β-activity. In most cases, the VNA eluted in fractions 2-4. These fractions were concentrated to 1 ml and aliquots were counted for determining the recovery rate; other aliquots were analyzed by GC-TEA to determine the VNA (10).

In two cases we used GC-MS in order to confirm the identity of the compound which had the retention time of NMOR in the GC-TEA system. These analyses were started with 50g snuff. The VNA fraction was further enriched by a second chromatographic step using 650g of silica gel prior to GC-MS analyses.

In order to determine whether artifacts led to formation of NMOR (and of other VNA) during the extraction and analysis, we added 31.2mg or 3.12mg of cis-2,6-dimethylmorpholine to the snuff in two separate analyses. This agent was recommended by Mirvish et al. for monitoring artifact formation (11). The average retention time for NMOR in the GC-TEA system was 14 min. while that of NDMMOR was 11 min., thus allowing distinction.

NMOR Analysis of Packaging Material

About 10g of container material (brown cardboard or colored plastic) were homogenized in a blender, then added to 200ml of citrate buffer (pH 4.5) containing 0.7g ascorbic acid and [^{14}C]-NMOR as an internal standard. After two hours of mixing, the homogenate was filtered and extracted four times with 200 ml DCM. The analyses of container wax extracts

for NMOR (and other VNA) were continued in the same manner as those of the snuff samples.

Determination of Morpholine

Twenty-five grams of snuff were suspended in 200ml water containing [^{14}C]morpholine (173,000 dpm = 0.30µg) and were extracted overnight under magnetic stirring. The extract was filtered through Celite, washed with water and acidified with 18 N acetic acid to pH 4.5. The acidic solution was extracted three times with 200 ml ether. Two grams of $NaNO_2$ were then added to the extracted aqueous layer. After 2 hrs. of magnetic stirring the aqueous solution was extracted three times with DCM. The combined DCM extracts were dried (Na_2SO_4), concentrated to about 2ml, and subsequently analyzed for NMOR as described above.

The morpholine analyses of the containers required about 10g of ground materials. The procedure was the same as that for snuff.

Model Study for Transfer of Morpholine

Twenty cardboard snuff containers USA III (~170g) were extracted in a soxhlet with 3000ml n-hexane. The extract was dried (Na_2SO_4) and reduced to about 300ml. The extracted material (~40g) had a waxy consistency. Four aliquots were placed into Petri dishes (i.d. 85mm) together with 0.5ml each of a solution of [^{14}C]morpholine in ethanol (1,516,000 dpm = 2.6µg). After the solvent had evaporated,

about 17g of snuff were placed on top of the wax layer of each Petri dish. The dishes were covered and stored in the dark. The snuff used for this test was free of NMOR. The Petri dishes were left at ambient temperatures (~20°C) for one month; then the snuff was removed and analyzed for [^{14}C]NMOR.

RESULTS

Figure 1 presents a GC-TEA trace of the VNA concentrate from one of the U.S. snuff samples. Significantly, this chromatogram is from one of 7 VNA concentrates among the analyzed snuff samples which had a major peak with the retention time of NMOR. Two analyses and extensive clean-up of 50g snuff samples each were carried out to enable us to identify NMOR in these VNA concentrates by GC-MS (Figure 2). Table 1 lists the results for VNA and morpholine in the 10 snuff samples including the NMOR and morpholine data for the snuff containers. The detection limit for individual VNA varied between 0.5-1 ppb for snuff samples of 20g. According to repeated analyses the standard deviation for NMOR was less than ±10%. In the presence of relatively high concentrations of NMOR (>100 ppb) standard deviations were within ±7%. The recovery rate of NMOR was between 50-70% as measured with [^{14}C]NMOR as an internal standard. NMOR was the major nitrosamine in the snuff containers but, in a few cases, traces of NDMA and NPYR (<3ppb) were also found. As described under MATERIALS AND METHODS, the morpholine analyses were carried out with the N-nitrosation method and with [^{14}C]morpholine as an internal standard. The detection limit was 2 ppb and the recovery rates varied between 70-80%. Since we found morpholine in snuff as well as in the containers, we investigated the possibility of artifactual formation of NMOR

Figure 1. A GC-TEA Trace of the VNA Concentrate from a U.S. Snuff Sample

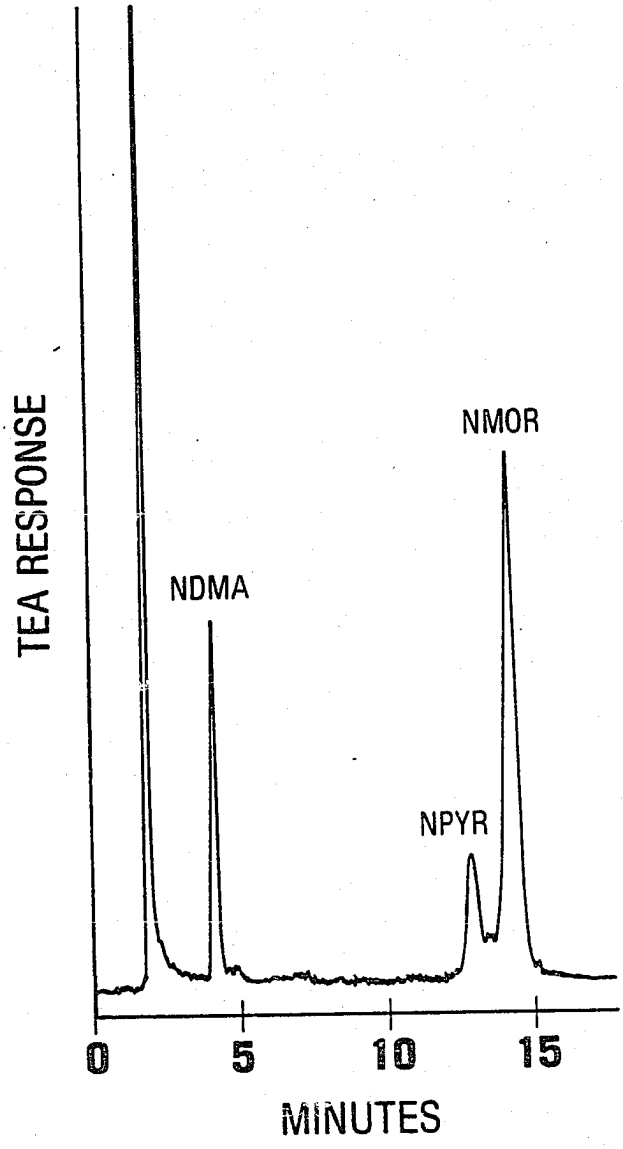

Figure 2. Mass Spectra of Reference and Isolate from Snuff Tobacco Identifying N-Nitrosomorpholine

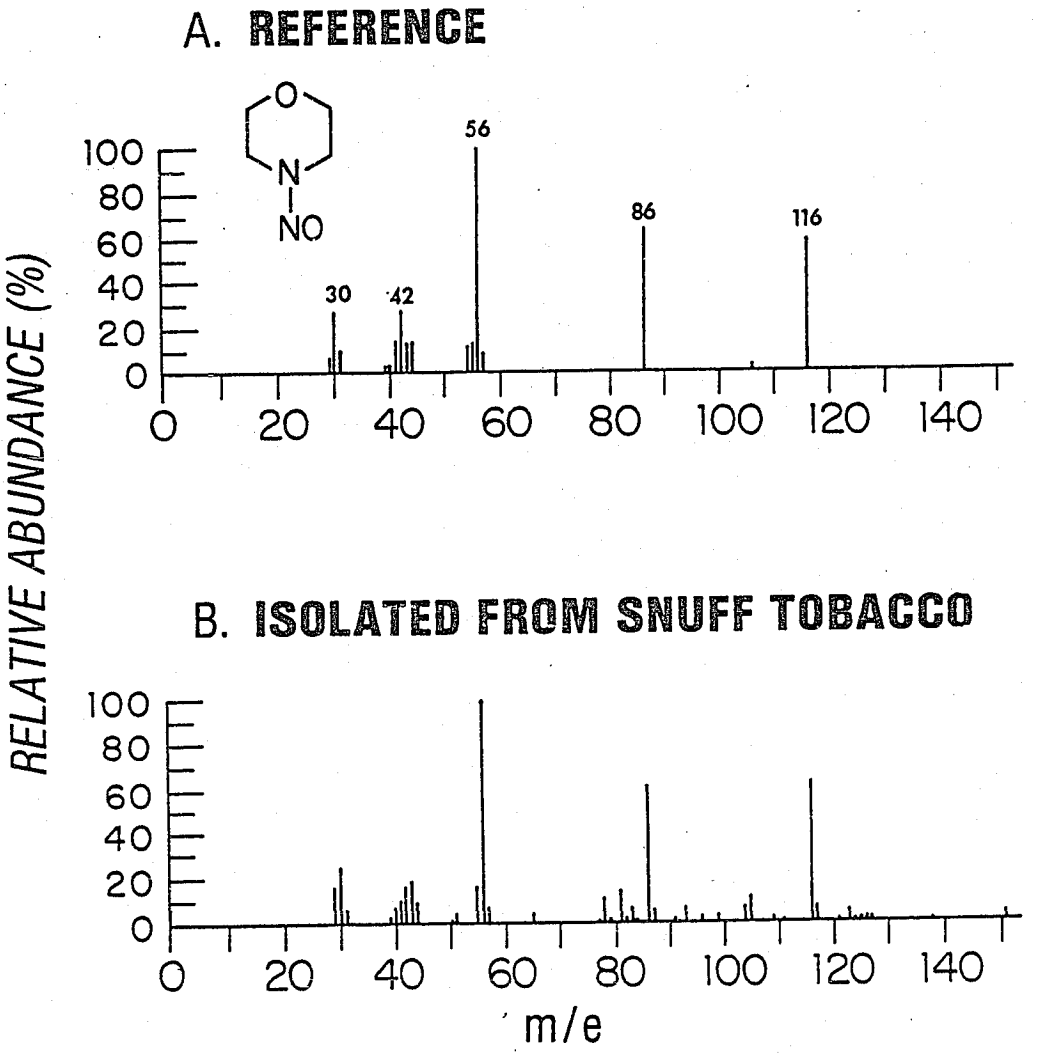

Table I

Nitrosomorpholine and Morpholine in Snuff and Snuff Containers (ppb)[a]

Snuff Brand		Snuff Tobacco		Snuff Container	
		NMOR	Morpholine	NMOR	Morpholine
USA	I	24	2,800	34	845
	II	690	1,500	10	170
	III	690	4,000	230	4,740
	IV	630	3,200	4	90
	V	31	2,200	3	140
Sweden	I	44	820	4	1,750
	II	(-)	200	3	460
	III	(-)	780	13	4,830
	IV	10	940	23	4,290
	V	(-)	2,500	N.D.	N.D.

[a]Based on dry weight in case of snuff; uncorrected for moisture in case of snuff containers. The latter had contained snuff previously.
Containers of USA I-III and Sweden I-IV were cardboard boxes with a metal lid, USA IV plastic container with individual snuff portions in porous paper bags; USA V plastic container; Sweden V individual snuff portions in Al-bags.

(-) Below detection limit (<2 ppb)

N.D. = not determined.

during extraction and analysis (11). When 31.2mg or 3.12mg of cis-2,6-dimethylmorpholine were added to the snuff, 1.62µg or 0.21µg of NDMMOR, respectively, were found. This demonstrates that 0.0052% and 0.0067% of the added amine have been N-nitrosated. This low degree of N-nitrosation indicates that the NMOR values observed in this study are not significantly increased by artifacts (<0.4 ppb). Table 1 shows that the concentration of morpholine in the snuff and snuff containers is not the yield determining factor for NMOR. Based on past experiences we know that the processing of snuff tobacco and its aging affect the N-nitrosamine yield (8). Table 1 indicates also that the containers are a possible source of morpholine in the snuff. In order to verify this concept, the waxy extracts from the snuff containers, [^{14}C]morpholine and moist snuff which was free of NMOR (brand II, Sweden), were incubated together at room temperature in sealed Petri dishes for one month (see MATERIALS AND METHODS). Analysis of the snuff for [^{14}C]NMOR showed in 2 parallel experiments that 0.60% and 0.51% of the [^{14}C]morpholine had diffused from the waxy layer in the bottom of the dish into the snuff and had been nitrosated to [^{14}C]NMOR. In the absence of [^{14}C]morpholine, however, the incubation of the waxy materials with snuff did not yield significant amounts of NMOR.

Table II presents an overview on VNA including NMOR, NDELA (9) and the four major tobacco-specific N-nitrosamines

Table II

N-Nitrosamines in Snuff (ppb)[a,b]

Snuff Brand		Volatile N-Nitrosamines				Tobacco-Specific N-Nitrosamines			
		NDMA	NPYR	NMOR	NDELA	NNN	NNK	NAT	NAB
USA	I	215	(-)	24	760	2,200	600	1,700	100
	II	37	120	690	1,700	19,000	2,400	19,000	800
	III	100	360	690	3,300	33,000	4,600	40,000	1,900
	IV	92	110	630	290	20,000	8,300	9,100	500
	V	(-)	(-)	31	600	830	210	240	10
Sweden	I	22	(-)	44	240	5,700	1,700	900	140
	II	60	(-)	(-)	225	6,100	1,000	2,200	80
	III	14	210	(-)	390	5,300	1,400	2,400	70
	IV	30	50	10	310	4,000	610	1,400	80
	V	(-)	(-)	(-)	290	2,000	800	1,400	40

[a]Values are based on dry weight.
(-) Below detection limit (<2ppb)
[b]Values for NDEA in snuff were below detection limit (<2ppb) except Sweden I, II, III and IV which had values of 6, 4, 12, and 5 ppb, respectively.

in snuff (7,8) For the analyses of NDELA and TSNA we applied previously published methods (9,15). NDELA derives from the sucker growth inhibitor maleic hydrazide diethanolamine residue on tobacco (9).

DISCUSSION

A few years ago we detected traces of VNA in snuff in a preliminary investigation (10). This study confirms that 5 popular U.S. snuff brands and 5 Swedish snuff products contain N-nitrosamines. Seven out of 10 snuff brands contained NMOR (10-690 ppb). Only one report in the literature refers to morpholine, the precursor for NMOR, as being detected in tobacco samples (12). Since we found appreciable amounts of morpholine (90-4,830 ppb) in the waxed cardboard containers which are used to package the snuff, we hypothesized that this compound may have partially diffused from the containers into the snuff where it may have contributed to morpholine and NMOR. This is of significance because NMOR is a strong animal carcinogen and morpholine is known to be N-nitrosatable *in vivo* (13). The diffusion of morpholine into the snuff was then demonstrated in a model experiment with [^{14}C] morpholine. However, the finding of 19,400 ppb of morpholine in a sample of loose leaf chewing tobacco, which was packaged in an aluminum pouch, indicates that there are other conceivable sources of morpholine contamination in processed tobacco, such as "casing solutions" (14). The latter are mixtures of hygroscopic agents and volatile and nonvolatile flavoring components which are applied to snuff. The exact composition of casing solutions are trade secrets.

The data in Table II should be evaluated in light of the findings from bioassays in Syrian golden hamsters. These have documented that NDMA is a very strong carcinogen, NMOR and NNK are strong carcinogens of about equal potency, NPYR, NNN and NDELA are moderately active carcinogens and NAT and NAB are weakly active (13,15-16). The carcinogenic potential of snuff must be kept at a minimum because snuff products are proven human carcinogens (3,4,17). Processing methods and packaging materials for snuff should be carefully selected so as to preclude the presence of morpholine which can contribute to the formation of NMOR in snuff as well as in vivo (13).

As Table II demonstrates, it is possible to offer snuff which is significantly lower in N-nitrosamines (Sample V USA and Sample V Sweden). N-Nitrosamines are the only known genotoxic agents in this human carcinogen. Their reduction in, or practical elimination from snuff products is necessary as a measure of risk reduction.

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Part III. N-Nitrosoproline, an Indicator of N-Nitrosation of Amines in Processed Tobacco

INTRODUCTION

Tobacco and tobacco smoke contain three types of N-nitrosamines, namely the volatile nitrosamines, N-nitrosodiethanolamine, formed from the residue of an agricultural chemical, and the tobacco-specific N-nitrosamines (1,2). These agents are not present in freshly cut leaves but are formed during tobacco processing (3,4). All of the N-nitrosamines so far identified in tobacco products are known animal carcinogens (5).

Recently, N-nitrosoproline (NPRO) has become an important tool in nitrosamine research. NPRO is considered nonmutagenic and noncarcinogenic (6); it is formed in man by endogenous N-nitrosation of proline and is excreted in the urine in unchanged form (7). Upon administration of dietary proline supplements, higher amounts of NPRO were found in the urine of some cigarette smokers than in urine of nonsmokers (8), thus it was questioned whether tobacco products contain NPRO. It was the goal of this study to explore the possible presence of NPRO in tobacco and tobacco smoke and to investigate whether this compound could serve as an indicator for the formation of TSNA during tobacco processing and smoking. The advantage of measuring N-nitrosation potential by determining the noncarcinogenic N-nitrosoproline

lies in avoiding the carcinogenic tobacco-specific N-nitrosamines for which reference compounds are not always available.

EXPERIMENTAL SECTION

Materials

All commercial tobacco products were purchased on the open market in Westchester County, NY, during 1982. Green Burley 21 tobacco plants were made available by Dr. T.C. Tso, U.S.D.A., Beltsville, MD. The leaves were harvested when the plants had reached maturity.

Chemicals

NPRO was synthesized from proline according to Lijinsky (9). Its purity was ascertained by gas chromatography (GC) and mass spectrometry (MS). The standard mixture of volatile N-nitrosamines was purchased from Thermo Electron Corp., Waltham, MA, the tobacco-specific N-nitrosamines (TSNA) were synthesized according to earlier published methods (10). L-Proline [U-^{14}C] (spec. act. 250μCi/μM) was obtained from ICN, Irvine, CA, and 14% BF_3 in methanol from Pierce Chemical Co., Rockford, IL.

Apparatus

A Model 543 Thermal Energy Analyzer (TEA) from the Thermo Electron Corp., Waltham, MA, was interfaced directly with a Model 700 gas chromatograph (Hewlett-Packard), using modifications previously described (11). The mass spectral analysis was done with a Hewlett Packard Model 5982 GC-MS

instrument. Cigarettes were smoked with a 20-port automatic smoker with a rotating head (H. Borgwaldt, Hamburg, FRG). Every second port of the smoking machine was connected to a nitrogen source which replaced the air with nitrogen in order to prevent the possible artifactual formation of nitrosamines in the headspace of the device and the trap (12).

Determination of NPRO in Tobacco

Five grams of tobacco were extracted for 2 h with 100ml water containing 6ml AS solution (20% ammonium sulfamate in 3.6N sulfuric acid) and NPRO-U-^{14}C (96,500 dpm/27ng) as an internal standard. Subsequently, the mixture was filtered through Celite 545. After adding 20g of NaCl, the filtrate was partitioned with 4 × 150ml ethyl acetate. The combined organic layers were dried (Na_2SO_4) and reduced to 0.1ml in a rotary evaporator. One ml of 14% BF_3 in MeOH was added to the residue and the mixture was heated at 60°C for 1 h. After cooling to ambient temperature, 1ml dichloromethane and 4ml water were added. The organic phase was removed and placed into a 1ml serum vial together with 100mg Na_2SO_4. An aliquot was used to determine the recovery rate by scintillation counting, and a second aliquot was analyzed by GC-TEA on a 6 ft × 1/4" o.d. (2mm i.d.) glass column packed with 10% Carbowax 20M on Chromosorb W (oven temperature 150°C).

For the mass spectral identification of NPRO, 100 g of snuff tobacco were extracted in 2000 ml water and the extract

was worked up as described under "Determination of NPRO in Tobacco" and further enriched by column chromatography on 60g of silica gel with a 1:1 mixture of methanol-ethyl acetate. The radioactive fraction was purified by preparative TLC. After extraction of the NPRO band, the final fraction was further concentrated and methylated prior to GC-MS identification. We used a 25m × 0.24mm (i.d.) fused silica capillary column (WCOT) coated with chemically bonded methyl silicone (film thickness 0.25μ). The GC program was 4 min. at 50°C, then 2°/min to 250°C. Under these conditions, the methyl ester of NPRO had a retention time of 11.3 min.

Determination of Free Proline in Tobacco as N-Nitrosoproline (NPRO)

One gram of processed tobacco was stirred for 2 h with 50ml water containing 1g $NaNO_2$, 2ml of 1N acetic acid, and 1ml proline-U-^{14}C (84,000 dpm/19ng). The determination of proline as NPRO was then carried out with the analytical procedure described above. In the case of green tobacco we homogenized ~25g of leaves, immediately after their removal from the stalk, in 150ml of diluted AS solution (see "Determination of NPRO in Tobacco") in a blender. The analysis was completed in the same manner as in the case of processed tobacco.

Analysis of NPRO and N-Nitrosopyrrolidine in Tobacco Smoke

For the untreated control, 100 cigarettes were smoked

with a 20-unit automatic smoker (see "Apparatus") and the mainstream smoke was led through 2 gas wash bottles containing citrate buffer and 20 mM ascorbic acid (10) with NPRO-U-^{14}C serving as internal standard (96,500 dpm/27ng). After addition of 80g of NaCl, the buffer solution was extracted with ethyl acetate and the latter was dried (Na_2SO_4). After concentrating to 2ml, the extract was further enriched by column chromatography (60g silica gel, mesh 40-140, Baker Chemicals) and then analyzed for NPRO (see "Determination of NPRO in Tobacco") and N-nitrosopyrrolidine (13). Only 10 cigarettes per analysis were smoked in the case of applications of 5 mg of either proline or NPRO in 50µl water by microsyringe technique (14).

Other Analytical Methods

Tobacco was analyzed for VNA and TSNA using earlier published methods (10,13). For the determination of moisture in tobacco, a modified Dean-Stark procedure was used (15). Nitrate in tobacco was determined by the specific ion electrode method (16).

Statistical Evaluation

Linear regression models were calculated on an IBM 4341 model II mainframe using a statistics analysis system (SAS Institute, Cary, North Carolina).

RESULTS AND DISCUSSION

Analysis of Tobacco for NPRO

A schematic representation of the NPRO analysis in tobacco is shown in Fig. 1. It involves extraction, solvent partition, and subsequent methylation as well as GC-TEA analysis. Figure 2 shows the GC-TEA trace of a concentrate from a cigarette tobacco extract depicting the methylester of NPRO (retention time 5.5 min.). In one case NPRO was enriched from 100g of snuff tobacco following the analytical scheme (Figure 1) with an additional column chromatographic step and preparative TLC; NPRO was then identified as the methyl ester by capillary GC-MS (Figure 3).

The identity of NPRO isolated from each tobacco sample was assured by established confirmatory methods (17). When treating both standard solution of NPRO and the concentrates from the tobacco extracts with HBr, the NPRO peak in the GC-TEA disappeared completely. For a more positive confirmation we oxidized NPRO to the corresponding nitramine (C_4H_7-(COOH)N-NO $\longrightarrow$ C_4H_7-(COOH)N-NO_2) using the method of Emmons (18), modified to include acetic anhydride and 50% hydrogen peroxide in place of trifluoroacetic acid anhydride and 90% H_2O_2 (19). (This treatment had to precede the methylation in order to avoid hydrolysis of the methyl ester of NPRO prior to GC-TEA analysis). A NPRO standard solution treated with BF_3/MeOH showed that approximately

Figure 1. Schematic for the Analysis of NPRO

ANALYSIS OF NPRO

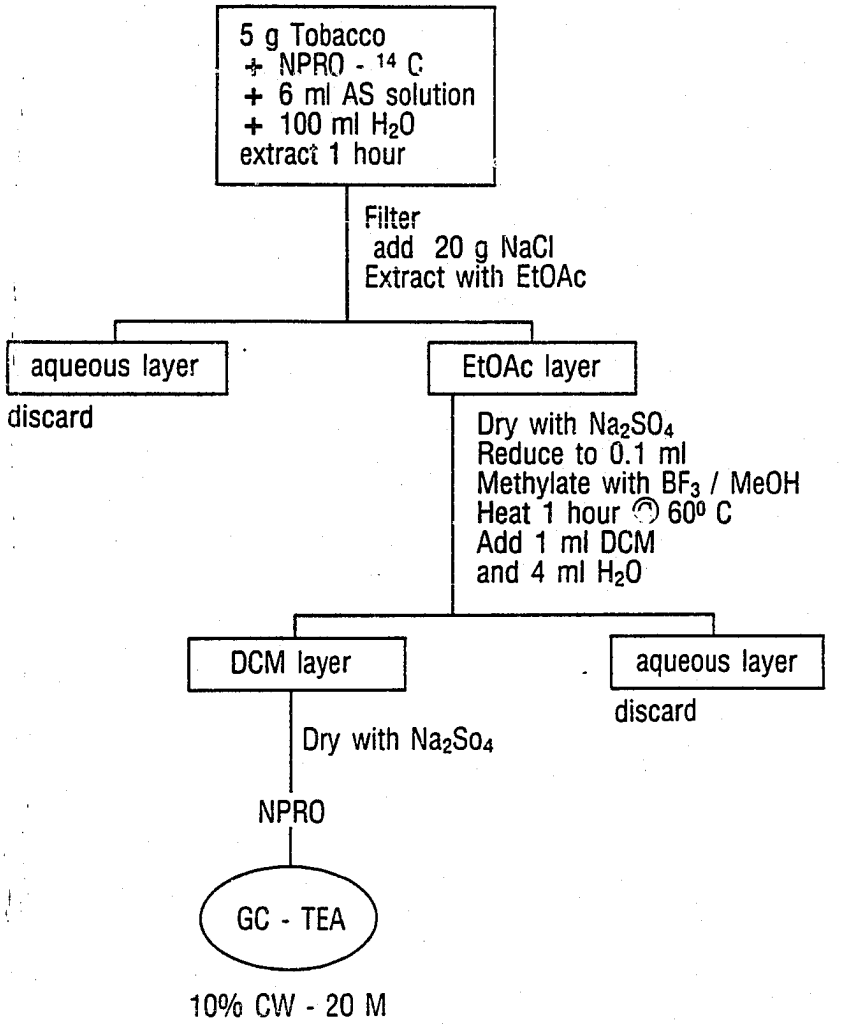


Figure 2. GC-TEA Trace of Cigarette Tobacco Extract (US, F, 85mm, Ultra Light) Showing the Presence of NPRO

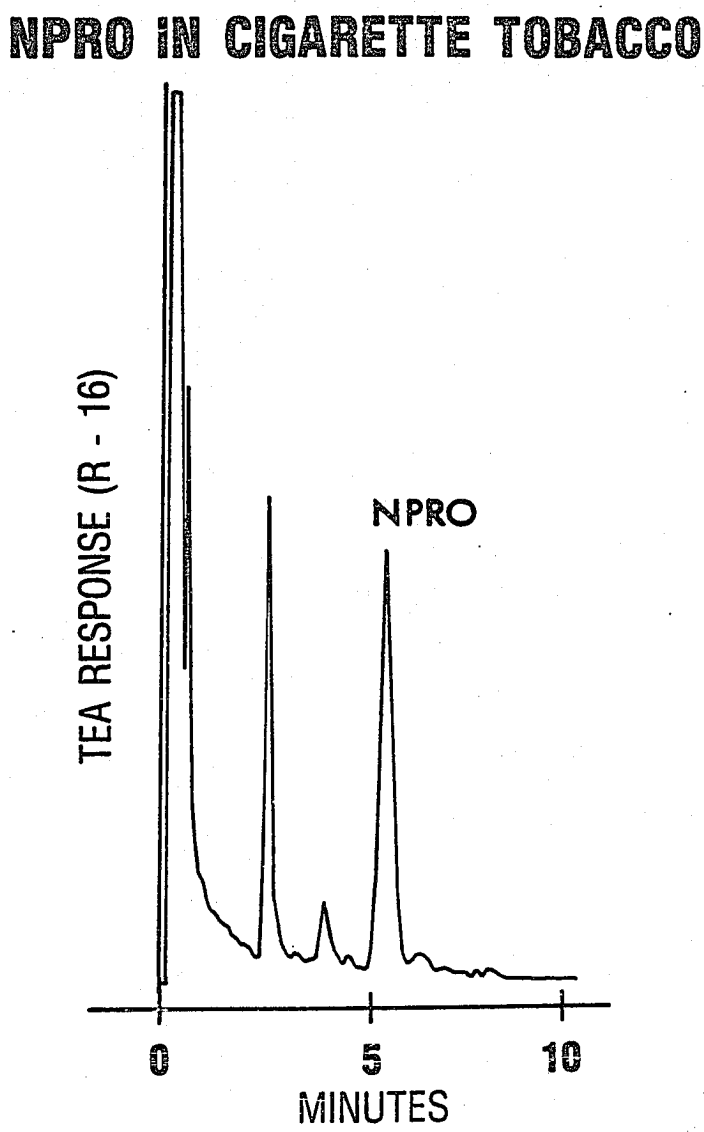
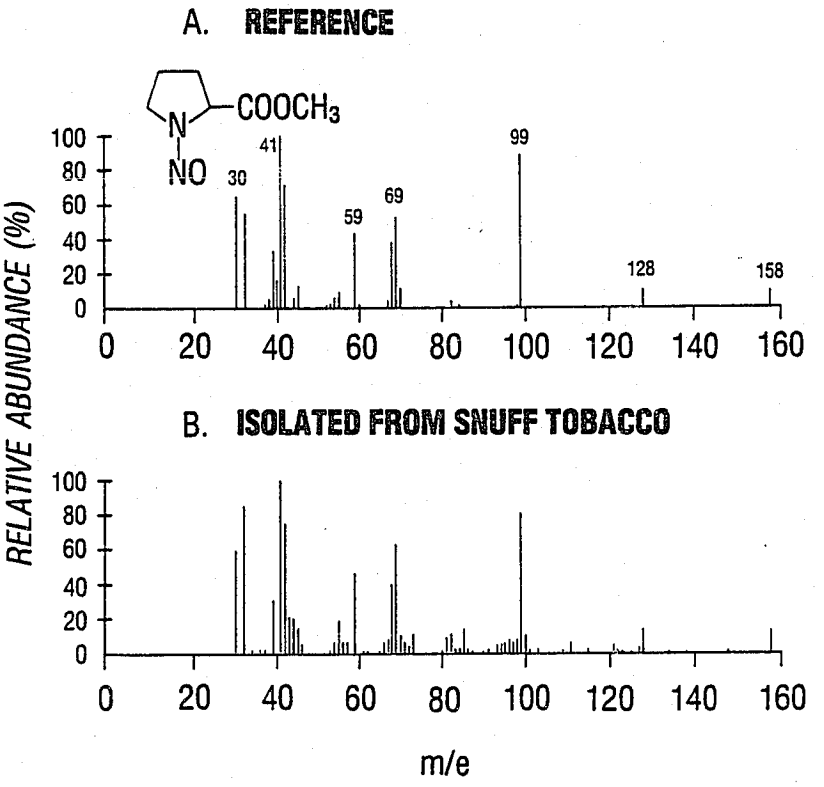


Figure 3. Mass Spectra of Reference and Isolate from Snuff Tobacco Showing NPRO as the Methyl Ester.



half of the NPRO was oxidized to the corresponding nitramine as detected by GC-TEA at a TEA pyrolyzer temperature of 800°C. The retention time of the methylated nitramine was exactly twice that of the methyl ester of NPRO.

Based on 5 analyses of the Kentucky reference cigarette, the relative standard deviation for NPRO and PRO was ±8%. According to the loss of the ^{14}C-labeled internal standard, 65-75% of NPRO and proline were enriched in the concentrate from which NPRO was determined as the methyl ester. Starting with 5g of processed tobacco, the detection limit was about 0.2ng/g.

When using 25g of green leaves (nitrate content 0.2%) which were analyzed immediately after removal from the stalk of a Burley 21 tobacco plant, we were unable to detect measurable amounts of NPRO (<5 ppb). This result supports our earlier observation (3,4) that N-nitrosamines are primarily formed during tobacco processing.

Table I lists the analytical data from 13 commercial tobacco products and from the Kentucky reference cigarette 1R1 for nitrate, nicotine, N'-nitrosonornicotine, the percentage of nicotine which is N-nitrosated to N'-nitrosonornicotine, total TSNA [N'-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, N'-nitrosoanatabine plus N'-nitrosoanabasine], proline, NPRO, and the percentage of N-nitrosated proline. The results for up to seven volatile N-nitrosamines in the tobacco of the same 14 tobacco products

Table I

Analytical Data for Commercial Tobacco Products[a]

Tobacco Product[b]	NO_3 (%)	Nicotine (%)	NNN (ppm)	% Nitrosation × 10^{-2}[c]	Total TSNA (ppm)	PRO (ppm)	NPRO (ppm)	% Nitrosation[d]
US,F,85(A)	0.81	1.82	2.64	1.33	3.73	1620	1.50	0.075
US,F,85(B)	1.23	1.45	2.17	1.37	4.95	1070	1.60	0.12
US,NF,85	0.70	2.05	1.83	0.81	3.61	990	0.88	0.071
US,NF,70	1.08	1.81	1.96	0.99	4.09	860	1.20	0.11
US,F,85, menthol	1.14	2.04	1.94	0.87	4.09	800	1.45	0.14
US,F,85, light	0.89	1.66	4.44	2.44	8.90	960	2.30	0.19
US,F,85, ultra lt	0.74	1.72	3.20	1.70	6.44	890	1.58	0.14
French,NF,70	0.93	1.25	1.80	1.32	2.70	980	1.55	0.13
French,F,70	0.98	1.20	0.64	0.48	0.98	565	1.43	0.20
Kentucky 1R1,85	0.53	2.30	0.68	0.27	1.08	1280	0.33	0.021
Cigar	1.98	1.10	2.94	2.43	4.78	100	1.13	0.92
Fine cut Snuff	3.48	2.42	20.5	7.71	33.3	480	21.8	3.66
Snuff (pouches)	2.73	1.91	6.63	3.17	15.1	400	3.48	0.70
Loose Leaf	2.20	0.92	1.16	1.16	1.98	160	0.45	0.22

[a]Based on dry tobacco weight.

[b]Numbers indicate length in mm of cigarettes; F = filter, NF = non-filter

[c]N-nitrosation of nicotine to NNN; [d]N-nitrosation of PRO to NPRO

Abbreviations: NNN = N'-nitrosonornicotine; TSNA = tobacco-specific N-nitrosamines; PRO = proline; NPRO = N-nitrosoproline

are summarized in Table II.

It was the goal of this study to explore whether the noncarcinogenic N-nitrosoproline could serve as an indicator for the formation of the tobacco-specific N-nitrosamines of which 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone is a strong animal carcinogen (5). As summarized in Table III, the data from these 14 tobacco products support the concept that NPRO can serve as an indicator for the formation of N'-nitrosonornicotine (r^2 = 0.961) and of TSNA (r^2 = 0.899). These statistically significant correlations are not much further enhanced by adding nitrate as a second variable (r^2 = 0.966 and 0.918) or proline (r^2 = 0.962 and 0.903) or both variables (r^2 = 0.966 and 0.918).

Fine Cut Snuff

In agreement with earlier findings (2,5), the highest amounts for N'-nitrosonornicotine and other tobacco-specific N-nitrosamines are found in fine cut snuff (Table I). During the processing of tobacco to this product, including fire curing, in this sample about 0.07% of nictoine have been N-nitrosated to carcinogenic N'-nitrosonornicotine. As was to be expected, the nitrosation of proline to NPRO is also proportionately high (3.7%), demonstrating further that the processing to fine cut snuff enhances the potential for the formation of N-nitrosamines. This observation cannot be explained by the relatively high nitrate content of the snuff

Table II

Volatile N-Nitrosamines in commercial Tobacco Products (ppb)[a]

Tobacco Product[b]	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR	Total VNA
US,F,85(A)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
US,F,85(B)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
US,NF,85	280	47	n.d.	n.d.	15.3	4.9	3.7	350
US,NF,70	250	n.d.	n.d.	1.5	5.5	n.d.	4.1	260
US,F,85, menthol	6.0	n.d.	n.d.	n.d.	n.d.	3.6	n.d.	10
US,F,85, light	6.7	2.0	2.3	5.0	7.0	9.9	10	43
US,F,85, ultra light	4.4	n.d.	n.d.	2.8	n.d.	5.9	n.d.	13
French,NF,70	42	7080	n.d.	n.d.	n.d.	7.5	1.1	7120
French,F,70	58	7870	n.d.	n.d.	11	n.d.	n.d.	7940
Kentucky 1R1,85	12.7	2.7	2.7	4.4	4.0	n.d.	7.6	34
Cigar	n.d.	3.2	11.8	0.9	22	20	6.4	64
Fine cut snuff	74	n.d.	n.d.	n.d.	14	115	44	247
Snuff (pouches)	15	n.d.	2.7	n.d.	13	140	36	207
Loose leaf	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-

[a] based on dry tobacco weight.

[b] Numbers indicate length in mm of cigarettes; F=filter; NF = nonfilter
Abbreviations: NDMA = nitrosodimethylamine, NDEA = nitrosodiethylamine, NDPA = nitrosodipropylamine, NDBA = nitrosodibutylamine, NPIP = nitrosopiperidine, NPYR = nitrosopyrrolidine, NMOR = nitrosomorpholine, VNA = volatile nitrosamines, n.d. = not detected (<0.5 ppb)

Table III

Statistical Evaluations (Commercial Tobacco Products)

Number in Model	Independent Variable	Dependent Variable	r^2
1	NO_3	NNN	0.592
1	nicotine	NNN	0.238
1	NO_3	NPRO	0.522
1	PRO	NPRO	0.052
1	NPRO	NNN	0.961
1	NPRO	TSNA	0.899
2	NPRO, NO_3	NNN	0.966
2	NPRO, NO_3	TSNA	0.918
2	NPRO, PRO	NNN	0.962
2	NPRO, PRO	TSNA	0.903
3	NPRO, PRO, NO_3	NNN	0.966
3	NPRO, PRO, NO_3	TSNA	0.918

Abbreviations: See footnote of Table I

tobacco (~3.5%). The values for volatile N-nitrosamines are not especially high, with the possible exception of N-nitrosopyrrolidine (0.12 ppm), and N-nitrosomorpholine (0.04 ppm). These two compounds are the least volatile ones of the group of volatile N-nitrosamines. Since the amounts of carcinogenic nitrosamines are about two orders of magnitude higher in fine cut snuff than in any other nonoccupational materials in man's environment, this observation strengthens our working hypothesis that the nitrosamines, especially the tobacco-specific N-nitrosamines, contribute significantly to the increased risk of snuff dippers for cancer of the oral cavity (1,21). While it may be difficult to document the role of TSNA in oral cancer risk of snuff dippers, it is a fact that they are the only known carcinogens in snuff. Therefore, the reduction of the carcinogenic nitrosamines deriving from the tobacco alkaloids should certainly be an important goal for the modification of snuff products.

Cigarette Smoke Analysis

Finally, it was explored whether cigarette smoke contains NPRO and whether proline or NPRO in tobacco give rise to NPRO in cigarette smoke. One hundred cigarettes were smoked under standard conditions with a 20-port automatic smoker (see "Experimental Section") and the mainstream smoke was trapped in two gas wash bottles containing a buffer solution with ascorbic acid (pH 4.5; 10). The actual analytical procedure

beginning with repeated ethyl acetate extraction was identical to the procedure used for the tobacco analysis as outlined in Figure 1. Despite repeated analysis we were unable to detect NPRO in tobacco smoke. When 5mg of proline or 5mg of NPRO were added to the 62mm cigarette column with a microsyringe and when the mainstream smoke of 100 cigarettes spiked with proline or NPRO was analyzed, we found that PRO and especially NPRO serve as precursor for N-nitrosopyrrolidine in the smoke (Table IV). Upon spiking of the cigarette with 5mg NPRO we found 7,140ng NPRO per cigarette in the mainstream smoke corresponding to a transfer rate of 0.14%. Since this test cigarette contained 0.88 ppm (680 ng) NPRO in the 62mm tobacco column smoked, an 0.14% transfer rate would account for 0.9ng/cigarette of the nitroso compound in the smoke. Since this calculated amount is close to the detection limit, one may conclude that if cigarette smoke contains any NPRO, it is less than 1.0ng per cigarette.

Table IV

The Fate of PRO and NPRO During Smoking

Cigarette[1]	Mainstream Smoke		
	NPYO		NPYR
	ng/Cig.	% Transfer	ng/Cig.
Control	n.d.[2]	-	5
+5mg PRO	trace[3]	-	49
+5mg NPRO	7,140	0.14	2,530[4]

[1] 85mm non-filter cigarette; 0.70% NO_3, 990 ppm PRO, 0.88 ppm NPRO

[2] n.d. = not detected (detection limit 0.5ng/cig.)

[3] Approx. 1ng/cig.

[4] 0.073% by decarboxylation.

Abbreviations: See footnotes of Tables I and II.

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